Growth performance, jejunum morphology and mucin-2 gene expression of broiler Japanese quails fed low-protein diets supplemented with threonine

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Abstract

The present experiment was conducted to examine the effects of threonine supplementation in low-protein diets on growth performance, jejunum morphology, and mucin-2 gene expression in meat-type Japanese quails. A total of 800 broiler quails were assigned to 8 dietary treatments based on a factorial arrangement (2 x 4) across 1-35-day period. Experimental treatments consisted diets with 180 g/kg (CP180), 200 g/kg (CP200), 220 g/kg (CP220), and 240 g/kg (CP240) crude protein content each supplemented with 100% and 110% threonine requirement of broiler quails. Growth performance of broiler quails was not affected by interaction of dietary protein and threonine contents while daily weight gain was higher in birds received CP240 than those given CP180 and CP200 (p < .05). Carcase yield increased in response to consumption of diets with 200 g/kg protein content and 110% threonine requirement compared to those receiving the other dietary treatments (p < .05) except for birds in CP240 group. Villus height to crypt depth ratio was higher in birds subjected to CP200 and CP240 each supplemented with 100% and 110% threonine requirement of quails, respectively, than the other groups (p < .05). Digestibility of protein was not affected by experimental treatments. Expression of mucin-2 gene decreased by dietary supplementing graded levels of protein (p < .05) while increased by graded levels of threonine (p < .05). In conclusion, reduction of dietary protein content did not affect the growth of broiler quails whenever diets added with 100% of their digestible threonine requirement. Furthermore, dietary protein content below 220 g/kg adversely affected DWG of quails.

Highlights

- Reduction of dietary CP content did not affect the growth of broiler quails whenever diets added with 100% of their threonine requirement.
- Morphology of the small intestine was influenced by variations in dietary energy and protein contents.
- Expression of mucin-2 gene decreased when quails fed on diets with graded levels of CP while increased with graded levels of threonine.

Introduction

In the poultry industry, nutritionists prefer to decrease dietary crude protein (CP) content to decrease feed cost and reduce total nitrogen output to the environment (Sigolo et al. 2017). However, dietary essential amino acid content decreases accompanied with a reduction of CP level in the feed. Nevertheless, reduction of dietary CP might impair the growth performance of poultry even with providing indispensable amino acids (Holsheimer and Janssen 1991; Bregendahl et al. 2002). On the other hand, some researchers have reported that crystalline amino acid supplementation can fairly ameliorate partial CP reduction in the diet of broiler chickens (Corzo et al. 2007a; Allameh and Toghyani 2019). Generally, adequate and accurate preparation of amino acids in the diet is necessary for protein accretion and maintenance in the body of broiler quails. Therefore, supplementation of feed grade amino acids in low CP feed might help to meet the need and avoid...
overconsumption of dietary protein sources in quail’s nutrition.

Threonine is an indispensable amino acid for poultry because they are not able to produce Threonine de novo (Waguespack et al. 2009). Therefore, this amino acid is needed to be exogenously prepared for broiler quails. Furthermore, threonine is the third limiting amino acid, particularly in low CP diets for poultry (Rezaeipour et al. 2012), participating in remarkable metabolic processes, such as formation of uric acid and synthesis of protein. Also, threonine is important for maintenance of gut barrier integrity and has an important role in the structure and function of gastrointestinal tract (Wils-Plotz and Dilger 2013). Threonine is a key component of mucus in the digestive tract, providing 40% of protein in mucus glycoproteins (Carlstedt et al. 1993; Corzo et al. 2007b). Indeed, threonine assists the mucin to remain intact and subsequently improves the immunity of animals. Schaar et al. (2005) by conducting an in vivo experiment have shown that between 80% and 90% of dietary threonine is used by the intestine, most of which is incorporated into mucosal proteins. Published data on the effect of dietary threonine regarding intestinal morphology of broiler quails is insufficient. It has been documented that optimal dietary content of total threonine for broiler quails is 10.6 g/kg during 1 to 28 days of age Baylan et al. (2006). However, Ton et al. (2013) found that nutritional requirement of digestible threonine for broiler quails is 12.60 g/kg across 1 to 14 days of age. Although digestible threonine requirement of Japanese quails recommended by Rostagno et al. (2011) is 7.9 g/kg, Rasheed et al. (2018) believed that supplementation of threonine up to 20% higher than 7.9 g/kg during 1 to 35 days of age improved growth performance, feed conversion ratio (FCR), gut health, and breast meat yield of broiler-type Japanese quails. All noted studies examined the effect of dietary threonine in CP adequate diets, but more research on the effect of dietary supplemental threonine in broiler quails received low CP diets is required. The effect of threonine supplementation in diet of poultry on the expression of mucin-2 gene has been examined by the researchers. Moghaddam et al. (2011) did not observe any effect of dietary threonine supplementation in graded levels on mucin-2 gene abundance of broiler chickens. Similarly, dietary threonine supplementation did not affect mucin-2 gene abundance of boiler chickens in the work of Horn et al. (2009). Conversely, Azzam et al. (2011) reported a linear increase in expressions of jejunal and ileal mucin-2 mRNA of laying hens by increasing dietary threonine level. Thus, further research in warranted to examine the effect of dietary threonine supplementation on mucin-2 gene expression in broiler quails.

We hypothesised that supplementation of diets containing different CP levels with 100% and 110% threonine requirement may influence gut development, CP digestibility, and growth performance in broiler quails. Thus, the objective of this study is to evaluate the effect of threonine supplementation (100% and 110% threonine requirement of broiler quails) to diets with 180 g/kg, 200 g/kg, 220 g/kg and 240 g/kg CP content on growth performance, morphology of jejunum, CP digestibility and mucin-2 gene expression in broiler quails.

Materials and methods

All experimental procedures were evaluated and approved by the Institutional Animal Care and Ethics Committee of the Islamic Azad University, Isfahan (Khorasgan) Branch.

Birds and management

A total of 800 1-day-old broiler Japanese quails (Coturnix japonica) were weighed and randomly assigned to 8 dietary treatments, each containing 5 replicate pens with 20 birds based on a factorial arrangement of treatments (2²×2⁴) in a completely randomised design. Experimental treatments included 4 diets with 180 g/kg (CP180), 200 g/kg (CP200), 220 g/kg (CP220) and 240 g/kg (CP240) CP each contained 100% (7.90 g/kg) or 110% (8.69 g/kg) digestible threonine requirement of broiler quails. Diets were formulated to meet or exceed nutrient requirement of broiler quails according to Rostagno et al. (2011) and were fed during 35 days of the experiment (Table 1). Dietary treatments were fed in mash form and offered ad libitum throughout the study. All birds had free access to water during the experiment. Ambient temperature was kept at 34°C (at floor level) from one to 3 days of age, at 31°C from 4 to 7 days of age, at 28°C from 8 to 14 days of age, and then gradually decreased to 25°C until the end of the experiment. The lighting programme consisted of 23-hour light and one-hour darkness.

Growth performance and carcase components

daily feed intake (DFI) and daily weight gain (DWG) of broiler quails in each pen were recorded during 1-35-day period and FCR (feed intake/weight gain) was
calculated accordingly. On day 35 of experiment, two quails close to the mean body weight of pen were slaughtered by cervical dislocation after 4 hours feed deprivation to evaluate carcass traits. Carcass, gizzard, duodenum, jejunum, ileum and caecum were collected, weighed, and expressed as a percentage of live body weight.

Morphology of jejunum

Two birds from each pen were slaughtered by cervical dislocation on day 20. Intestine was sampled from jejunum; midway between the point of entry of the bile ducts and Meckel’s diverticulum and ileum; 10 cm proximal to the ileo-cecal junction. Jejunum samples were taken to evaluate the villus height, crypt depth, villus height: crypt depth ratio and number of goblet cells. Samples were rehydrated and stained with Alcian Blue (pH 2.5) and periodic acid-Schiff’s reagent (Sigma Chemical Company, St. Louis, MO, USA). The samples were rinsed in distilled water before storage in a 45% acetic acid solution until analysis. Optical microscope (Olympus CX31, Tokyo, Japan) was used for morphological examination of small intestine samples. A total of 10 intact, well-oriented villus-crypt units were selected for each intestinal cross-section (3 cross-sections/sample). Villus height (μm) was measured from the tip of the villus to the villus crypt junction, and crypt depth was defined as the depth of the invagination between two villi. Villus height to crypt depth ratio (V/C) was then calculated. The number of goblet cells in the intestinal segments was determined from the number of goblet cells in 5 villi per segment/bird. The average of values for each cross-section was used for further analysis.

Digestibility of protein

On day 33 post-hatch, 4 Japanese quails of each pen (20 quails overall) were transferred to separate cages where all birds had free access to the experimental diets for a preliminary 2-days adaptation period followed by 3 days of excretion collection in triplicate. A source of acid-insoluble ash was added to all diets (10 g/kg) as an indigestible marker. Excreta were collected using trays located beneath each cage and stored at −20 °C. All samples were dried in an oven of 80 °C for 24 h and the crude protein was determined using Kjeldahl method (Ratriyanto and Indreswari 2014).

### Table 1. Dietary composition and nutrients.

| Items                        | CP180 | CP200 | CP220 | CP240 | CP180 | CP200 | CP220 | CP240 |
|------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|
| Ingredients (g/kg)           |       |       |       |       |       |       |       |       |
| Corn (850 g/kg crude protein)| 658.1 | 603.5 | 551.2 | 498.0 | 656.9 | 602.2 | 550.4 | 496.7 |
| SBM (440 g/kg crude protein) | 260.0 | 311.0 | 354.0 | 395.0 | 260.0 | 311.0 | 354.0 | 395.0 |
| Wheat bran (157 g/kg crude protein)| 25.0 | 20.0 | 17.0 | 15.0 | 25.0 | 20.0 | 17.0 | 15.0 |
| CGM (600 g/kg crude protein) | 10.0 | 15.0 | 25.0 | 36.0 | 10.0 | 15.0 | 25.0 | 36.0 |
| Soybean oil                  | 1.0   | 7.5   | 13.0  | 18.5  | 1.4   | 8.0   | 13.0  | 19.0  |
| Dicalcium phosphate          | 15.0  | 14.6  | 14.0  | 14.0  | 15.0  | 14.6  | 14.0  | 14.0  |
| Calcium carbonate            | 12.7  | 12.7  | 12.6  | 12.6  | 12.7  | 12.7  | 12.6  | 12.6  |
| DL-Methionine                | 2.5   | 2.0   | 1.5   | 1.0   | 2.5   | 2.0   | 1.5   | 1.0   |
| L-lysine HCL                 | 4.1   | 2.7   | 1.4   | 0.2   | 4.1   | 2.7   | 1.4   | 0.2   |
| L-Threonine                  | 2.2   | 1.6   | 0.9   | 0.26  | 3.0   | 2.4   | 1.7   | 1.1   |
| Vitamin and mineral premix   | 5.0   | 5.0   | 5.0   | 5.0   | 5.0   | 5.0   | 5.0   | 5.0   |
| Sodium chloride              | 2.4   | 2.4   | 2.4   | 2.4   | 2.4   | 2.4   | 2.4   | 2.4   |
| Sodium bicarbonate           | 2.0   | 2.0   | 2.0   | 2.0   | 2.0   | 2.0   | 2.0   | 2.0   |
| Calculated nutrient level (as fed basis) |       |       |       |       |       |       |       |       |
| ME (Kcal/kg)                 | 2900  | 2900  | 2900  | 2900  | 2900  | 2900  | 2900  | 2900  |
| Crude protein (g/kg)         | 180   | 200   | 220   | 240   | 180   | 200   | 220   | 240   |
| Digestible lysine (g/kg)     | 11.2  | 11.2  | 11.2  | 11.2  | 11.2  | 11.2  | 11.2  | 11.2  |
| Digestible Met + Cys (g/kg)  | 7.6   | 7.6   | 7.6   | 7.6   | 7.6   | 7.6   | 7.6   | 7.6   |
| Digestible threonine (g/kg)  | 7.9   | 7.9   | 7.9   | 7.9   | 8.69  | 8.69  | 8.69  | 8.69  |
| Calcium (g/kg)               | 9.0   | 9.0   | 9.0   | 9.0   | 9.0   | 9.0   | 9.0   | 9.0   |
| Available phosphorous (g/kg) | 3.75  | 3.75  | 3.75  | 3.75  | 3.75  | 3.75  | 3.75  | 3.75  |

SBM: soybean meal; CGM, Corn gluten meal; ME, Metabolisable energy.

*Vitamin premix provided per kg of diet: vitamin A (retinol), 2.7 mg; vitamin D3 (Cholecalciferol), 0.05 mg; vitamin E (tocopherol acetate), 18 mg; vitamin K3, 2 mg; thiamine 1.8 mg; riboflavin, 6.6 mg; panthothenic acid, 10 mg; pyridoxine, 3 mg; cyanocobalamin, 0.015 mg; niacin, 30 mg; biotin, 0.1 mg; folic acid, 1 mg; choline chloride, 250 mg; antioxidant 100 mg; Mineral premix provided per kg of diet: Fe (FeSO4.7H2O, 20.09% Fe), 50 mg; Mn (MnSO4.H2O, 32.49% Mn), 100 mg; Zn (ZnO, 80.35% Zn), 100 mg; Cu (CuSO4.5H2O), 10 mg; l (K1, 58% l), 1 mg; Se (NaSeO3, 45.56% Se), 0.2 mg.

bCP180: a diet with 180 g/kg crude protein content.

cCP200: a diet with 200 g/kg crude protein content.

dCP220: a diet with 220 g/kg crude protein content.

Table 1. Dietary composition and nutrients.
Table 2. Primers used for quantitative real-time PCR.

| Target gene | Gene bank accession | Forward sequence (5’ to 3’) | Reverse sequence (5’ to 3’) |
|-------------|---------------------|-----------------------------|----------------------------|
| Mucin-2     | NM_001318434        | CCACAAGTCTCCAGTACTCAACA     | AGGTTTCATAGTCAACCCACACCTTC |
| β-actin     | NM_205518           | CTGGCACCTAGCACAATGAA        | CTGGTTCATGATCACCACCATCT    |

**Quantitative real-time PCR**

At the end of the experiment, two quails from each pen were slaughtered after 6 hours of feed withdrawal. Samples from jejunum were collected and quickly snap-frozen in liquid nitrogen that was further used to measure mucin-2 mRNA levels. Total RNAs were extracted from jejunum using Iraizol reagent (RNA Biotechnology Co., Isfahan, Iran). RNA concentration was quantified by spectrophotometer nano-drop (MD-1000) in wavelength of 250 nm. Complementary (c) DNA was synthesised from 1 μg of RNA samples with an MScript II RT kit (QiAGENE, Germany), according to the manufacturer’s recommended protocol. All primers were synthesised and purified by Sigma Company. The β-actin was used as reference gene to normalise the expression of target gene. The primer pairs for the amplification of mucin-2 and β-actin cDNA fragments are listed in Table 2. Quantitative real-time PCR (qRT-PCR) was performed to determine the levels of inducible mucin-2 mRNA. Two microliters of cDNA fragments are listed in Table 2. Quantitative real-time PCR (qRT-PCR) was performed to determine the levels of inducible mucin-2 mRNA. Two microliters of tenfold dilution reverse transcription products were used for PCR in a final volume of 25 μL containing 0.4–0.8 μM primers and 12.5 μL of QuantiTect SYBR Green master mix (Life Technologies, Cat # 4367659). Cycling parameters were as follows: 10 min at 95°C, then 40 cycles of 95°C for 30 s, annealing temperature for 30 s, and 72°C for 30 s, and extension for 2 min at 72°C. To confirm amplification specificity, the PCR products from each primer were subjected to a melting curve analysis and subsequent agarose gel electrophoresis. The final mucin-2 concentrations were calculated as arbitrary unit of band density relative to total protein concentration of each sample.

**Statistical analysis**

Data were analysed considering all birds in a pen as an experimental unit for different parameters. When a significant F-test was detected (P < 0.05), corresponding means were separated by Tukey’s test, and the interaction between treatments was analysed using a least square means test adjusted for Tukey’s test. Whenever the interaction effects of main factors were significant, the main effects were not further discussed. For all statistical analyses, significance was declared at P ≤ 0.05 unless otherwise stated.

**Results**

**Growth performance and carcase components**

Results on the effect of dietary treatments on growth performance of broiler quails are summarised in Table 3. None of growth-related parameters including DFI, DWG, and FCR were affected by interaction of dietary supplemental threonine level and CP content during 1–35 d of rearing period. Main effect of treatments showed that feeding broiler quails by CP240 significantly increased DWG compared to the birds received diets with 200 g/kg and 180 g/kg CP content (p < .05). Threonine level in the feed did not affect growth performance-related parameters in broiler quails.

According to Table 4. Effects of dietary CP content and threonine level were interacted on carcase yield (p < .05). The greatest carcase yield observed when quails were subjected to diets with 200 g/kg CP content and 110% threonine requirement which was higher than those received the other dietary treatments (p < .05) except for birds in CP240 group. Feeding broiler quails with diets containing 220 g/kg and 240 g/kg CP caused a higher gizzard weight than 180 g/kg as shown by main effect of dietary CP content (p < .05). Furthermore, graded levels of CP content higher than 180 g/kg in the feed increased relative weight of duodenum (p < .05).

**Morphology of jejunum and digestibility of crude protein**

There was a significant interaction between dietary CP content and threonine level on the jejunal morphometric features (p < .05; Table 5). Jejunal villus height was higher in birds fed on diets containing 180 g/kg CP and 110% threonine, 200 g/kg CP and 100% threonine, 220 g/kg CP with either 100% or 110% threonine requirement of broiler quails than those received the other dietary treatments (p < .05; Table 5). On the other side, crypt depth significantly decreased in birds given CP240 supplemented with 110% threonine requirement compared to the other experimental groups (p < .05; Table 5) except for diets with 200 g/kg CP and 110% threonine level. These variations in villus height and crypt depth of jejunum resulted in higher V/C in birds subjected to CP200 and CP240 supplemented each with 100% and 110% threonine requirement of quails, respectively, than the other groups (p < .05; Table 5).
Regardless of dietary CP level, goblet cell count was higher in birds given 110% dietary threonine requirement than those received 100% (p < .05; Table 5) except for birds fed with CP240 which increased goblet cell count with 100% dietary threonine requirement (p < .05; Table 5). Digestibility of CP was not affected by dietary CP content and threonine level (Table 6).

**Quantitative real-time PCR**

Effect of experimental treatments on the expression of mucin-2 gene is shown in Table 7. Expression of mucin-2 gene was not influenced by the interaction of dietary CP content and threonine level. However, graded levels of CP in the feed from CP18 to CP24...
significantly decreased expression of mucin-2 gene ($p < .05$). Additionally, feeding quails with diets added with 110% threonine requirement increased mucin-2 gene expression compared to 100% ($p < .05$).

### Discussion

In the present experiment, growth performance of broilers was not affected by the interaction of dietary CP content and threonine level. It may show that reduction of CP content in the feed does not deleteriously affect growth of broiler quails whenever diets at least added with 100% of their digestible threonine requirement. On the other hand, quails fed on diets containing 240 g/kg CP had higher growth rate than the birds received CP180 and CP200, irrespective of dietary threonine. Obtained results might indicate that

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**Table 5.** Effects of dietary treatments on morphology of jejunum in broiler quails.

| CP level (g/kg) | Threonine level (%) | Jejunum, μm | Villus height | Crypt depth | V/C | Goblet cell count |
|----------------|---------------------|-------------|---------------|-------------|-----|------------------|
|                |                     |             |               |             |     |                  |
| 180            | 100                 | 531.25$^b$  | 55.62$^{bc}$  | 10.09$^{bc}$| 8.76$^b$|                   |
| 180            | 110                 | 648.57$^a$  | 60.00$^{ab}$  | 10.81$^{ab}$| 10.96$^b$|                   |
| 200            | 100                 | 707.50$^a$  | 60.00$^{ab}$  | 12.14$^a$   | 8.76$^b$|                   |
| 200            | 110                 | 495.00$^b$  | 51.25$^{d}$   | 9.70$^{bc}$ | 10.96$^a$|                   |
| 220            | 100                 | 650.00$^a$  | 63.75$^{d}$   | 9.44$^{bc}$ | 6.60$^c$ |                   |
| 220            | 110                 | 646.87$^a$  | 67.50$^a$     | 8.73$^c$    | 11.29$^a$|                   |
| 240            | 100                 | 486.25$^b$  | 56.25$^{bc}$  | 8.72$^c$    | 12.22$^a$|                   |
| 240            | 110                 | 531.25$^a$  | 43.75$^a$     | 12.05$^a$   | 6.05$^c$ |                   |
| SEM            |                     |             |               |             |     |                  |

**Table 6.** Effects of dietary treatments on ileal protein digestibility.

| CP level (g/kg) | Threonine level (%) | CP digestibility |
|----------------|---------------------|------------------|
|                |                     |                  |
| 180            | 100                 | 82.71            |
| 180            | 110                 | 81.98            |
| 200            | 100                 | 82.49            |
| 200            | 110                 | 82.57            |
| 220            | 100                 | 82.52            |
| 220            | 110                 | 82.73            |
| 240            | 100                 | 82.37            |
| 240            | 110                 | 82.64            |
| SEM            |                     |                  |

**Table 7.** Effects of dietary treatments on mRNA expression of mucin-2 gene.

| CP level (g/kg) | Threonine level (%) | Mucin-2 expression |
|----------------|---------------------|--------------------|
|                |                     |                    |
| 180            | 100                 | 4.34               |
| 180            | 110                 | 4.40               |
| 200            | 100                 | 100                |
| 200            | 110                 | 200                |
| 220            | 100                 | 220                |
| 220            | 110                 | 240                |
| 240            | 100                 | 240                |
| 240            | 110                 | 240                |
| SEM            |                     |                    |

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*$a,b,c,d$Values in the same column not sharing a common superscript differ significantly ($p < .05$).

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dietary CP content below 220 g/kg adversely affect DWG in broiler quails owing to lower dietary CP intake of them. Wen et al. (2017) reported that dietary CP reduction from 253.2 g/kg to 176.1 g/kg impaired growth but increased DFI of French meat quails during 15 to 35 days of age. However, DFI was not influenced in our experiment. On the contrary, reduction of dietary CP from 260 g/kg to 220 g/kg across 2 to 6 weeks of age did not affect DWG and FCR of bobwhite quails but decreased DFI of them (Blake and Hess 2013). Growth performance of broiler quails was not affected by supplementation of threonine to the feed exceeding 100% requirement of them. It can be explained that supplementation of dietary 7.9 g/kg digestible threonine is an optimal level for quails in this study. Nevertheless, improved growth performance of broiler quails was observed in response to dietary supplementation of threonine higher than recommended demand by the other researchers (Baylan et al. 2006; Rasheed et al. 2018). These contradictory results might be due to difference exist in strain of birds and supplemented amino acid levels in their diets.

Muscle meat is an edible part of a meat-type quail with high economic value for producers. Therefore, nutritionists try to direct nutrition towards the improvement in carcase yield. It has been reported that dietary protein levels affect muscle yield in broiler chickens (Rezaei et al. 2004) and dietary threonine plays an important role in the development of breast muscle as a desired part of carcase (Rasheed et al. 2018). In the present experiment, the greatest carcase yield obtained when quails fed on diets containing 200 g/kg CP in conjunction with 110% of their digestible threonine requirement. In accordance with our results, Sigolo et al. (2017) observed that dietary threonine inclusion at 110% requirement level increased eviscerated carcase weight in broiler chickens fed on low CP diets. However, Abbasi et al. (2014) did not see any effect of feeding low or adequate CP diets containing threonine beyond 100% requirement on carcase yield of broiler chickens. On the other side, Baylan et al. (2006) reported that carcase yield remained statistically unaffected in response to supplementation of threonine in the feed of broiler quails. Reports have shown that requirement of threonine for carcase gain differs, depending on age, strain, sex and crude protein content of the feed (Barkley and Wallis 2001), which may justify obtained results of these experiments. Feeding broiler quails with diets containing higher than 180 g/kg and 200 g/kg CP increased proportional weight of duodenum and gizzard, respectively. On the contrary, Incharoen et al. (2010) observed the increased weight of intestinal segments by feeding low-protein diets in broiler chickens. Presumably, discrepancies in the obtained results of different studies imply that different magnitude of incorporated dietary protein may have affected gizzard or intestinal weight which make it difficult to conclude rigidly about the effect of dietary CP or threonine on digestive organs.

Generally, morphology of small intestine is affected by the nutrition of poultry (Laudadio et al. 2012; Ale Saheb Fosoul et al. 2016). In this regard, certain amino acids were reported to influence morphology of small intestinal segments (Murakami et al. 2007; Zaefarian et al. 2008). Threonine is one of the main constitutes of mucin structure, providing 11% of its amino acid content as encoded by mucin-2 gene (Gum et al. 1992). As such, mucin dynamics in the gut might be sensitive to threonine availability. Furthermore, dietary protein content has a critical role in the small intestinal features (Incharoen et al. 2010). In the present experiment, V/C was greater in quails fed on diets containing 200 g/kg and 240 g/kg CP along with 100% and 110% threonine requirement of broiler quails, respectively. Moreover, dietary supplementing threonine beyond the requirement of quails (110%) increased number of goblet cells irrespective of dietary CP content. The V/C is a criterion to evaluate digestive capacity in the small intestine. Additionally, goblet cell count is an index for evaluating intestinal mucin secretion since goblet cells secret mucin in the digestive tract to protect the intestinal membrane from degradation of digestive enzymes and invasion of pathogens (Montagne et al. 2003). Findings on V/C and number of goblet cells are against the results about lack of interaction effect between dietary CP content and threonine levels on CP digestibility and subsequently growth performance of quails in this experiment. It seems that, when the quail nutrient demand was met through diet, gastrointestinal tract development could not further improve growth performance of them. In agreement with the present study, Chen et al. (2017) observed that threonine supplementation increased intestinal goblet cell density in broiler chickens. Similarly, an increased jejunal goblet cell number was observed as dietary threonine content increased in diet of broiler chickens (Horn et al. 2009).

Proteins and certain amino acids have been shown to change mucin secretion and may interact directly with goblet cells or with the enteric nervous system to apply an alteration in mucin production (Montagne
et al. 2000; Claustre et al. 2002; Faure et al. 2005). In gallus gallus, mucin-2 gene is on chromosomes 5, encoding a gel-forming protein and is expressed in the small intestine and other mucous membrane-containing organs. As found herein, the expression of mucin-2 gene increased by reduction of CP content in the feed of broiler quails. It is assumed to be a physiological response of quail’s body to use more nutrients in response to dietary CP reduction. Alternatively, dietary supplementation of 110% digestible threonine requirement increased mucin-2 gene expression since threonine has a prominent role in forming backbone protein of mucin (Montagne et al. 2004). In contrast, Moghaddam et al. (2011) did not observe any effect of dietary threonine supplementation (8 g/kg, 8.7 g/kg, and 9.4 g/kg) on mucin-2 mRNA abundance in broiler chickens. Also, Horn et al. (2009) reported that mucin-2 mRNA abundance was not changed in broiler chickens received diets containing 3.3 g/kg and 8.2 g/kg of threonine. Therefore, more research is needed to study the effect of threonine on mucin-2 gene expression in broiler quails.

Conclusions
Results of the present experiment demonstrated that reduction of CP content in the feed did not affect growth of broiler quails whenever diets added with 100% of their threonine requirement. In this regard, digestibility of CP and relative weight of digestive organs were not affected by the interaction of dietary CP content and threonine levels but morphology of small intestine was influenced. It seems that when the quail nutrient demand was met through diet, gastrointestinal tract development could not further improve growth performance of them. Regardless of dietary threonine, quails fed on diets containing 240 g/kg CP had higher growth rate than the birds received CP180 and CP200. It may show that reduction of dietary CP content below 220 g/kg adversely affect DWG in broiler quails.

Geolocation information
The research was performed in Iran, Islamic Republic of, Isfahan (Latitude: 32.65722, Longitude: 51.67761), Coordinates: 32°39′26″N 51°40′39″E.

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