Acta Scientiarum
http://periodicos.uem.br/ojs/acta
ISSN on-line: 1807-8672
Doi: 10.4025/actascianimsci.v42i1.48734

Effects of antibiotic replacement with garlic powder and probiotic on performance, carcass characteristics, oxidative enzymes and intestinal morphology of broiler chickens

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ABSTRACT. An experiment was conducted to evaluate the effects of probiotic and garlic powder instead of antibiotic on performance, carcass characteristics, oxidative enzymes and intestinal morphology of broiler chickens. A total of 200 one-day-old male broiler chicks were used in a completely randomized design. The experimental groups were including control group (without any additives) or CG, antibiotic group or AG, garlic powder group or GG, probiotic group or PG and garlic powder plus probiotic group or GPG. The broilers were weighted at the end of days 10, 24 and 42 to evaluate the body performance. At the end of experiment, four broilers randomly selected from each replicate to blood sampling and carcass traits measurement (2 chickens for each one). The use of GG significantly decreased feed intake than AG ($p < 0.05$) which approved in GPG when probiotic added to GG ($p < 0.05$). All groups, exception GG showed less feed intake than CG between days 0 to 42 of experiment ($p < 0.05$). GPG shows significant differences than CG between days 21 to 42 and 0 to 42 and also than GG between days 0 to 42. The use of GG and GPG significantly increased liver enzyme activities ($p < 0.05$). AG, PG and GPG showed a higher height and width villi than CG. So simultaneous use of probiotic and garlic powder can be a suitable alternative to antibiotics to normal performance and liver function

Keywords: virginamycin; Ross 308; medicinal plants; villi; perimalac.

Received on July 14, 2019.
Accepted on October 4, 2019.

Introduction

Protein is an important ingredient in human foods which is necessary to more produce of it along with other nutritious items. Antibiotics have always been used as the most important growth factor in poultry feed to produce more protein and also they were used to treatment and protection the animal bodies against diseases (Revolledo & Ferreira, 2010). They also used as a growth stimulus; but the resistance of microorganisms against their antibiotics is the major problem to use them (Diaz-Sanchez, D'Souza, Biswas, & Hanning, 2015). The studies have been done on animals clearly shows this problem and they found several kinds of microorganisms resistant to antibiotics in their products (Caly, D'Inca, Auclair, & Drider, 2015). Because the microorganisms can transfer from one environment to another, so resistant can spread to other animal bodies or humans (Braykov et al., 2016). Antibiotic resistant microorganisms can be transmitted through oral and non-oral routes which lead to ineffective of many types of antibiotics (Abujradah, Pandey, & Pandey, 2018). Therefore, researchers are looking for materials or methods to use instead of antibiotics that microorganisms can not resist against it and can also perform antibiotic activities. In this respect probiotics that are producing from the beneficial microorganisms of digestive tract showed the suitable results to be replaced with antibiotics (Teshfam, Nodeh, & Hassanzade, 2005; Bednarczyk, 2016).

Garlic as an available plant is also a good choice to use. Garlic has different properties such as antimicrobial, immunostimulant, reducer of fat, cholesterol (Jarriyawattanachaikul, Chaveerach, & Chokesajjawatee, 2016) and low density lipoprotein (LDL) (YIN & Cheng, 2003), therefore that is suitable to use in this regard. Adding one percent of garlic in broiler diet improves their performance (Karangiya et al., 2016). This study was conducted to determine the effects of antibiotic replacement with garlic powder and probiotic on body performance, carcass characteristics and small intestinal tissue of broiler chicks.

Material and methods

This experiment was conducted at the farm of Islamic Azad University of Darab, Fars, Iran. 200 one-old-day male broiler chicks (Ross, 308) weighted on arrival (average weight of 46.3 ± 0.5) and randomly assigned to five treatments and -four replicates of 10 birds in a completely randomized design. The study performed a 42- day trial with broilers housed on floor pens. Broiler chickens were fed by corn-soybean based diets during rearing period. Food and water were supplied *ad-libitum*. Weighting of fed and broilers were done at the end of days 10, 24 and 42 to evaluate body performance. At the end of experiment, two birds from each replicate of treatments were slaughtered to evaluate carcass characteristics. The composition of the basal ration is given in Table 1. Treatments included: control group [no antibiotic, probiotic and garlic powder] or CG, group 2 [containing antibiotic (16.5g ton^{-1} virginamycin)] or AG, group 3 [containing garlic powder (1%)] or GG, group 4 [containing probiotic (0.9 g /kg diet)] or PG and group 5 [containing garlic powder (1%) plus probiotic (0.9 g kg^{-1} diet)] or GPG. The probiotic used in this study was *Perimalac* (1×10^8 cfu g^{-1}). Diets were formulated according to the nutritional requirements of chickens Ross (Aviagen, 2014). The broilers fed into three nutritional periods contain: starter (0-10 days), grower (11-24 days) and finisher (25 – 42 days).

Body performance

Body performance of each replicate including feed intake (FI), body weight gain (BWG) and feed conversion ratio (FCR) was calculated at the end of days 10, 24 and 42.

Carcass characteristics

Randomly selected two birds from each replicate and slaughtered after a fasting period (four hours) and evaluated the changes of carcass traits.

Blood sampling and measurements

On day 42, two broilers randomly selected from each replicate and blood samples were taken from wing's vein (3 mL/bird) and then the blood serum was separated with centrifuge at 2000 × g for 15 min and stored at -20°C. Individual serum samples were analyzed for aspartate aminotransferase (AST), alkaline aminotransferase (ALT) and alkaline phosphatase (ALP) using an automated analyzer (Technicon RA-1000, Tarrytown, USA) according to the commercial kit protocol (Pars Azmoon Company; Tehran, Iran). Isolated serum was transferred to laboratory of Kharazmi University on day 42 to determine the activity of oxidative enzymes.

Table 1. Ingredients and nutrient composition of basal ration

Ingredient	Starter (0-10 days)	Grower (11-24 days)	finisher (25-42 days)
Corn	52.86	58.13	60.24
Oil, vegetable	3.83	4.57	5.32
Soybean meal (44%)	37.35	28.99	30.86
DL-Methionine	0.36	0.28	0.25
L-Lysine HC	0.25	0.15	0.14
L-Threonine	0.1	0.00	0.04
Limestone	1.6	0.97	1.43
Fish meal	2	5	0
NaCl	0.25	0.27	0.3
Vit. & Min. permix	0.5	0.5	0.5
Dicalcium Phosphate	0.9	1.13	0.93
Total	100	100	100
Nutrients Analysis			
Crude Protein%		21	19
Energy (kcal/kg)	3025	3150	3200
Lysine(SID)%	1.43	1.24	1.09
Met+Cys(SID)%	1.07	0.93	0.86
Threonine(SID%)	0.78	0.71	0.62
Tryptophan(SID)%	0.32	0.45	0.26
Calcium%	1	0.9	0.85
Ava. Phosphorus%	0.5	0.46	0.42

Vitamin & mineral premix (each kg contained): vitamin A, 1800000 IU; vitamin D3 , 400000 IU; vitamin E, 3600 IU; vitamin K3 , 400 mg; thiamine, 360 mg; riboflavin, 1320 mg; niacin, 6000 mg; vitamin B6 , 600 mg; vitamin B5 , 2000; vitamin B12, 3 mg; Folic acid, 200 mg; biotin, 20 mg. Choline 80 g; zinc, 17 g; iron, 10 g; copper, 2 g; manganese, 20 g; selenium, 40 mg; iodine, 200 mg.

Small intestinal tissue morphology

At the end of the experiment (end of day 42), after a period of starvation (four hours), two chicks from each replicate randomly selected and slaughtered after emptying the contents of the intestine and then several pieces were cut in sizes of 2 cm. Intestinal pieces were placed in 10% formalin buffer. Morphological studies of the intestine were performed based on the method of the Teshfam et al. (2005).

Statistical analysis

Statistical analysis was performed using the software Statistical Analysis Software (SAS, 2000). Means were compared using Duncan`s multiple-range test and significance was determined at $p < 0.05$ (Duncan, 1955).

Results

Body performance

Tables 2, 3 and 4 respectively show the effects of treatments on, feed intake, body weight gain and feed conversion ratio of broiler chickens. Respectively the highest and lowest feed intake were observed in GPG and CG in the first stage of the rearing period (days 0 to 10). The difference of these two averages shows statistically significant ($p < 0.05$).

Table 2. Effects of antibiotic replacement with probiotic and garlic powder on feed intake of broiler chickens.

Treatments	0-10	11-24	25-42	0-42
CG	382.22^{b}	1069.65^{b}	3108.29^{a}	4656.16^{a}
AG	396.58^{ab}	1103.85^{a}	3053.79^{a}	4554.22^{b}
GG	388.72^{ab}	1112.16^{a}	3094.96^{a}	4595.84^{ab}
PG	391.28^{ab}	1059.29^{b}	3066.12^{a}	4516.69^{b}
GPG	401.52^{a}	1058.30^{b}	3044.91^{a}	4504.73^{b}
Standard error	11.59	17.39	44.62	53.38

Means with different superscripts in the same column are significantly different at $p < 0.05$.

In the second stage of the rearing period (11 to 24 days), respectively the highest and lowest feed intake were observed in GG and GPG. AG and GG showed statistically higher feed intake than other experimental groups ($p < 0.05$). The use of antibiotic in this stage significantly increased feed intake in AG than CG, PG and GPG ($p < 0.05$). There were no significant differences in feed intake between the averages obtained from different groups in the last stage of the experiment (25 to 42 days) ($p > 0.05$). Between the days 0 to 42 the highest feed intake showed in control group which created significant differences with PG and GPG ($p < 0.05$) exception GG ($p > 0.05$). There were no significant differences between all other experimental groups ($p > 0.05$).

The maximum of the body weight gain showed in GPG.

Table 3. Effects of antibiotic replacement with probiotic and garlic powder on body weight gain of broiler chickens.

Treatments	0-10	11-24	25-42	0-42
CG	268.24^{b}	668.53^{c}	1387.63^{b}	2324.40^{c}
AG	278.38^{a}	721.47^{a}	1468.17^{a}	2477.02^{ab}
GG	279.66^{a}	708.38^{b}	1432.85^{b}	2420.89^{b}
PG	283.54^{a}	715.74^{a}	1453.14^{ab}	2452.42^{ab}
GPG	293.08^{a}	734.93^{a}	1485.32^{a}	2513.33^{a}
Standard error	10.39	14.75	20.51	41.32

Means with different superscripts in the same column are significantly different at $p < 0.05$.

The means of feed intake showed in GPG, PG and AG showed significant differences with control group at the period between days 0 to 10 and with groups of control and GG at the periods between days 11 to 24 and 25 to 42 ($P < 0.05$). AG has shown significant differences with GG at the periods of days 11 to 24 and 25 to 42. AG had no significant differences with GG and GPG who used garlic powder in their diets ($p > 0.05$). Concomitant use of probiotics and garlic powder (GPG) in various stages of experiment (0 to 10, 11 to 24, 25 to 42 and 0 to 42 days) showed no difference of body weight gain with the birds used antibiotic in their diets ($p > 0.05$). Also comparison the mean of body weight gain of GPG with the mean of GG showed significant increases at the periods of days 11 to 24, 25 to 42 and 0 to 42 ($p < 0.05$) which are represents of the incremental effects of these two factors.

Despite improvements in FCR in the first stage of the rearing period (0 to 10 days), no significant difference was observed between the averages of experimental groups ($p > 0.05$). The best and worst FCR generated in the second stage of the rearing period (11 to 24 days), respectively created in the experimental groups of GPG and CG. Comparison of the averages of these two groups show a statistical significant difference ($p < 0.05$). There were no significant differences between of all other experimental groups ($p > 0.05$). One thing to note at this stage of rearing period is the no significant differences between AG with GG ($p > 0.05$). Unlike the second stage of the rearing period (days 11 to 24), in the third stage (days 25-42), AG shows significant difference with CG ($p < 0.05$). There is also a significant difference between CG and GPG ($p < 0.05$). But there is no difference between GG and PG with CG ($p > 0.05$). Respectively the best FCR showed in GPG and AG and the worst, created in CG in this stage of experimental period (days 11 to 24). Several significant differences can be seen between 0 and 42 days of age. In this period, CG showed significantly worse performance than other experimental groups ($p< 0.05$). The best performance is also created in GPG which shows a significant difference with GG ($p < 0.05$) and these observations reflect the effects of garlic powder in collaboration with probiotic to improve FCR between days 0 to 42. The groups of GG and PG didn't show any significant difference with AG ($p < 0.05$).

Table 4. Effects of antibiotic replacement with probiotic and garlic powder on feed conversion ratio of broiler chickens.

Treatments	0-10	11-24	25-42	0-42
CG	1.41^{a}	1.60^{a}	2.24^{a}	2.00^{a}
AG	1.38^{a}	1.53^{ab}	2.08^{b}	1.84^{bc}
GG	1.39^{a}	1.57^{ab}	2.16^{ab}	1.90^{b}
PG	1.38^{a}	1.48^{ab}	2.11^{ab}	1.84^{bc}
GPG	1.37^{a}	1.44^{b}	2.05^{b}	1.79^{c}
Standard error	.03	.04	.07	.05

Means with different superscripts in the same column are significantly different at $p < 0.05$.

Carcass characteristics

There were no significant differences between the average weights of the organs of breast, gizzard, neck, carcass, wings and thigh ($p > 0.05$), but the average weights of heart, spleen, liver and abdominal fat showed several significant differences ($p < 0.05$).

Heart

The use of antibiotic, garlic powder and probiotic significantly decreased the average weight of the heart tissue of the different experimental groups than control group ($p < 0.05$); however, the use of these factors didn't cause any significant differences to each other ($p > 0.05$).

Table 5. Effect of antibiotic replacement with garlic powder and probiotic on various slaughter traits of broiler chickens (g/kg pre-slaughter live body weight) (0 to 42 d of age).

Treatments	carcass	heart	gizzard	spleen	neck	breast	Abdominal fat	Liver	wings
CG	73.23^{a}	$.48^{a}$	1.16^{a}	0.06^{c}	5.27^{a}	24.79^{a}	2.11^{a}	1.67^{b}	7.41^{a}
AG	74.81^{a}	$.42^{b}$	1.14^{a}	0.08^{bc}	5.19^{a}	25.51^{a}	1.88^{ab}	1.75^{b}	7.33^{a}
GG	74.20^{a}	$.41^{b}$	1.22^{a}	0.10^{ab}	5.39^{a}	25.77^{a}	1.37^{b}	2.27^{a}	7.54^{a}
PG	72.96^{a}	$.44^{ab}$	1.23^{a}	0.08^{bc}	5.24^{a}	26.14^{a}	1.56^{ab}	1.79^{b}	7.29^{a}
GPG	75.37^{a}	$.43^{ab}$	1.19^{a}	0.12^{a}	5.16^{a}	24.96^{a}	1.24^{b}	2.46^{a}	7.38^{a}
Standard error	4.11	.03	.18	.003	.34	1.38	.16	.22	.42

Means with different superscripts in the same column are significantly different at $p < 0.05$.

Abdominal fat

The use of garlic powder or probiotic with garlic powder decreased average weights of abdominal fat ($P < 0.05$); but AG and PG didn't show any significant difference than CG ($p > 0.05$).

Spleen

The spleen average weight of GPG shows significant differences with groups PG, AG and control ($p < 0.05$). Also another significant different can show between the spleen average weights of GG and control ($p < 0.05$).

Liver

GPG and GG showed significant differences with all other groups ($p > 0.05$).

Liver enzymes

Liver enzymes appeared in serum at day 42 of age are presented in Table 6. According of this table the use of garlic powder and probiotic significantly has decreased the activity of all three liver enzymes including AST, ALT and ALP ($p < 0.05$). Also the use of probiotic has decreased enzyme activities of AST and ALT ($p < 0.05$).

Table 6. Effects of antibiotic replacement with probiotic and garlic powder on liver oxidative enzyme activities.

Treatments	ALT	ALP	AST
CG	12.72^{a}	1659.31^{a}	40.47^{a}
AG	12.43^{a}	1434.60^{b}	38.03^{ab}
GG	11.21^{bc}	1297.58^{c}	35.19^{b}
PG	11.68^{b}	1337.38^{c}	35.82^{b}
GPG	10.86^{c}	1208.71^{d}	33.64^{c}
Standard error	0.79	29.37	2.91

Means with different superscripts in the same column are significantly different at $p < 0.05$

Small intestinal morphology

Figure 1 shows the villi height differences between the different parts of the small intestine. According to observations in duodenum and jejunum, the chicks that used antibiotic and garlic powder with probiotic in their diet had the highest height of villi in their intestinal duodenum part; and they show no significant difference with each other ($p < 0.05$). Also, as it can be seen, the height of the small intestinal villi in groups using probiotic and garlic powder were significantly higher than the control group ($p < 0.05$). In ileum part, the observed statistical differences were similar of duodenum and jejunum except there were no statistically significant differences between the groups using diets include antibiotics, probiotic and probiotic with garlic powder with each other ($p > 0.05$). The Figure 2 shows the villi width differences between the different sections of small intestine. Based on this Figure, in the intestinal segments of duodenum and jejunum, the chicks have used antibiotic, garlic powder and garlic powder plus probiotic have the maximum width of villi; and AG and GPG have significant statistical differences with the control and probiotic groups ($p <0.05$). The results of the villi width in ileum part indicate that, like the duodenum and jejunum, antibiotics and garlic powder plus probiotic show the most intestinal widths of villi in this part; But unlike those two previous parts (duodenum and jejunum), these two treatments have significantly increased wider villi than those have used only probiotic in their diet ($p <0.05$); However, all treatments have a larger width than the control group ($p < 0.05$).

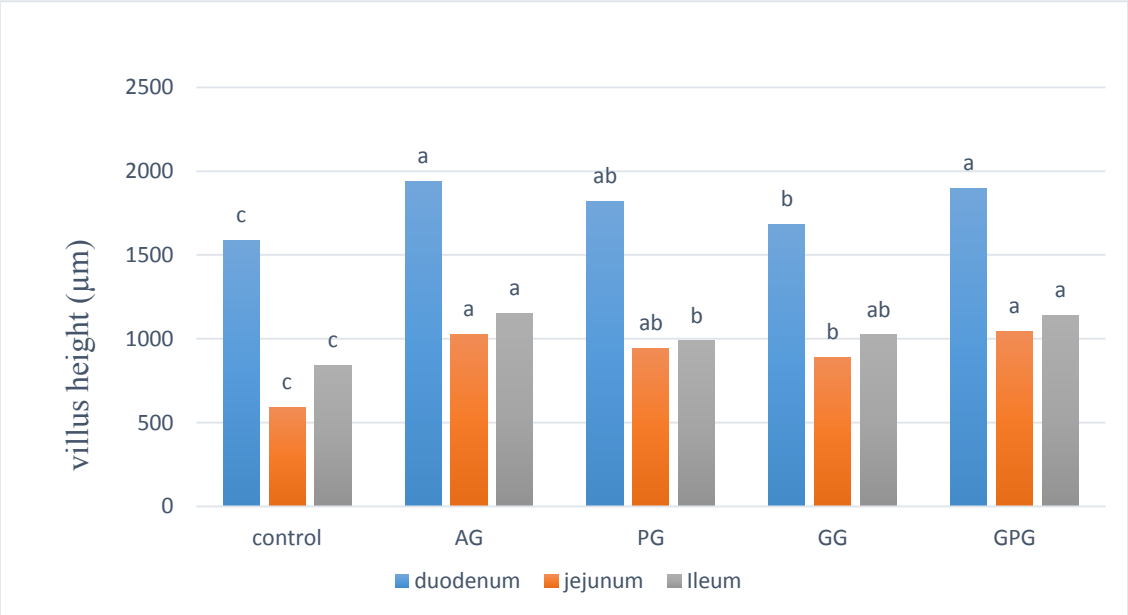

Figure 1. Villi height differences in different parts of the small intestine.

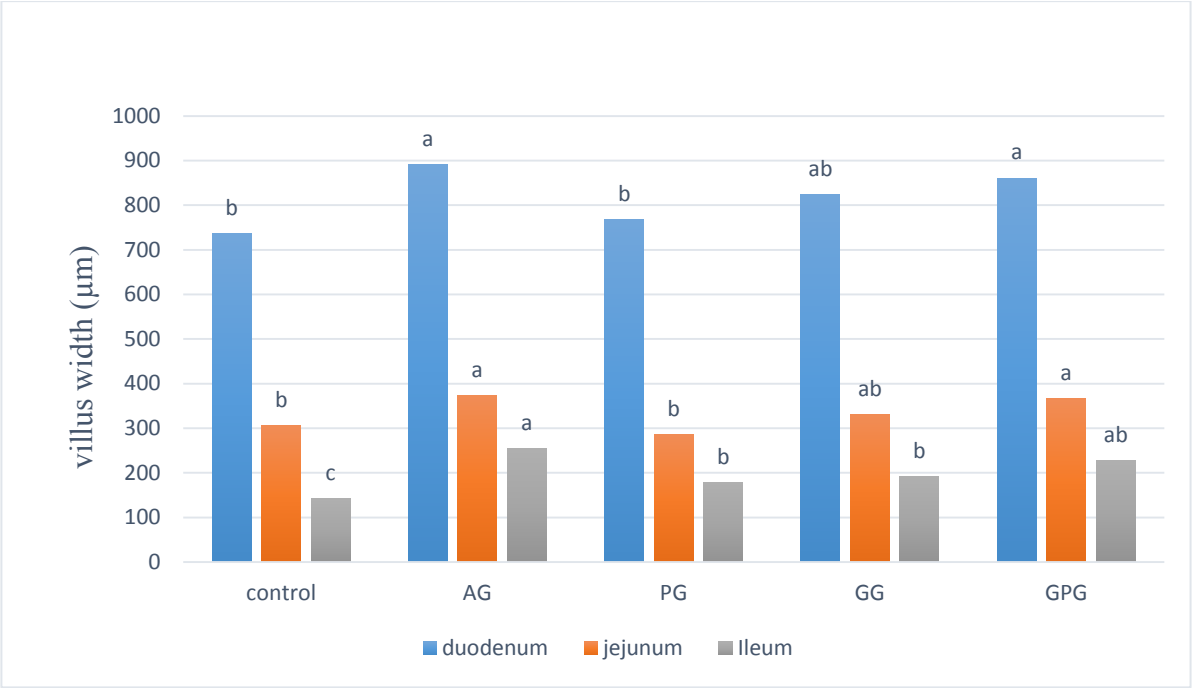

Figure 2. Villi width differences in different parts of small intestine.

There was no statistically significant difference in depth of crypts lieberkuhn between the averages obtained from different treatments ($p > 0.05$).

Discussion

Body performance

Respectively the most increases BWG have been created in GPG, AG, PG and GG. Several studies have been reported the effects of antibiotics on BWG (Fasina, Newman, Stough, & Liles, 2016; Salim, Hugue, Kamaruddin, & Beg, 2018; Hyun et al., 2018). This effect of antibiotics increased the body's growth in chickens and they achieved the second rank of body weight gain in this experiment. Antibiotics kill harmful microorganisms in the digestive tract and other body tissues and as a result increase body growth (Sanjay et al., 2018). Reducing the number of microbes in the digestive tract and other parts of the body by antibiotics lead to healthy, reducing energy consumption and thus lead to increased growth (Onyimonyi, Chukwuma, & Igbokwe, 2012). Probiotics stimulate the immune system. Also they prevent harmful microorganisms by binding to gastrointestinal tract and occupy the colonization sites (Sergeant et al., 2014). Probiotics produce some antimicrobial substances to eliminate the harmful microbes which finally lead to improved health, decreased digestive tract energy consumption and increased body growth (Adil, Banday, Bhat, Mir, & Rehman, 2010). Antimicrobial compounds found in garlic powder and the effects of this additive improve the functioning of the body's growth by strengthening the cardiovascular system – vessel (Elagib, El-Amin, Elamin, & Malik, 2013). These reasons improved the body performance of group GPG than all other groups.
The use of antibiotic (AG), garlic powder (GG), probiotic (PG) and garlic powder with probiotic (GPG) at the first stage of rearing period (days 0 to 10) and also the use of antibiotic (AG) and garlic powder (GG) at the second stage of rearing period (days 11 to 24) have increased feed intake than control group. Stimulation of body growth by antibiotics and probiotics have been proven and this reason causes increased body growth (Carvalho & Santos, 2016). Also all three factors of probiotic (Sanders, 1999), antibiotic (Seal, 2013) and garlic powder (Abujradah, Pandey, & Pandey, 2018) can eliminate pathogenic microbes and improve body health which can finally increase the growth and feed intake. Non-use of antibiotic, probiotic and garlic powder to make the diet of control group increased pathogenic microorganisms in intestinal tract and other places of the body which decreased the health (Tabidi, Mukhtar, & Hassan, 2013; Teshfam, Nodeh, & Hassanzade, 2005). Unlick of all groups, increase the body's need to energy and the production of hormones and enzymes to withstand against harmful microorganisms leaded to increase feed intake in control group ($p < 0.05$).

The best FCR between days of 0 to 42 respectively showed in GPG, AG, PG and GG. Effects of garlic powder, antibiotic and probiotic on body weight gain and daily feed intake can be reasons of these observed (Tabidi, Mukhtar, & Hassan, 2013). Also garlic powder and probiotic can promote several body systems including enzyme secretion, absorption and immune (Ronquillo & Hernandez, 2017) which can help to body to catch better body weight gain.

Carcass characteristics

Groups AG and GG created the lowest percentages of heart weight and showed significant differences with the control group ($p < 0.05$). The use of probiotic increased average weights of the heart. Also mentioned average weight differences can be due to increased activity of the heart (due to stimulation of the immune system by microbes in probiotic employed) (Shams Shargh, Dastar, Zerehdaran, Khomeiri, & Moradi, 2012) and antibacterial, anti-stress and lowering blood pressure effects of the garlic powder Garlic effects on stress reduction have been reported by several researchers. Garlic can prevent the body against free radicals; also it can reduce blood cholesterol and triglyceride which lead to health and weight loss of the heart (Ali, Qota, & Hassan, 2010).

Several studies have clearly demonstrated the effects of garlic (Kim, Jin, & Yang, 2009) and probiotics (Puspani, Candrawati, & Bidura, 2016) to reduce cholesterol and body fat levels which have created significant differences between the various groups ($p < 0.05$) (Issa & Abo Omar, 2011).

Probiotics and garlic powder effects on white blood cells are the main cause of differences between the spleen average weights of experimental groups. Lymphocytes should move to spleen to full maturity which lead to change the spleen weight (Hascik et al., 2016).

The use of garlic reduces stress in broilers (Zekic et al., 2014). Stress lead to produce free radicals. The effects of free radicals on liver cell membranes cause damage and lipid peroxidation; which may lead to change the average of liver weights (Gbenga, Adebisi, Fajemisin, & Adetunji, 2009).

Oxidative enzymes (AST, ALT and ALP)

Garlic inhibits the free radicals in the body by its antioxidant activity (Gbenga, Adebisi, Fajemisin, & Adetunji, 2009). Free radicals are one of the factors that could cause serious damages to liver cells which lead to release three enzymes including AST, ALT and ALP into the bloodstream; so garlic powder decreases the entry of these enzymes into blood circulation by protect the liver cells against free radicals (Issa & Abo Omar, 2011).

Small intestinal morphology

The presence of pathogenic bacteria increase the mucus secretion in the gastrointestinal mucosal layer and decrease both of height and width of small intestinal villi as defense mechanisms (Castanon, 2007). Therefore, increasing the number of pathogenic bacteria leads to an increase in the diameter of the wall and the height of the digestive system. The use of probiotics increase villi length in the all intestinal three parts. This increase is probably due to fermentation in narrow gut (Latha, Vinothini, Calvin, & Dhanasekaran, 2016); the production of volatile fatty acids and the reduction of pH can made an unsuitable environment to prevent the growth of pathogenic bacteria and as a result, it has reduces the renovations in the intestinal wall and creates greater length and width villi (Daneshmand, Sadeghi, Karimi, Vaziry, & Ibrahim, 2015; Quintana-Hayashi, Padra, Padra, Benktander, & Linden, 2018). Garlic also has antibacterial properties (Jimoh, Ibitoye, Dabai, & Garba, 2013). Pathogenic microorganisms decrease the rate of nutrient absorption in the small intestine through four ways include: increasing the thickness of the intestinal wall, reducing passage rate of material in digestive system, degrading nutrients, and increasing the need for nutrients due to increased intestinal mucus motility (Dkhil, Abdel-Baki, Wunderlich, Sies, & Al-Quraishy, 2011**)**. Increases in mucus turnover and gastrointestinal enterocytes is due to increased production of polyamines and volatile fatty acids. Therefore, garlic antibacterial activity can increase the length and width of the small intestine (Rehman & Munir, 2015).

Conclusion

This study showed that the use of probiotics and probiotics with garlic powder can provide a suitable growth performance and feed conversion ratio in the absence of antibiotics; but does not improve feed intake. The use of garlic powder, in particular with probiotics, protects the liver cells; and creates villi with a

good width and height in the small intestine; but they have not shown a good effect on the depth of crypts of lieberkulhn. Therefore, the simultaneous use of garlic powder and probiotics can be a good alternative to antibiotics to improve body performance and maintain liver function of broiler chickens.

Acknowledgements

The author is grateful to the Islamic Azad University of Darab for research funding support.

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