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Dietary Garlic Powder Supplementation Could Ameliorate Unfavorable Effects of Choline Deficiency on the Liver Health and Immune System of Broiler Chickens

Research Article

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ABSTRACT

Effects of different levels of choline and garlic powder on performance, liver health and immune response in broiler chickens were evaluated in this study. A factorial experiment with 2 dietary choline levels (the recommended level in Ross 308 broiler guidelines and 25 % less than the recommendation) and 3 dietary garlic powder levels (0, 5 or 10 g/kg diet) was conducted using Ross 308 broiler chickens. Four hundred eighty 1-d-old Ross 308 broiler chickens were randomly divided into 6 dietary treatments, each containing 4 replicate pens, with 20 birds each. During the finisher phase (d 25 to 42), dietary supplementation with 5 and 10 g/kg of garlic powder increased feed intake. At finisher phase and the whole experimental period, dietary supplementation with 10 g/kg of garlic powder improved the daily weight gain. During the finisher phase and the whole experimental period, the daily weight gain of birds fed low choline diets was less than that of birds fed recommended choline level. During the finisher phase and the whole experimental period, dietary choline deficiency increased feed conversion ratio. Choline deficiency decreased serum IgM level, and IgM concentration was higher in chicks fed diet with 5 g/kg garlic powder. However, dietary garlic powder at 10 g/kg level increased the total white blood cells and reduced the heterophyles numbers. Diets deficient in choline and also 10 g/kg dietary garlic powder increased the liver fat deposits. The liver Creatine phosphokinase (CPK) enzyme levels in the serum of chicks fed the choline-deficient diets were higher compared to birds fed the diets with recommended choline level. Consumption of garlic powder at 10 g/kg level, lowered serum CPK enzyme levels compared with the diets containing zero or 5 g/kg garlic powder. Choline deficiency and also dietary garlic regardless of its dosage were reduced the levels of serum triglycerides and very low density lipoproteins (VLDL). However, serum HDL were decreased in chickens fed 5 g/kg garlic powder compared with those fed garlic free diets. The findings of this study suggest that the effect of garlic powder in addressing the adverse effects caused by deficiency of choline are not through lipid metabolism improvement and more likely are due to the beneficial effects of garlic on increased feed intake , which increased choline intake and improved immune system function.

KEY WORDS broilers, choline, garlic powder, immune system, performance.

INTRODUCTION

In recent decades the application of antibiotics as livestock's growth promoters has been restricted, because of increased fear of pathogens resistance (Simon *et al.* 2003). Then the scientists focused on finding in acceptable alternatives for antibiotics. Medicinal plants have been the base of traditional medicine in all around the worlds. Herbal products are usually available with low price and low risk of toxicity and are environment-friendly (Windisch *et al.* 2008). Garlic (*Allium sativum*) is a usual flavoring agent with well-known therapeutic properties (Amagase *et al.* 2001). The medicinal effects of garlic are because of its flavonoid and organo-sulphur components (Kim *et al.* 2013), more than 33 sulphur-containing compounds, enzymes, 17 amino acids, and minerals such as selenium (Newall *et al.* 1996). Allicin (of which allin is the precursor) is the most important active constituent of garlic, consists up to 70% of the total thiosulfinates (Rybak *et al.* 2004). However, allicin is not stable and also is inadequately absorbable in intestinal lumen (Lawson *et al.* 1992).

There are reports on the antioxidant effects (Chowdhury *et al.* 2002), lowering plasma and meat cholesterol (Konjufca *et al.* 1997), immunomodulating and antimicrobial activities (Kyo *et al.* 2001) of garlic in poultry. On the other hand, the majority of previous reports have confirmed the improved performance traits in birds fed garlic (Tollba and Hassan, 2003), as well as an improvement in the small intestine morphology (Adibmoradi *et al.* 2006; Incharoen *et al.* 2010).

Choline is a major part of membrane phospholipids (Hollenbeck, 2010), an essential methyl-group donor (Zeisel and Blusztajn, 1994) and is part of the neurotransmitters (Wessler *et al.* 2001). Choline deficiency in poultry decreases feed intake and growth rate (Tsiagbe *et al.* 1982; Ryu *et al.* 1995) and dietary choline supplementation can improve the situation (Jukes, 1940; Rama Rao *et al.* 2001).

Moreover, there are reports that suggest inadequate choline consumption could increase the risk of fatty liver in poultry (Cooke *et al.* 2007; Pickens *et al.* 2009). The growth promoting effects of dietary garlic in broiler chickens have been confirmed using nutritionally adequate diets and we are unaware of any previous research in which choline deficiency in broiler diet have been accompanied with dietary garlic supplementation. The present study was designed to survey this hypothesis that if the affected growth rate and fatty liver symptoms in choline deficient broilers could be alleviated by dietary garlic consumption?

MATERIALS AND METHODS

All procedures of this research were approved by the animal welfare committee of animal science department of UMA University, Iran. A dose-response study with 2 dietary choline levels (the recommended level in Ross 308 broiler guidelines and 25% less than the recommendation) and 3 dietary garlic powder levels (0, 5 or 10 g/kg diet) was conducted. The 1-d-old mixed sex Ross 308 broiler chickens were randomly divided into 6 dietary treatments, containing 4 replicate pens, with 20 birds each. These birds were kept in wood shavings litter floor pens (200×150×40 cm) in an environmentally controlled poultry house. The temperature was kept at 31 ˚C (at floor level) from 1 to 7 d of age, and then it was reduced gradually to room temperature (20-22 ˚C) until 42 d of age. The average humidity was 65% in whole the experimental period. Water and feed were provided *ad libitum*, and lighting was 23 L: 1D from hatch to 42 d of age.

The corn-soybean meal experimental diets containing 0, 5 or 10 g/kg garlic powder were formulated (Table 1). Two dosages of choline chloride (purity≥98%, Vetaque Animal Health Co., Ltd., Tehran, Iran) were used to formulate experimental diets containing the recommended choline concentrations (1700, 1600 or 1500 mg/kg choline for starter, grower and finisher diets, respectively), or 25% less choline supply (1275, 1200 or 1125 mg/kg choline for starter, grower and finisher diets, respectively). Except for the choline content of the half of the experimental diets, all nutrients met the Ross 308 guidelines (2014) recommendations for starter (1-10 d), grower (11-24 d) and finisher (25-42 d) phases of rearing period. The diets were prepared in mash form (Aviagen, 2014).

At 10, 24 and 42 d of age, daily weight gain (DWG), feed intake (FI), and feed conversion ratio (FCR) of chickens from each pen were calculated for feeding period. Feed intake and feed conversion ratio were all corrected for mortality. At 23 d of age, 2.5 mL of 0.5% sheep red blood cell (SRBC) solution was injected into wing vein of two birds (one male and one female) per replicate and a weeks later blood samples were prepared and serums were separated.

On 35 d of the experiment, blood samples were taken from the wing vein of two birds per replicate. The serum samples were used to measure the antibodies titer against the Newcastle disease virus (NDV) and avian Influenza virus (AIV) by hemagglutination inhibition test according to International Epizootic Office (OIE) recommendation (Office International Epizootic, 2012). Aspartate aminotransferase (AST) and creatine phosphokinase (CPK) concentrations, and very low density lipoproteins (VLDL), low density lipoproteins (LDL), high density lipoproteins (HDL), total cholesterol (Chol) and triglycerides (TG), were determined using an auto-analyzer system (Bio Systems Co, Spain) and commercially available kits (Bio Systems Co. Spain).

At 35 d of age, blood samples with ethylenediaminetetraacetic acid (EDTA) anticoagulant also were taken from the wing vein of experimental birds to determine the number of white blood cells (WBC). One hundred white blood cells were counted to record the differential WBC, and to determine the H/L ratio.

At 42 d of age, two birds (one male and one female) were randomly selected from each replicate.

Table 1 Composition of the basic experimental diets containing normal or 25% less choline level

Ingredient (g/kg)	Starter		Grower		Finisher	
	Recommended choline	25% less choline	Recommended choline	25% less choline	Recommended choline	25% less choline
Corn	459.2	459.2	485.5	485.5	519.6	519.6
Soybean meal	434.8	434.8	402.9	402.9	373.4	373.4
soybean oil	58.8	58.8	71.9	71.9	69.6	69.6
Dicalcium phosphate (DCP)	20.9	20.9	17.2	17.2	16.0	16.0
Calcium carbonate	11.9	11.9	10.7	10.7	10.2	10.2
Common salt	2.3	2.3	4.4	4.4	4.4	4.4
Vitamin premix[1]	2.5	2.5	2.5	2.5	2.5	2.5
Mineral premix[2]	2.5	2.5	2.5	2.5	2.5	2.5
DL-methionin	3.2	3.2	2.5	2.5	1.8	1.8
HCl-lysin	0.4	0.4	0	0	0	0
Choline (mg/kg)	1060	360	960	295	846	222
Chemical analysis (calculated) (%)						
Metabolizable energy (kcal/kg)	2980	2980	3100	3100	3130	3130
Crude protein	23.27	23.27	22	22	21	21
Ca	1.033	1.033	0.9	0.9	0.85	0.85
Av. P	0.52	0.52	0.45	0.45	0.42	0.42
Na	0.198	0.198	0.20	0.20	0.20	0.20
Lys	1.41	1.41	1.29	1.29	1.22	1.22
Met	0.69	0.69	0.60	0.60	0.52	0.52
Met + Cys	1.06	1.06	0.95	0.95	0.86	0.86
Crude fiber	4.70	4.70	4.49	4.49	4.31	4.31

[1] Vitamin premix provided the following per kilogram of diet: vitamin A (retinyl acetate): 9000 IU; vitamin D (cholecalciferol): 5500 IU; vitamin E (DL-α-tocopheryl acetate): 68 IU; Menadione: 9.0 mg; Pyridoxine: 7.0 mg; Riboflavin: 26.0 mg; Ca-pantothenate: 26.3 mg; Biotin: 0.41 mg; Thiamine: 3.66 mg; Niacin: 75 mg; Cobalamin: 0.03 mg and Folic acid: 3.70 mg.

[2] Mineral premix provided the following per kilogram of diet: Fe: 82 mg; Mn: 60 mg; Zn: 115 mg: Cu: 15 mg; I: 0.85 mg and Se: 0.4 mg.

The livers, spleen and bursa of fabricius samples were removed immediately and stored in 10% buffered neutral formalin for fixation and were quietly shaken to eliminate any adhering intestinal contents. Cross sections (5 µm thick) of each tissue were processed in low-melt paraffin and stained with hematoxylin and eosin. The micrometrical analysis was carried out using Dino-Lite Digital Microscope, Digital Dino-Lite Eye-Piece and Dino-Capture 1 software on microphotographs. For detection of lipids in the liver tissue samples, a saturated solution (3%) of Sudan Black B in 70% ethanol was used (Lison and Dagnelie, 1935) and the periodic acid schiff's methods was used to determine the carbohydrate concentration in liver tissue samples (McManus, 1948).

To express the qualitative results obtained from periodic acid-schiff (PAS) and Sudan black staining, the results were scored in range 1-5. In this way, the group that had the lowest response to staining was scored with number 1 and those who showed the greatest response were expressed with the number 5.

Statistical analyses

Data were analyzed with the general linear model procedure and differences between treatments means were compared using Duncan's multiple range test (SAS, 1996).

RESULTS AND DISCUSSION

The effects of dietary choline and garlic powder levels on growth performance of broilers are presented in Table 2. During the finisher phase (d 25 to 42), dietary supplementation with 5 and 10 g/kg of garlic powder increased the feed intake (FI) (P<0.05) when compared with the birds fed the garlic free diets. However, the FI of birds at starter, grower and whole the experimental period was not affected. Furthermore, during the grower phase (d 11 to 24) and whole the experimental period, the choline deficiency has resulted in less FI (P<0.05). No interaction was observed between dietary choline and garlic powder levels on bird's FI.

Dietary garlic had no effect on DWG during the starter and grower phases. However, at finisher phase and whole the experimental period, dietary supplementation with 10 g/kg of garlic powder improved the DWG (P<0.05) when compared with that of birds fed the garlic free diets. Choline deficiency during starter and grower phases had no effect on DWG, however, at finisher phase and whole the experimental period, the body weight gain (BWG) of birds fed low choline diets was less than that of birds fed recommended choline level (P<0.05). No interaction was detected between dietary choline and garlic levels on DWG. Dietary garlic had no effect on birds FCR.

Table 2 Performance traits of broilers fed experimental diets contained different levels of choline and garlic powder levels

Measured parameter	Dietary garlic, g/kg					Dietary choline				Garlic × choline
	0	5	10	P-value	SEM	Recommended choline	25% less choline	P-value	SEM	P-value
Daily feed intake (g/bird/d)										
Starter	32.08	32.06	31.57	0.698	0.48	32.37	31.44	0.106	0.39	0.887
Grower	69.48	69.24	70.35	0.920	2.00	73.71[a]	65.67[b]	0.003	1.64	0.793
Finisher	141.57[b]	145.82[a]	148.18[a]	0.006	1.28	146.12	144.26	0.225	1.05	0.595
Total	91.47	93.21	94.47	0.134	1.00	94.90[a]	91.20[b]	0.005	0.82	0.881
Daily weight gain (g/bird/d)										
Starter	17.48	17.21	17.67	0.790	0.47	17.53	17.38	0.785	0.38	0.358
Grower	40.61	43.07	44.71	0.124	1.35	44.02	41.57	0.133	1.10	0.413
Finisher	72.24[b]	76.49[ab]	80.49[a]	0.047	2.17	81.80[a]	71.01[b]	0.0004	1.77	0.549
Total	48.66[b]	51.24[ab]	53.61[a]	0.016	1.08	53.91[a]	48.43[b]	0.0004	0.88	0.364
Feed conversion ratio										
Starter	1.84	1.87	1.79	0.543	0.05	1.86	1.81	0.416	0.04	0.339
Grower	1.72	1.62	1.58	0.362	0.07	1.69	1.58	0.196	0.06	0.882
Finisher	1.99	1.93	1.85	0.283	0.06	1.79[b]	2.05[a]	0.001	0.05	0.328
Total	1.89	1.83	1.77	0.153	0.04	1.77[b]	1.89[a]	0.018	0.04	0.381

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

During the starter and grower phases, dietary choline level did not affect the FCR of birds. However, at the finisher phase and whole the experimental period, dietary choline deficiency increased FCR (P<0.05).

Immune system and white blood cells

As shown in Table 3, choline deficiency had no effect on antibody titer against sheep red blood cells (SRBC), but dietary garlic powder significantly increased this parameter (P<0.05).

Serum IgG level was not affected by dietary levels of choline and garlic powder, but choline deficiency decreased serum IgM level; IgM concentration was higher in chicks fed diet with 5 g/kg garlic powder compared to those fed the garlic-free diets (P<0.05).

Choline deficiency has no effect on white blood cell parameters of broiler chickens, however, dietary garlic powder at 10 g/kg level increased the total white blood cells and reduced the heterophils numbers compared with the birds fed the diets with no added garlic powder (P<0.05). The number of lymphocytes, monocytes, and heterophils to lymphocytes ratio were not affected by the consumption of garlic powder. No significant interaction was observed between dietary choline and garlic on the characteristics of white blood cells.

Liver fat, liver enzymes and serum lipid in broilers

The results of liver fat content, liver enzymes and serum lipid profile are demonstrated in Table 4. Diets deficient in choline increased the liver fat deposits (P<0.05); on the other hand, 10 g/kg dietary garlic powder also increased liver fat concentration compared with birds fed diets without garlic (P<0.05).

The liver CPK enzyme levels in the serum of chicks fed the choline-deficient diets were higher compared to birds fed the diets with recommended choline concentration (P<0.05). Consumption of garlic powder at 5 or 10 g/kg level, lowered serum CPK enzyme levels compared with the diets containing no garlic powder (P<0.05). The level of liver AST enzyme in serum was not changed in birds fed choline deficient diet. Consumption of 5 g/kg garlic powder increased serum AST level compared to those fed with diets containing 10 g/kg or no garlic powder (P<0.05). Significant interactions were observed between dietary choline and garlic powder effects on liver fat and serum CPK and AST concentrations (P<0.05).

Choline deficient diet increased the levels of serum cholesterol and decreased serum LDL in chickens (P<0.05), but the presence of garlic powder in the diets had no effect on serum LDL or cholesterol concentrations. Choline deficiency and also dietary garlic regardless of its dosage, reduced the serum triglyceride and VLDL concentrations (P<0.05). Dietary choline level had no effect on serum HDL concentration. Serum HDL was decreased in chickens fed 5 g/kg garlic powder compared with those fed garlic free diets (P<0.05). There were significant interactions between dietary choline and garlic powder effects on serum triglycerides and VLDL concentrations (P<0.05).

Qualitative evaluation of the distribution of proteoglycans and fat in the liver

The highest tonality to dye PAS was observed in the liver tissue of group fed choline deficient, garlic free diet and the lowest tonality was recorded in the group fed the choline deficient diet containing 1% garlic powder (Table 5, Figure 1).

Table 3 Effect of dietary choline and garlic powder levels on blood antibodies and with blood cells in broiler chickens

Measured parameter	Dietary choline				Dietary garlic (g/kg)					Choline × garlic
	25% less choline	Recommended choline	P-value	SEM	0	5	10	P-value	SEM	P-value
SRBC	7.68	7.67	0.99	0.22	6.69[b]	8.05[a]	8.06[a]	0.001	0.31	0.50
IgG	4.31	4.80	0.20	0.25	4.15	4.50	5.00	0.15	0.36	0.23
IgM	3.36[a]	2.88[b]	0.04	0.15	2.54[b]	3.55[a]	3.06[ab]	0.004	0.22	0.50
Total white blood cells	27792	26683	0.39	932	25950[b]	26519[ab]	29413[a]	0.08	1115	0.81
Lymphocytes	69.21	70.34	0.55	1.37	69.56	68.00	71.87	0.26	1.65	0.56
Heterophyles	29.29	28.74	0.68	0.96	31.25[a]	28.75[ab]	26.93[b]	0.04	1.15	0.19
Monocytes	1.47	1.50	0.92	0.34	1.50	1.40	1.57	0.91	0.44	0.20
H:L ratio	0.42	0.44	0.53	0.023	0.46	0.45	0.38	0.10	0.027	0.13

SRBC: sheep red blood cells.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 Effects of dietary choline and garlic on liver fat and enzymes and serum lipid in broilers

Measured parameter	Dietary choline				Dietary garlic(g/kg)					Choline × garlic
	25% less choline	Recommended choline	P-value	SEM	0	5	10	P-value	SEM	P-value
Liver fat (%)	18.84[b]	25.65[a]	0.0001	0.87	19.93[b]	22.43[ab]	25.01[a]	0.006	1.08	0.007
CPK	7619.6[b]	11141.7[a]	0.0002	504.7	504.8[b]	10783.9[a]	7187.9[a]	0.001	677.20	0.0001
AST	218.2	227.8	0.11	4.14	216.5[b]	235.0[a]	218.5[b]	0.028	4.81	0.0001
TChol	131.4[b]	138.1[a]	0.03	1.97	138.4	136.6	130.7	0.13	2.61	0.16
TG	52.82[a]	45.92[b]	0.005	1.48	57.87[a]	44.62[b]	44.57[b]	0.0001	1.81	0.003
HDL	85.86	85.71	0.95	1.86	90.92[a]	81.41[b]	85.04[ab]	0.027	2.89	0.23
LDL	53.27[a]	43.17[b]	0.01	1.88	42.71	39.00	36.87	0.31	2.49	0.22
VLDL	10.16[a]	8.62[b]	0.01	0.42	11.03[a]	8.52[b]	8.92[b]	0.02	0.53	0.0008

CPK: creatine phosphokinase; AST: aspartate aminotransferase; TChol: total cholesterol; TG: triglycerides; HDL: high density lipoproteins; LDL: low density lipoproteins and VLDL: very low density lipoproteins.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The most tonality to the Sudan black staining was observed in the liver tissue of group fed choline deficient garlic free diet and the least amount of tonality was related to group fed the choline deficient 0.5% garlic powder containing diet (Table 5, Figure 2).

Micrometry parameters of spleen, liver and bursa of Fabricius tissues

Table 6 show the effects of dietary choline and garlic powder levels on the micrometry parameters of spleen, liver and bursa of fabricius. The parameters of spleen follicle diameter and the diameter of the spleen follicular capsule were not affected by dietary choline or garlic powder levels. In bursa of fabricius, reduced choline consumption significantly decreased the follicle diameter, height of epithelium and thickness of the capsule of follicle (P<0.05), the diameter of zaygr area of follicle was not affected by dietary choline level. The 5 and 10 g/kg dietary garlic powder dosages increased and decreased the thickness of capsule of follicle bursa of fabricius compared to the garlic free group, respectively (P<0.05). Choline deficiency had no effect on hepatocytes, however 5 g/kg dietary garlic powder increased diameter of hepatocytes as compared to the group fed 10 g/kg garlic powder (P<0.05).

On the other hand, 10 g/kg dietary garlic increased the diameter of hepatocytes nucleus compared to the groups fed 5 g/kg garlic powder or garlic free diets (P<0.05).

The dietary requirement of choline is considerably more than other water soluble vitamins. The structural role of choline in phospholipids synthesis and its methyl group donor role in the body explain the unusual dietary requirements as a water soluble vitamin. Then, the depressing effects of choline shortage on broilers performance in this study do not seem unexpected.

Attia *et al.* (2005) attributed the negative effects of choline deficiency on broiler performance traits to less methyl-group supply in the body.

There are huge numbers of reports on the positive effect of garlic on FI (Shi *et al.* 1999; Javandel *et al.* 2008; Horton *et al.* 1991; Cullen *et al.* 2005). In the present study, birds fed 5 or 10 g/kg garlic powder had higher feed intake in the finisher period.

However this effect was not enough to influence the FI in the whole experimental period. The broilers with choline deficiency supplemented with 10 g/kg garlic powder showed weight gain as good as the broilers fed the diet with recommended choline level and without additive garlic powder.

Table 5 The qualitative results of periodic acid-schiff (PAS) and Sudan black staining of broiler chickens liver tissue

Dietary garlic (g/kg)	Recommended choline			25% less choline		
	0	5	10	0	5	10
PAS	+4	+3	+3	+5	+2	+1
Sudan black	+2	+4	+3	+5	+1	+2

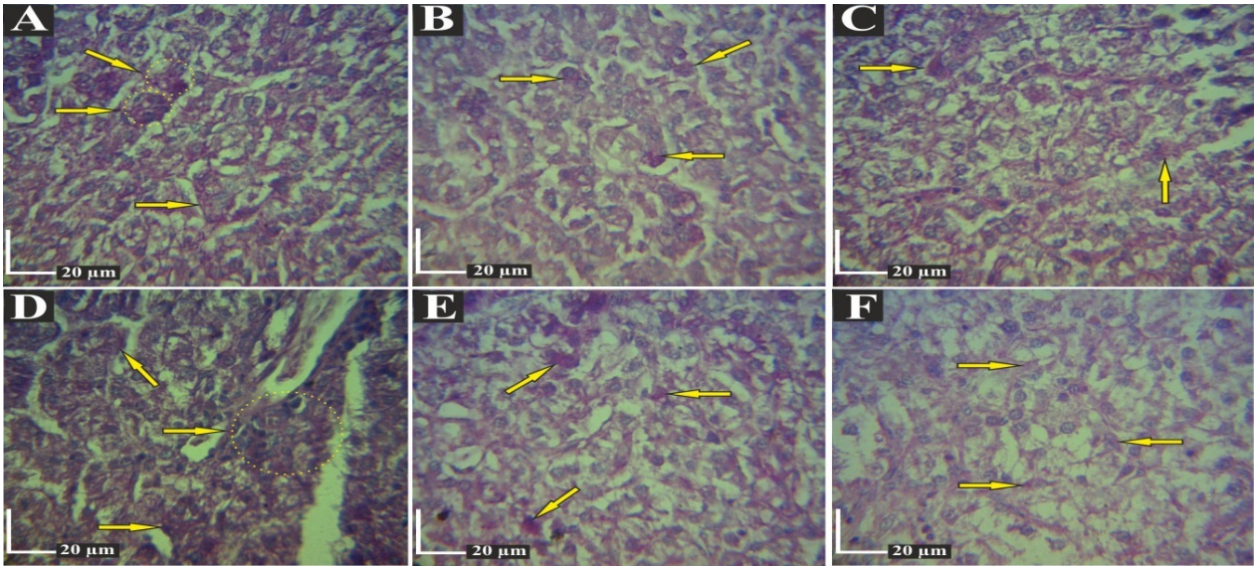

Figure 1 The liver tissue response to PAS staining (arrows show the positive interactions)
A: choline (+), garlic (0 g/kg); B: choline (+), garlic (5 g/kg); C: choline (+), garlic (10 g/kg); D: choline (-), garlic (0 g/kg); E: choline (-), garlic (5 g/kg) and F: choline (-), garlic (10 g/kg)

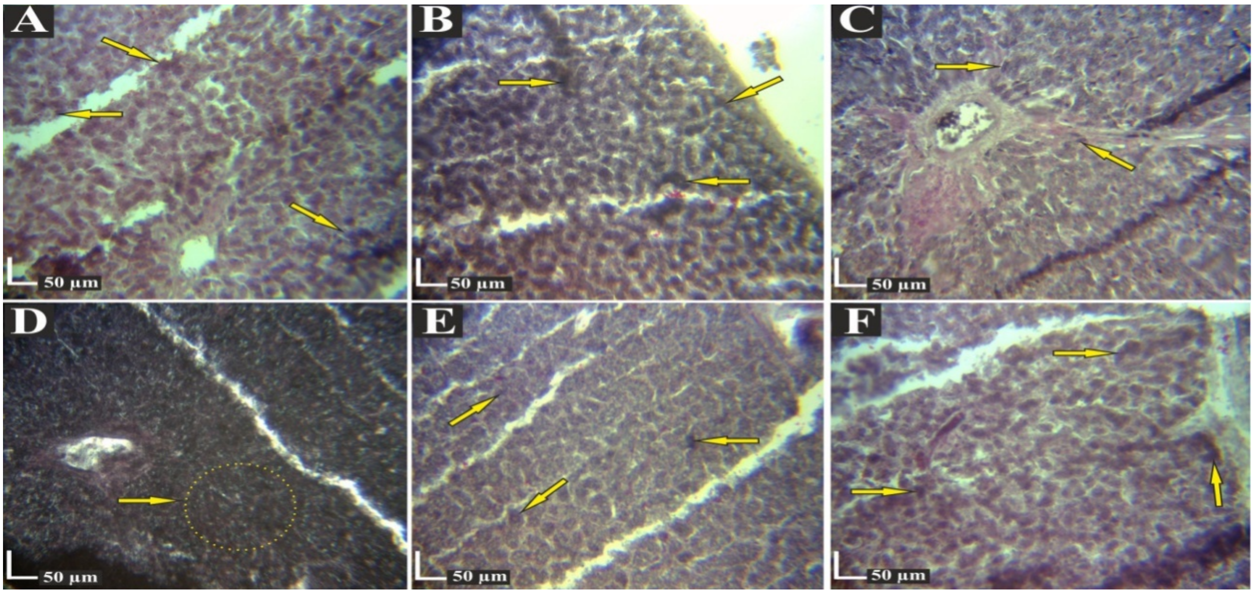

Figure 2 The liver tissue response to Sudan black staining (Arrows show the positive interactions)
A: choline (+), garlic (0 g/kg); B: choline (+), garlic (5 g/kg); C: choline (+), garlic (10 g/kg); D: choline (-), garlic (0 g/kg); E: choline (-), garlic (5 g/kg) and F: choline (-), garlic (10 g/kg)

Mohammadi Motamedi and Takalomi (2014) reported that adding 10 g/kg garlic in the diet increased the antibody titer against SRBC and the serum's IgG and IgM concentrations. These reports are largely consistent with the findings of the present study.

We couldn't found any report on the effects of choline consumption levels on white blood cells of chickens; however, Mohammadi Motamedi and Takalomi (2014) stated that garlic reduced the heterophile to lymphocyte ratio. Eid and Iraqi (2014) also reported that garlic consumption increased the white blood cells, heterophile, lymphocytes and heterophile to lymphocyte ratio, and attributed it to a stimulatory effect of garlic on the immune system. The differences in the reports may be due to differences in dosage, or the type of feeding or processing of garlic (Khan *et al.* 2012).

Based on the previous reports, it was expected that choline deficiency may reduce the methyl groups supple and leads to fatty liver disease in the broiler chickens (Kettunen *et al.* 2001). The findings of this study confirmed this view so that consumption the choline-deficient diet increased chicken's liver fat. But contrary to expectations, consumption of garlic not only did not reduce the liver fat, but also had an intensifying effect.

Liver's CPK and AST enzymes act as a marker of liver injury and are increased in incidence of fatty liver disease (Cray *et al.* 2008). In the present experiment, serum AST levels of chicken fed choline deficient diet were not changed, but the level of liver CPK enzyme in serum of chickens fed a choline-deficient diet was higher than those fed recommended choline level, which suggests the symptoms of fatty liver in choline-deficient birds. The interesting finding was the effect of garlic on liver enzymes, such that 10 g/kg dietary garlic powder decreased serum CPK concentration. Accordingly, it seems that choline was more effective on serum CPK, while garlic influenced the AST concentration. The significant interaction between the effects of choline and garlic on liver enzymes also confirms this fact, such that without dietary garlic, choline deficiency increased the level of serum CPK, whereas in garlic fed birds, choline deficiency increased serum AST. There are previous supporting reports (Ajayi and Ajayi, 2014). In another study, Eidi *et al.* (2006) stated that garlic was effective on lipid profile and also decreased serum AST level. Dietary garlic reduced serum LDL, cholesterol and triglycerides and increased HDL levels, and also reduced the serum AST concentration (Krauze *et al.* 2012).

Serum lipoproteins play a key role in the transfer of lipids synthesized in the liver to other parts of the body and especially abdominal fat. Due to the observed effects of choline and garlic on lipid metabolism in broiler chickens, the alterations in the serum lipids in this study were not unexpected. VLDL plays an important role for transferring fat from liver to adipose tissue (Hermier, 1997), the effect of choline deficiency in reducing serum VLDL levels in this study, explains the reason of the increased liver fat. Interestingly, dietary garlic regardless of its dosage, decreased serum triglycerides and VLDL and as a result, as mentioned earlier, exacerbated fatty liver. According to observations of this study, choline deficiency decreased the levels of serum LDL and cholesterol of broilers and dietary garlic had no effect on these parameters.

Ajayi and Ajayi (2014) reported that dietary garlic reduced serum cholesterol, triglycerides and LDL and increased serum HDL levels.

The mentioned results can be attributed to the inhibitory effect of garlic on the acetyl coenzyme A enzyme carboxylase which is responsible for the biosynthesis of fatty acids. The cholesterol and triglyceride lowering effects of garlic reduces the activity of hepatic lipogenic and cholestrogenic enzymes such as malic enzyme, fatty acid synthase and glucose-6-phosphate dehydrogenase (Qureshi *et al.* 1983a; Chi *et al.* 1982). It was also reported that garlic compounds can inhibit cholesterol bio-synthesis in the liver through inhibiting the enzyme 3-hydroxy-3-methyl-glutaryl-CoA reductase, the rate limiting enzyme in the biosynthesis of cholesterol and other lipogenic enzymes (Lin and YEN, 2002).

In a study in broiler chickens, Qureshi *et al.* (1983b) added 3.8% garlic to the basal diet for 4 weeks and observed that the activity of the enzyme 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA Reductase) 54% and serum cholesterol level 18% reduced. In another experiment, feeding 3% garlic powder, up to 40% lowered the HMG-CoA reductase and cholesterol 7 alpha-hydroxylase activities (Konjufca *et al.* 1997). The hypocholesterolemic effects of garlic can be attributed to the reduced activity of the main enzymes in cholesterol, lipid and bile acid synthesis (Konjufca *et al.* 1997), and also an increased bile acid excretion rate because of an interference with the cholesterol uptake (Shin *et al.* 2004).

According to the observations of this experiment, 10 g/kg dietary garlic powder consumption increased the liver fat compared to birds fed the diets without garlic, so that the highest levels of liver fat were observed in birds fed diets deficient in choline and supplemented with garlic powder. The possible cause of increased fat in the liver of birds fed garlic could be the increased feed intake and thereby stimulating greater energy intake which triggers hepatic lipogenesis.

PAS staining is used to determine the carbohydrate storages of cells and the intensity of the stain is an indicator of more carbohydrate storages. Sudan black staining determines the amount of fats in liver cells and high staining intensities can be a symptom of fatty liver disease. In the present experiment, the most staining to PAS dye was observed in the group fed the diet deficient in choline and without garlic and the least staining was observed in the liver tissue of birds fed the diet containing 10 g/kg garlic with choline deficiency. On the other hand, the most Sudan black staining of liver tissue was found in birds fed diets deficient in choline and without garlic and the lowest staining was observed in the group fed a diet of containing 5 g/kg garlic powder with choline deficiency.

Table 6 The effect of choline deficiency and dietary garlic powder on micrometry parameters of spleen, liver and bursa of fabricius tissues in broiler chickens

Micrometry parameters	Effects of choline				Effects of garlic (g/kg)					Choline × garlic
	Recomended choline	25% less choline	P-value	SEM	0 garlic	5 garlic	10 garlic	P-value	SEM	P-value
DSF	190.4	202.1	0.58	14.7	187.8	184.2	216.7	0.39	18.0	0.32
DSFC	40.1	39.0	0.71	2.2	39.0	42.1	37.5	0.48	2.7	0.14
DBF	359.6[a]	320.2[b]	0.04	13.1	321.4	341.7	356.58	0.32	16.1	0.007
DZBF	245.7	253.7	0.78	20.8	218.3	237.4	293.5	0.12	25.6	0.16
HBE	44.8[a]	37.6[b]	0.004	1.6	39.4	41.9	42.4	0.50	1.9	0.0009
TCBF	200.2[a]	108.7[b]	0.0001	5.2	148.6[b]	190.8[a]	124.1[c]	0.0001	6. 4	0.0001
HD	7.5	7. 9	0.28	0.25	7.8[ab]	8.3[a]	6.9[b]	0.02	0.32	0.015
DHN	4.3	4.4	0.58	0.16	4.0[b]	4.2[b]	4.9[a]	0.011	0.19	0.22

DSF: diameter of spleen follicle; DSFC: diameter of spleen follicle capsule; DBF: diameter of bursa of fabricius follicle; DZBF: diameter of zaygr area of bursa of fabricius follicle; HBE: height of bursa of fabricius epithelium; TCBF: thickness of the capsule of follicle of bursa of fabricius; HD: hepatocytes diameter and DHN: diameter of hepatocytes nucleus.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

In other word, the finding of the present study suggest that choline deficiency and no added dietary garlic resulted in both carbohydrate and fat accumulation in liver cells of chicks. The follicle diameter measurement is performed to demonstrate the activity of immune organs. For example, increasing the diameter of the follicles in the spleen could indicate an increase in the proliferation and growth of immune cells such as lymphocytes, especially type B, and an increased spleen activity.

In the present study, the parameters of lymphoid follicle diameter and thickness of the capsule (DSF and DSFC) in the spleen did not change due to the changing dietary levels of choline or garlic powder. There is a similar interpretation to the changes in the follicle diameter in bursa of fabricius and increased Zaygr area diameter in the lymphatic follicles represents a greater involvement of the lymphatic system by experimental treatments.

Parameters of capsules in bursa of fabricius like the spleen are indicators of immune system activity and more epithelial height suggest a more activity of antigen receptor cells in bursa of fabricius. In the present study, decreased choline consumption significantly decreased parameters of lymphatic follicle diameter, height of epithelium and capsule thickness of bursa of fabricius, which clearly indicates a reduction in the activity of the body's immune system. Garlic powder consumption at 5 g/kg level increased the diameter of hepatocytes compared to the group fed 10 g/kg garlic powder.

The consumption of 10 g/kg dietary garlic powder also increased the diameter of hepatocytes compared to the groups fed diets containing no garlic or 5 g/kg garlic. Increasing in the core diameter and size of hepatocytes could be signs of increased activity, and particularly more protein synthesis in the cells.

CONCLUSION

This study clearly showed that reducing dietary choline up to 75% of the recommended level reduces the growth of broiler chickens. On the other hand, adding garlic supplements to the diet largely offsets the adverse impact of the growth slowdown and this is partly due to increased feed intake, only during the finisher phase of rearing period. Choline deficiency also affected the immune system organs. On the other hand, the adverse effect of choline deficiency reduced the level of serum IgM and dietary garlic increased antibody titer against SRBC. Another positive effect of dietary garlic powder was the increase in total number of white blood cells, particularly the heterophyles. In this study, choline deficiency stimulated fat liver disorder caused by fat accumulation in hepatocytes and increased level of serum CPK, however, unexpectedly, the situation were severed by garlic powder consumption. Garlic powder at 5 g/kg level also increased serum AST enzyme levels as an indicator of liver damage. The lower serum VLDL level because of choline deficiency and garlic consumption, also confirm the fatty liver occurrence, because it affect the transport of the synthesised fat from liver. Therefore, it can be concluded that the effect of garlic powder in addressing the adverse effects caused by deficiency of choline are not through lipid metabolism improvement and more likely are due to the beneficial effects of garlic on increased feed intake, which increased choline intake and improved immune system function.

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