Effect of threonine and potassium carbonate supplementation on performance, immune response and bone parameters of broiler chickens

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ABSTRACT
This study aimed to investigate the effect of different levels of dietary threonine (i.e. 100%, 110% and 120% of Ross recommendations) and potassium (i.e. 0.85% and 0.94% of diet) on performance, immune response, and bone parameters broiler chickens. Three hundred one-day-old male broiler chickens (Ross 308) were randomly assigned to 1 of 6 dietary treatments in a completely randomized design with a 3 × 2 factorial arrangement. Growth performance was not affected by dietary treatments. Birds fed diet containing 120% threonine and high potassium level were exhibited a lower immune response to phytohemagglutinin-P (PHA-P) as compared to control group (P < .05). Addition of 120% threonine with 0.94% potassium decreased heterophil percentage and heterophil to lymphocyte ratio and increased lymphocyte percentage (P < .05). Tibia and femur width and ash, Ca and P percentage of tibia, and breaking strength of bones were not influenced by dietary potassium level, whereas the width of tibia and femur was increased in broiler chickens received diet containing 120% threonine and 0.94% potassium compared to control group (P < .05). It is concluded that broiler chickens FI during starter period, cell mediated immune response and blood hematology were influence by dietary threonine and potassium interaction.

Introduction
Synthetic essential amino acids (EAAs) play a critical role in poultry nutrition providing nutritionists with considerable flexibility in feed formulation to improve the overall amino acid (AA) balance (Moghadam et al. 2011) and immunoglobulin (Ig) synthesis (Kogut 2009). Threonine is the third limiting AA after methionine and lysine in conventional poultry diets and directly involved in mucin synthesis that is required for intestinal immune function, inhibition of apoptosis, stimulation of lymphocyte proliferation and enhancement of antibody production (Kidd 2000). Therefore, taking threonine into the feed formulation matrix would be a critical limitation in order to use of other EAAs such as total sulphur AAs and Lys in the body efficiently (Kidd 2000). Some researcher have shown that excess dietary threonine promoted broiler chicken performance (Rezaeipour et al. 2012), immunological responses through increasing the relative weight of immune organs, synthesis of immunoglobulin A (IgA), immunoglobulin G (IgG), and secretory IgA (Corzo et al. 2007; Maroufy an et al. 2010; Ren et al. 2014).

Besides L-threonine, rapidly growing animals have a relatively high potassium requirement, which could be limiting element with high protein diets (Hays and Swenson 1993). Potassium is the major intracellular cation that plays a pivotal role in fluid movement and maintaining the membrane potential and influencing the fate of AAs in the cell. Persistence of the electrolyte balance of diet may indirectly affect the electrolyte balance of body cells where in imbalance situations the incidence of metabolic disorders is possible (Oviedo-Rondon et al. 2001).

Insufficient bone mineralization is one of the reasons for leg bone abnormalities (Jankowski et al. 2011) that often caused by mineral metabolism disorders, in particular, a deficiency of Ca and P (Venäläinen et al. 2006). Sodium metabolism disorders lead to a decrease in the ash content of bones, thus exerting a negative effect on the bone mineralization process (Murakami et al. 2000). Sodium-calcium interactions also play a vital role in the pathogenesis of skeletal muscle damage in broiler chickens (Sandercock and Mitchell 2004) and reduction of sodium and increasing potassium content of broiler chicken diets with constant dietary electrolyte balance may be beneficial for bone mineralization. On the other hand, it is established that there is a relation between dietary or serum AA and serum potassium levels on rats but not in broiler chickens. The effect of an AA on steady-state potassium content depends in part upon the charge on the AA so that cationic AAs had a marked and significant depressant effect on potassium content of rats muscles while neutral AAs like threonine had a significant but less marked depressant effect on potassium content and anionic AAs had no effect (Levinsky et al. 1962). To date, there has been no reliable evidence to show the potassium and protein or threonine (as neutral AAs) interaction in broiler chickens. Based on the previous literatures, we hypothesized reduction or threonine (as neutral AAs) interaction in broiler chickens. This study aimed to investigate the effect of threonine and potassium carbonate supplementation on performance, immune response, and bone parameters broiler chickens.
potassium of broiler chickens. Therefore, the aim of this study was to explore the interaction between dietary threonine and potassium levels on performance, immune response, and bone parameters of broiler chickens.

**Material and methods**

**Animals, diets and treatment**

This experiment was carried out in broiler chicken farm of Ilam University (Iran). A total of 300 one-day-old male broiler chicks (Ross 308) were purchased from a commercial hatchery. Environmental temperature was set at 31°C and 28°C for the first and second weeks, respectively, decreased by 2.5°C per week up to 22°C. The relative humidity was 55 ± 5% throughout the study and the birds were kept in floor pens. Birds had access to 1 of 6 dietary treatments, with 5 replicates and 10 birds each, in a completely randomized design with 3×2 factorial arrangement. Dietary treatments consisted of 3 levels of threonine (100%, 110% and 120% of Ross 308 recommendations) and 2 levels of potassium (0.85 and 0.94% of diet as NaHCO3) respectively). Feed ingredients (i.e. corn, corn gluten meal, and soybean meal) were analysed for AA normal and high, respectively). Feed ingredients (i.e. corn, corn gluten meal, and soybean meal) were analysed for AA.

| Table 1. Ingredient and nutrient composition of basal diet. |
|---------------------------------|
| Ingredient (g/kg) | Nutrient composition (g/kg) |
|-------------------|-------------------------------|
| Corn              |                                |
| Soybean meal (44% CP) |                                |
| Vegetable oil     |                                |
| DL-Methionine     |                                |
| L-Lysine HCl      |                                |
| L-Threonine       |                                |
| Di-Calcium Phosphate |                                |
| Oyster shell      |                                |
| Salt              |                                |
| NaHCO3            |                                |
| Vitamin premix    |                                |
| Mineral premix    |                                |
| Calculated nutrients (as % unless otherwise stated) | |
| Ingredient (g/kg) | Nutrient composition (g/kg) |
|-------------------|-------------------------------|
| Corn (g/kg)       | 12.47                         |
| Soybean meal (44% CP) | 20.60                         |
| Vegetable oil     | 2.4                           |
| DL-Methionine     | 2.37                          |
| L-Lysine HCl      | 2.79                          |
| L-Threonine       | 0.47                          |
| Di-Calcium Phosphate | 15.14                        |
| Oyster shell      | 12.88                         |
| Salt              | 2.75                          |
| NaHCO3            | 1.65                          |
| Vitamin premix    | 2.5                           |
| Mineral premix    | 2.5                           |
| Calculated nutrients (as % unless otherwise stated) | |
| Ingredient (g/kg) | Nutrient composition (g/kg) |
|-------------------|-------------------------------|
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| Soybean meal (44% CP) | 20.60                         |
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| DL-Methionine     | 2.37                          |
| L-Lysine HCl      | 2.79                          |
| L-Threonine       | 0.47                          |
| Di-Calcium Phosphate | 15.14                        |
| Oyster shell      | 12.88                         |
| Salt              | 2.75                          |
| NaHCO3            | 1.65                          |
| Vitamin premix    | 2.5                           |
| Mineral premix    | 2.5                           |
| Calculated nutrients (as % unless otherwise stated) | |

**Performance, hemagglutination inhibition (HI) test and white blood cells count**

During the study, feed intake (FI) and body weight (BW) were recorded weekly and then feed conversion ratio (FCR) was calculated accordingly. European production efficiency factor (EPEF) was calculated as (Awad et al. 2008):

\[
\text{EPEF} = \left[ \frac{\text{Viability}\% \times \text{BW(kg)} / \text{age(d)} \times \text{FCR(kgfeed/kggain)}}{100} \right]
\]

The blood samples were collected from the wing vein of 10 birds (those subjected for injection of phytohemagglutinin) from each treatment in non-heparinized collecting tubes at d 28 of age. Heterophil (H) and lymphocyte (L) percentage were determined and H:L ratio was calculated accordingly.

**Toe web swelling test**

The lymphoproliferative response to phytohemagglutinin (PHA-P: L1668 Sigma), as an indicator of a T-cell-induced delayed-type hypersensitivity reaction, was assessed as described previously (Corrier and DeLoach 1990). The toe web swelling reaction to PHA-P was measured in 2 birds from each replicate (marked with a black colour) at d 32 of age. One-tenth millilitre of a PHA-P solution (1 mg/mL in phosphate buffer saline: PBS) was injected subcutaneously into 2 sites on the left toe web. As a sham control, 0.1 mL of PBS was injected into 2 sites on the right toe web. The thickness of each injection site was measured using a pressure-sensitive micrometre before

| Table 2. Amino acid profile and standardized ileal digestible (SID) amino acids of ingredients. |
|---------------------------------|
| Ingredient (g/kg) | Nutrient composition (g/kg) |
|-------------------|-------------------------------|
| Corn (g/kg)       | 12.47                         |
| Soybean meal (44% CP) | 20.60                         |
| Vegetable oil     | 2.4                           |
| DL-Methionine     | 2.37                          |
| L-Lysine HCl      | 2.79                          |
| L-Threonine       | 0.47                          |
| Di-Calcium Phosphate | 15.14                        |
| Oyster shell      | 12.88                         |
| Salt              | 2.75                          |
| NaHCO3            | 1.65                          |
| Vitamin premix    | 2.5                           |
| Mineral premix    | 2.5                           |
| Calculated nutrients (as % unless otherwise stated) | |
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| L-Threonine       | 0.47                          |
| Di-Calcium Phosphate | 15.14                        |
| Oyster shell      | 12.88                         |
| Salt              | 2.75                          |
| NaHCO3            | 1.65                          |
| Vitamin premix    | 2.5                           |
| Mineral premix    | 2.5                           |
| Calculated nutrients (as % unless otherwise stated) | |
injection and at 4, 24, and 48 h after injection. The toe web swelling reactions to PHA-P were calculated using the following swelling index:

\[
\text{Swelling index} = \frac{(\text{thickness of left toe web after PHA-P injection} - \text{initial thickness of left toe web}) - (\text{thickness of right toe web after PBS injection} - \text{initial thickness of right toe web})}{\text{(thickness of left toe web after PBS injection) - (thickness of right toe web after PBS injection)}}
\]

**Bone breaking strength, diameters, width, length and ash content**

At d 42 of age, five birds from each treatment were chosen at random, weighed and then slaughtered according to the Islamic method (Halal) and the right tibia and femur were excised. Soft tissues were removed manually and the bone cleaned with gauze. Bone samples were stored in plastic bags and frozen at -20°C until analysis for breaking strength. Bone diameter, width and length were measured using a Lutron digital caliper (model DC-515, Lutron Electronic Enterprise Co., Ltd, Taiwan) at the narrowest and widest points. SEM 100, 110 and 120% of ross broiler threonine amino acid recommendation (2014). A round-based probe was attached to a 50-kg load cell with automated materials test system software. Bone breaking strength, diameters, width, length and ash content, the right tibia bone was used to measure the concentration of phosphorus (P) (Latimer 2012).

**Statistical analysis**

Statistical analysis was conducted using GLM procedure (SAS 2001) to evaluate the main and interactive effects of treatments on growth performance, immune response, and bone traits of broiler chickens. The orthogonal polynomial contrasts were used to assess the linear and quadratic effects of dietary treatments. The means of treatments were compared by Tukey test at P < .05.

**Results**

**Growth performance and immune response**

Data on average BW, FI and FCR and UPEF are presented in Table 3. There was no significant difference in broiler chickens growth performance between the groups fed different levels of dietary potassium during the starter, growing, finisher and entire period of experiment. In regards to dietary threonine level, the lowest FCR during starter period and the highest FI and BW during grower period and BW during entire period of experiment. In regards to dietary threonine level, the lowest FCR during starter period and the highest FI and BW during grower period and BW during entire period of experiment. In regards to dietary threonine level, the lowest FCR during starter period and the highest FI and BW during grower period and BW during entire period of experiment. In regards to dietary threonine level, the lowest FCR during starter period and the highest FI and BW during grower period and BW during entire period of experiment. In regards to dietary threonine level, the lowest FCR during starter period and the highest FI and BW during grower period and BW during entire period of experiment.
Threonine (%) B Means 100%, 110% and 120% of Ross broiler threonine amino acid recommendation (2014).

Table 4. Effect of treatments on toe web thickness index of broiler chickens against PHA-P injection (mm).

| Potassium | Threonine (%) |
|-----------|---------------|
| Normal (0.85%) | 100 | 1.322<sup>a</sup> |
| High (0.94%) | 100 | 0.847<sup>b</sup> |

Threonine (%)<sup>b</sup>

| 100 | 1.156<sup>a</sup> |
| 110 | 0.999<sup>a</sup> |
| 120 | 0.817<sup>b</sup> |

Potassium source was potassium carbonate.

Table 5. Effects of treatments on blood haematology and lymphoid organs of broiler chickens.

| Blood cell count | H% | L% | H:L ratio |
|------------------|-----|----|-----------|
| Potassium<sup>a</sup> | Normal (0.85%) | 32.58<sup>a</sup> | 64.42<sup>b</sup> | 0.54<sup>a</sup> |
|                   | High (0.94%) | 18.75<sup>a</sup> | 79.25<sup>b</sup> | 0.24<sup>b</sup> |
| Threonine (%)<sup>b</sup> | 100 | 22.62<sup>a</sup> | 74.50<sup>b</sup> | 0.31<sup>b</sup> |
|                   | 110 | 33.12<sup>a</sup> | 64.62<sup>b</sup> | 0.57<sup>a</sup> |
|                   | 120 | 21.25<sup>a</sup> | 76.38<sup>b</sup> | 0.28<sup>b</sup> |

SEM = 0.115

Table 6. The effects of treatments on bone mineralization of broiler chickens (mg/g).

| Potassium | Threonine (%) |
|-----------|---------------|
| Normal (0.85%) | 100 | 1.275<sup>a</sup> |
| High (0.94%) | 100 | 1.154<sup>ab</sup> |
|              | 110 | 0.969<sup>abc</sup> |
|              | 120 | 1.037<sup>abc</sup> |

Threonine × Potassium<sup>***</sup>

Table 7. The effects of treatments on bone mechanical properties of broiler chickens.

| Bone parameter | Probability |
|----------------|-------------|
|                | *           |
| Potassium      | **          |
| Threonine      | **          |
| Threonine × Potassium | **          |
| SEM            | NS          |

Discussion

Bone parameters

The results indicated that broiler chicken received the higher level of threonine (120%) and potassium (0.94%) had thicker tibia, whereas as compared to other groups, feeding diet containing 110% threonine with 0.94% potassium to broiler chickens led to higher femur width (Table 6; <.05). Tibia and femur mineral concentration and bone mechanical properties are presented in Tables 7 and 8. No significant differences were observed in the tibia and femur maximum breaking strength and tibia Ca, P and total ash percentage were observed among treatments while broiler chickens fed diet containing 120% threonine had lower femur breaking strength and tibia P percentage (<.05).

Discussion

Growth performance and immune response

Recently, it has been well documented that supplemented dietary threonine levels from 8.0 to 10.5 g/kg of diet (Kidd et al. 2005; Rezaeipour and Gazani 2014; Eftekhari et al. 2015; Chen et al. 2016) while increasing of L-threonine in diets improved FCR and BWG of broiler chickens (Rezaeipour et al. 2012) did not improve BWG, FI and FCR of broiler chickens. Similarly, our results indicated that different levels of dietary threonine (7.9–9.3 g/kg) did not affect growth performance of broiler chickens. Therefore, the similar growth performance among treatments in the current study suggested that the minimum levels of threonine (0.79%, 0.69% and 0.61% in starter, grower and finisher phases, respectively) might be adequate in order to maintain broiler chicken FCR and BWG. In addition, lack of positive response with supplemented threonine in the current experiment might be due to proportionately reduced requirement of threonine in relation to the lower CP and Lys levels in our diets. On the other hand, increased FI in chicks fed diet containing higher level of potassium and 120% threonine during starter period may be explained by the lower dietary sodium concentration and high amount of endogenous AA loss during this period (Adedokun et al. 2011).

In the present study, it was observed that supplemented threonine and potassium interaction decreased cell-mediated immunity in response to PHA-P injection of broiler chickens. Similarly, immune response to PHA-P of broiler chickens fed graded levels (0.07%, 0.14%, 0.21% and 0.28%) of L-threonine increased linearly and quadratically by increasing threonine levels in the diet (Khalaji et al. 2011). It can, therefore, be speculated that addition of higher level of potassium and threonine together had negative effect on broiler chickens immune response to PHA-P and the possible reason of these results is unknown. The lower H/L ratio in chickens fed diet containing 120% threonine in our study was in contrast to previous study (Corzo et al. 2007) who reported that white blood cell count (absolute and ratio) did not differ between broiler chickens fed different threonine levels.

It was reported that neutral AAs like threonine reduce potassium content of muscles (Levinsky et al. 1962) and it may affect potassium requirement. There is lack of adequate data about threonine and potassium interaction in broiler chickens,
therefore, possible explanation of our results is almost complicated. On the other hand, little information is available on the effects of relatively high dietary concentrations of potassium on performance of boiler chickens. Clinical signs of potassium deficiency in avian species are overall muscle weakness, characterized by weak extremities and poor intestinal tone with distention (Austic and Scott 1997). Feeding diets containing

| Table 6. Effect of treatment on broiler chicken bone length and width (mm). |
|------------------|------------------|------------------|------------------|------------------|------------------|
|                  | **Tibia**        |                  | **Femur**        |                  |                  |
|                  | Length           | Width            | Diameter         | Length           | Width            | Diameter         |
| **Potassium**    |                  |                  |                  |                  |                  |                  |
| Normal (0.85%)   | 32.04            | 33.80            | 7.19             | 73.80            | 9.48             | 9.62             |
| High (0.94%)     | 30.79            | 33.36            | 7.38             | 73.34            | 9.82             | 9.92             |
| SEM              | 0.62             | 0.94             | 0.11             | 0.47             | 0.12             | 0.11             |
| **P-value**      | 0.93             | 0.20             | 0.19             | 0.51             | 0.08             | 0.06             |
| **Threonine (%)**|                  |                  |                  |                  |                  |                  |
| 100              | 97.14            | 7.02             | 7.02             | 73.36            | 9.57             | 9.57             |
| 110              | 99.40            | 7.48             | 7.47             | 73.82            | 10.13            | 10.12            |
| 120              | 100.07           | 7.36             | 7.36             | 73.53            | 9.62             | 9.62             |
| SEM              | 0.89             | 0.12             | 0.12             | 0.59             | 0.02             | 0.14             |
| **P-value**      | 0.08             | <0.05            | 0.07             | 0.85             | 0.14             | 0.63             |

**SEM** Mean values within a column with unlike superscript letters were significantly different (P<0.05).

**Threonine (%)** Mean values within a column with unlike superscript letters were significantly different (P<0.05).

Potassium source was potassium carbonate.

Means 100, 110 and 120% of Ross broiler threonine amino acid recommendation (2014).

| Table 7. Effect of treatments on bones breaking strength (BBS) (N/m²). |
|------------------|------------------|------------------|------------------|------------------|------------------|
|                  | **BBS max**      | **BBS break**    |                  |                  |                  |
|                  | Tibia            | Femur            | Tibia            | Femur            |                  |
| **Potassium**    |                  |                  |                  |                  |                  |
| Normal (0.85%)   | 499.38           | 241.58           | 516.86           | 258.17           |                  |
| High (0.94%)     | 367.94           | 258.22           | 416.48           | 268.22           |                  |
| SEM              | 44.38            | 15.04            | 51.87            | 16.52            |                  |
| **P-value**      | 0.06             | 0.45             | 0.19             | 0.67             |                  |
| **Threonine (%)**|                  |                  |                  |                  |                  |
| 100              | 417.40           | 268.87           | 484.77           | 275.62           | 11.00            |
| 110              | 518.96           | 298.70           | 546.37           | 299.11           | 13.01            |
| 120              | 364.63           | 182.13           | 368.77           | 214.86           |                  |
| SEM              | 54.36            | 18.42            | 63.53            | 20.23            |                  |
| **P-value**      | 0.16             | <0.05            | 0.17             | <0.05            |

**SEM** Mean values within a column with unlike superscript letters were significantly different (P<0.05).

Potassium source was potassium carbonate.

Means 100%, 110% and 120% of Ross broiler threonine amino acid recommendation (2014).

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**Ca** % of Ash | % of DM | % of Ash | % of DM | Ash (gr) | Ash (%)
---|---|---|---|---|---
Normal (0.85%) | 42.84 | 16.68 | 13.52 | 5.21 | 2.56 | 49.25
High (0.94%) | 45.64 | 18.36 | 11.66 | 4.71 | 2.74 | 51.65
**SEM** | 1.69 | 0.75 | 0.91 | 0.38 | 0.08 | 1.14
**P-value** | 0.26 | 0.14 | 0.17 | 0.37 | 0.11 | 0.16

**Threonine (%)** Mean values within a column with unlike superscript letters were significantly different (P<0.05).

Potassium source was potassium carbonate.

Means 100%, 110% and 120% of Ross broiler threonine amino acid recommendation (2014).

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**P** % of Ash | % of DM | % of Ash | % of DM | % of Ash | % of DM | Ash (gr) | Ash (%)
---|---|---|---|---|---|---|---
Normal (0.85%) | 42.75 | 17.72 | 13.94 | 5.76 | 2.57 | 52.24
High (0.94%) | 42.94 | 16.58 | 14.54 | 5.52 | 2.64 | 49.36
**SEM** | 2.07 | 0.92 | 1.12 | 0.47 | 0.09 | 1.40
**P-value** | 0.28 | 0.44 | <0.05 | <0.05 | 0.50 | 0.31

**SEM** Mean values within a column with unlike superscript letters were significantly different (P<0.05).

Potassium source was potassium carbonate.

Means 100%, 110% and 120% of Ross broiler threonine amino acid recommendation (2014).

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Threonine × Potassium

**Linear** | <0.05 | 0.07 | <0.05 | 0.84 | 0.18 | 0.78
**Quadratic** | 0.47 | 0.07 | 0.59 | 0.61 | 0.66 | <0.05

**SEM** Mean values within a column with unlike superscript letters were significantly different (P<0.05).

Potassium source was potassium carbonate.

Means 100, 110 and 120% of Ross broiler threonine amino acid recommendation (2014).
higher potassium content (1.22% and 1.27%) with dietary electrolyte balance (DEB) values from 329 to 349, decreased broiler chickens performance (Koreleski et al. 2010) and inclusion of 25% and 50% more than basal diet to male turkey diets reduced weight gain but had no effect on FCR (Reece et al. 2000). Nevertheless, in other experiment broiler chickens fed diet containing 0.18% sodium, 1.22% potassium and 0.22% chloride with DEB value of 330, achieved high weight gain (Borgatti et al. 2004). However, in our experiment, potassium and sodium levels were not more than 0.95 and 0.17%, respectively, and it was suggested that when dietary potassium was 1.01%, sodium is not needed more than 0.20% for 0–21d chickens (Murakami et al. 1997).

It was reported that potassium salts may ameliorate the stress effects in broiler chickens (Borges et al. 2004; Ahmad and Sarwar 2005). In regards to this statement, addition of 0.50% and 1% potassium chloride (KCl) to diet or drinking water had no effect on blood haematology (Borges et al. 1999). In contrast, our result showed that the addition of potassium increased lymphocyte percentage and decreased H and H:L ratio. In regards to our results, it seems that 10% substitution of sodium with potassium has no detrimental effect on broiler chicken performance and probably make no deficiency of potassium and sodium in broiler chickens (Koreleski et al. 2010).

**Bone traits**

It has been well documented that there is a positive effect of dietary protein on bone metabolism. Higher dietary AA levels reduced bone ash but did not affect tibia breaking strength (Skinner et al. 1991). In the current study, threonine supplementation (0.93%) decreased bone P concentration while did not affect bone mineralization and bone Ca concentration. Similar results were reported that feeding diets with high (21%) and low (19%) protein did not affect toe ash content (Coto et al. 2009). On the other hand, higher protein and threonine levels decreased blood sodium (our unpublished data) and potassium and increased uric acid concentration in broiler chickens (Darsi et al. 2012). The metabolism of majority of AAs such as glutamic acid, lysine, arginine, serine, glycine and the branched chain AAs appears to be influenced by acid-base balance (Patience 1990) and it may be suggested that threonine influence bone traits but its mechanism may be different from acid-base balance.

In the current study, addition of potassium carbonate to low-sodium diet resulted in no difference in bone traits and Ca and P content compared to those of other group. Little information is available on the effects of relatively high dietary concentrations of K on bone traits of broiler chickens. Research with high sodium content (0.34–1.34 or 2.82 g/kg) with higher potassium (11 g/kg) did not show any differences in turkey total ash, calcium and phosphorus in tibia dry matter while increased the bone density index, the maximum bending moment and the minimum breaking strength of tibia (Jankowski, Lichtorowicz, et al. 2012; Jankowski, Żduńczyk, et al. 2012). In our study extra potassium or reduction of dietary sodium did not affect broiler chickens bone traits while in previous study, inclusion of 25% and 50% potassium more than basal diet, led to higher incidence of turkey leg weakness at 12 wk (Reece et al. 2000). They suggested that dietary potassium concentrations greater than those usually present in corn-soybean meal basal diets for growing turkeys should be avoided. Higher potassium concentrations more than NRC requirements led to develop hyperkalemia either from diminished renal excretion or excessive diffusion from cells to the extracellular fluid (Reece et al. 2000). Renal Ca reabsorption is directly proportional to sodium reabsorption. When dietary sodium chloride is increased, the fractional reabsorption of sodium is decreased, leading to a parallel reduction in Ca reabsorption (Sellmeyer et al. 2002). In these situations, it is possible to hypothesize that addition of potassium carbonate may be beneficial to improve bone quality by decreasing Ca excretion. This effect may be partly responsible for the numerically higher Ca content of tibia in chicks fed higher potassium level in the present study.

**Conclusion**

In conclusion, it is identified that there is no interaction between threonine and potassium in performance and humoral immunity of broiler chickens. Nevertheless, it is concluded that higher potassium and threonine levels improved broiler chickens FI during starter period, cell-mediated immune response and blood haematology. It is suggested that higher level of potassium with threonine supplementation may be applicable in ameliorating stresses in broiler production.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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