Effect of different levels of lysine and threonine on carcass characteristics, intestinal microflora and growth performance of broiler chicks

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ABSTRACT
The aim of current study was to evaluate the effect of different levels of lysine and threonine on performance, carcass characteristics and intestinal microflora of broiler. Nine hundred and sixty Ross 308 one-day-old female broiler chicks were selected, and they were randomly divided in a factorial design with 12 treatments, four replicates and 20 chicks in each replicate. The tested diets included four levels of lysine (100, 110, 120 and 130%, suggested by NRC) and three levels of threonine (100, 110 and 120%, suggested by NRC). Two phase (starter 0–21d; finisher 22–42d) feeding schedule was followed. Results showed that of 21 days of age, 120% of lysine and threonine the live and carcass weights and breast, thigh and liver yields in chicks increased (p < .05). At the age of 42 days those treatments which received 120% of threonine gained the highest rate of live and carcass weights and yield of carcass characteristics. The intestinal microflora, in 42 days of age, with the increase in concentration of lysine significantly decreased in the intestinal microflora. The level of threonine in diet, and the interaction between threonine and lysine at 42 days of age effect on intestinal microflora. The best feed conversion ratio was related to 120% lysine. According to the results of this study, the carcass characteristics and growth performance can be improved with increasing in lysine and threonine levels to 120% above from NRC recommendations for commercial broilers.

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Introduction
Broiler chicks do not require to crude protein inherently, but they are required to accurate and balance amounts of amino acids. Nowadays, researchers are trying to formulate diets based on digestible amino acids (Creswel & Swick 2001) because it seems that digestible amino acids in feed are available part of protein to meet the requirements of maintenance and production (Mehri et al. 2012). Partial replacement of intact protein (e.g. soybean meal) with crystalline, free amino acids, excesses of dietary amino acids are minimised in relation to their requirement, bringing the dietary protein closer to ideal protein and, in turn, decreasing the dietary crude protein content (Bregendahl et al. 2002). The use of supplemental amino acids in poultry diets has provided nutritionists with considerable flexibility in formulation. The availability of lysine, methionine and threonine at competitive prices has enabled the vast majority of the poultry industry to include them as supplements in their diets and, therefore, allow for a reduction in pressure points caused by their degree of limitation in practical broiler diets (Corzo et al. 2007). Lysine and threonine are generally considered to be the first and second-limiting amino acids in practical broiler diets after the sulphur amino acids (Corzo et al. 2007; Samadi & Liebert 2007).

Lysine is an important amino acid in protein synthesis. The presence of this amino acid is lower in metabolic reactions and it mainly participates in body protein structure. A good number of studies showed that increasing dietary levels of lysine improved breast meat production (Mack et al. 1999; Barboza et al. 2000). Lysine also impacts on the improvement of functional traits such as decreasing feed conversion and increasing feed intake.

Unlike lysine, threonine is not only used for protein deposition, but also plays several other significant metabolic roles. Threonine is particularly involved in maintenance processes, like the renewal of intestinal mucus and the synthesis of immune proteins. It has been estimated that more than half of the dietary threonine consumed by a piglet (Stoll et al. 1998) or a
human (Fuller et al. 1994) is retained at the intestinal level to fulfil these gut-maintenance functions and is primarily used in the synthesis of mucins (Corzo et al. 2007). This study was designed to evaluate the effect consuming duration of lysine and threonine (21 and 42 days) on growth performance, carcass characteristics and intestinal microflora on female broiler chicks.

Materials and methods

A total of 960 Ross 308 one-day-old female broiler chickens (mean weight 43 ± 5 g) were selected and randomly distributed in a factorial design with 12 treatments, four replicates and 20 chicks in each replicate. Temperature was recorded daily and managed to provide thermoneutral conditions to the birds throughout the study. The lighting schedule consisted of 23 h of light and 1 h of darkness. Cross-ventilation was accomplished by negative air pressure. Chicks were vaccinated for infectious bronchitis (at days 1 and 7), Newcastle disease (at days 10 and 25) and infectious bursal disease (at day 17). The experimental diets include four levels of lysine (100, 110, 120 and 130% suggested by National Research Council 1994) and three levels of threonine (100, 110 and 120% suggested by National Research Council 1994). The diets in two starter (0 to 21 days of age) and grower (21 to 42 days of age) periods were fed ad libitum to the birds. For each of the periods, diets were formulated to meet the (National Research Council 1994) nutrient recommendations and lysine and threonine were supplemented to the diets to achieve the desired values of these two amino acids in various treatments (Table 1). The body weight gains, feed intake and feed conversion ratio of chicks were calculated throughout the experiment. On days 21 and 42, two birds per replicate with mean weight close to the mean weight of the replicate were selected and slaughtered by cutting the jugular vein after 3 h of fasting. After slaughter and measuring the carcass weight, carcass components weight (breast, thigh, liver, heart and abdominal fat) were measured and calculated (%) relative to live weight. In each slaughter, a sample of the ileum contents was removed and to determine the total count of intestinal microflora, the sample was transferred to the microbiology laboratory of Islamic Azad University, Shahrekord branch. To calculate the total count of intestinal microflora, surface plate method was used. Ten grams of each sample of ileal faeces were dissolved in 90 ml of distilled water and from $10^{-1}$ dilution made, a serial dilution from $10^{-2}$ to $10^{-6}$ were prepared in distilled water (for this purpose, 1 ml of each dilution was added to 9 ml of distilled water).

### Table 1. Ingredient composition of the experimental diets (% as-fed basis).

| Ingredients, % | Starter | Grower |
|---------------|---------|--------|
| Maize         | 56.70   | 65.50  |
| Soybean meal  | 30.05   | 21.35  |
| Safflower meal| 4.38    | 5.52   |
| L-Lys HCl     | 0.02    | 0.18   |
| L-Thr         | 0.02    | 0.08   |
| DCP           | 0.17    | 0.17   |
| Oyster shell  | 2.90    | 2.60   |
| Salt          | 0.57    | 0.57   |
| Soybean oil   | 0.42    | 0.30   |
| Mineral premix| 3.19    | 1.94   |
| Vitamin premix| 0.25    | 0.25   |
| Sand          | 1.08    | 1.29   |

From three final dilutions of each sample, 1 ml in dense form was cultured on surface of two Brain Heart Infusions (BHI) (Purchased from HiMedia Company, India). After 24 h incubating the plates in 37°C, the number of colonies on the plates that were between 30 and 300 were counted. The average number of colony counts from each dilution was calculated and multiplied in the inverse of the dilution factor and reported as Colony Forming Unit (CFU) (Downes & Ito 2001). The obtained data were analysed as a 3 x 4 factorial experiment in a completely randomised design with four replications using General Linear Models (GLM) procedure of (Statistical Analysis Systems Institute 2001). The significance of difference between means was determined by Duncan’s multiple-range-test at $(p < .05)$.

### Results and discussion

Results of the effects of different levels of lysine and threonine on carcass characteristics and intestinal microflora in the starter and grower periods have been reported in Tables 2-4. Most live weight, carcass weight, and breast, thigh and liver yields at the age of 21 days were due to the treatment with lysine content, 120% of which was recommended by NRC.
In agreement with our results, Nasr and Kheiri (2011) reported that there was a significantly higher body weights in broilers fed with 120% of lysine relative to NRC recommendations. Kidd et al. (1998) reported that the breast meat weight and yield increased when broilers received the higher lysine levels in the starter diets. Labadan et al. (2001) suggested that broilers in the first two weeks of their age need more lysine than that of recommended by NRC for maximum breast meat production. In contrast to our finding Zaboli et al. (2012) reported that the levels of 0.4 and 0.5% of supplemental lysine are the best

### Table 2. Effect of different levels of lysine and threonine on carcass characteristics at day 21.

| Treatments | Live weight, g | Carcass weight, g | Breast, % | Thigh, % | Liver, % | Heart, % | Abdominal fat, % |
|------------|----------------|-------------------|-----------|---------|----------|----------|-----------------|
| **Lys effect** |                |                   |           |         |          |          |                 |
| 100%       | 568.55         | 326.44            | 16.93     | 13.24   | 1.40     | 0.30     | 0.570           |
| 110%       | 575.89         | 327.11            | 17.46     | 13.67   | 1.55     | 0.34     | 0.570           |
| 120%       | 585.55         | 350.00            | 18.02     | 14.26   | 1.60     | 0.34     | 0.574           |
| 130%       | 596.44         | 341.33            | 17.44     | 13.94   | 1.44     | 0.31     | 0.537           |
| **Thr effect** |                |                   |           |         |          |          |                 |
| 100%       | 578.42         | 331.08            | 17.40     | 13.61   | 1.49     | 0.32     | 0.568           |
| 110%       | 590.00         | 337.33            | 17.35     | 13.79   | 1.50     | 0.33     | 0.572           |
| 120%       | 587.67         | 342.50            | 17.62     | 13.94   | 1.56     | 0.33     | 0.546           |
| **Lys and Thr interactions** |                |                   |           |         |          |          |                 |
| 100% Lys + 100% Thr | 574.33  | 324.33            | 17.30     | 13.90   | 1.40     | 0.33     | 0.563           |
| 100% Lys + 110% Thr | 554.33  | 322.33            | 17.09     | 13.76   | 1.37     | 0.32     | 0.599           |
| 100% Lys + 120% Thr | 563.33  | 338.33            | 16.89     | 13.80   | 1.36     | 0.34     | 0.548           |
| 110% Lys + 100% Thr | 566.00  | 326.67            | 17.22     | 13.30   | 1.56     | 0.32     | 0.571           |
| 110% Lys + 110% Thr | 584.67  | 338.67            | 17.58     | 13.44   | 1.44     | 0.35     | 0.581           |
| 110% Lys + 120% Thr | 592.33  | 313.33            | 17.51     | 13.85   | 1.53     | 0.34     | 0.593           |
| 120% Lys + 100% Thr | 600.33  | 342.33            | 17.47     | 13.85   | 1.53     | 0.34     | 0.593           |
| 120% Lys + 110% Thr | 584.00  | 337.33            | 17.70     | 13.84   | 1.53     | 0.34     | 0.593           |
| 120% Lys + 120% Thr | 608.33  | 367.33            | 17.60     | 14.08   | 1.66     | 0.35     | 0.572           |
| **SEM** | 1.604 | 1.388 | 0.147 | 0.120 | 0.051 | 0.014 | 0.007 |

Means within a column with no common superscript differ significantly. SEM: standard error of means.

### Table 3. Effect of different levels of lysine and threonine on carcass characteristics at day 42.

| Treatments | Live weight, g | Carcass weight, g | Breast, % | Thigh, % | Liver, % | Heart, % | Abdominal fat, % |
|------------|----------------|-------------------|-----------|---------|----------|----------|-----------------|
| **Lys effect** |                |                   |           |         |          |          |                 |
| 100%       | 1909.22        | 1164.44           | 24.88     | 21.14   | 2.25     | 0.44     | 1.39            |
| 110%       | 2068.67        | 1268.55           | 25.31     | 21.87   | 2.31     | 0.46     | 1.34            |
| 120%       | 2202.33        | 1399.33           | 26.32     | 23.73   | 2.34     | 0.47     | 1.33            |
| 130%       | 2190.55        | 1372.55           | 25.89     | 21.68   | 2.28     | 0.49     | 1.33            |
| **Thr effect** |                |                   |           |         |          |          |                 |
| 100%       | 2067.25        | 1273.33           | 25.42     | 22.10   | 2.27     | 0.45     | 1.35            |
| 110%       | 2078.67        | 1290.33           | 25.56     | 21.87   | 2.30     | 0.44     | 1.36            |
| 120%       | 2132.17        | 1340.00           | 25.82     | 22.33   | 2.30     | 0.45     | 1.37            |
| **Lys and Thr interactions** |                |                   |           |         |          |          |                 |
| 100% Lys + 100% Thr | 1901.67  | 1148.67           | 25.12     | 20.72   | 2.21     | 0.40     | 1.39            |
| 100% Lys + 110% Thr | 1889.67  | 1140.67           | 24.88     | 21.87   | 2.25     | 0.43     | 1.38            |
| 100% Lys + 120% Thr | 2033.33  | 1243.33           | 24.77     | 21.53   | 2.25     | 0.40     | 1.40            |
| 110% Lys + 100% Thr | 1936.33  | 1290.00           | 25.30     | 21.44   | 2.27     | 0.42     | 1.35            |
| 110% Lys + 110% Thr | 2043.33  | 1342.67           | 26.26     | 21.06   | 2.23     | 0.45     | 1.35            |
| 110% Lys + 120% Thr | 2125.33  | 1243.33           | 26.25     | 21.87   | 2.31     | 0.45     | 1.35            |
| 120% Lys + 100% Thr | 2032.00  | 1365.67           | 25.89     | 22.94   | 2.32     | 0.46     | 1.34            |
| 120% Lys + 110% Thr | 2121.67  | 1363.00           | 26.17     | 23.33   | 2.33     | 0.46     | 1.33            |
| 120% Lys + 120% Thr | 2209.67  | 1429.33           | 27.03     | 24.91   | 2.37     | 0.50     | 1.33            |
| **SEM** | 1.941 | 2.375 | 0.091 | 0.108 | 0.016 | 0.009 | 0.025 |

Means within a column with no common superscript differ significantly. SEM: standard error of means.
levels for maximum weight of breast and thigh at the starter period, the researchers also observed that carcass yield was not significantly affected by different levels of lysine. In their studies, liver weight in these two levels of supplemental lysine is significantly higher than the other levels. Hickling et al. (1990) reported that body weight at the end of three weeks of age is reduced slightly by increasing dietary lysine level. Chickens which received 100 and 110% lysine, had a higher liver yield. Carew et al. (2005) observed that true liver weights were significantly decreased at each step of the lysine deficiency, but when they expressed the liver weight relative to the body weight, most of these differences disappeared. Tesserand et al. (1996) reported no significant difference in liver weight by changing dietary lysine level. In the present study, different levels of lysine had no significant effect on heart yield at day 21. In agreement with our findings, Cengiz et al. (2008) observed no significant effect on heart yield by different levels of lysine in the starter period, but Bouyeh (2013) reported that increasing levels of lysine caused a significant increase in heart weight. According to our findings, only 130% lysine reduced the abdominal fat percentage significantly, and other lysine levels had no significant effect on the percentage of abdominal fat. Along with our findings, Holsheimer and Ruesink (1993) also observed that incremental levels of lysine, in the starter period, did not significantly affect the abdominal fat percentage. Grisoni et al. (1991) used 8 levels of lysine and reported that different levels of lysine had no significant effect on abdominal fat weight. However, increasing dietary lysine level reduced the percentage of abdominal fat relative to body weight and carcass weight. Inconsistencies observed in previous studies may be due to the effects of sex, physiological status, ingredient composition, dose of usage, environment and duration of used amino acids. Improved carcass traits were caused by increase in the level of lysine and threonine, which is dependent on the mechanism of these metabolites in their bodies. Lysine plays main role in protein synthesis that could affected on improving carcass characteristics. Threonine involved in the synthesis and renewal of the intestinal mucosa, which may be affected on improving the carcass traits.

Chicks that received threonine (Table 2), 120% of the NRC recommendations, had higher body and carcass weight and breast, thigh yields. In agreement with our results, Hosseinipour et al. (2012) reported that higher body weight in the starter period belonged to the treatment that threonine contents are higher than that of NRC recommendations. Hosseinipour et al. (2012) also observed that the addition of threonine to the diet, in the starter period, causes a significant increase in carcass yield. Edwards et al. (1997) expressed that the use of 5.73 g/kg of diet threonine resulted in maximum body weight. The best level of threonine for achieving the highest carcass weight, and breast, thigh and liver yields was observed in chickens that received threonine in accordance with the recommendations of NRC. In contrast to our findings, Rezaeipour et al. (2012) expressed that the results of carcass characteristics and internal organ weights of broilers at the starter period, was not significantly changed as a result of different levels of dietary L-threonine. Threonine levels had no significant effect on the liver and heart yields. Alizadehmazraeh et al. (2013) observed that different levels of threonine had no significant effect on the yield at the starter period. They also found that level of 110% of threonine, compared to 100%, did not create a significant change in the liver and heart yields. Ciftci and Ceylan (2004)

### Table 4. Effect of different levels of lysine and threonine on intestinal microflora.

| Treatments          | Total intestinal microflora |
|---------------------|----------------------------|
|                     | Day 21, cfu | Day 42, cfu |
| Lys effect          |             |             |
| 100%                | 37.75 × 10^{4} | 32.74 × 10^{4} |
| 110%                | 38.24 × 10^{4} | 32.19 × 10^{4} |
| 120%                | 37.84 × 10^{4} | 32.04 × 10^{4} |
| 130%                | 37.30 × 10^{4} | 32.00 × 10^{4} |
| Thr effect          |             |             |
| 100%                | 37.84 × 10^{4} | 32.29 × 10^{4} |
| 110%                | 37.65 × 10^{4} | 32.32 × 10^{4} |
| 120%                | 37.87 × 10^{4} | 32.12 × 10^{4} |
| Lys and Thr         |             |             |
| 100% Lys +100% Thr  | 36.93 × 10^{4} | 32.97 × 10^{4} |
| 110% Lys +110% Thr  | 38.00 × 10^{4} | 33.03 × 10^{4} |
| 120% Lys +120% Thr  | 38.33 × 10^{4} | 32.23 × 10^{4} |
| 110% Lys +100% Thr  | 38.43 × 10^{4} | 32.30 × 10^{4} |
| 120% Lys +110% Thr  | 37.80 × 10^{4} | 32.10 × 10^{4} |
| 110% Lys +120% Thr  | 38.50 × 10^{4} | 32.17 × 10^{4} |
| 120% Lys +100% Thr  | 38.70 × 10^{4} | 31.90 × 10^{4} |
| 110% Lys +110% Thr  | 37.73 × 10^{4} | 32.07 × 10^{4} |
| 120% Lys +120% Thr  | 37.10 × 10^{4} | 32.17 × 10^{4} |
| 110% Lys +100% Thr  | 37.30 × 10^{4} | 32.09 × 10^{4} |
| 120% Lys +110% Thr  | 37.67 × 10^{4} | 32.07 × 10^{4} |
| Lys SEM             | 1105.54     | 13088.02    |

Means within a column with no common superscript differ significantly.
SEM: standard Error of Means.
reported that incremental levels of threonine increased breast yield, but it decreased the thigh yield and liver weight at the starter period. Rosa et al. (2001) observed no significant effect on liver yield by different levels of threonine. Khan et al. (2006) reported that breast meat yield is affected significantly by different levels of threonine. According to our results, only 120% threonine significantly decreased the abdominal fat percentage. Rezaeipour et al. (2012) expressed that dietary threonine levels had no significant effect on abdominal fat percentage. Hosseinpour et al. (2012) observed that the levels of 90 to 120% threonine, relative to NRC recommendations, had no significant effect on abdominal fat percentage.

Concerning the interactions between the different levels of lysine and threonine, the higher live and carcass weights, and breast, thigh and liver yields belonged to chickens that received 120% lysine and 120% threonine relative to NRC recommendations. Kidd et al. (1997) suggested that the NRC recommendations of lysine at 1.10% of diet from 1 to 21 days of age are too low. Rezaeipour et al. (2012) indicated that NRC recommendations of threonine at the starter period are enough for optimal carcass performance. But in agreement with the results of this study, Kidd et al. (1997) demonstrated that lysine and threonine interacted to affect breast yields and feeding high dietary lysine without consideration to dietary threonine may limit breast yields. Intake 130% lysine and 120% threonine decreased abdominal fat. Lysine is a precursor of L-carnitine. L-Carnitine causes beta-oxidation and changes in lipid metabolism. L-Carnitine can decrease the abdominal fat (Kheiri et al. 2011), which might be the reason for the abdominal fat reduction in our experiment.

Live and Carcass weight, breast, thigh, liver and heart yields, by level of 120% lysine, were significantly higher than other treatments at the grower period (42 days). In agreement with our results, Mukhtar et al. (2010) showed that highest live weight in 42 days of age obtained with the consumption of 1.2 and 1.3% lysine. Hickling et al. (1990) reported that no significant differences in body weight were observed in chickens that consumed different levels of lysine at the end of 6th week. Han and Baker (1994) observed that breast meat yield increased quadratic ally in response to lysine addition. Tahmasbi et al. (2006) found that supplementing lysine in the broiler diets at the grower period improves carcass weight, carcass and breast meat yields, but thigh, liver and heart weights are not affected significantly. Bouyeh (2013) in his experiment concluded that lysine levels of 1.3 and 1.4% of NRC recommendations, at 42 days of age, resulted in a significant increase in carcass, breast, liver and heart yields, but no significant effect on thigh yield was observed. Mukhtar et al. (2010) showed that incremental levels of lysine had no significant effect on liver and heart yields at the day 42 of age. Nasr and Kheiri (2011) reported that highest liver and heart yields are belonged to chickens that received 120% lysine. The abdominal fat percentage by levels of 110 and 120% lysine was declined significantly. Han and Baker (1994) reported that the levels of 1.01 and 1.11% of dietary lysine caused further decrease in abdominal fat percentage. Increasing threonine level (120%), at the grower period, increased live and carcass weights, as well as breast, thigh and liver yields. In contrast with our findings, Rezaeipour et al. (2012) indicated that the best level of threonine to obtain higher carcass components yields (carcass, breast, thigh and liver) is equal to NRC recommendations, and in agreement with our findings, Hosseinpour et al. (2012) reported that using 110% threonine, relative to NRC recommendations, improved live weight, carcass and breast meat yields at the grower period. Ayasan et al. (2009) found that there is a linear response to dietary threonine levels for final body weight of broilers at the grower period. Corzo et al. (2009), by increasing levels of threonine at the days 21 to 42, observed a significant increase in carcass yield and breast meat weight, but no significant changes in the breast meat yield were observed. Moradi et al. (2013) suggested 115% level of threonine relative to Ross 308 recommendations for a significant increase in carcass yield at the day 42. Kidd and Kerr (1997) demonstrated that the best level of threonine to reach the highest breast yield is the level of 0.75% of diet which is almost similar to NRC recommendations, and it is also in disagreement with our findings. In this period (Table 3), heart yield and abdominal fat percentage were not affected by threonine levels. Moradi et al. (2013) observed that the level 105% of threonine, relative to 100% of Ross 308 recommendations, caused a significant reduction in abdominal fat percentage, while the levels of 110 and 115% increased the abdominal fat percentage significantly. They also reported that the level of 105% of Ross 308 recommendations causes a higher heart yield. In agreement with our results, Corzo et al. (2003, 2009) indicated no significant effect on abdominal fat percentage by different levels of threonine.

According to the interactions between lysine and threonine and its effects on carcass traits at the grower period (Table 3), the results were largely close to the results of the starter period, so that the most favourable results appertains to the treatment that
**Table 5. Effect of different levels of lysine and threonine on growth performance at day 42.**

| Treatments | Feed intake, g day\(^{-1}\) | Body weight gain, g day\(^{-1}\) | FCR |
|------------|-----------------------------|-------------------------------|-----|
| **Lys effect** | | | |
| 100% Lys | 88.11 | 45.47\(^{a}\) | 1.94\(^{a}\) |
| 110% Lys | 88.67 | 48.80\(^{b}\) | 1.82\(^{b}\) |
| 120% Lys | 90.75 | 51.79\(^{a}\) | 1.74\(^{a}\) |
| 130% Lys | 90.35 | 49.17\(^{b}\) | 1.81\(^{b}\) |
| **Thr effect** | | | |
| 100% Thr | 89.98 | 49.20 | 1.80 |
| 110% Thr | 89.45 | 49.47 | 1.80 |
| 120% Thr | 89.98 | 50.76 | 1.77 |
| **Lys and Thr interactions** | | | |
| 100% Lys + 100% Thr | 88.55 | 45.20\(^{c}\) | 1.95\(^{c}\) |
| 100% Lys + 110% Thr | 88.80 | 44.99\(^{c}\) | 1.97\(^{c}\) |
| 100% Lys + 120% Thr | 89.05 | 48.88\(^{b}\) | 1.83\(^{b}\) |
| 110% Lys + 100% Thr | 88.84 | 46.10\(^{c}\) | 1.92\(^{c}\) |
| 110% Lys + 110% Thr | 89.06 | 48.60\(^{b}\) | 1.84\(^{b}\) |
| 110% Lys + 120% Thr | 89.33 | 50.55\(^{ab}\) | 1.77\(^{ab}\) |
| 120% Lys + 100% Thr | 89.50 | 52.47\(^{a}\) | 1.71\(^{a}\) |
| 120% Lys + 110% Thr | 89.60 | 52.61\(^{a}\) | 1.70\(^{a}\) |
| 120% Lys + 120% Thr | 89.92 | 54.52\(^{a}\) | 1.67\(^{a}\) |
| 130% Lys + 100% Thr | 88.72 | 52.19\(^{a}\) | 1.71\(^{a}\) |
| 130% Lys + 110% Thr | 88.90 | 51.30\(^{ab}\) | 1.74\(^{ab}\) |
| 130% Lys + 120% Thr | 89.20 | 50.21\(^{ab}\) | 1.78\(^{ab}\) |
| **SEM** | 0.56 | 2.375 | 0.091 |
| **p Value** | | | |
| Lys | .501 | .001 | .001 |
| Thr | .061 | .561 | .165 |
| Lys + Thr | .155 | .001 | .001 |

Means within a column with no common superscript differ significantly.
SEM: standard error of means.

received 120% NRC recommendations lysine and threonine. Obviously, the much more amount of lysine in the protein tissue of the body, especially in the presumable carcass compared with other amino acids, can be a reason for achieving such results. According to the results of the current study and in agreement with Kidd et al. (1997), the dietary lysine and threonine levels can be raised to 20% higher than NRC recommendations, to obtain the optimum live and carcass weights and carcass component yields (breast, thigh, liver and heart), but adding more than this amount to the level of lysine limits the desirability of live and carcass traits especially for live and carcass weights and breast and thigh yields. High concentrations of lysine in the body protein tissues, particularly, in the edible carcass such as breast and thigh muscles influenced the bioavailability of nutrients. Also, effective participation of threonine in metabolic reactions of body and also intestinal mucus health which affect the absorption of ingredients can be the cause of these results.

Effects of different levels of lysine and threonine on intestinal microflora are shown in Table 4. At 21 days used 120% and 130% lysine can be decreased total intestinal microflora. At the age 42 days, with the increase use of lysine created a significant decreased in the intestinal microflora. At the starter period 110% threonine decreased total intestinal microflora. The interaction between threonine and lysine in 21 days showed significant effect on intestinal microflora. At 42 days, it was found the interaction between threonine and lysine which was used higher than NRC recommendation level can be reduced total intestinal microflora, which there was a lack of research in this area. Intestinal microflora can be affected by diet. But of course, nutrition is one of the most important factors that can affect the intestinal microbial population. Among the nutrients, amino acids are the most functional ones which are involved in the biological and metabolic reactions and compounds in different parts of the body which play an important role in this case, including lysine and threonine amino acids. Hence, the effect of intestinal microflora, which is created in this study by different levels of lysine and threonine for the health and functional specifications of broiler chicks.

Table 5 shows the effect of different levels of lysine and threonine on the growth performance. The different levels of lysine and threonine did not signify their effect on feed intake. The interaction between lysine and threonine showed non-significant effect on feed intake.

By increasing the lysine level (120%), the body weight increased. Using lysine level (120%) and different levels of threonine (interaction lysine and threonine) were related to the highest body weight gain. Threonine levels had no significant effect on body weight gain.

The best feed conversion ratio was related to 120% of lysine. The interaction between lysine and threonine, by increasing lysine level (120%) with different levels of threonine improved the feed conversion ratio. The higher lysine requirement was related to improved body weight gain and feed conversion ratio (Nasr & Kheiri 2011). These results showed that lysine requirement of Ross female broilers for maximum body weight gain were higher than those of the levels suggested in National Research Council (1994).

**Conclusions**

The best levels of lysine and threonine to achieve the most favourable values of carcass traits at the starter and grower periods of broiler chicks are the level of 120% relative to NRC recommendations. By increasing lysine level (120%), and different levels of threonine (interaction lysine and threonine) was related to the highest body weight gain. According to the result of this study, an improvement in carcass traits, growth performance and reduction of the intestinal microflora due to the usage of 120% lysine and threonine, was
assumed. There was probably a relation between the reduction of intestinal microflora, and improving carcass traits and growth performance.

**Disclosure statement**

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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