Effect of arginine and threonine in ovo supplementation on immune responses and some serum biochemical attributes in broiler chickens

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ABSTRACT
This experiment was conducted to investigate effects of in ovo injecting arginine (Arg), threonine (Thr) and Arg + Thr on humoral immunity, serum differential leukocyte counts and serum biochemical attributes in broiler chickens. Four hundred fertile eggs were randomly assigned to five experimental treatments including: control (none injected), sham (0.5 ml of 0.5% saline), Arg (35 mg/egg), Thr (25 mg/egg) and Arg + Thr (35 + 25 mg/egg) which injected in the amniotic fluid on day 14 of incubation. After hatching, broiler chicks were fed a commercial corn–soya bean diet up to 42 days of age. Broilers received Arg and Thr had higher body weight ($p < .05$) and daily feed intake ($p < .05$) than those supplemented with control or sham. Antibody titre against sheep red blood cells increased in broilers in ovo supplemented with amino acids compared to the birds in control group ($p < .05$) while no beneficial effect of applied treatments observed on the antibody titre against influenza and Newcastle disease viruses. Moreover, in ovo injection of Arg significantly increased spleen and bursa of Fabricius relative weights compared to sham on day 11 of age ($p < .05$). In ovo administration of Thr resulted in higher serum glucose concentration than the other groups ($p < .05$). Furthermore, serum albumin concentration increased in response to Thr administration compared to chickens in control group ($p < .05$). In conclusion, Arg and Thr in ovo supplementation improved humoral immunity of broilers and increased proportional weight of their lymphoid organs in post-hatch period.

HIGHLIGHTS
- In ovo injection of amino acids may improve immune responses in broiler chickens.
- Supplementation of amino acids during embryonic stage could increase growth performance of chickens.
- Meet the nutrient requirement of broiler embryo is important for its later growth and immunity.

Introduction
In modern poultry production, this is of crucial importance to keep balance between growth and immune-competence because broiler chickens are mainly bred for greater growth rate rather than better immunity. In early stage of growth, commercial broiler chicks are susceptible to stress due to limited egg nutrient contents across final period of incubation (Gonçalves et al. 2013) and also delayed access to dietary nutrients within first hours post-hatch as the result of hatchery operating procedures (Batal and Parsons 2002). Therefore, pathogens might adversely damage immune function of chickens and consequently compromise their growth performance. As such, providing a bird with a proper starting point is very important (Kadam et al. 2013).

From the nutritional attitude, application of immunomodulatory nutrients such as carbohydrates, amino acids, vitamins and enzymes help in synthesis of mediators immune effectors which contribute to the clonal proliferation of antigen-driven lymphocytes and the enrolment of monocytes and heterophils from bone marrow (Kidd 2004; Kogut and Klasing 2009). Dietary supplementation of amino acids such as methionine, arginine (Arg), threonine (Thr), glycine, serine and valine in early ages post-hatch was shown to improve humoral immune responses in broiler chicks (Bhargava 2018).
et al. 1971; Lee et al. 2002). On the other hand, in ovo administration of amino acids may improve intestinal development, compensate the probable deficiency of nutrients across early ages post-hatch period and subsequently enhance immunity of broiler chicks (Kadam et al. 2013). Threonine and Arg are indispensable amino acids for broilers because they are not able to produce Thr and Arg de novo (Waguespack et al. 2009). Therefore, these amino acids are needed to be exogenously prepared for broiler chickens. In ovo supplementing blend of amino acids have been reported to improve humoral and cell-mediated immunity (Bhanja and Mandal 2005). Kadam et al. (2008) revealed that in ovo supplementation of 20–30 mg Thr into the yolk sac enhances antibody titre against sheep red blood cells (SRBC) in broiler chicks. Recently, the expression of genes related to humoral immunity, interleukin-6 and tumour necrosis factor-α increased after that broiler chicks were in ovo injected with Arg, Thr or methionine and cysteine (Bhanja et al. 2014). Bhargava et al. (1971) observed that broiler chicks which infected with Newcastle disease virus (NDV) needed more Thr to obtain optimal antibody titre against NDV than for maximum growth. However, data on the effect of in ovo injecting amino acids on the antibody titres against NDV and influenza disease viruses (IDV) is very little. Moreover, the effect of amino acids on serum biochemical parameters and serum differential leukocyte counts are equivocal. Azzam et al. (2011) observed that supplementing dietary graded levels of Thr increased serum total protein, however, Azzam and El-Gogary (2015) failed to show similar results with dietary inclusion of Thr. It has been reported that Arg inclusion in the feed increased the heterophil to lymphocytes ratio (H/L) (Lee et al. 2002) while Maroufyen et al. (2010) reported that dietary Thr had no effect on blood differential leukocyte counts. As such, more research is needed to clarify the effect of in ovo supplementation of these amino acids individually or in combination in broiler chickens.

The objective of present experiment was to investigate in ovo injection effect of Arg, Thr and Arg + Thr on humoral immunity, serum differential leukocyte counts and serum biochemical attributes in broiler chickens.

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.

Experimental treatments and injection procedure

A total number of 400 fertile commercial eggs of broiler chicks (Ross 308) with an average weight of 71.0 ± 0.4 g were collected and incubated under the standard condition (temperature 37.5 ± 0.2 °C, relative humidity 65 ± 2%). All the eggs were candled on day 14 of embryotic age, the injection points were located and shell surface was disinfected. Four hundred fertile eggs were allotted to 5 treatments of 80 eggs with 4 replicates (20 eggs per replicate) including: control (none injected); sham group (0.5 ml of 0.5% saline); 35 mg Arg (L-arginine, #A5006; Sigma-Aldrich Inc., St. Louis, MO, USA) dissolved in 0.5% saline (0.5 ml/egg); 25 mg Thr (L-Threonine, #T 8625; Sigma-Aldrich Inc., St. Louis, MO, USA) dissolved in 0.5% saline (0.5 ml/egg); 35 mg Arg + 25 mg Thr dissolved in 0.5% saline (0.5 ml/egg) which was confirmed by the preliminary experiment in (Tahmasebi and Toghyani 2016). Moreover, saline concentration is based on the concentration applied in the work of Uni et al. (2005). Before injection, a small tiny hole was drilled on the surface of the air sac and injections were made into the amnion under the air sac using 23-gauge needles cut to 19 mm based on the method standardised by Ohta and Kidd (2001). Afterwards, holes were sealed with liquid paraffin and the eggs were relocated into the incubator.

Animals and housing

After hatching, 300 chicks which had assigned to 5 different treatments were selected, weighed, sexed and distributed among 5 replicates, comprising 20-floor pens and 15 chicks per pen in completely randomised design and reared for 42 days. Male and female chicks were mixed at a ratio of 1:1. Regarding that all fertilised eggs had the same initial weight, selecting 300 out of all hatched chicks was due to the fact that we wanted to continue the experiment with this number of birds. Broiler chicks were grown on floor pens furnished with paper roll as bedding material. Furthermore, chicks had free access to feed and water throughout different periods of the experiment. Diet was fed in pellet form and formulated to meet or exceed the nutrient requirements of broilers offered by the Ross Broiler Manual (Aviagen 2009; Table 1). The lighting programme considered as a period of 23 h light and 1 h darkness. Broilers were kept in a temperature-controlled house at 32 °C from day 1 to 7, 29°C for day 8 to 14, 26 °C for day 15 to 21, and 22 °C for day 22 to the end of the trial.
Growth performance

Records of body weight (BW) gain and daily feed intake (DFI) were obtained throughout the trial (1 to 42 days). Feed conversion ratio was also calculated.

Antibody titre, lymphoid organs and serum differential counts

On day 9 of age, Newcastle and influenza antigens were injected subcutaneously with 0.2 ml per chick with dual vaccine of Newcastle-influenza (H9N2 sub-type). Also, chicks were orally vaccinated against Newcastle disease (Lasota) on day 19 of age. Two chicks per pen were randomly selected for intraperitoneal injection with 1.0 ml of SRBC suspension diluted with phosphate buffer saline (PBS) on day 25. Five days later, the same wing-banded birds were bled to determine antibody titres against SRBC, IDV and NDV. Subsequently, antibody titre against SRBC was measured by hemagglutination assay method (HA). Antibody titres against IDV and NDV were separately measured by hemagglutination inhibition method (HI). The HI antibodies were then converted to log2. Antibody titre against SRBC was measured by the microtiter procedure described by Wegmann and Smithies (1966) Lymphoid organs including spleen and bursa of Fabricius were evaluated after slaughter of two birds close to the average BW of the pen on days 11 and 42 of the experiment.

Differential leukocyte counts and heterophil to lymphocyte ratio (H/L) were determined by blood sampling on day 31. Blood samples were taken from wing vein using syringes containing heparin to avoid blood clot formation. Blood smears were stained by May–Greenwald–Giemsa stain (Lucas and Jamroz 1961). One hundred leukocytes per samples including granular (heterophils and eosinophils) and non-granular (lymphocytes and monocytes) were counted under an optical microscope (Nikon, Japan) with 100× oil immersion lens, and H/L was calculated and recorded (Gross and Siegel 1983).

Serum biochemical parameters

On day 14 post-hatch, blood samples were taken from 2 birds of each pen and collected in non-heparinized tubes by brachial vein puncture. Serum was separated via 2000 × g centrifuge of blood samples for 15 minutes (SIGMA 4-15 Lab Centrifuge, Germany). Individual serum samples were analysed for glucose, total protein, albumin, total cholesterol, triacylglycerol, aspartate aminotransferase (AST) enzymes with a spectrophotometer using the kit package (Pars Azmoon CO; Tehran, Iran). Globulin concentration in

### Table 1. Ingredients and composition of the diets.

| Diet ingredients, %          | Starter (1–11 d) | Grower (12–24 d) | Finisher (25–42 d) |
|-------------------------------|------------------|------------------|--------------------|
| Corn                          | 47.90            | 41.18            | 41.79              |
| Soybean meal (42% CP)         | 41.22            | 37.35            | 31.22              |
| Wheat                         | 5.00             | 15               | 20                 |
| Soybean oil                   | 1.67             | 2.90             | 3.62               |
| Calcium carbonate             | 1.14             | 0.99             | 0.96               |
| Dicalcium phosphate           | 1.84             | 1.47             | 1.45               |
| α-Methionine                  | 0.29             | 0.21             | 0.16               |
| L-Lysine                      | 0.12             | 0.01             | 0.00               |
| Sodium chloride               | 0.27             | 0.34             | 0.25               |
| Vitamin premix                | 0.25             | 0.25             | 0.25               |
| Mineral premix                 | 0.25             | 0.25             | 0.25               |
| Multi enzyme                  | 0.05             | 0.05             | 0.05               |
| Calculated composition        |                  |                  |                    |
| ME, Kcal/Kg                   | 2850             | 2950             | 3050               |
| Crude protein, %              | 21.92            | 20.62            | 18.56              |
| Lysine, %                     | 1.34             | 1.16             | 1.01               |
| Methionine + Cystine, %       | 1.00             | 0.89             | 0.79               |
| Threonine, %                  | 0.85             | 0.80             | 0.71               |
| Arginine, %                   | 1.62             | 1.51             | 1.34               |
| Calcium, %                    | 0.98             | 0.84             | 0.81               |
| Available phosphorous, %      | 0.49             | 0.42             | 0.40               |

*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; pantothenic 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; I (KI, 58% I), 1 mg; Se (NaSeO3, 45.56% Se), 0.2 mg.* The multienzyme constitutions were composed of xylanase, b-glucanase, cellulase, pectinase, phytase, protease, lipase, and α-amylase.
serum was computed by subtracting albumin concentration from proteins. Subsequently, albumin to globulin ratio (A/G) was determined.

**Statistical analysis**

Data were analysed using the general linear model procedure of SAS (SAS/STAT Version 9.2, SAS Institute Inc., Cary, NC). Pen was considered as the experimental unit for different parameters. The Fisher’s protected least significant difference (LSD) test was used for multiple treatment comparisons. Means were presented with their standard error of means (SEM). Statements of statistical significance were declared \( p < .05 \) unless noted otherwise.

**Results**

**Hatchability and growth performance**

Hatchability was significantly decreased in response to in ovo injection of Arg and Arg + Thr compared to control group \( (p < .05; \text{Table 2}) \) whereas no such detection observed by Thr in ovo injection (Table 2). Across the entire rearing period, broiler chickens in ovo supplemented with Thr had higher BW gain than broilers in the other groups \( (p < .05; \text{Table 3}) \). Also, in ovo injection of Arg increased BW gain of broiler chickens compared to those in control or sham experimental groups \( (p < .05; \text{Table 3}) \). Similarly, the greatest DFI observed in chickens administrated with Thr which was higher than those received Arg, sham and control \( (p < .05; \text{Table 3}) \). Furthermore, DFI of

| Table 2. Effects of in ovo feeding arginine (Arg) and threonine (Thr) on hatchability of broiler chicks. |
|---------------------------------------------------|
| Treatments                  | Control | Sham | Arg   | Thr | Arg + Thr | SEM  | \( p \)-value |
|-----------------------------|---------|------|-------|-----|-----------|------|--------------|
| Hatchability, %             | 89.10   | 86.20| 77.40 | 89.90| 74.70     | 6.31 | .009         |
| \( a,b,c \)Values in the same row not sharing a common superscript differ significantly \( (p < .05) \); Arg: arginine; Thr: threonine; BWG: body weight gain; F: daily feed intake; FCR: feed conversion ratio. |

| Table 3. Effects of in ovo feeding arginine (Arg) and threonine (Thr) on performance of broilers during the entire rearing period. |
|---------------------------------------------------|
| Treatments                  | Control | Sham | Arg   | Thr | Arg + Thr | SEM  | \( p \)-value |
|-----------------------------|---------|------|-------|-----|-----------|------|--------------|
| BWG, g/bird 1–42            | 2155.60 | 2073.80| 2322  | 2569.40| 2279.40   | 143.05| .001         |
| FI, g/bird 1–42             | 3700.40 | 3750.60| 4061.40| 4414.20| 4170.60   | 176.25| .006         |
| FCR                         | 1.71    | 1.80 | 1.74  | 1.71| 1.82      | 0.07  | .055         |
| \( a,b,c \)Values in the same row not sharing a common superscript differ significantly \( (p < .05) \); Arg: arginine; Thr: threonine; BWG: body weight gain; F: daily feed intake; FCR: feed conversion ratio. |

| Table 4. Effects of in ovo feeding arginine and threonine on the antibody titre (day 30), lymphoid organs (days 11 and 42) and heterophil to lymphocyte ratio (day 31). |
|---------------------------------------------------|
| Treatments                  | Control | Sham | Arg   | Thr | Arg + Thr | SEM  | \( p \)-value |
|-----------------------------|---------|------|-------|-----|-----------|------|--------------|
| Humoral immunity            |         |      |       |     |           |      |              |
| IDV, log2                   | 6.13    | 6.63 | 6.26  | 5.88| 6.51      | 0.11 | .337         |
| NDV, log2                   | 7.51    | 7.88 | 7.51  | 7.76| 8         | 0.18 | .468         |
| SRBC, log2                  | 7.01    | 7.21 | 8     | 7.86| 7.63      | 0.45 | .026         |
| Bursa of Fabricius d 11, % BW | 0.15    | 0.14 | 0.18  | 0.17| 0.17      | 0.01 | .048         |
| Spleen d 11, % BW           | 0.07    | 0.06 | 0.09  | 0.07| 0.08      | 0.05 | .036         |
| Bursa of Fabricius d 42, % BW | 0.20    | 0.18 | 0.27  | 0.20| 0.19      | 0.02 | .690         |
| Spleen d 42, % BW           | 0.11    | 0.10 | 0.10  | 0.13| 0.10      | 0.01 | .455         |
| H/Lc                        | 0.36    | 0.34 | 0.35  | 0.34| 0.35      | 0.01 | .067         |
| \( a,b \)Values in the same row not sharing a common superscript differ significantly \( (p < .05) \); Arg: Arginine, Thr: Threonine; IDV: Influenza disease virus; NDV: Newcastle disease virus; SRBC: Sheep red blood cells; BW: body weight; \( ^{c} \)Heterophil to lymphocyte ratio. |
broilers increased in response to Arg in ovo supplementation compared to sham and control (p < .05; Table 3). However, FCR of broiler chickens remained unaltered after application of experimental treatments.

**Antibody titre, lymphoid organs and serum differential counts**

Antibody titre against SRBC significantly increased in broilers supplemented with amino acids compared to control (p < .05) while no influential effect of applied treatments observed on the antibody titre against IDV, NDV and H/L (Table 4). Moreover, Arg administration considerably increased proportional weight of the spleen and bursa of Fabricius compared to sham on day 11 of age (p < .05). Relative weights of the spleen and bursa of Fabricius were not affected upon the in ovo injection of sham or amino acids on day 42 of age (Table 4). Differential leukocyte counts including lymphocytes, monocytes, eosinophils and heterophils remained statistically unaffected after in ovo injection of sham or amino acids (Table 5).

**Serum biochemical parameters**

According to Table 6, concentration of serum triacylglycerol decreased in chickens in ovo supplemented with Arg + Thr compared to sham (p < .05). Furthermore, chickens in ovo administered with Thr possessed the greatest serum glucose concentration in which glucose was substantially higher than sham, control, Arg and Arg + Thr (p < .05). In comparison to control, serum albumin concentration increased in response to Thr supplementation (p < .05). Moreover, chickens in control and sham groups had higher concentration of AST than broilers with amino acids in ovo injection (p < .05).

**Discussion**

Generally, unaffected hatchability is a prerequisite for in ovo injection. However, Arg decreased hatchability in this experiment. Similarly, Gao et al. (2017) observed that in ovo injection of 2% Arg solution significantly decreased hatchability of broiler chicks. Furthermore, Sanami et al reported a remarkable decrease in hatchability when broiler eggs in ovo injected with high concentration of Arg solution. On the contrary, Tangara et al. (2010) showed that injecting Arg solution into amnion had no effect on hatchability in duck neonates. Compromising impact of Arg on hatchability might be due to toxicity of Arg or the imbalance of internal amino acids caused by excessive intake of exogenous Arg, which could induce the overload burden of amino acid metabolism in chicken embryos. In the present trial, the improved BW gain of broilers in response to in ovo injection of Thr, Arg and Arg + Thr might be in part due to increased DFI of them. This is partially relating to the prominent role of Thr in production of mucin and gastric enzymes (Kadam et al. 2013) which consequently develop gastrointestinal tract of chickens. Furthermore, increasing effect of Arg on DFI of broilers has been observed in the literature (Kadam et al. 2013; Saki et al. 2013).

**Table 5. Effects of in ovo feeding arginine and threonine on differential leukocyte counts on day 31 of age.**

| Treatments | Lymphocytes, % | Monocytes, % BW | Eosinophils, % BW | Heterophils, % BW |
|------------|----------------|-----------------|------------------|------------------|
| Control    | 72.76          | 2.13            | 1.17             | 25.51            |
| Sham       | 72.88          | 2.26            | 1.21             | 24.13            |
| Arg        | 72.40          | 2.13            | 1.00             | 24.88            |
| Thr        | 72.60          | 1.13            | 1.00             | 24.88            |
| Arg + Thr  | 72.80          | 1.88            | 1.00             | 25.01            |
| SEM        | 0.54           | 0.07            | 0.07             | 0.49             |
| p-value    | .396           | .528            | .314             | .112             |

Arg: Arginine; Thr: Threonine; BW: body weight.

**Table 6. Effects of in ovo feeding arginine and threonine on serum biochemical parameters on day 14 of age in broiler chickens.**

| Treatments | Serum biochemical parameters | Control | Sham | Arg | Thr | Arg + Thr | SEM | p-value |
|------------|-----------------------------|---------|------|-----|-----|-----------|-----|---------|
| Total protein, g/dL | 4.24 | 4.27 | 4.04 | 4.38 | 4.02 | 0.06 | .091  |
| Total cholesterol, g/dL | 109 | 111 | 113 | 113 | 115 | 1.41 | .112  |
| Triacylglycerol, g/dL | 100<sup>ab</sup> | 104<sup>a</sup> | 88<sup>ab</sup> | 99<sup>ab</sup> | 87<sup>b</sup> | 2.37 | .043  |
| Glucose, g/dL | 189<sup>b</sup> | 201<sup>b</sup> | 200<sup>b</sup> | 216<sup>a</sup> | 195<sup>b</sup> | 1.99 | .002  |
| Albumin, g/dL | 1.71<sup>b</sup> | 1.77<sup>b</sup> | 1.74<sup>b</sup> | 1.92<sup>a</sup> | 1.78<sup>b</sup> | 0.03 | .042  |
| Globulin, g/dL | 2.54 | 2.50 | 2.31 | 2.46 | 2.24 | 0.55 | .921  |
| Albumin/globulin | 0.67 | 0.70 | 0.75 | 0.79 | 0.80 | 0.55 | .930  |
| AST, IU/L | 312<sup>a</sup> | 300<sup>a</sup> | 212<sup>b</sup> | 254<sup>ab</sup> | 210<sup>b</sup> | 10.52 | .031  |

<sup>a,b</sup>Values in the same row not sharing a common superscript differ significantly (p < .05); Arg: Arginine; Thr: Threonine; AST: aspartate aminotransferase.
On the other hand, Thr is a precursor of glycine; an amino acid which is more required in early stage of embryonic growth (Ohta et al. 1999). Also, Arg is a limiting amino acid for poultry since they are not able to produce Arg de novo (Sung et al. 1991). Thereby, promotion in growth performance of broilers received amino acids is owing to increased DFI of them as well as amino acid utilisation for anabolic functions in early ages.

In the present study, the increased antibody titre against SRBC in broiler chicks in ovo administrated with amino acids might stand to the reason that Thr provides a main component of intestinal mucin and plasma γ-globulin in animals. Also, Thr was able to prevent cellular apoptosis, stimulate the cell growth and increase antibody production in lymphocytes when added to the culture medium (Duval et al. 1991). On the other hand, Arg has been shown as an immunological modulator due to its effect on nitric oxide production (Collier and Vallance 1989) and increase the thymic function (Efron and Barbul 1998; Evoy et al. 1998). In agreement with our results, Kadam et al. (2008) reported that 20–30 mg injection of Thr into the yolk sac increased antibody titre against SRBC in broiler chicks. Also, antibody titre against SRBC increased when eggs administrated with 0.5 ml mix of Thr, glycine and serine on day 14 of embryonic age in broiler chickens (Bhanja and Mandal 2005). Bhanja et al. (2014) claimed that expression of genes related to humoral immunity, interleukin-6 and tumour necrosis factor-α increased after that chicks in ovo injected with lysine, threonine or methionine and cysteine. Conversely, Deng et al. (2005) demonstrated that feeding dietary graded levels of Arg to leghorn chickens had no effect on the antibody titre against SRBC in their fourth week of age. Moreover, in ovo inoculating blend of 25 mg Thr, Arg, glycine, serine and valine into amniotic cavity of broiler chickens did not affect the antibody titre against SRBC as compared to broilers in untreated group (Bakyaraj et al. 2012). In this study, antibody titre against NDV and IDV was not influenced by amino acid supplementation. Likewise, Kidd et al. (2001) indicated that dietary Arg had no effect on the antibody titre against NDV and IDV in broiler chicks. However, Bhargava et al. (1971) fed chickens with graded levels of dietary Thr and observed the increased antibody titre against NDV, although they believed that more Thr is required to obtain optimum antibody titre. It seems that discrepancies regarding the effect of amino acids on the antibody titre, in part accounts for combination of administrated different levels of Thr and various technical injecting or feeding procedures.

The proportional weight of the spleen and bursa of Fabricius increased following Arg in ovo supplementation. Development of the lymphoid organs is important for sufficient immune responses. The weight of spleen is considered to be correlated with the proliferation of immune cells within its tissue because avian have no lymph nodes (Elmore 2006). Also, increased bursa of Fabricius proportional weight could be a positive sign for the improvement in immunity of broiler chickens (Rajput et al. 2013). It shows that Arg affects cellular proliferation and weight of the lymphoid organs. In this regard, Kwak et al. (1999) supplemented 0.53–1.53% graded levels of Arg to Arg deficient diets and reported the increased relative weight of the spleen, bursa of Fabricius and thymus in Cornell K strain chickens. Nevertheless, Kidd et al. (2001) failed to observe the effect of dietary Arg supplementation near the NRC (1994) recommendations on lymphoid organs. The increased spleen weight of broilers in this study supports the significant increased antibody titre against SRBC after Arg supplementation. Lymphoid organs in this trial remained unaltered following Thr administration that is in agreement with findings reported in some other experiments (Rao et al. 2011; Taghinejad-Roudbaneh et al. 2013; Azzam and El-Gogary 2015).

Research evaluating the effect of Arg and Thr on leukocyte population is sparse. In the present trial, blood leukocyte counts were not influenced through Arg and Thr in ovo administration. On the contrary, Lee et al. (2002) demonstrated that dietary Arg inclusion led to an altered profile of circulating leukocytes after inoculation with infectious bronchitis virus in peripheral blood of Leghorn-type chickens. The lack of amino acid impact in this study may show that protective effects of amino acids are more pronounced in birds which exposed to an infectious challenge. Results of the current experiment are in line with the findings of Maroufyan et al. (2010) who declared that blood differential leukocyte counts were not affected by dietary supplementation of Thr in broiler chickens.

In this experiment, serum glucose concentration of broiler chickens augmented in response to Thr in ovo supplementation. Embryo of chicken needs a source of energy for glycogen accumulation to be used in hatching activities. Accordingly, because the egg is not a rich source of carbohydrates, glycogenic amino acids such as Thr might be used for gluconeogenesis. On the contrary, Bhanja et al. (2012) reported that plasma glucose concentration decreased after Thr
in ovo inoculation. Furthermore, Azzam and El-Gogary (2015) observed that dietary Thr supplementation had no effect on blood glucose concentration. These contradictory results show the need of further research in this field. The abnormal increase of AST concentration would be an indicator of liver damage. Thereby, reduction in serum AST concentration might provide evidence that Arg supplementation induces hepatoprotective effects. Increasing effect of Thr on serum albumin concentration compared to the birds in control group may be related to the mild increase of blood total protein. Accordingly, there is a relationship between blood concentration of total protein and serum concentration of albumin and globulin (Hunt and Hunsaker 1965), supporting the significant increase of albumin in the current study. Serum total protein level is always an indicator for immune function in poultry. Similarly, feeding graded levels of Thr has been reported to increase the concentration of serum total protein (Azzam et al. 2011). In contrast, Azzam and El-Gogary (2015) failed to show significant effect of dietary 0.25, 0.50, 0.75 and 1.00 g/kg Thr supplementation on serum total protein concentration.

Conclusions

In ovo injection of amino acids impaired hatchability but improved growth performance and increased antibody titre against SRBC in broiler chickens. This is supported by the improved proportional weight of the bursa of Fabricius and spleen on day 11 of age following Arg in ovo injection. Furthermore, leukocyte differential counts were not influenced by amino acid supplementation that might be due to the absence of applying an infectious challenge in this study. Furthermore, in ovo administration of Thr increased serum albumin and total protein concentration that might be indicator of an immune function.

Disclosure statement

No potential conflict of interest was reported by the authors.

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