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Effects of Dietary Protein and *Macleaya Cordata* Alkaloid Extract Supplementation on Growth Performance, Apparent Ileal Digestibility of Protein and Plasma Amino Acid Concentration in Broiler Chickens

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Abstract

The current study evaluated the effects of feeding a phytogenic substance, *Macleaya cordata* alkaloid extract (MCAE), on the growth performance and protein digestibility of broiler chickens fed two dietary concentrations of protein. A total of 560, one-day-old male broiler chicks (initial body weight = 39.9 ± 0.1) were randomly assigned to eight dietary treatments with five replicates (14 broiler chickens per replicate) in a 2 × 4 factorial arrangement including two concentrations of dietary crude protein (100 and 95% of established requirements; control and low-protein diets, respectively) and four inclusion rates of MCAE (0, 180, 360, and 540 mg/kg diet). Crude protein (CP) and MCAE interactions occurred on the apparent ileal digestibility of CP (P = 0.05), organic matter (P = 0.01), and ash (P = 0.01) at d 35. However, no interaction between CP and MCAE occurred for body weight, average daily gain, and the feed conversion ratio. Dietary MCAE supplementation increased body weight (P = 0.01) at d 35, average daily feed intake (P = 0.04), and average daily gain (P = 0.02) from d 1 to 35 of the broiler chickens linearly. Greater carcass yield and reduced abdominal fat were observed with a higher level of dietary protein intake (P = 0.01). Dietary supplementation with MCAE also reduced the relative weight of the ceca linearly (P = 0.01) without affecting carcass yield at d 35. Dietary treatments had no effects on plasma free Met, Thr, and Gly concentrations at d 35 and serum uric acid and creatinine concentrations at d 15 and 35. In summary, these results indicated that dietary supplementation with MCAE may improve body weight and average daily gain of broiler chickens at both dietary protein levels, by enhancing apparent ileal digestibility of dietary CP and organic matter, as well as by increasing feed intake.

Introduction

For more than half a century, in-feed antibiotics have been used in poultry diets to improve the performance, intestinal health, and survivability of broilers (Allen *et al.,* 2013; Pluske, 2013). The presence of antibiotic residues in poultry products can lead to antibiotic resistance and is a food safety concern. Hence, in recent years, antibiotic use in poultry production has been restricted or banned world-wide (Diarra and Malouin, 2014). Thus, alternatives to antibiotics are needed to minimize the effects of sub-optimal management on broiler productivity in commercial settings. In recent years, special attention has been given to phytogenic products as feed additive growth promotors because of their effects on the productivity and well-being of animals (Kolpin *et al.,* 2002; Windisch *et al.,* 2008; Kantas *et al.,* 2015).

Phytogenic compounds, such as the quaternary benzophenanthridine alkaloids (QBA) sanguinarine and chelerythrine, and protopine alkaloids (PA) extracted from plants, such as *Macleaya cordata*,

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have antimicrobial, anti-inflammatory, anti-fungal, adrenolytic, and sympatholytic properties and thus can improve the productivity of broiler chickens (Kantas *et al.,* 2015; Niu *et al.,* 2012, Liu *et al.,* 2013b). Furthermore, these bioactive compounds can improve feed intake (FI), ileal digestibility of dietary CP, and the diversity of intestinal microbiota in swine and other species (Kosina *et al.,* 2004; Vieira *et al.,* 2008a, b; Liu *et al.,* 2013a). Additionally, Dršata *et al.* (1996) found that sanguinarine inhibits the aromatic L-amino acid decarboxylase (AAD*) in vitro*, probably due to the similarity of its chemical structure to aromatic amino acids (AAs). Therefore, reduced intestinal decarboxylation of aromatic AAs, such as phenylalanine, tyrosine, and tryptophan, through the inhibition of amino acids decarboxylase may improve protein and AA digestibility. However, little is known about the effects of dietary MCAE supplementation on production and carcass traits of broiler chickens fed a low-crude protein diet.

Therefore, the objective of the present study was to evaluate the effects of dietary MCAE (a commercial product containing a mixture of phytogenic compounds) supplementation on growth performance in broiler chickens fed varying levels of CP. We hypothesized that MCAE can improve growth performance by improving the ileal digestibility of dietary CP.

Materials and methods

Birds and diet preparation

Experimental protocols were reviewed and approved by the animal care and use committee of the Isfahan Agricultural and Natural Resources Research and Education Center (protocol No. 2594-264-1). The present study was conducted at the research farm of the Isfahan Agricultural and Natural Resources Research and Education Center, Isfahan, Iran. A total of 560 one-day-old male Ross 308 broiler chicks (initial weight. 39.9 ± 0.1) were purchased from a commercial hatchery and assigned to eight dietary treatments in a 2 × 4 factorial arrangement (five cages per treatment; 14 birds per cage). The main effects were two concentrations of dietary protein, i.e. control diets, 100% of requirements (CON) and low-crude protein diets, 95% of requirements (LCP); and four inclusion rates of MCAE (Sangrovit X10®, Animal Nutrition Center, Phytobiotics Futterzusatzstoffe GmbH, Eltville, Germany) i.e. 0, 180, 360, and 540 mg/kg diet. Two starter and grower diets were formulated to be iso-energetic and to meet or exceed nutrient requirements provided by Ross recommendations (Aviagen, 2014; Table 1) during 1 to 15 and 16 to 35 days of age, respectively.

Macleaya cordata alkaloid extract is a commercial product that contains a mixture of QBA and PA, as well as other bioactive compounds, such as sanguinarine and chelerythrine alkaloids (Tschirner *et al.,* 2003). The diet ingredients included 20 g celite per kg (Celite 545®; Samchun Pure Chemical Co; Seoul, Korea) as an indigestible marker for determining the apparent ileal digestibility (AID) of dietary nutrients. Broiler chickens had free access to mash feed and water throughout the study. The broiler chickens were randomly distributed in an environmentally controlled broiler facility equipped with 40 battery cages (length 124 cm × width 65 cm). Bird densities were 7.7 to 23.4 kg/m^2 during d 1 to15 and 16 to 35, respectively. The lighting program consisted of 23-h light and 1-h dark during the whole experimental period. The light was provided with incandescent bulbs and the light intensity at the broiler chicken level was 30 lx. Ambient temperature was kept at 33 °C for d 1 to 3, which gradually decreased thereafter to 24 °C by the end of the study. Relative humidity was maintained between 45 to 65%.

Growth performance and carcass traits

Body weight (BW) and FI was measured for each cage at d 15 and 35. Average daily feed intake (ADFI) and average daily gain (ADG) were determined for the same periods to calculate the feed conversion ratio (FCR). At d 35, two broiler chickens per replicate cage, with BW closest to the group average, were randomly chosen and individually weighed and slaughtered by cervical dislocation to determine the weights of the carcass, abdominal fat, intestine, and ceca. All carcass data were expressed as a percentage of live BW. The small intestine of each chicken was also excised and the length was measured from the gizzard junction to the ileocecal junction. The relative length of the intestine was calculated as a function of BW and expressed in percentage.

Blood biochemical analysis

All blood samples were collected via wing vein puncturing. Serum uric acid and creatinine concentrations were determined at d 15 and 35 by collecting blood samples into serum tubes from two broiler chickens selected randomly from each cage. Plasma AA concentrations were determined at d 35, by collecting samples into two EDTA-containing tubes from two broiler chickens randomly selected from each cage. To separate serum and plasma blood samples were centrifuged at 2000 × *g* for 20 min and 3000 × g for 15 min at 4 °C, respectively. Plasma and serum aliquots were pooled for each cage and were kept frozen at – 30 °C until further analyses (Boogers *et al.,* 2008).

For AA analysis, plasma samples were deproteinized with sulfosalicylic acid, as described by Teerlink *et al.* (1994), centrifuged for 15 min at

15000 × g and 4 °C, aliquoted, and stored at -20°C until further analysis. Pre-column derivatization with phenylisothiocyanate was performed for 30 min at 40 °C and a 20-µL aliquot of the derivative sample was then analyzed by high-performance liquid chromatography (HPLC, Model RF20A, Shimadzu Corporation, Kyoto, Japan) to determine plasma free AA concentrations (Anderson and Moore, 2004). Serum uric acid and creatinine were measured using commercial colorimetric kits (Pars Azmoon Co; Tehran, Iran), according to the manufacturer's instructions (Lin *et al.,* 2004).

Table 1. Ingredients and chemical composition of the basal diets during the starter (1 to 14 d) and grower (15 to 35 d) periods (as-fed basis).

Item	Dietary CP (% Requirement)			
	100% of the recommended CP		95% of the recommended CP	
	Starter	Grower	Starter	Grower
Ingredients (g/kg)				
Yellow corn (8% CP)	502	572	542	603
SBM [1] (44% CP)	415	345	380	318
Soybean oil	34.0	37.5	29.0	33.5
Limestone	16.0	16.0	16.0	16.0
Monocalcium phosphate	16.0	12.4	16.0	12.4
DL-Methionine	3.7	3.4	3.6	3.1
L-Lys HCl	2.1	2.4	2.3	2.5
L-Threonine	1.2	1.2	1.1	1.1
Sodium chloride	3.5	2.3	3.5	1.6
$NaHCO_3$	-	1.8	-	2.8
Vitamin and mineral premix [2]	5.0	5.0	5.0	5.0
Choline chloride	1.5	1.0	1.5	1.0
Calculated chemical composition (g/kg)				
ME (kcal/kg)	2900	3000	2900	3000
Crude protein	225	200	213	190
Calcium	10.0	9.1	10.0	9.1
Available Phosphorus	5	4	5	4
Sodium	1.6	1.6	1.6	1.6
DEB [3] (meq/kg)	231	220	216	220
Apparent ileal digestible (g/kg)				
Lysine	12.8	11.4	12.2	10.8
Methionine	6.6	6.0	6.3	5.7
Methionine + Cystine	9.5	8.7	9.0	8.3
Threonine	8.1	7.3	7.7	6.9
Tryptophan	2.5	2.2	2.4	2.1
Arginine	13.9	12.8	13.2	12.2
Valine	9.5	8.8	9.0	8.4
Isoleucine	8.6	7.9	8.2	7.5
Determined chemical composition (g/kg)				
Crude protein	221	204	218	193
Calcium	11.5	9.5	11.0	9.7
Total phosphorus	8.1	6.8	7.9	6.5
Sodium	1.7	1.8	1.7	1.7

[1] SBM, soybean meal.
[2] Vitamin and mineral premix provided per kilogram of diet: vitamin A (retinyl acetate), 12,000 IU; cholecalciferol, 5,000 IU; vitamin E (alpha-tocopherol acetate), 70 IU; vitamin K_3 (menadione), 3.5 mg; thiamin, 3.2 mg; riboflavin, 8 mg; pyridoxine, 4.3 mg; cyanocobalamin, 0.025 mg; niacin, 65 mg; D-pantothenic acid, 30 mg; choline chloride, 800 mg; folic acid, 2 mg; biotin, 0.3 mg; Fe, 70 mg as $FeSO_4.H_2O$; Cu, 16 mg as $CuSO_4.5H_2O$; Mn, 120 mg as MnO; Zn, 110 mg as $ZnSO_4.H_2O$; Se, 0.35 mg as Na_2SeO_3; I, 1.3 mg as KI.
[3] Dietary electrolyte balance: (Na^+, meq/kg + K^+, meq/kg) – Cl^-, meq/kg.

Apparent ileal digestibility

At d 35, two broiler chickens randomly selected from each replicate were euthanized by cervical dislocation. Then, the contents of the ileum (from Meckel's diverticulum to 1 cm above the ileocecal junction) were gently expelled into special containers (Gheisari *et al.,* 2011). Ileal digesta samples from all broiler chickens within a cage (2 to 3 g/broiler) were

pooled, flash-frozen in liquid nitrogen, and stored at –20 °C until being processed later.

Ileal digesta and feed samples were freeze-dried, ground, and pulverized. Feed and digesta samples were analyzed for nitrogen (AOAC, 2006; method 990.03), organic matter (OM; the percentage of OM was calculated as the weight loss upon ash), and ash (AOAC, 2005; method 942.05). To determine the acid insoluble ash content of diets and digesta, the method of Siriwan *et al.,* (1993) was used. The AID coefficients for CP, OM, and ash were calculated using the following equations (Van Keulen and Young, 1977):

$$AID\ (\%) = 100 - [100 \times (\%Marker_{diet} / \%Marker_{digesta}) \times (\%Nutrient_{digesta} / \%Nutrient_{diet})]$$

where $Marker_{diet}$ is the concentration of indigestible marker in the diet, $Marker_{digesta}$ is the concentration of indigestible marker in the ileal digesta, $Nutrient_{diet}$ is the concentration of nutrients in the diet and $Nutrient_{digesta}$ is the concentration of nutrients in the digesta (Stein *et al.,* 2007).

Statistical analysis

The data were checked for normal distribution and then analyzed as a 2 × 4 factorial arrangement in a completely randomized design using a 2-way GLM procedure and the Statistical Analysis System version 9.2 (SAS, 2008) to determine the main effects of dietary CP and MCAE, as well as the interaction effects (CP × MCAE) on measured parameters. Also, three preplanned contrasts, i.e. MCAE 0, vs. other concentrations of MCAE (180, 360, or 540 mg/kg diet), linear and quadratic effects of supplemental MCAE, were used to assess the effect of MCAE supplementation on measured traits. The results of blood analysis for each cage were pooled and represented one replicate, so that cage was considered as the experimental unit for different traits. For all statistical analyses, significant differences were considered at $P \leq 0.05$ and trends at $0.05 \leq P \leq 0.10$.

Results

Measures of growth performance, carcass traits, and intestinal characteristics

The effects of dietary treatments on the growth performance and carcass traits of broiler chickens are presented in Table 2. No interactive effect between CP and MCAE (CP × MCAE) on BW, ADFI, ADG, and the FCR was observed at d 15 and 35. However, regardless of the dietary protein level, MCAE linearly increased the BW, ADG, and ADFI of broiler chickens at d 35 ($P < 0.05$), but did not affect the overall FCR. Overall ADFI was higher in the LCP group (approximately 1%) compared to the CON group ($P = 0.04$). Relative to 0 mg/kg dietary MCAE inclusion, dietary supplementation with MCAE improved BW, ADG, and ADFI of broiler chickens at d 35 by about 1.9, 2.1, and 1.3%, respectively ($P < 0.05$). No interaction between CP and MCAE was observed on carcass traits at d 35. Dietary protein intake increased the carcass yield and reduced the abdominal fat ($P = 0.01$), but had no effects on other measures of carcass characteristics. Relative to MCAE 0, dietary supplementation with MCAE reduced the abdominal fat of 35-day-old chickens by about 11% (0.90 vs. 0.79%; $P = 0.05$). No interaction effect (CP × MCAE) was observed on the relative weights of the ceca and the small intestine, nor intestine length. Dietary MCAE supplementation reduced the relative weight of the ceca linearly ($P= 0.01$). Neither CP nor MCAE supplementation affected the intestinal weight or length.

Plasma free amino acid concentrations and serum biochemistry

Table 3 presents the effects of dietary CP, the CP × MCAE interaction, and contrast analysis on the concentrations of selected plasma AA and serum biochemical parameters. The interaction effects of CP × MCAE did not change the concentrations of plasma free AA. However, the result of contrast analysis revealed that chickens fed diets containing supplemental MCAE had a higher plasma concentration of Trp compared with those fed a diet without MCAE ($P = 0.05$). The plasma Lys concentration was lower in chickens fed the LCP diet, compared to those that received the CON diet ($P = 0.01$). Dietary CP intake did not affect the concentrations of other studied AA. No interaction effects were observed on measures of serum biochemistry at d 15 and 35.

Nutrient digestibility

Data on nutrient digestibility are presented in Table 4. An interaction effect between CP and MCAE was observed for the AID of CP, OM, and ash ($P \leq 0.05$) at d 35. Dietary MCAE reduced the AID of dietary ash at inclusion rates of 180 and 360 mg/kg, but improved the AID of ash at an inclusion rate of 540 mg/kg in the CON group. Dietary supplementation with MCAE improved the AID of dietary CP, OM, and ash linearly ($P \leq 0.05$). However, orthogonal contrast analysis revealed that relative to MCAE 0, dietary supplementation with MCAE improved the AID of dietary CP and OM ($P = 0.01$).

Table 2. Effects of dietary crude protein (CP) and *Macleaya cordata* alkaloid extract (MCAE) supplementation on measures of growth performance, carcass characteristics, and intestine characteristics in broiler chickens during a 35 d study.

Item	CP MCAE [1], mg/kg	100% of the recommended CP				95% of the recommended CP				*SEM* [2]	p value for MCAE				
		0	180	360	540	0	180	360	540		CP	MCAE			CP × MCAE
												0, vs. others [3]	Lin.[4]	Quad.[4]	
Growth performance															
Body weight, d 15, (g/bird)		472	493	487	499	483	498	498	495	10.9	0.47	0.05	0.11	0.36	0.90
Final body weight, d 35, (g/bird)		2220	2252	2261	2291	2226	2253	2258	2300	25.55	0.86	0.03	0.01	0.84	0.99
ADG [1], (g/bird/day)		62	63	63	64	62	63	63	64	0.78	0.94	0.05	0.02	0.79	0.95
Feed intake, d 1 to 35, (g/bird/day)		93.1	92.3	94.3	93.6	92.9	94.9	94.5	94.9	0.63	0.04	0.04	0.04	0.48	0.14
FCR [1], (g/g)		1.5	1.4	1.5	1.4	1.5	1.5	1.5	1.5	0.01	0.14	0.24	0.22	0.89	0.29
Carcass components															
Carcass yield [5]		72.6	73.6	72.2	72.4	70.9	71.5	70.5	72.0	0.63	0.01	0.65	0.99	0.94	0.54
Abdominal fat [5]		0.8	0.7	0.8	0.7	1.0	0.8	1.1	0.7	0.08	0.01	0.05	0.21	0.64	0.51
Intestine components															
Ceca [5]		0.8	0.7	0.6	0.6	0.8	0.7	0.6	0.6	0.05	0.51	0.01	0.01	0.13	0.81
Intestine [5]		3.7	4.1	3.8	4.0	4.1	3.6	4.1	4.07	0.06	0.49	0.68	0.28	0.57	0.08
Intestine length [6]		8.1	8.3	8.3	8.2	8.3	8.0	8.3	8.27	4.49	0.92	0.16	0.09	0.74	0.71

[1] *Macleaya cordata* alkaloid extract (MCAE), ADG = average daily gain, FCR = feed conversion ratio.

[2] *SEM* = pooled standard error of the mean (n = 5 replicates/treatment).

[3] Contrast analysis performed to determine the difference between the MCAE 0 diet and all three MCAE diets (MCAE 0, vs. 180, 360, and 540 mg/kg of MCAE).

[4] Orthogonal contrast analyses were performed to assess the effect of MCAE inclusion on growth performance, carcass, and intestine components coefficients. Comparisons were made between 0, 180, 360, and 540 mg/kg MCAE treatments; Lin.: linear, Quad.: quadratic.

[5] Carcass components and intestine components (ceca and intestine) were weighed and expressed as a percentage of live weight.

[6] Values expressed as relative length (cm/100 g BW; Lee *et al.,* 2015).

[a-d] Values in the same row with different superscripts are significantly different at $P < 0.05$.

Table 3. Effects of dietary crude protein (CP) and *Macleaya cordata* alkaloid extract (MCAE) supplementation on plasma free amino acids (AA), creatinine, and uric acid in broiler chickens during a 35 d study.

Item	CP MCAE [1], mg/kg	100% of the recommended CP				95% of the recommended CP				*SEM* [2]	p value for MCAE				
												MCAE			
		0	180	360	540	0	180	360	540		CP	0, vs. others [3]	Lin. [4]	Quad. [4]	CP × MCAE
Plasma AA, µmol/L															
	Lys	338	263	317	290	234	219	200	268	17.4	0.01	0.11	0.98	0.11	0.06
	Met	77.0	79.5	79.2	82.5	80.5	78.5	77.5	86.7	3.87	0.68	0.59	0.20	0.30	0.85
	Thr	592	595	601	630	596	597	621	606	44.5	0.99	0.72	0.55	0.94	0.97
	Trp	76.7	82.2	86.2	87.2	80.7	85.2	83.0	82.5	3.20	0.92	0.05	0.09	0.34	0.53
	Gly	728	590	744	750	617	755	1039	679	78.6	0.43	0.40	0.39	0.32	0.32
Serum biochemical profile, mg/dL															
	Creatinine 15 d	0.27	0.26	0.29	0.24	0.29	0.24	0.28	0.36	0.01	0.19	0.96	0.30	0.28	0.06
	Uric acid 15 d	4.49	5.62	4.99	5.30	4.67	3.93	4.82	5.15	0.21	0.29	0.44	0.29	0.88	0.42
	Creatinine 35 d	0.29	0.32	0.30	0.29	0.30	0.29	0.30	0.29	0.01	0.59	0.71	0.64	0.26	0.43
	Uric acid 35 d	4.40^{a}	4.22^{a}	4.08^{ab}	3.34^{b}	4.09^{ab}	4.69^{a}	3.41^{b}	3.72^{b}	0.18	0.87	0.13	0.09	0.17	0.03

[1] *Macleaya cordata* alkaloid extract (MCAE).

[2] *SEM* = pooled standard error of the mean (n = 5 replicates/treatment).

[3] Contrast analysis performed to determine the difference between the MCAE 0 diet and all three MCAE diets (MCAE 0, vs. 180, 360, and 540 mg/kg of MCAE).

[4] Orthogonal contrast analyses were performed to assess the effect of MCAE inclusion on the plasma free amino acids coefficients. Comparisons were made between 0, 180, 360, and 540 mg/kg MCAE treatments; Lin.: linear, Quad.: quadratic.

[a-d] Values in the same row with different superscripts are significantly different at $P < 0.05$.

Table 4. Effects of dietary crude protein (CP) and *Macleaya cordata* alkaloid extract (MCAE) supplementation on apparent ileal digestibility coefficients of CP, OM, and ash in broiler chickens at 35 d of age.

Item	CP MCAE [1], mg/kg	100% of the recommended CP				95% of the recommended CP				*SEM* [2]	p value for MCAE				
		0	180	360	540	0	180	360	540		CP	MCAE			
												0, vs. others [3]	Lin.[4]	Quad.[4]	CP × MCAE
CP		0.717	0.771	0.723	0.820	0.737	0.786	0.773	0.752	0.84	0.77	0.01	0.05	0.67	0.05
OM [1]		0.631^{b}	0.723^{a}	0.643^{b}	0.760^{a}	0.718^{a}	0.747^{a}	0.748^{a}	0.728^{a}	1.69	0.01	0.01	0.03	0.71	0.01
Ash		0.432^{bcd}	0.394^{cd}	0.382^{d}	0.539^{a}	0.447^{bc}	0.419^{bcd}	0.467^{b}	0.433^{bcd}	1.97	0.76	0.92	0.05	0.01	0.01

[1] *Macleaya cordata* alkaloid extract (MCAE), OM = organic matter.

[2] *SEM* = pooled standard error of the mean (n = 5 replicates/treatment).

[3] Contrast analysis performed to determine the difference between the MCAE 0 diet and all three MCAE diets (MCAE 0, vs. 180, 360, and 540 mg/kg of MCAE).

[4] Orthogonal contrast analyses were performed to assess the effects of MCAE inclusion on the apparent ileal digestibility coefficients of CP, OM, and ash. Comparisons were made between the 0, 180, 360, and 540 mg/kg MCAE treatments; Lin.: linear, Quad.: quadratic.

[a-d] Values in the same row with different superscripts are significantly different at $P < 0.05$.

Discussion

Growth performance and carcass traits

In the current study and based on contrast analysis, MCAE supplementation increased BW, ADG, and ADFI. Our results are in general agreement with other studies in broiler chickens and other species that used various inclusion rates of MCAE in the diet (Moser *et al.,* 2003; Kozlowski *et al.,* 2008; Zdunczyk *et al.,* 2010; Karimi *et al.,* 2014; Goodarzi Boroojeni *et al.,* 2018;). However, the findings of the current study are in contrast to those of Vieira *et al.* (2008c) who reported an improved FCR in broiler chickens that received 50 and 25 ppm of MCAE from d 1 to 21 and 22 to 42, respectively. These seemingly contradictory results may be due in part to the composition of the experimental diets, age and sex of the birds, dosage of MCAE, and the alkaloid extraction method used, which can alter QBA and PA concentrations, as well as those of other bioactive compounds. Another possible mechanism through which MCAE might improve growth performance in chickens is through the upregulation of neurotransmitters. For example, a previous study showed that MCAE can upregulate serotonin synthesis and secretion and thus, improve FI in broiler chickens (Le Floch and Seve, 2007). Hence, the linear increase in ADFI that was seen with MCAE supplementation in the present study might reflect an enhanced production of serotonin in these birds. Furthermore, the improved growth performance due to MCAE supplementation that was observed in the current study may also, in part, be attributed to the anti-inflammatory properties of MCAE, since MCAE can inhibit the activation of NF-κB, a key regulator of inflammation (Wullaert *et al.,* 2011). Sanguinarine can also inhibit the lipopolysaccharide-induced nitric oxide response of macrophages *in vitro*, which might also limit immune system activation, thus improving growth performance (Niewold and De Backer, 2010). Thus, future studies that directly assess the activity level of the immune system are warranted, since we did not do so in the present study. Collectively, in the present study, supplementation of MCAE improved measures of FI and growth performance, as determined by contrast analysis. These beneficial effects of MCAE can be associated with improved nutrient digestibility and increased appetite and likely with reduced inflammation.

In the current study, dietary MCAE supplementation reduced the percentage of abdominal fat, but MCAE supplementation did not affect carcass yield, intestinal weight and intestinal length, a measure of absorptive surface area in broiler chickens (Wu *et al.,* 2013; Mabelebele *et al.,* 2014). Similarly, Khadem *et al.* (2014) reported that the percentage of abdominal fat in broiler chickens linearly decreased with increasing concentrations of MCAE in the drinking water, perhaps due to a suppressing effect of MCAE on the production of glucocorticoids (i.e., stress hormones) and increased lipid deposition in broiler chickens, especially in the abdominal cavity (Peng *et al.,* 2016). Findings of the current study are not, however, in agreement with the findings of others who reported no effects of dietary MCAE supplementation on the relative weights of abdominal fat in broiler chickens (Karimi *et al.,* 2014; Xue *et al.,* 2017; Sedghi *et al.,* 2018). Again, these differences may be due to differences in the basal diet composition, duration of the experimental periods, bird age, and the dosages of MCAE that were used in the different studies. The decreased ceca relative weight seen with MCAE supplementation may also be associated with a general improvement in intestinal and cecal health, as MCAE supplementation can improve the cecal environment by modulating the cecal microbiota population in favor of butyric acid-producing microbes (Zdunczyk *et al.,* 2010). The increased butyric acid concentration in the ceca of poultry is associated with reduced colonization of pathogenic *Salmonella* species (Van Immerseel *et al.,* 2005). Also, the decreased ceca relative weight in the present study can in part be attributed to increased digestibility of CP in the upper gut. Improved digestibility of CP in the upper part of the intestine can then lead to the decreased passage of unabsorbed peptides and AA into lower parts of the gut. The latter results in diminished production of bacterial ammonia, SCFAs, and branched-chain fatty acids in the ceca and colon (Juskiewicz *et al.,* 2005, 2013). Production of these microbial by-products is associated with the degradation and fermentation of undesirable nutrients in the lower part of the gut (Libao-Mercado *et al.,* 2006). Thus, MCAE supplementation in the current study may have suppressed cecal fermentation, which in turn, resulted in lower relative cecal weight due to less cellular hypertrophy and hyperplasia (Mateos *et al.,* 2012). In the current study, MCAE supplementation did not affect the carcass yield, which is in agreement with the findings of Karimi *et al.* (2014) and Kozlowski *et al.* (2008). Rather, carcass yield was improved by the dietary CP contents in the present study. The latter is consistent with the findings of other workers (Sabino *et al.,* 2004; Lima *et al.,* 2008). However, Zdunczyk *et al.* (2010) and Xue *et al.* (2017) reported that feeding MCAE at an inclusion rate lower than the rates that were used in the present study (30 and 150 mg/kg), resulted in improved carcass yield. Intestinal relative length is associated with the digestive capacity of the intestine, as a higher relative length of the intestine may reflect a higher surface area for absorption (Wijtten *et al.,* 2012). Lee *et al. (*2015) previously showed that feeding MCAE increases the relative length of the small intestine while reducing its relative weight. However, in the current study, feeding MCAE did not affect intestine relative length

and weight. This finding suggests that the improved productivity and nutrient digestibility observed in the present study was not due to an increased surface area for nutrient absorption. Further study is needed to clarify the effects of MCAE supplementation on the morphology of the intestinal mucosa and its relationship with nutrient digestibility and growth performance in broiler chickens. Taken together, dietary supplementation of MCAE reduced the abdominal fat contents and relative weight of the ceca without affecting the relative weight of the intestine and overall carcass yield. The latter suggests that the effect of MCAE on the relative weights of the ceca and abdominal fat is not quantitatively significant enough to affect the overall carcass yield of broiler chickens. Although MCAE supplementation linearly increased AID and ADFI, it did not affect plasma free AA concentrations, except Trp, at d 35 in the present study. The latter can in part be associated with higher levels of ADFI, and hence CP intake, in birds fed the LCP diet.

Plasma free amino acid concentrations and serum biochemistry

Blood creatinine is an indicator of increased skeletal muscle degradation and has been associated with reduced efficiency of AA utilization and protein accretion (Washington and Van Hoosier, 2012; Thongprayoon *et al.,* 2016). In the present study, serum creatinine concentration was affected by neither dietary protein nor MCAE supplementation at d 15 and 35, suggesting that protein degradation and the efficiency of AA utilization were not affected by dietary treatments. Measures of serum uric acid concentration reflect the nutritional and physiological state of birds. Changes in serum uric acid concentrations during the fed state are associated with the efficacy of protein and AA utilization (Corzo *et al.,* 2005; Donsbough *et al.,* 2010). In the current study, serum uric acid concentration was not affected by the dietary treatments, suggesting a lack of treatment effect on the efficacy of protein utilization. Surprisingly, the main effect of dietary protein did not change the serum uric acid concentration in the present study, which can likely be attributed to the higher ADFI in the LCP group. However, it is worth mentioning that MCAE supplementation tended to linearly increase plasma Trp concentration and decrease the serum uric acid concentration. Increased plasma Trp concentration in MCAE fed birds can likely be associated with the reduced intestinal decarboxylation of aromatic Trp through the inhibition of the aromatic L-amino acid decarboxylase by MCAE (Dršata *et al.,* 1996). Furthermore, lower uric acid concentrations in MCAE fed broilers potentially suggest that MCAE supplementation improved dietary CP and AA utilization, which in part explains the better growth performance in broiler chickens fed MCAE in the current study. In sum, the results of the current study do not provide solid evidence for the beneficial effects of MCAE on the post-absorptive utilization of proteins and AA.

Nutrient digestibility

The ultimate goal of the current study was to evaluate the effectiveness of dietary MCAE supplementation when CP intake was lower than recommended levels for optimum growth. In the current study, AID of CP and OM improved significantly when compared to the broiler chickens fed diets without MCAE. Several studies have suggested that MCAE might improve the activity of digestive enzymes and result in increased ileal digestibility of nutrients (Windisch *et al.,* 2008; Goodarzi Boroojeni *et al.,* 2018). One study demonstrated the inclusion of 25 to 50 mg/kg of sanguinarine in animal diets resulted in an increased secretion of pancreatic enzymes (Vienna *et al.,* 2007). Jankowski *et al.* (2009) also showed that broilers fed diets supplemented with MCAE had a lower pH in the proventriculus digesta and a significant increase in the activity of mucosal maltase. They also reported that the cecal short-chain fatty acid (SCFA) concentration significantly decreased in the sanguinarine group. A decrease in SCFA in the ceca is associated with an improved beneficial microbiota population and overall intestinal health in chickens (Walugembe *et al.,* 2015). Also, Zdunczyk *et al.* (2010) indicated that supplementation of broiler diets with 30 mg/kg of an alkaloid-containing preparation of MCAE can promote a beneficial cecal environment by reducing the activities of bacterial β-glucosidase and β-glucuronidase, as well as decreasing the pH of the digesta. Lee *et al.* (2015) indicated that dietary MCAE altered gut microbiota, specifically increasing cecal lactic acid bacteria when compared with chickens fed diets without MCAE. Therefore, in addition to altering the production and activity of digestive enzymes, alkaloid extracts from *Macleaya cordata* might influence gastrointestinal functions, particularly fermentation in the lower part of the gastrointestinal tract. Also, dietary alkaloids affect gastrointestinal motility (Wu *et al.,* 2002). For example, Panwar *et al.,* (2014) showed that alkaloids reduced the rate of gastric emptying and slowed the transit of a test substance through the small intestine in rats. Our results are also in agreement with some studies performed in other species in which supplementation of post-weaning piglet diets with a *Macleaya cordata* extract at 120 mg/kg improved their growth performance and apparent pre-cecal digestibility of nutrients (Goodarzi Boroojeni *et al.,* 2018). Taken together, our results suggest that MCAE can improve the AID of nutrients in both groups fed LCP and CON diets, likely by improving intestinal health and

digestive capacity. However, no similar trend was observed for the improving effects of MCAE on AID of CP in the LCP and CON groups.

Conclusion

In conclusion, the results of the present study showed that dietary supplementation with MCAE improves the growth performance of broiler chickens when dietary protein is moderately reduced. The current findings suggest that MCAE affects the growth performance of broilers, mainly by improving the ileal digestibility of dietary protein, as well as by increasing FI and possibly the post-absorptive utilization of AA. Although, it seems that trend of MCAE effects on ileal nutrients digestibility is specific for each LCP and CON groups. The present findings warrant further studies to evaluate the effect of MCAE supplementation on performance, measures of protein and amino acids utilization, and nitrogen excretion in broilers chickens fed dietary protein levels lower than that used in the current study.

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