The compensation effect of dietary garlic on chicken consuming a minimal level of choline

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ABSTRACT
An experiment with two dietary choline levels (recommended level or 75\% of the recommended level) and three dietary garlic powders (0, 5 or 10 g/kg) was conducted on broiler chickens. Three hundred thirty mixed sex 1-day-old Ross 308 broiler chickens were randomly assigned to six dietary treatments, each housed in four replicate pens with 14 birds per pen. Dietary garlic increased the finisher phase’s feed intake. Dietary 10 g/kg of garlic powder and recommended choline level improved body weight gain. Choline deficiency increased feed conversion ratio (FCR). The jejunum villi height was decreased in birds consuming the choline deficient diets; however, villi height was increased by garlic powder. In the duodenum and ileum, 10 g/kg diet garlic powder significantly increased crypt depth compared to those of the treatments with no garlic. This study showed that reducing dietary choline to 75\% of the recommended level reduced the growth rate of broilers that could be compensated by dietary garlic. This effect was partly due to higher finisher phase feed intake.

Introduction
The choline deficiency in poultry usually decreases feed intake and growth rate and dietary choline supplementation can alleviate these problems (Wen et al. 2014). Choline is synthesised in the body of the chicken in inadequate amounts (McDowell 2000). Garlic (\textit{Allium sativum}) is a medicinal herb that has acquired an important role as a medicinal agent in many countries. Substantial evidence indicates that allicin is the main active component of garlic, consisting of up to 70\% of total thiosulfinates (Rybak et al. 2004). Garlic has been confirmed to have antioxidant effects (Chowdhury et al. 2002) and immunomodulating and antimicrobial activities (Kyo et al. 2001) in poultry. The majority of research on the growth-promoting effects of garlic in broiler chicken has been done using nutritionally adequate diets. The present study was, therefore, designed to investigate the possible growth promoting effects of dietary garlic powder in broilers fed a choline-deficient diet.

Materials and methods
All procedures of this research were approved by the Animal Welfare Committee of the Animal Science Department of the University of Mohaghegh Ardabili. A study with two dietary choline dosages, including the recommended dosage in Ross 308 broiler guidelines or 75\% of the recommended choline level; and 0, 5 or 10 g/kg dietary garlic powder (Table 1) was conducted. Three hundred and thirty-six 1-day-old mixed sex Ross 308 broiler chickens were randomly allotted to six dietary treatments, each containing four replicate pens with 14 birds per pen. The birds were rear based on Ross 308 guidelines. Water and feed were provided \textit{ad libitum}. The fresh garlic bulbs were separated, peeled, sliced and air dried for 24 h, then placed in an 80\(^\circ\)C oven and heated until dry. The dried garlic was ground using a laboratory mill. Choline chloride (purity \textgeq 98\%, Vetaque Animal Health Co., Ltd., Tehran, Iran) was used to formulate the experimental diets containing the recommended choline concentrations (1700, 1600 and 1500 mg/kg choline for starter,
grower or finisher diets, respectively), or 75% of the recommended level (1275, 1200 or 1125 mg/kg choline for starter, grower and finisher diets, respectively) and all the other nutrients met the Ross 308 Broiler Nutrition Specification (2014) recommendations for the starter (1–10 d), grower (11–24 d) and finisher (25–42 d) phases of the rearing period.

At 12, 24 and 42 d of age, body weight gain (BWG), feed intake (FI) and FCR of chickens from each pen were calculated for feeding period. FI and FCR were all corrected for mortality rate. On day 42 of the study, two birds from each pen (a male and a female) were randomly selected and fasted for 12 h. The selected birds were slaughtered and the small intestine morphology traits were determined as described by Adibmoradi et al. (2006). Data were analysed with the general linear model procedure and differences between treatment means were compared using Duncan’s multiple range test (v. 7.0, SAS Institute 1996).

Results

The effects of dietary choline and garlic powder on growth performance in broilers are presented in Tables 2–4. During the finisher phase, dietary supplementation with 5 and 10 g/kg of garlic powder increased FI (p < .05), when compared with that of birds fed garlic-free diets. However, FI of the birds at starter, grower and the entire experimental period did not differ. Furthermore, during the grower phase and throughout the experimental period, choline deficiency decreased the FI (p < .05). No interaction was observed between dietary choline and garlic powder levels on FI. Dietary garlic had no effect on the BWG during the starter and grower phases. However, during the finisher phase and the entire experimental period, consumption 10 g/kg of garlic powder improved the BWG (p < .05) when compared with that of birds fed the garlic-free diets. Choline deficiency during starter and

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

| Ingredient, % | Normal choline | Choline deficient | Normal choline | Choline deficient | Normal choline | Choline deficient |
|---------------|----------------|-------------------|----------------|-------------------|----------------|-------------------|
| Corn          | 45.92          | 45.92             | 48.55          | 48.55             | 51.96          | 51.96             |
| Soybean meal  | 43.48          | 43.48             | 40.29          | 40.29             | 37.34          | 37.34             |
| Soybean oil   | 5.88           | 5.88              | 7.19           | 7.19              | 6.96           | 6.96              |
| DCP           | 2.09           | 2.09              | 1.72           | 1.72              | 1.60           | 1.60              |
| Calcium Carbonate | 1.19   | 1.19              | 1.07           | 1.07              | 1.02           | 1.02              |
| Common salt   | 0.23           | 0.23              | 0.44           | 0.44              | 0.44           | 0.44              |
| Vitamin preixa | 0.25   | 0.25              | 0.25           | 0.25              | 0.25           | 0.25              |
| Mineral preixa | 0.25   | 0.25              | 0.25           | 0.25              | 0.25           | 0.25              |
| DL-Methionine | 0.32           | 0.32              | 0.25           | 0.25              | 0.18           | 0.18              |
| HCl-lysine    | 0.04           | 0.04              | 0              | 0                 | 0              | 0                 |
| Choline chloride | 0.1060 | 0.0360           | 0.0960         | 0.0295            | 0.0846         | 0.0222            |

### Table 2. Daily feed intake (g/bird/d) of broilers fed experimental diets contained different levels of choline and garlic powder levels.

| Effect of garlic | Starter | Grower | Finisher | Total |
|-----------------|---------|--------|----------|-------|
| p Value         | .698    | 2.00   | .006     | 1.28  |
| SEM             | 0.39    | 2.00   | 1.00     | 0.82  |

| Effect of choline | Starter | Grower | Finisher | Total |
|-------------------|---------|--------|----------|-------|
| p Value           | .08     | 1.05   | .005     | 0.82  |
| SEM               | 0.39    | 1.05   | 0.82     |       |

Within a row not sharing a common superscript differ significantly at p < .05.
grower phases had no effect on BWG; however, throughout the finisher phase and the entire experimental period, the BWG of birds fed low choline diets were less than that of birds fed recommended choline level \( (p < .05) \). No interaction was detected between dietary choline and garlic levels on BWG. Dietary garlic had no effect on the birds’ FCR. During starter and grower phases, dietary choline level did not affect the birds’ FCR. However, during the finisher phase and throughout the experimental period, dietary choline deficiency increased FCR \( (p < .05) \).

The effects of dietary treatments on small intestine morphology in broilers are presented in Table 5. The choline content of the diet did not affect the VH in duodenum and ileum, however, jejunum’s VH was decreased in birds consuming choline-deficient diets \( (p < .05) \). In the duodenum, VH was increased by the addition of 10 g/kg garlic powder to the diet, as opposed to the garlic-free diet \( (p < .05) \). In the jejunum, garlic consumption increased VH regardless of consumption dosage, when compared with the garlic-free diet \( (p < .05) \). In the ileum, the diet containing 5 g/kg garlic powder increased the VH compared to the garlic-free diet \( (p < .05) \). Choline deficiency significantly increased epithelium thickness in the duodenum and ileum \( (p < .05) \), but did not affect the jejunum. Dietary garlic significantly decreased the epithelium thickness in all small intestine sections \( (p < .05) \); however at the jejunum, the observed difference between the diets containing zero or 5 g/kg garlic powder was not significant. Choline deficiency significantly increased goblet cell numbers in all small intestine sections \( (p < .05) \). Both dietary garlic powder dosages decreased goblet cell numbers in the ileum \( (p < .05) \); however, in the duodenum and jejunum only the 10 g/kg garlic powder consumption resulted in the same effect \( (p < .05) \). In the duodenum and ileum, choline deficiency decreased crypt depth \( (p < .05) \), but no differences were detected for the jejunum. In the duodenum and ileum, 10 g/kg diet garlic powder increased crypt depth compared with diets with no garlic \( (p < .05) \). No differences were detected for the jejunum. Choline deficiency significantly increased crypt depth: villi height ratio (CD:VH) in the ileum \( (p < .05) \), but no differences were detected for the other two small intestinal sections \( (p < .05) \). In the duodenum, dietary garlic had no effect on the CD:VH ratio. In the jejunum, 10 g/kg diet garlic decreased the CD:VH ratio compared with the garlic-free diets \( (p < .05) \), and in the ileum, 10 g/kg diet garlic increased the CD:VH ratio compared with zero or 5 g/kg diet garlic consumption \( (p < .05) \).
No significant interactions were detected between dietary choline and garlic levels on small intestine morphological traits.

**Discussion**

The negative effect of choline deficiency on chicken growth rate in the present experiment is not surprising. In a comparable report, Attia et al. (2005) observed weight loss in poultry deficient in choline and attributed it to the reduced supply of methyl groups in the body. An interesting observation in the study was the negative effect of choline deficiency on bird growth rate at the end of the trial. This means that by increasing body weight and daily growth rate of the finisher phase in rearing period, the birds were not able to consume sufficient choline from diets with 75% of the recommended choline level. This effect was so remarkable that the average daily growth rate of birds in the entire rearing period was affected. In this study, both the 5 and 10 g/kg levels of garlic powder in the diets increased birds’ feed intake in the finisher period. However, this effect was not sufficient to affect feed consumption through the entire rearing period. In previous reports, the 0.25% and 1% dietary garlic dosages increased broilers’ feed intake (Shi et al. 1999; Javandel et al. 2008). Issa and Omar (2012) stated that garlic powder consumption has no significant effect on weight gain or feed conversion ratio in broilers.

Considering that a significant portion of nutrient absorption in the small intestine takes place in the jejunum, the shorter villus height in the jejunum due to choline deficiency could be a factor in lower feed intake and growth rate in chickens (Adibmoradi et al. 2016). The effect of garlic powder in increasing the VH seems a remarkable finding, and suggests an offsetting effect of garlic on the negative effect of choline deficiency through improved absorption in the small intestine. Adibmoradi et al. (2006) reported the increasing effects of dietary garlic on VH and CD in the intestine of broilers which confirms our findings in the duodenum and ileum. Crypts are considered a villi-producing factory; shallower crypt indicates slower turnover and less need for new tissue synthesis. A lower CD:VH ratio is an indicator of improved intestinal digestive capacity, indicating improved digestion and absorption rates (Montagne et al. 2003). It seems that the increase in goblet cell numbers is a response to intestinal lumen distress (De Santis et al. 2015). In the present study, choline deficiency has increased the number of goblet cells in all three sections of the small intestine, and dietary garlic powder reduced the number of goblet cells. These observations suggest that choline deficiency caused unfavourable changes in the intestine, and that dietary garlic could modulate the stressful conditions. Adibmoradi et al. (2006) also reported that garlic reduced goblet cell numbers in the intestine of broilers. In this study, consumption of diets containing garlic reduced the thickness of the intestinal epithelium, which is a positive indicator of the digestion process and confirms the results of Adibmoradi et al. (2006).

**Table 5.** Small intestine morphology of broilers fed experimental diets contained different levels of choline and garlic powder levels.

| Recommended choline| 25% less choline |
|--------------------|------------------|
| **Garlic powder dosage, g/kg** | **Garlic powder dosage, g/kg** | 0 | 5 | 10 | SEM | 0 | 5 | 10 | SEM |
| VH | 1802.06a | 1809.00a | 1822.80a | 1695.60b | 1797.60a | 1807.20a | 12.76 |
| ET | 44.80bc | 40.80cd | 39.40d | 52.40a | 43.80bc | 51.00cd | 0.92 |
| GN | 8.60bc | 8.60bc | 7.40 | 10.20a | 9.00bc | 8.60bc | 0.21 |
| CD | 148.80ab | 150.20ab | 153.20a | 145.40a | 147 | 150.00ab | 0.77 |
| CD:VH | 0.082 | 0.083 | 0.084 | 0.086 | 0.081 | 0.082 | 0.0007 |
| VH | 832.20bc | 840.80ab | 842.00a | 825.80c | 832.80abc | 838.60ab | 1.53 |
| ET | 41.20abcd | 40.60ab | 36.80cd | 42.60a | 40.00a | 40.20ab | 0.65 |
| GN | 9.20abcd | 9.40abcd | 8.00 | 10.60a | 10.00ab | 8.60cd | 0.22 |
| CD | 142.8 | 144.6 | 143.4 | 143.6 | 146.2 | 142.4 | 0.56 |
| CD:VH | 0.17ab | 0.17ab | 0.17b | 0.17ab | 0.17a | 0.17b | 0.0006 |
| VH | 792.00ab | 819.80a | 805.20b | 783.20a | 793.80ab | 800.40ab | 1.13 |
| ET | 40.00bc | 37.40b | 35.80bc | 45.80a | 39.60a | 38.00b | 0.8 |
| GN | 10.40ab | 8.80cd | 8.00 | 11.20a | 9.20bc | 9.80bc | 0.24 |
| CD | 99.80bc | 102.00bc | 110.00 | 95.00a | 96.60bc | 100.80bc | 1.13 |
| CD:VH | 0.13b | 0.12b | 0.14a | 0.12b | 0.12b | 0.13b | 0.0014 |

*Within a row not sharing a common superscript differ significantly at p < .05.

VH: villus height, μm; ET: epithelial thickness, μm; GN: goblet cell numbers; CD: crypt depth, μm.*
Conclusions
This study clearly showed that reducing dietary choline to 75% of the recommended level significantly reduces the growth rate of broilers. On the other hand, adding garlic supplements to the diet largely compensates for the depressed growth rate. This effect was partly due to higher feed intake in birds fed garlic powder, especially during the finisher phase of the rearing period. Therefore, it can be concluded that dietary garlic powder addressed the adverse effects caused by choline deficiency.

Disclosure statement
No potential conflict of interest was reported by the authors.

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