Effect of dietary protein concentrations, amino acids and conjugated linoleic acid supplementations on productive performance and lipid metabolism of broiler chicks

Youssef A. Attia, Fulvia Bovera, Abd-El-Hamid E. Abd-El-Hamid, Abd-Elrazk E. Tag EL-Din, Mohammed A. Al-Harthi, Antonino Nizza and Raesa M. Elharidy

Arid Land Agriculture Department, King Abdulaziz University, Jeddah, Saudi Arabia; Dipartimento di Medicina Veterinaria e Produzioni Animali, University of Napoli Federico II, Napoli, Italy; Department of Animal and Poultry Production, Damanhour University, Damanhour, Egypt

ABSTRACT

The study investigated the effect of low-crude protein (CP), essential amino acids (EAAs) and conjugated linoleic acid (CLA) on broiler growth performance. From 1 to 6 days of age, 196 male broiler chicks fed the same basal diet containing 22.5% of crude protein. From 7-28 days the chicks were assigned to 7 treatments (4 replicates, 7 birds/replicate). The control group fed a 21.5% CP diet, supplemented with DL-methionine (Met) and L-lysine (Lys). The low-CP diet (19% CP) was supplemented with Met + Lys (2EAAs group) or with Met, Lys, L-arginine, L-tryptophan and L-threonine (5EAAs group); 2EAAs and 5EAAs groups were also supplemented with 0, 2 or 4% CLA. The body weight gain (BWG) was the biggest \( (p < .01) \) in the control group; 2EAAs and 2EAAs 4% CLA groups had lower BWG than the three groups fed 5EAAs diets. Diet 5EAAs + 2% CLA induced lower \( (p < .01) \) feed conversion ratio (FCR) than 5EAAs + 4% CLA and also showed the lowest \( (p < .01) \) protein intake. Reducing CP in broiler diet increased \( (p < .01) \) the digestibility of CP. The total protein content of plasma was greater \( (p < .01) \) in 2EAAs + 4%CLA and 5EAAs + 4%CLA than in the control. Decreasing the protein content of the diet from 21.5 to 19.0% penalised the growth performance of broilers from 7 to 28 days of age and the administration of CLA at 2 or 4% was not able to support growth. However, addition of 5EAAs without or with 2 or 4% of CLA resulted in recovery of FCR and PCR.

INTRODUCTION

Worldwide, protein nutrition represents a major challenge to poultry production. To reduce the feed cost, the protein content of the diets can be reduced with an appropriate amino acid supplementation, giving positive effects also on environmental pollution (Attia 2003; Attia et al. 2006). However, the use of low-crude protein (CP) diet leads to a decrease of body weight gain (BWG), increase of fat deposition and impairs feed conversion ratio (FCR) according to the level of protein reduction (Attia et al. 1998). So, even if the use of low-CP diet is very interesting in poultry production under an economical and environmental point of view but, solutions need to avoid their negative effects on poultry growth and carcase traits. The addition of essential amino acids to low-CP diets can help to counteract the negative effects on poultry growth. The use of methionine, L-lysine and L-threonine in broiler feed is well established, while other amino acids as tryptophan and arginine are generally considered as the next limiting amino acids in broiler feed. The arginine (Arg) is a ‘next limiting’ amino acid due its antagonism with lysine (Lys) and if Lys was added to a diet, also the requirements of Arg increases (Jones et al. 1967; Austic & Scott 1975) and this can affect broiler performance (Balnave & Barke 2002; Atencio et al. 2004) and muscle development (Fernandes et al. 2009). Tryptophan, apart from being a structural component of all protein is a precursor of serotonin and melatonin, regulating the broiler circadian rhythm and thus feed intake and growth (Emadi et al. 2011). In addition, the negative effects of low-CP diets on poultry carcase traits could be overcome by supplementation with conjugated linoleic acid (CLA). CLA is a collective term for a series of conjugated dienoic positional and geometrical isomers of linoleic acid (C18:2),
which refers to a slight rearrangement of the molecular structure (conjugation) resulting in a fatty acid with altered chemical functions (Terpstra 2004; Doreau et al. 2011). Linoleic acid can be found in vegetable oils, whereas the CLA, is found primarily in meat and dairy products. The most commonly form of CLA found in dietary supplements is manufactured from vegetable oils such as sunflower or safflower oils. The interest in CLA administration is tied to its effects decreased fat deposition, increased energy expenditures and thermogenesis (trans-10 cis-12) which could mitigate the negative effects of low-CP diets. CLA reduces the carcass fat content in mice (West et al. 1998), the back fat thickness in pigs (Cook et al. 1998), increase lean tissue deposition and decreases fat deposition in pigs (Ostrowska et al. 1999). These effects can be due to an adipogenesis inhibition which suppresses preadipocyte differentiation (Kennedy et al. 2010). The increase of energy expenditure and thermogenesis was largely studied in mice and rodents (West et al. 2000; Miner et al. 2001; Nagao et al. 2003) and was ascribed to an increase of the basal metabolic rate (Kennedy et al. 2010). In addition, the antioxidants proprieties of CLA improved immunity and fights cancer (cis-9, trans 11) (Doreau & Chilliard 1997; Zhang et al. 2005; Zanini et al. 2006). Thus, this paper aims to investigate the effect of low-CP diets supplemented with methionine and lysine without or with arginine, threonine and tryptophan and the role of CLA on performance, meat quality and plasma constituents of broilers during 7–28 days of age. The expected result was that the addition of CLA to low-protein diet may improve broiler growth rate and decrease fat deposition.

Materials and methods
A total of 196, seven days old male Hubbard broiler chicks (average body weight 97 ± 3.5 g) were obtained from a commercial hatchery. Birds were kept in battery brooders under similar hygienic and environmental conditions: the temperature of the building was 32, 30 and 27°C for 1st, 2nd and 3rd weeks of age and ~24°C thereafter; the light programme provided 23 h of light daily. During 1–6 days of age, chicks were fed the pre-experimental diet shown in Table 1.

Chicks were randomly assigned to 7 dietary treatments (28 chicks/treatment as 4 replicates of 7 chicks) in a complete randomised experimental design. The control group fed a maize-soybean based diet containing 21.5% of CP and supplemented with DL-methionine and L-lysine in order to meet poultry requirement (Hubbard broiler Management Guide 2006). The other six treatments fed a maize-soybean based diet formulated to have a lower protein content than the control diet (low-CP diet, 19% CP). The low-CP diet was supplemented with DL-methionine and L-lysine (2AAs group) or with DL-methionine L-lysine; L-threonine, L-arginine, and L-tryptophan (5AAs group). All the amino acids supplementations were made to keep their levels similar to those of the control. The broilers fed 2EAAs and 5EAAs diets were supplemented with 0, 2 and 4% CLA (LUTA®-CLA60, BASF, Germany; containing approximately 600 g/kg conjugated isomers, as a 50-50 mixture of 18:2 cis-9, trans-11 and 18-2 trans-10, cis –12). The diets (Table 1) were formulated according to the analysed (Association of official analytical chemists, AOAC 2004) crude protein content of yellow maize (7.5%) and soybean meal (41%) and using appropriate equations (National Research Council 1994) for estimation of amino acids from chemical composition. The other nutrients profiles were determined (Association of official analytical chemists, AOAC 2004) crude protein content of yellow maize (7.5%).

### Table 1. Composition and calculated and measured analyses of the starter diet fed from 1 to 6 d of age and the basal experimental diet fed from 7 to 28 d of age.

| Ingredients, % | Starter diet* | 19% | 19% CP |
|----------------|--------------|-----|-------|
| Yellow maize  | 44.0         | 48.6| 54.5  |
| Soybean meal (44% CP) | 47.2 | 43.3| 35.5  |
| Soybean oilc | 2.25         | 5.50| 5.50  |
| L-lysine      | 0.0          | 0.00| 0.26  |
| DL-methionine | 0.225        | 4.55| 4.20  |
| L-Arginine    | 0.00         | 0.225| 0.30  |
| L-Threonine   | 0.00         | 0.225| 0.30  |
| L-Tryptophan  | 0.00         | 0.225| 0.30  |
| Vitamin and minerals premix | 0.30 | 0.30| 0.30  |
| NaCl          | 0.30         | 0.30| 0.30  |
| Bone meal     | 2.475        | 2.76| 2.76  |
| Wash building sand | 0.00 | 0.03| 1.86  |
| Calculated values |            |     |       |
| Crude protein, % | 22.6 |     | 19.1  |
| Methionine, %  | 0.60         | 0.58| 0.62  |
| Methionine + cysteine, % | 0.90 | 0.95| 0.95  |
| Lysine, %      | 1.38         | 1.29| 1.29  |
| Arginine, %    | 1.82         | 1.54| 1.32  |
| Tryptophan, %  | 0.38         | 0.35| 0.30  |
| Threonine, %   | 1.06         | 0.89| 0.77  |
| Available P, % | 0.90         | 0.99| 0.95  |
| ME, kcal/kg diet | 3018 | 3034| 3039  |
| Dry matter, %  | —            | 91.8| 90.9  |
| Analysed values, % |      |     |       |
| Crude protein  | —            | 21.21| 18.93|
| Ether extract  | —            | 3.55| 5.67  |
| Crude fibre    | —            | 2.46| 2.63  |
| Crude ash      | —            | 7.63| 7.52  |

*Starter diet fed from 1 to 6 days of age

*Basal experimental diets fed from 7 to 28 days of age

CLA was replaced soybean oil at 2% and 4% in the basal on weight basis assuming similar MEn according to Sell et al. (2001).

Vitamins and minerals mixture provide per kilogram of diet vitamin A (as all-trans-retinyl acetate); 12,000 IU; vitamin D3 (all rac-α-tocopheryl acetate); 1000 IU; vitamin E (all rac-α-tocopheryl acetate); 10 IU; k 3 mg; Vit.D3, 2200 ICU; riboflavin, 10 mg; Ca pantothenate, 10 mg; niacin, 20 mg; choline chloride, 500 mg; vitamin B12, 0.1 mg; vitamin B6, 1.5 mg; thiamine (as thiamine mononitrate); 2.2 mg; folic acid, 1 mg; β-Biotin, 0.5 mg. Trace mineral (milligrams per kilogram of diet) Mn, 55; Zn, 50; Fe, 30; Cu, 10; Se, 0.1; I, 20 and ethoxyquin 3 mg.
Concentration was calculated as the difference between total protein and albumin. Also, plasma albumin to globulin and HDL/LDL ratios were calculated.

At day 28 of age, 4 chicks from each treatment were housed individually in separate cages for 5 days. Birds were allowed to the experimental diets for 4 days as preliminary period followed by 3 days as a collection period, in which quantities of feed intake and excreta were determined. The proximate analyses of feed and dried excreta and meat were carried out determined using the following procedures (Association of official analytical chemists, AOAC 2004): dry matter, method number 934.01; crude protein, method number 954.01; ether extract, method number 920.39; crude fibre, method number 954.18; ash, method number 942.05. Digestibility of nutrients was calculated according to Attia et al. (2006).

Data were analysed using one-way analyses of variance of the GLM procedure of statistical analysis software (SAS) version 6.11 (SAS Institute 2002) according to the following model: \( y_{ij} = \mu + A_i + e_{ij} \) whereas \( \mu \) = overall mean; \( A_i \) = effect of treatment and \( e_{ij} \) = random error.

Differences among means were compared \((p < .05)\) using student Newman–Keuls Test (SAS Institute 2002).

Results

The body weight gain (Table 2) was the biggest \((p < .01)\) in the control group; 2EAs and 2EAs +4%CLA groups had lower values than the three groups fed 5EAs diets. Feed intake of the low-CP groups supplemented with 2 EAs was higher \((p < .01)\) than the other groups (Table 2). 5EAs +2%CLA diet induced lower \((p < .01)\) FCR than the 5EAs +4%CLA diet but the FCR obtained with both diets was not different from the control and lower \((p < .01)\) than 2EAs groups; in addition all the 2EAs groups had a worse \((p < .01)\) FCR than the control (Table 2). 5EAs +2%CLA group had the lowest \((p < .01)\) protein intake (Table 2). The diets 2EAs, 5EAs and 5EAs +4%CLA had a lower \((p < .01)\) protein intake than the control group. The 5EAs diets had a better \((p < .01)\) PCR than all the groups fed 2EAs diets. Mortality rate was unaffected by dietary treatments.

The effect of dietary treatments on nutrient digestibility of broilers is reported in Table 3. The ash digestibility was unaffected by dietary treatments, while the reduction of protein percentage in the diet increased \((p < .01)\) the digestibility of crude protein. The dry matter digestibility of 5EAs diets and that of low-CP 2EAs group supplemented with 2% CLA was higher than the control. Also the organic matter digestibility was increased \((p = .01)\) due to use of low-protein diets.
excluding the 2EAAs +4%CLA for which the organic matter digestibility was not different from the positive control and the other groups. Excluding the 2EAAs and the 5EAAs diet unsupplemented with CLA (for the last no differences were recorded among all the other groups), the other low-CP diets increased (p < .01) the ether extract digestibility of broilers. The reduction of protein percentage in the diets always reduced (p < .01) the nitrogen excretion and the most effective were 5EAAs + 2%CLA and 5EAAs +4%CLA diets, followed by 2EAAs and 5EAAs diets and by 2EAAs +2%CLA and +4%CLA.

Table 4 reports the effect of dietary treatments on carcase traits of broilers. Carcass yield, breast, thigh, pancreas, heart and gizzard percentages were unaffected by dietary treatments. The abdominal fat percentage was the biggest (p < .01) when broilers fed the 2EAAs +2%CLA diet, while no differences were
recorded among the other groups. The percentage of liver was higher \( (p < .01) \) in the 2EAAs +4%CLA than the control, 2EEAs and 5EAAs groups. The spleen percentage was lower \( (p < .05) \) in the 5EAAs +4%CLA than in 2EAAs +2%CLA group; no differences were recorded among the other groups.

Table 5 shows that no differences were recorded among the dietary treatments on meat chemical characteristics of breast and thigh. The percentage of liver fat (Table 5) was higher \( (p < .01) \) in the 5EAAs +4%CLA diet than the others (with the exception of 2EAAs +2%CLA diet); 2EAAs +2%CLA had the smallest \( (p < .01) \) fat percentage followed by 2EAAs and the other diets.

The effect of CLA addition to low-protein diets on blood profiles is reported in Table 6. The total protein content was greater \( (p < .01) \) in 2EAAs +4%CLA and 5EAAs +4%CLA than in the control and the others 2EAAs groups; the control group had also a lower \( (p < .01) \) plasma protein content than the 5EAAs group. 2EAAs +4%CLA group had the highest albumin level \( (p < .01) \); group fed 21.5% CP had higher albumin level than low-CP 2EAAs unsupplemented group and all the 5EAAs groups; among 5EAAs groups, that supplemented with 2 or 4% CLA had lower albumin content than the unsupplemented group. The globulin content of low-protein groups supplemented with 4% CLA was greater \( (p < .01) \) than the positive control and the 2EAAs groups. All the dietary treatments reduced \( (p < .05) \) the albumin/globulin ratio in respect of the control group. The AST level was the greatest \( (p < .01) \)

### Table 5. Effect of low-protein amino acid supplemented-diets without or with different levels of conjugated linoleic acid additions during 7–28 days of age on chemical composition of breast and thigh muscle (% DM basis) and lipids of liver of 28 d old broiler chickens.

| CP,% | 21.5 | 19.0 | 19.0 | 19.0 | 19.0 | 19.0 | 19.0 | 19.0 | SEM | p Value
|------|------|------|------|------|------|------|------|------|-----|------|
| AAS  | 0    | 2    | 2    | 2    | 5    | 5    | 5    | 5    |     |      |
| CLA, % | 0 | 2 | 2 | 4 | 0 | 2 | 4 | SEM | p Value |

Breast, %
- Moisture: 72.4, 73.2, 74.2, 73.1, 73.8, 73.8, 73.8, 73.1, 0.8, ns
- Protein: 61.2, 60.2, 61.1, 60.5, 60.8, 61.2, 61.3, 0.08, ns
- Lipid: 32.3, 33.4, 31.2, 31.7, 32.6, 31.8, 31.5, 0.6, ns
- Protein/Lipid: 2.15, 2.14, 2.19, 2.17, 2.11, 2.15, 2.17, 0.13, ns
- Ash: 2.08, 3.06, 2.36, 2.48, 2.78, 2.63, 2.59, 0.11, ns

Thigh, %
- Moisture: 69.8, 70.1, 71.4, 70.0, 70.2, 70.1, 71.1, 0.7, ns
- Protein: 58.1, 57.3, 57.7, 57.7, 57.8, 57.4, 57.5, 0.6, ns
- Lipid: 36.6, 37.4, 36.9, 37.0, 37.5, 36.9, 36.7, 0.7, ns
- Protein/lipid: 1.85, 1.78, 1.82, 1.82, 1.79, 1.81, 1.81, 0.11, ns
- Ash: 2.35, 3.67, 2.45, 2.32, 2.89, 2.64, 2.82, 0.11, ns

Liver, %
- Lipid: 24.0, 21.3, 19.7, 22.8, 23.8, 25.2, 25.9, 0.5, 0.0001

### Table 6. Effect of low-protein amino acid supplemented-diets without or with different levels of conjugated linoleic acid additions during 7–28 days of age on blood biochemical constituents of 28 d old broiler chickens.

| CP,% | 21.5 | 19.0 | 19.0 | 19.0 | 19.0 | 19.0 | 19.0 | 19.0 | SEM | p Value |
|------|------|------|------|------|------|------|------|------|-----|------|
| AAS  | 0    | 2    | 2    | 2    | 5    | 5    | 5    | 5    |     |      |
| CLA, % | 0 | 2 | 2 | 4 | 0 | 2 | 4 | SEM | p Value |

Total protein: 3.15, 3.37, 3.85, 5.04, 4.54, 4.06, 4.82, 0.72, 0.0002
Albumin: 1.02, 0.87, 0.96, 1.12, 0.91, 0.82, 0.79, 0.08, 0.0001
Globulin: 2.13, 2.51, 2.89, 3.92, 3.63, 3.24, 4.04, 0.73, 0.0002
Albumin/globulin: 0.63, 0.37, 0.34, 0.30, 0.28, 0.26, 0.20, 0.18, 0.02
AST, U/l: 86.3, 80.8, 122.1, 145.6, 45.4, 82.1, 125.4, 13.0, 0.0001
ALT, U/l: 18.4, 22.2, 14.6, 11.2, 6.3, 4.6, 6.2, 1.0, 0.0001
Total lipids: 871.8, 700.3, 723.3, 836.7, 557.7, 628.6, 346.2, 66.4, 0.0001
Total cholesterol: 81.5, 136.3, 67.4, 71.8, 129.2, 126.3, 65.7, 3.7, 0.0001
HDL: 60.0, 62.3, 59.8, 52.8, 60.0, 53.5, 59.0, 9.2, ns
LDL: 86.4, 67.3, 80.5, 70.5, 46.0, 88.5, 67.0, 10.0, 0.0001
HDL/LDL: 0.68, 0.94, 0.75, 0.75, 1.72, 0.61, 0.90, 0.91, 0.0001
in the 2EAAs +4%CLA group followed by: 5EAAs +4%CLA and 2EAAs +2%CLA; positive control, 2EAAs and 5EAAs +2%CLA; 5EAAs groups. The level of plasma ALT was the greatest ($p < .01$) in the 2EAAs group followed, in order, by: positive control; 2EAAs +2 and +4% CLA; 5EAAs; 5EAAs +4%CLA and 5EAAs +2%CLA groups. Excluding 2EAAs +4%CLA, all the diets reduced ($p < .01$) total lipids in respect of the control group and the lowest value was recorded in 5EAAs +4%CLA group. 2EAAs group had the highest ($p < .01$) value of plasma cholesterol followed by 5EAAs and 5EAAs +2%CLA groups which had a cholesterol level higher than the positive control group; the other low-CP groups had a lower content of cholesterol than the positive control and the lowest value was recorded for 2EAAs +4%CLA group. No effect of CLA addition to low-CP diet was observed on plasma HDL content, while 2EAAs, 5EAAs and 5EAAs +4%CLA groups reduced ($p < .01$) the LDL content in respect of the control group and the minimum value was recorded for 5EAAs diet. The HDL to LDL ratio was the highest ($p < .01$) in 5EAAs group and no differences were recorded among the other groups.

Discussion

Growth performance of broiler in our trial was lower than that reported in the Hubbard Broiler Management Guide (2006). In particular, the average body weight at 28 days in the control group was around 865 g, instead of 1662 and this was related to a lower cumulative feed intake (1160 g from 7 to 28 days in the control group, 2240 from 1 to 28 d as reported in Hubbard guide. Worldwide, the weight differences of broilers in comparison to the standard reference are a common problem, and this expected due to different environmental, nutritional and hygienic conditions and this is particularly true in the developing countries. Our results indicated that the reduction of protein level in a maize-soybean based diet penalised the body weight gain of chicks and, in general, essential amino acids supplementation is not able to recover the performance losses, but 5EAAs gave better results than 2EAAs, indicating that also the supplementation of threonine, tryptophan and arginine is very important to sustain chick growth when protein level is reduced. This is particularly true in our trial, as it is well known (National Research Council 1994) that amino acids requirements of poultry are higher in a younger age. In respect of the effect of lowering diet protein on broiler performance, there are contradictory data in literature. Aletor et al. (2000) observed that decreasing CP diet of broiler chicken from 22.5 to 15.3% did not affect the growth rate when the diet was correctly balanced with essential amino acids. Tetteh et al. (2010) found that 28 days old chicks fed low-protein diet (16.0 vs. 20.0% CP) had a lower weight gain than the control group, due to a lower feed intake and this effect was more evident in males than in females. These discrepancies can be related to several factors as the degree of CP reduction, the amino acids supplementation, the level of metabolisable energy, the class and age of the chickens (Aletor et al. 2000). In the present trial, the importance of an adequate amino acids supplementation is confirmed by the recorded FCR values that worsened in 2EAAs diets, but were not different from the control group in 5EAAs diets for which the protein intake is lower than that of the control. In addition, also the efficiency of protein utilisation was better in low-CP 5EAAs than that of 2EAAs diets.

Regarding the effect of CLA on broiler growth performance, the expected result of an improvement of feed conversion ratio in comparison to the unsupplemented groups, due to a reduction of fat deposition was not obtained. This partly agree with the findings of Buccioni et al. (2009) and Cho et al. (2013) who found a negative effect of CLA supplementation on broiler growth and was in line with the findings of Martínez-Aispuro et al. (2014) who found that the addition of CLA to low-protein or standard diets of growing pigs did not improve the growth performance or carcass characteristics. Several studies (Terpstra 2001; Javadi et al. 2007) have shown that the specific mechanisms by which dietary CLA reduces the body fat content can vary from one animal species to another. In broilers fed CLA, Badinga et al. (2003) observed that the reduction of liver lipid accumulation reflected enhanced b-oxidation or reduced de novo lipid synthesis, while Javadi et al. (2007) indicates higher de novo synthesis and lower desaturase activity. However, further investigations need to better clarify the exact way of action of CLA on broiler lipid metabolism.

As expected, the decrease of protein level in the diets increased the protein digestibility and this mainly contributed to the increase of dry matter and organic matter digestibility. However, also the digestibility coefficients of lipids were increased in the chick fed low-protein diets, probably due to the general decrease in feed intake and, as a consequence, in fat intake.

Several authors (Fanche & Jensen 1989; Rosebrough & McMurtry 1993) observed that chicks fed low-protein diets had a concomitant increase in carcass fat deposition and, as the liver is the primary site of lipogenesis in chickens (Rosebrough & Steele 1985) the increase of
liver percentage observed by the same authors, suggests that the tendency for increased liver weight in chick fed low-protein diets can be related to an increase in lipogenic activity. In our trial, in general, there are few differences among groups in terms of fat deposition and percentage of liver. The results on abdominal fat deposition are in line with the findings of Widyaratne & Drew (2011). Also the effect of liver fat percentage is not linear but the groups supplemented with 4% CLA had a higher liver fat percentage than the correspondent low-CP groups no supplemented with CLA, and, for the 5EAAs group, also higher than the control. This result agree with the conclusion of other authors (Doreau & Chilliard 1997; Shin et al. 2011) confirming that although its potential role in fat reduction, the effect of CLA on lipid metabolism is not clear and, in particular, seems to be tied to the animal sex: in fact female broilers have been shown to accumulate more abdominal fat that male broiler and thus the CLA effect is more evident (Twining et al. 1978). It’s not easy to explain the reduction of the spleen relative weight with low-CP diet supplemented with 5EAAs and 4% CLA in comparison to the diet supplemented with 2 EAAs and 2% CLA. Jahanian (2009) stated that when low-protein diets were fed to broiler, the spleen weight can decrease due to a not sufficient level of arginine. In our trial, the arginine was supplemented when spleen weight decrease, so further investigation need to evaluate the effect of CLA level on this parameter.

The use of low-protein diet was able to affect most of the plasma constituents. In particular, the total protein level was, in general, increased and the increasing was tied to an increase of globulin and a decrease in albumin, so that the albumin/globulin ratio was decreased due to use of 19% CP-diet even if there is a trend in decreasing albumin to globulin ratio in groups supplemented by 5EAAs. Both increase of globulin and decrease in albumin to globulin ratio are tied to an improved immune system function on broiler (Bovera et al. 2015, 2016) and can positively affect the health status of birds. The increase in protein and globulin due to the diet supplemented with 5EAAs may be partially due to arginine supplementation: in fact arginine was reported to protect animals against ammonia intoxication and disease and oxidative stress (Basiouni et al. 2006; Bautista-Ortega et al. 2013) and/or methionine (Tsiagbe et al. 1987).

Regarding the liver function, ALT level was in general reduced by low-CP diets but this trend is not evident for AST that showed a wide variation among groups. However, AST (mitochondrial enzyme) is considered a less specific of liver function than other enzymes since it can also be found in many peripheral tissues (as muscles) and hence had a very high variability (Moniello et al. 2005; Bovera et al. 2007).

Plasma lipids and LDL were, in general, decreased due to feeding low-CP diet, in particular, when the diet was supplemented with 5EAAs. This revealed the beneficial effects of feeding low-CP diet supplemented with methionine, lysine, arginine, threonine and tryptophan on lipid metabolism. In this regard, Attia et al. (2001) found that low-CP methionine and lysine supplemented-diet resulted in lower serum total protein, higher plasma total lipids, triglycerides and cholesterol.

Results indicated that both levels of CLA had similar potential effect for reducing plasma total cholesterol of the group supplemented with methionine and lysine while, 4% CLA was more efficient than 2% when added to low-CP diet supplemented with 5EAAs. However, plasma HDL was not affected by CLA level, while plasma LDL was increased when 2 and 4% CLA was supplemented to the low-CP diet supplemented with 5EAAs. Nonetheless, the values were lower than those of the positive control. Thus, HDL/LDL ratio in 2EAAs diets was not affected by CLA level. However, the ratio was similarly decreased when both CLA levels were added to low-CP diet 5EAAs diet. The effect of CLA on lipid and cholesterol metabolism has been suggested by Badinga et al. (2003) who found that hepatic lipid and triacylglycerols concentrations were significantly reduced by dietary CLA, while the proportions of SFA in liver lipids increased. In addition, Aydin (2005) indicated the existence of a correlation between delta-9 desaturase enzyme activity and secretion of very low-density lipoproteins from the liver. In fact, certain fatty acids (i.e. sterculic acid, CLA) inhibit the activity of this enzyme in the liver and cause a decrease in liver oelic acid compared to other fatty acids such as linoleic or palmitic acids; oleic acid has significant role in the secretion of triacylglycerols from chicken hepatocytes. Also, Szymczyk et al. (2001) reported that total serum cholesterol concentrations reached a maximum in broilers fed 1.0% CLA and then decreased ($p < .01$) slightly from 141.73 to 136.47 mg/dl. The same was true also for HDL-cholesterol (from 113.58 to 109.97 mg/dl; $p < .01$), but LDL cholesterol/total cholesterol ratio and serum triacylglycerol concentration were unaffected by CLA. In addition, Badinga et al. (2003) revealed that hepatic lipid and triacylglycerols concentrations were significantly reduced by dietary CLA. On the other hand, Aletor et al. (2003) showed that broilers fed the low-CP diets supplemented with CLA had a higher cholesterol concentration in plasma, lower hepatic triglyceride concentrations than broilers fed low-CP diets without CLA.
supplement. Du & Ahn (2002) found that CLA increased triglyceride, total cholesterol, and HDL cholesterol levels in plasma and the increased plasma triglyceride level could be caused by increasing fatty acid synthesis in the liver after CLA feeding due to increasing hepatic fatty acid synthesis in the liver. Along the same side, Javadi et al. (2004) found that prolonged feeding of 0.5 g CLA/kg increased triacylglycerol accumulation in the liver. However, key enzymes for fatty acids synthesis such as acetyl CoA carboxylase and fatty acid oxidation e.g. 3-hydroxy acetyl-COA dehydrogenase and citrate synthesis were not significantly affected. Terpstra (2004) concluded that CLA may have effects on plasma lipids, however, only one study in humans showed a significant HDL-cholesterol-lowering effect of CLA. On the other studies, there were no indications of the effect of CLA on plasma total, LDL- and HDL-cholesterol or plasma triacylglycerol concentrations. In this regard, Azain et al. (2000) found that serum metabolites such as triglycerides, free fatty acids and cholesterol were not affected by dietary addition of 0.5% CLA to rats. These indicated that the effect of CLA on lipid metabolism was controversial in literature and warrant further investigation.

Conclusions

Decreasing the protein content of a maize-soybean meal-based diet from 21.5 to 19.0% penalised the growth performance of broilers from 7 to 28 days of age and the supplementation of two or five essential amino acids and CLA at 2 or 4% diet are not able to improve growth rate. However, the addition of methionine, lysine, arginine, threonine and tryptophan without or with 2 or 4% of CLA resulted in recovery of FC and PC. On the other hand, the higher digestibility of nutrients, and in particular, of protein, contributes to reduce the excreta nitrogen percentage by-25%. In addition, no changes were observed for carcase and meat traits.

Disclosure statement

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

ORCID

Youssef A. Attia http://orcid.org/0000-0001-6505-3240

References

Aletor VA, Eder K, Becker K, Brigitte R, Paulicks R, Roth FX, Roth-Maier DA. 2003. The effects of conjugated linoleic acids or an alpha-glucosidase inhibitor on tissue lipid concentration and fatty acid composition of broiler chicks fed a low-protein diet. Poul Sci. 82:111–116.

Aletor VA, Hamid I, Niess E, Pfeffer E. 2000. Low-protein amino acids-supplemented-diets in broiler-chickens: effects on performance, carcass characteristics whole-body composition and efficiencies of nutrient utilization. J Sci Food Agric. 80:547–554.

Association of official analytical chemists, AOAC. 2004. Official methods of analysis of the Association of Official Analytical Chemists. 18th ed. Washington (DC): AOAC.

Attia YA. 2003. Performance, carcass characteristics, meat quality and plasma constituents of meat type drakes fed diets containing different levels of lysine with or without a microbial phytase. Arch Anim Nutr. 66:39–48.

Attia YA, Abd El-Rahman SA, Qota EMA. 2001. Effects of microbial phytase without or with cell-wall splitting enzymes on the performance of broilers fed marginal levels of dietary protein and metabolizable energy. Egypt Poult Sci J. 21:521–547.

Attia YA, Böhmer BM, Roth-Maier DA. 2006. Responses of broiler chicks raised under constant relatively high ambient temperature to enzymes, amino acid supplementations, or diet density. Arch Geflügel. 70:80–91.

Atencio A, Albino LFT, Rostagno HS, Oliveira DC, Veites FM, Pupa JMR. 2004. Exigência de arginina digestível para frangos de corte machos em diferentes fases. Rev Bras Zootec. 33:1456–1466.

Attia YA, Nawar ME, Mona O, Salwa AB. 1998. Optimum levels of metabolizable energy and crude protein in the rations for Avian-34 broiler chicks. Proceedings of the 10th Conference of The Egyptian Society of Animal Production, Assiut University, Egypt; p. 223–241.

Austic RE, Scott RL. 1975. Involvement of food intake in the lysine-arginine antagonism in chicks. J Nutr. 105:1122–1131.

Aydin R. 2005. Type of fatty acids, lipoprotein secretion from liver and fatty liver syndrome in laying hens. Int J Poult Sci. 4:917–919.

Azain MJ, Hausman DB, Sisk MB, Flatt WP, Jewell DE. 2000. Dietary conjugated linoleic acid reduces rat adipose tissue cell size rather than cell number. J Nutr. 130:1548–1554.

Badinga LKT, Selberg AC, Dinges CW, Comer A, Miles RD. 2003. Dietary conjugated linoleic acid alters hepatic lipid content and fatty acid composition in broiler chickens. Poult Sci. 82:111–116.

Balnave D, Barke J. 2002. Re-evaluation of the classical dietary arginine-lysine interaction for modern poultry diets: a review. Worlds Poult Sci J. 58:275–289.

Basuoni G, Najib H, Zaki MM. 2006. Influence of extra supplementation with arginine and lysine on overall performance, ovarian activities and humoral immune response in local Saudi hens. Int J Poult Sci. 5:441–448.

Bautista-Ortega J, Ruiz-Feria JN, Stallone CA. 2013. Effects of arginine and antioxidant vitamins on pulmonary artery reactivity to phenylephrine in the broiler chicken. Poult Sci. 92:1062–1072.

Bovera F, Moniello G, De Riu N, Di Meo C, Pinna W, Nizza A. 2007. Effect of diet on the metabolic profile of ostriches (Struthio camelus var. domesticus). Trop Anim Health Prod. 39:265–270.
Bovara F, Loponte R, Marono S, Piccolo G, Parigi G, Iaconieri M, Gasco L, Nizza A. 2016. Use of Tenebrio molitor larvae meal as protein source in broiler diet: effect on growth performance, nutrient digestibility, and carcass and meat traits. J Anim Sci. 94:639–647.

Bovara F, Piccolo G, Gasco L, Marono S, Loponte R, Vassalotti G, Mastellone V, Lombardi P, Attia YA, Nizza A. 2015. Yellow mealworm larvae (Tenebrio molitor, L.) as a possible alternative to soybean meal in broiler diets. Br Poul Sci. 56:569–575.

Buccioni A, Antongiovanni M, Mele M, Gualtieri M, Minieri S. 2009. Effect of oleic and conjugated linoleic acid in the diet of broiler chickens on the live growth performances, carcass traits and meat fatty acid profile. Ital J Anim Sci. 8:603–614.

Cho S, Ryu C, Yang J, Mbiriri DT, Choi CW, Chae JI, Kim YH, Shim KS, Kim YJ, Nag-Jin C. 2013. Effect of conjugated linoleic acid feeding on the growth performance and meat fatty acid profiles in broiler: meta-analysis. Asian Austral J Anim Sci. 26:995–1002.

Cook ME, Miller CC, Park Y, Pariza MW. 1998. Immune modulation by altered nutrient metabolism: nutritional control of immune-induced growth depression. Poult Sci. 72:1301–1305.

Doreau M, Bauchart D, Chiilliard Y. 2011. Enhancing fatty acid composition of milk and meat through animal feeding. Anim Prod Sci. 51:19–29.

Doreau M, Chiilliard Y. 1997. Digestion and metabolism of dietary protein and arginine concentrations in starting broiler chicks. Poult Sci. 88:1818–1824.

Javanian R. 2009. Immunological responses as affected by dietary protein and arginine concentrations in starting broiler chicks. Poult Sci. 88:1818–1824.

Vassalotti G, Mastellone V, Lombardi P, Attia YA, Nizza A. 2015. Yellow mealworm larvae (Tenebrio molitor, L.) as a possible alternative to soybean meal in broiler diets. Br Poul Sci. 56:569–575.

Emadi M, Jahanshiri F, Kaveh K, Hair-Bejo M, Ideris A, Du M, Ahn DU. 2002. Effect of dietary conjugated linoleic acid on the growth rate of live birds and on the abdominal fat content and quality of broiler meat. Poult Sci. 81:428–433.

Emadi M, Jahanshiri F, Kaveh K, Hair-Bejo M, Ideris A, Alimon AR. 2011. Nutrition and immunity: the effects of the combination of arginine and tryptophan on growth performance, serum parameters and immune response in broiler chickens challenged with infectious bursal disease vaccine. Avian Pathology. 40:63–72.

Fanche BI, Jensen LS. 1989. Influence on performance of three to six-week-old broilers of varying dietary protein contents with supplementation of essential amino acid requirements. Poult Sci. 68:113–123.

Fernandes JI, Murakami AE, Martins EN, Sakamoto MI, Garcia ER. 2009. Effect of arginine on the development of the pectoralis muscle and the diameter and the protein:deoxygenyribonucleic acid ratio of its skeletal myofibers in broilers. Poultry Science. 88:1399–1406.

Hubbard broiler Management Guide. 2006. Available from: http://www.winmixsoft.com/files/info/Hubbard%20Broiler%20Management%20Guide.pdf

Jahanian R. 2009. Immunological responses as affected by dietary protein and arginine concentrations in starting broiler chicks. Poult Sci. 88:1818–1824.

Javadi M, Beynen AC, Hovenier R, Lankhorst AE, Lemmens AG, Terpstra AHM, Geelen MJH. 2004. Prolonged feeding of mice with conjugated linoleic acid increases hepatic fatty acid synthesis relative to oxidation. J Nutr Biochem. 15:680–687.

Javadi M, Geelen MJH, Everts H, Hovenier R, Javadi S, Kappert H, Beynen AC. 2007. Effect of dietary conjugated linoleic acid on body composition and energy balance in broiler chickens. Br J Nutr. 98:1152–1158.

Jones JD, Petersburg SJ, Burnett DC. 1967. The mechanism of the lysine-arginine antagonism in the chick: effect of lysine on digestion, kidney arginase, and liver transamidinase. J Nutr. 93:103–116.

Kennedy A, Martinez K, Schmidt S, Mandrup S, LaPoint K, McIntosh M. 2010. Antiobesity mechanisms of action of conjugated linoleic acid. J Nutr Biochem. 21:171–179.

Martínez-Aispuru M, Figueroa-Velasco JL, Zamora-Zamora V, Cordero-Mora JL, Narciso-Gaytán C, Sánchez-Torres MT, Carrillo-Domínguez S, Castillo-Domínguez RM. 2014. Effect of CLA supplementation to low-protein diets on the growth performance, carcass characteristics, plasma urea nitrogen concentration, and fatty acid profile in the meat of pigs. Braz Arch Biol Technol. 57:742–754.

Miner JL, Cederberg CA, Nielsen MK, Chen X, Baie CA. 2001. Conjugated linoleic acid (CLA), body fat, and apoptosis. Obes Res. 9:129–134.

Moniello G, Bovara F, Solinas IL, Piccolo G, Pinna W, Nizza A. 2005. Effect of age and blood collection site on the metabolic profile of ostriches. S Afr J Anim Sci. 35:268–272.

Nagao K, Wang YM, Inoue N, Han SY, Buang Y, Noda T, Kouda N, Okamatsu H, Yanagita T. 2003. The 10trans, 12cis isomer of conjugated linoleic acid promotes energy metabolism in OLETF rats. Nutrition. 19:652–656.

National Research Council. 1994. Nutrient requirements of domestic animals. Nutrient requirements of poultry. 9th ed. Washington (DC): National Academy Press.

Ostrowska E, Muralitharan M, Cross R, Bauman D, Dunshea F. 2005. Dietary conjugated linoleic acids increase lean tissue and decrease fat deposition in growing pigs. J Nutr. 129:2037–2042.

Rosebrough RW, McMurtry JP. 1993. Protein and energy relationships in the broiler chicken. Effects of protein quantity and quality on metabolism. Br J Nutr. 70:667–678.

Rosebrough RW, Steele NC. 1985. Energy and protein relations in the broiler. 1. Effect of protein levels and feeding regimes on growth, body composition and in vitro lipogenesis in broiler chickens. Poult Sci. 64:119–126.

SAS Institute. 2002. SAS® User’s Guide: Statistics. Cary (NC): SAS Institute Inc.

Sell JL, Jin S, Jeffrey M. 2001. Metabolizable energy value of conjugated linoleic acid for broiler chicks and laying hens. Poult Sci. 80:209–214.

Shin D, Narciso-Gaytán C, Park JH, Smith SB, Sanchez-Plata MX, Ruiz-Feria CA. 2011. Dietary combination effects of conjugated linoleic acid and flaxseed or fish oil on the concentration of linoleic and arachidonic acid in poultry meat. Poult Sci. 90:1340–1347.

Terpstra AHM. 2001. Differences between humans and mice in efficacy of the body fat lowering effect of conjugated linoleic acid: role of metabolic rate. J Nutr. 131:2067–2068.

Terpstra AHM. 2004. Effect of conjugated linoleic acid on body composition and plasma lipids in humans: an overview of the literature. Am J Clin Nutr. 79:352–361.

Teteh A, Tona K, Aklikokou K, Gbeassor M, Buyse J, Decuyper E. 2010. Effect of low protein or high energy diets on layer type chick juvenile performance. Int J Poult Sci. 9:1156–1160.
Tsiagbe VK, Cook ME, Harper AE, Sunde ML. 1987. Enhanced immune responses in broiler chicks fed methionine-supplemented diets. Poult Sci. 66:1147–1154.

Twining PV, Jr, Thomas OP, Bossond EH. 1978. Effect of diet and type of birds on the carcass composition of broilers at 28, 49 and 59 days of age. Poult Sci. 57:492–497.

West DB, DeLany JP, Camet PM, Blohm F, Truett AA, Scimeca J. 1998. Effects of conjugated linoleic acid on body fat and energy metabolism in the mouse. Am J Physiol. 275:R667–R672.

West DB, Blohm FY, Truett AA, DeLany JP. 2000. Conjugated linoleic acid persistently increases total energy expenditure in AKR/J mice without increasing uncoupling protein gene expression. J Nutr. 130:2471–2477.

Widyaratne GP, Drew MD. 2011. Effects of protein level and digestibility on the growth and carcass characteristics of broiler chickens. Poult Sci. 90:595–603.

Zanini SF, Colnago GL, Pessotti BMS, Bastos MR, Casagrande FP, Lima VR. 2006. Body fat of broiler chickens fed diets with two fat sources and conjugated linoleic acid. Int J Poult Sci. 5:241–246.

Zhang HJ, Guo YM, Yuan JM. 2005. Conjugated linoleic acid enhanced the immune function in broiler chicks. Br J Nutr. 94:746–752.