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

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ABSTRACT

The study investigated the effect of low-crude protein (CP), essential aminoacids (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.

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Broilers; low-protein diets; essential amino acids; conjugated linoleic acids

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),

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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 carcase 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

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.

	Starter diet[a]	21.5%	19%	19% CP[b]
Ingredients, %				
Yellow maize	44.0	48.6	54.5	54.5
Soybean meal (44% CP)	47.2	43.3	35.5	35.5
Soybean oil[c]	5.50	4.55	4.20	4.20
L-lysine	0.0	0.00	0.26	0.26
DL-methionine	0.225	0.225	0.30	0.30
L-Arginine	0.00	0.00	0.00	0.22
L-Threonine	0.00	0.00	0.00	0.11
L-Tryptophan	0.00	0.00	0.00	0.05
Vitamin and minerals premix[d]	0.30	0.30	0.30	0.30
NaCl	0.30	0.30	0.30	0.30
Bone meal	2.475	2.76	2.76	2.76
Wash building sand	0.00	0.03	1.86	1.48
Calculated values				
Crude protein, %	22.6	21.5	19.1	19.0
Methionine, %	0.60	0.58	0.62	0.62
Methionine + cysteine, %	0.90	0.95	0.95	0.95
Lysine, %	1.38	1.29	1.29	1.29
Arginine, %	1.82	1.54	1.32	1.64
Tryptophan, %	0.38	0.35	0.30	0.35
Threonine, %	1.06	0.89	0.77	0.89
Ca, %	0.90	0.99	0.95	0.95
Available P, %	0.46	0.50	0.48	0.48
ME, kcal/kg diet	3018	3034	3039	3039
Dry matter, %	—-	91.8	90.9	90.1
Analysed values, %				
Crude protein	—-	21.21	18.93	19.27
Ether extract	—-	3.55	5.67	5.48
Crude fibre	—-	2.46	2.63	2.75
Crude ash	—-	7.63	7.52	7.86

[a]Starter diet fed from 1 to 6 days of age
[b]Basal experimental diets fed from 7 to 28 days of age
[c]CLA was replaced soybean oil at 2 and 4% in the basal on weight to weight basis assuming similar MEn according to Sell et al. (2001).
[d]Vitamins and minerals mixture provide per kilogram of diet vitamin A (as all-trans-retinyl acetate); 12,000 U; vitamin E (all rac-α-tocopheryl acetate); 10 U; k_3 3 mg; Vit.D_3, 2200 ICU; riboflavin, 10 mg; Ca pantothenate,10 mg; niacin, 20 mg; choline chloride, 500 mg; vitamin B_{12}, 10 μg; vitamin B_6, 1.5 mg; thiamine (as thiamine mononitrate); 2.2 mg; folic acid, 1 mg; D-Biotin, 50 μg. Trace mineral (milligrams per kilogram of diet) Mn, 55; Zn, 50; Fe, 30; Cu, 10; Se, 0.1; I, 20 and ethoxyquin 3 mg.

official analytical chemists, AOAC 2004) or calculated (National Research Council 1994). The CLA supplemented to the diets replaced soybean oil on weight to weight basis, assuming similar ME_n value (Sell et al. 2001). The experimental diets were fed from 7 to 28 d of age. Water and mash feed were offered *ad libtium*. The experimental protocols and management was approved by the council of the Department of Animal and Poultry Production, Damanhour University.

Birds were individually weighed at 7, 14, 21 and 28 d of age in the morning before offering the feeds to calculate body weight gain. Feed intake was recorded weekly according to the replicate feeding system followed in the present work. Each group was daily provided with enough pre weighed amount of its corresponding diet. The remainder and scattered feeds as well as the consumed feed were weekly calculated for each replicate and thereafter, the average weekly feed intake per bird was calculated. Protein intake was calculated by multiplying dietary CP by the amount of feed intake. Feed conversion and protein conversion ratios were calculated in the form of units of feed and protein intake, respectively required to produce one unit of live body weight gain.

At the end of trial (28 d of age) 5 birds/treatment (at least 1 per replicate, for a total of 35 chicks) were slaughtered after being fasted overnight according to the Islamic procedures. The birds were feather picked and total inedible parts (head, legs and viscera) were scattered. Then, the remaining carcase (dressed weight), thigh, breast, abdominal fat, pancreas, liver, gizzard, heart and spleen were weighed. The percentage of carcase yield and internal organs were expressed as relative to live body weight.

Five skinless boneless breast and thigh meat samples were weighed and dried in an electric drying oven at 70 °C until constant weight. The dried flesh was finely ground through a suitable mixer to pass through a sieve (1-mm^2) and then carefully mixed, and samples were kept into well tight glass container for subsequent chemical analysis.

Six blood samples were collected from each treatment (at least 1 per replicate). Blood samples were collected in heparinised tubes and plasma was separated by centrifugation at 3000 rpm for 15 min and stored at 20 °C until analysis. Blood biochemical parameters (total protein, albumin, total lipids, total cholesterol, activities of aminotransferase and aspartate aminotransferase, low-density and high-density lypoproteins) were determined using specific commercial diagnosing kits (Diamond Diagnostics Company, Egypt). Globulin 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

μ = 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; 2EAAs and 2EAAs +4%CLA groups had lower values than the three groups fed 5EAAs diets. Feed intake of the low-CP groups supplemented with 2 EAAs was higher ($p<.01$) than the other groups (Table 2). 5EAAs +2% CLA diet induced lower ($p<.01$) FCR than the 5EAAs +4% CLA diet but the FCR obtained with both diets was not different from the control and lower ($p<.01$) than 2EAAs groups; in addition all the 2EAAs groups had a worse ($p<.01$) FCR than the control (Table 2). 5EAAs +2%CLA group had the lowest ($p<.01$) protein intake (Table 2). The diets 2EAAs, 5EAAs and 5EAAs +4% CLA had a lower ($p<.01$) protein intake than the control group. The 5EAAs diets had a better ($p<.01$) PCR than all the groups fed 2EAAs 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 5EAAs diets and that of low-CP 2EAAs 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,

Table 2. Effect of low-protein amino acid supplemented-diets without or with different levels of conjugated linoleic acid additions on growth, feed intake, feed conversion, protein intake and protein conversion of broiler chicks from 7 to 28 d of age†.

CP, %	21.5	19.0	19.0	19.0	19.0	19.0	19.0		
AAS	0	2	2	2	5	5	5		
CLA, %	0	0	2	4	0	2	4	SEM	*p* Value
Body weight gain, g	768^{a}	706^{c}	715^{bc}	700^{c}	747^{b}	736^{b}	735^{b}	16	.0001
Feed intake, g	1160^{b}	1244^{a}	1295^{a}	1249^{a}	1146^{b}	1084^{b}	1159^{b}	40	.0005
Feed conversion, g/g	1.51^{bc}	1.76^{a}	1.81^{a}	1.78^{a}	1.53^{bc}	1.47^{c}	1.58^{b}	0.06	.0001
Protein intake, g	249^{a}	236^{b}	246^{ab}	237^{ab}	218^{b}	206^{c}	220^{b}	8	.0001
Protein conversion, g/g	0.33^{ab}	0.36^{a}	0.34^{a}	0.34^{a}	0.29^{b}	0.28^{b}	0.30^{b}	0.01	.0001
Dead, n	2	3	2	3	2	3	3	–	–

$^{a–c}$Means within the same row not having similar superscripts are significantly different ($p < .05$).
†n: 4 replicates of 7 chickens per treatment for body weight data and 4 replicates for other parameters.
CP: crude protein; AAS: amino acid supplementations; 1: methionine and lysine supplemented low-protein diet; 2 : methionine; lysine; arginine; threonine and tryptophan supplemented low-protein diet; CLA: conjugated linoleic acid; SEM : standard error of mean.

Table 3. Effect of low-protein amino acid supplemented-diets without or with different levels of conjugated linoleic acid additions on digestibility of nutrients of 28 d old broiler chickens†.

CP,%	21.5	19.0	19.0	19.0	19.0	19.0	19.0		
AAS	0	2	2	2	5	5	5		
CLA,%	0	0	2	4	0	2	4	SEM	*p* Value
Digestibility, %									
Dry matter	78.1^{b}	79.8^{ab}	80.3^{a}	79.9^{ab}	80.9^{a}	81.0^{a}	80.4^{a}	1.4	.001
Crude protein	76.8^{b}	78.1^{a}	79.3^{a}	79.0^{a}	80.3^{a}	80.6^{a}	79.9^{a}	1.9	.005
Ether extract	82.3^{b}	82.3^{b}	84.6^{a}	84.0^{a}	83.1^{ab}	84.2^{a}	84.3^{a}	1.6	.001
Organic matter	72.1^{b}	76.2^{a}	77.1^{a}	74.0^{ab}	75.6^{a}	77.4^{a}	78.8^{a}	1.9	0.01
Ash	25.2	24.4	24.6	25.4	23.9	25.1	24.8	1.3	ns
Excreted nitrogen, %‡	5.36^{a}	3.78^{c}	4.77^{b}	4.40^{b}	3.28^{c}	2.94^{d}	3.00^{d}	0.09	.001

$^{a–c}$Means within the same row not having similar superscripts are significantly different ($p < .05$).
CP: crude protein; AAS: amino acid supplementations; 1 : methionine and lysine supplemented low-protein diet; 2 : methionine; lysine; arginine; threonine and tryptophan supplemented low-protein diet; CLA: conjugated linoleic acid; SEM : standard error of mean; ns : not significant.
†n : 5 observations per treatment.
‡: excreta nitrogen voided in from of faecal and urine nitrogen as a percentage of dry matter basis regardless of nitrogen intake;

Table 4. Effect of low-protein amino acid supplemented-diets without or with conjugated linoleic acid during 7–28 days of age on carcase yield and carcase parts and inner organs (% of live weight) of 28 d old broiler chickens†.

CP,%	21.5	19.0	19.0	19.0	19.0	19.0	19.0		
AAS	0	2	2	2	5	5	5		
CLA,%	0	0	2	4	0	2	4	SEM	*p* Value
Carcass yield‡	66.5	65.9	65.5	65.5	66.5	65.2	64.5	1.8	ns
Breast	18.9	19.1	19.3	18.2	20.8	18.1	18.3	0.9	ns
Thigh	28.8	29.9	29.3	28.8	29.2	27.7	29.7	1.1	ns
Abdominal fat	0.69^{b}	0.57^{b}	1.13^{a}	0.75^{b}	0.41^{b}	0.41^{b}	0.60^{b}	0.12	.004
Pancreas	0.35	0.33	0.40	0.45	0.34	0.44	0.35	0.04	ns
Heart	0.74	0.71	0.76	0.74	0.65	0.63	0.54	0.06	ns
Gizzard	2.73	2.73	2.35	2.71	2.62	2.02	2.02	0.23	.08
Liver	2.90^{b}	3.03^{b}	3.89^{ab}	4.96^{a}	3.01^{b}	3.91^{ab}	3.64^{ab}	0.41	.007
Spleen	0.079^{ab}	0.079^{ab}	0.089^{a}	0.078^{ab}	0.072^{ab}	0.050^{ab}	0.041^{b}	0.012	.03

^{a,b}Means within the same row not having similar superscripts are significantly different ($p < .05$). ns $p \geq .05$.
CP: crude protein; AAS: amino acid supplementations; 1: methionine and lysine supplemented low-protein diet; 2 : methionine; lysine; arginine; threonine and tryptophan supplemented low-protein diet; CLA: conjugated linoleic acid; SEM : standard error of mean; ns : not significant.
†n : 5 observations per treatment;
‡carcase without internal organs and inedible parts as a percentage of live body weight.

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		
AAS	0	2	2	2	5	5	5		
CLA,%	0	0	2	4	0	2	4	SEM	*p* Value
Breast,%									
Moisture	72.4	73.2	74.2	73.1	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[4]	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^{bc}	21.3^{d}	19.7^{e}	22.8^{c}	23.8^{bc}	25.2^{ab}	25.9^{a}	0.5	.0001

[a–e]Means within the same row not having similar superscripts are significantly different ($p < .05$). ns $p \geq .05$.
CP: crude protein; AAS: amino acid supplementations; 1: methionine and lysine supplemented low-protein diet; 2: methionine; lysine; arginine; threonine and tryptophan supplemented low-protein diet; CLA: conjugated linoleic acid; P/L: protein to lipid ratio; SEM: standard error of mean; ns: not significant.
†n: 5 observations per treatment.

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		
AAS	0	2	2	2	5	5	5		
CLA,%	0	0	2	4	0	2	4	SEM	*p* Value
Total protein‡	3.15^{c}	3.37^{bc}	3.85^{ab}	5.04^{a}	4.54^{ab}	4.06^{abc}	4.82^{a}	0.72	.0002
Albumin‡	1.02^{b}	0.87^{cd}	0.96^{bc}	1.12^{a}	0.91^{c}	0.82^{d}	0.79^{d}	0.08	.0001
Globulin‡	2.13^{c}	2.51^{b}	2.89^{abc}	3.92^{a}	3.63^{a}	3.24^{abc}	4.04^{a}	0.73	.002
Albumin/globulin	0.63^{a}	0.37^{b}	0.34^{b}	0.30^{b}	0.25^{b}	0.26^{b}	0.20^{b}	0.18	.02
AST, U/l	86.3^{c}	80.8^{c}	122.1^{b}	145.6^{a}	45.4^{d}	82.1^{c}	125.4^{b}	13.0	.0001
ALT, U/l	18.4^{b}	22.2^{a}	14.6^{c}	11.2^{c}	6.3^{d}	4.6^{e}	6.2^{d}	1.0	.0001
Total lipids‡	871.8^{a}	700.3^{b}	723.3^{b}	836.7^{a}	557.7^{c}	628.6^{bc}	346.2^{d}	66.4	.0001
Total cholesterol‡	81.5^{c}	136.3^{a}	67.4^{d}	71.6^{d}	129.2^{b}	126.3^{b}	65.7^{e}	3.7	.0001
HDL‡	60.0	62.3	59.8	52.8	60.0	53.5	59.0	9.2	ns
LDL‡	90.0^{a}	67.3^{b}	80.5^{ab}	70.5^{ab}	36.0^{c}	88.5^{ab}	67.8^{b}	10.0	.0001
HDL/LDL	0.68^{b}	0.94^{b}	0.75^{b}	0.75^{b}	1.72^{a}	0.61^{b}	0.90^{b}	0.91	.0001

[a–e]Means within the same row not having similar superscripts are significantly different ($p < .05$). ns $p \geq .05$.
CP: crude protein; AAS: amino acid supplementations; 1: methionine and lysine supplemented low-protein diet; 2: methionine; lysine; arginine; threonine and tryptophan supplemented low-protein diet; CLA: conjugated linoleic acid; AST: aspartate aminotransferase; ALT: Alanine transaminase; HDL: high-density lipoprotein; LDL: low-density lipoprotein; HDL/LDL: high-density lipoprotein/low-density lipoprotein;
†n: 6 observations per treatment; SEM: standard error of mean; ns: not significant.
‡mg per 100 ml.

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. Teteh 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 carcase 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 carcase 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 lipogenetic 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 oleic 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 HDL 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-hydroy 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.

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