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Original Research Article

Nutrient density of prestarter diets from 1 to 10 days of age affects intestinal morphometry, enzyme activity, serum indices and performance of broiler chickens

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ABSTRACT

A total of 480 day-old Cobb 500 broilers were used to investigate the effects of different levels of digestible amino acids (DAA; 100%, 107% and 114% of Cobb recommendations) and ME (3,000 or 2,900 kcal/kg) of prestarter diet on mixed sex broilers performance, enzyme activity, small intestine morphology, and serum metabolites. Broilers were randomly allotted to 6 treatments, where each treatment applied to 4 pens with 20 birds in each. The birds were subjected to their respective treatment diets from 1 to 10 days of age. This was followed by feeding common starter and finisher diets for the last 29 days. The enzyme activity of the pancreas was measured at 10 days of age. Morphometric indexes of jejunum were measured at 10 days of age and the end of the feeding period. Our results showed that the body weight (BW) increased as the DAA density of the prestarter diet increased from 100% to 114% over the first 10 days and the entire period of the study. Birds fed 114% DAA presented a better feed conversion ratio on day 10 ($P < 0.05$). At day 39, carcass weight and breast yield increased as the DAA levels increased from 100% to 114% ($P < 0.05$). The whole intestine length, small intestine length, and weights of the pancreas were lower in birds fed 100% DAA-diets at 10 days of age ($P < 0.05$). Increasing the dietary DAA and ME did not affect serum amylase, lipase, and protease concentrations and pancreatic amylase and lipase activity ($P > 0.05$); however, the activity of pancreatic protease increased as the DAA level increased from 100% to 114% ($P < 0.05$). The villus width and villus surface area (VSA) increased as the DAA level increased from 100% to 114% on day 10 ($P < 0.05$). At 10 days of age, crypt depth was the lowest in the birds fed plenty DAA prestarter diets ($P < 0.05$). It was found that dietary treatments at 39 days of age did not affect intestinal morphology. The results of the present work indicate that DAA level of 114% of Cobb recommendations and energy level of 2,900 kcal/kg diet may be recommended for starting broiler chicks.

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1. Introduction

The early life nutrition of broilers plays a crucial role in their productivity because of their muscle cell proliferation and development of digestive tract during this period (Vieira and Moran, 1999). The transition from late embryogenesis to the early post-hatch period is characterized by major morphological and physiological changes allowing the bird to immediately consume nutrients after hatching (Willemsen et al., 2010). This transition is fundamental as the bird shifts from metabolism based on the lipid-rich yolk to a solid carbohydrate and protein based diet at hatch. Diet

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is an important characteristic of poultry production, and different poultry species or lines have different requirements depending on genetic, age, environment, and the health status of the poultry. In the recent years, interest in early nutrition research has increased regarding the strong positive correlation exists between early live weight and body weight (BW) at the end of the production cycle (Tona et al., 2004; Willemsen et al., 2008). In this connection, an increase in the average early weight will minimize the number of small birds that, for any reason, do not eat. As a result, the overall lifetime performance of the chickens would be seriously affected by posthatch nutrition (Geyra et al., 2001b). The ratio of macronutrients has a major influence on posthatch performance and body composition of broiler chickens (Collin et al., 2003; Swennen et al., 2007) and under- and over-formulation of macronutrients will decrease this performance (Kidd et al., 2004). Therefore, the composition of prestarter diet (the content of crude protein, amino acids, energy, macro, and microelements) can influence the subsequent growth and development of broiler chickens. In general, diets with high ME or high energy: protein ratio induces energy deposition as fat. Increasing the CP content of the diet beyond requirements will result in leaner birds but with poorer efficiency, as the elimination of excess nitrogen is an energy consuming process (Swennen et al., 2007). Amino acid balance and the ideal protein concept (Emmert and Baker, 1997) can also affect broiler response to dietary energy. Amino acids in excess of that required for protein synthesis and other aspects of body metabolism are catabolized; which, as a result, incurs an energy cost and metabolic stress (Sklan and Plavnik, 2002). Wang et al. (2015) reported that high nutrient density diets (high amino acid and AME) might improve broiler performance without affecting their intestinal structure during 8 to 21 days. In another study performed by Ullah et al. (2012), the performance of chicks in 35 days of age was significantly higher in broilers fed 2,850 ME and 1.4% total lysine during the first 10 days posthatch than other treatments. Although the advantages of broiler performance of feeding increased amino acid densities have been well documented, a limited number of works has been reported on the effects of manipulation of nutrient densities in prestarter diets on the enzyme activity and intestinal morphology.

Thus, the aim of the present study is to find the appropriate levels of ME and digestible amino acids (DAA) in prestarter phase to the optimum production at the lowest cost and determine whether providing dietary DAA higher and ME lower than Cobb recommendations affects the performance, enzymes activity, intestinal morphology, and serum indices of broilers.

2. Material and methods

2.1. Experimental design

In this study, 480 day-old Cobb 500 broilers (mixed sex) were housed in cage pens (Battery) according to a completely randomized design in factorial arrangement, consisting of 2 levels of ME (2,900 and 3,000 kcal/kg) and 3 levels of DAA (100%, 107%, and 114% of Cobb recommendations) during 1 to 10 days of age. A fixed proportion of DAA relative to CP were maintained in graded increments of CP from 21.4% to 24.6%. Water and feed were offered *adlibitum*. Birds were fed one of 6 experimental diets from 1 to 10 days of age, the same starter diet from 11 to 21 days of age, and same finisher diet from 22 to 39 days of age. During the prestarter phase, broilers were divided into 6 treatments as follows: 1) 3,000 kcal/kg ME and 100% of DAA and CP (normal) of Cobb recommendations; 2) 3,000 kcal/kg ME and 107% of DAA and CP (high) of Cobb recommendations; 3) 3,000 kcal/kg ME and 114% of DAA and CP (plenty) of Cobb recommendations; 4) 2,900 kcal/kg ME and 100% of DAA and CP (normal) of Cobb recommendations; 5) 2,900 kcal/kg ME and 107% of DAA and CP (high) of Cobb recommendations; 6) 2,900 kcal/kg ME and 114% of DAA and CP (plenty) of Cobb recommendations. Before formulation of the experimental diets, ingredients were analyzed for nutrient concentration by NIRS DS 2500 FOSS. The analytical values obtained from these values are shown in Table 1. The formula and chemical composition of the dietary treatments are shown in Table 2.

2.2. Productive performance and serum indices

Body weight and feed consumption were obtained weekly followed by calculating body weight gain (BWG), daily feed intake (FI), and feed conversion ratio (FCR) using these data. On day 10, one chicken from each replicate of each treatment with BW close to the mean replicate was selected and then sacrificed by neck dislocation and collected blood samples. The blood samples were transferred into tubes and centrifuged at 3,521 $\times$ *g* at 4 °C for 4 min using HETTICH EBA 280S. The sera were removed and stored at −20 °C for further analysis. Immediately after slaughter, the pancreas was removed, weighed, and stored in liquid nitrogen for subsequent analyses. The length of intestine and small intestine was individually measured. Serum protease was determined using BS 3000P spectrophotometer and Nαbenzoyl-D,L-arginine p-nitroanilide (BAPNA) according to the procedure described by Mikhailova et al. (2014). Moreover, lipase and amylase in serum were determined with commercial kits (Human company, Germany), using the Chem Well 2900 analyzer (Awareness Technology, Inc., USA). Pancreatic protease activity was measured calorimetrically based on casein hydrolysis method as described by Batoev (1971), amylase activity was determined based on starch hydrolysis by modified Smith-Roy's method (Merina-Gluzkina, 1965). Pancreatic lipase was analyzed with commercial kits (Diakon company, Russia) using semiautomatic spectrophotometer BS 3000P.

On day 39, 4 birds per treatment (had BW close to the mean replicate) were selected for carcass traits evaluation. Birds were weighed, sacrificed by neck dislocation, bled, plucked, and eviscerated. Carcass yield was calculated as hot eviscerated carcass weight (without feet, head, and abdominal fat) relative to live body weight. Prime cuts yield included whole breast yield (with skin and bones), legs yield (thighs and drumsticks with bones and skin), and abdominal fat (fat located around the cloaca, cloacal bursa, gizzard, proventriculus, and adjacent abdominal muscles).

2.3. Tissue sampling and analysis of histological samples

At days 10 and 39, 4 birds per treatment were sacrificed by neck dislocation and their jejunum (midpoint from the pancreatic duct to Meckel's diverticulum) was excised. The jejunum was of particular interest because it is a major site of nutrient absorption in poultry (Horn et al., 2009) and intestinal mass. In a study, it was observed that villus height and area increased several folds in the jejunum and duodenum and less in the ileum to 10 days of age (Uni et al., 1999). Tissue samples (5 cm) were removed and flushed with

Table 1
Nutrient analysis of ingredients used in experimental diets.

Analysis/ingredient	Corn	Soybean meal	Wheat
AME, kcal/kg	3,350	2,386	3,075
Crude protein, %	7.73	47.71	12.14
Digestible Lys, %	0.20	2.66	0.28
Digestible Met + Cys, %	0.26	1.11	0.41
Digestible Thr, %	0.25	1.54	0.31

AME = apparent metabolizable energy.

Table 2
Composition of the diets (as-fed basis).

Item	Pre-starter						Starter	Finisher
	1	2	3	4	5	6		
Ingredients, %/treatment								
Corn	44.33	39.44	34.56	46.78	41.90	37.01	23.45	22.14
Sunflower oil	2.59	3.36	4.14	0.55	1.32	2.10	3.97	4.54
Soybean meal	32.89	37.01	41.12	32.49	36.60	40.71	29.80	23.90
Wheat	15.00	15.00	15.00	15.00	15.00	15.00	38.00	45.00
DL-methionine	0.31	0.35	0.39	0.31	0.35	0.39	0.31	0.28
Lysine-HCl	0.26	0.25	0.25	0.27	0.26	0.25	0.28	0.30
L-threonine	0.14	0.15	0.16	0.14	0.15	0.16	0.14	0.13
Choline chloride	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Limestone	2.39	2.37	2.34	2.39	2.37	2.34	2.17	1.98
NaCl (salt)	0.23	0.24	0.23	0.22	0.23	0.23	0.21	0.20
Vitamin premix[1]	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Mineral premix[2]	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Sodium sulfate	0.13	0.12	0.11	0.13	0.12	0.11	0.12	0.13
Ammonium phosphate	1.54	1.52	1.51	1.53	1.51	1.51	1.35	1.20
CelloLux[3]	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.02
Calculated analyses								
ME, kcal/kg	3,000	3,000	3,000	2,900	2,900	2,900	3,050	3,130
Crude protein, %	21.40	23.00	24.60	21.40	23.00	24.60	21.15	19.10
Digestible Lys, %	1.19	1.28	1.37	1.19	1.28	1.37	1.15	1.03
Digestible Met + Cys, %	0.88	0.95	1.02	0.88	0.95	1.02	0.87	0.80
Digestible Thr	0.80	0.86	0.92	0.80	0.86	0.92	0.77	0.69
Calcium, %	0.96	0.96	0.96	0.96	0.96	0.96	0.88	0.80
Available P, %	0.48	0.48	0.48	0.48	0.48	0.48	0.44	0.40

ME = metabolizable energy.
[1] Vitamin premix provided 1 kg of diet with: vitamin A, 10,800 IU; vitamin D_3, 2,160 IU; vitamin E, 15 IU; vitamin K_3, 1.0 mg; vitamin B_1, 4 mg; riboflavin, 5 mg; pantothenic acid, 10 mg; niacin, 25 mg; vitamin B_6, 8 mg; folic acid, 0.4 mg; vitamin B_{12}, 0.08 mg; biotin, 0.15 mg.
[2] Mineral premix provided 1 kg of diet with: I, 0.35 mg; Se, 0.15 mg; Zn, 40 mg; Cu, 8 mg; Fe, 80 mg; Mn, 100 mg.
[3] CelloLux contains enzyme complex of cellulose, glucanase and xylanase.

0.9% NaCl and then fixed in 10% neutral buffered formalin solution for morphometric analysis. The tissues were processed by dehydration through a series of graded alcohols, cleared with xylene. Paraffin-embedded tissue sections (5 μm) were prepared using an HM-325 universal automated microtome (Microm international GmbH, Germany). Slides were stained by the Hematoxylin–Eosin method, as described by Uni et al. (1995). Micrographs were taken with a Jenamed-2 light microscope (Carl Zeiss, Jena, Germany) using Image Scope C (Systems for Microscopy and Analysis LLC, Russia) to calculate the morphometric variables.

Morphometric parameters, including villus height from the tip of the villus to the crypt, midpoint villus width, crypt depth from the base of the villi to the base of the crypt, and V/C ratio (calculated by dividing villus height by crypt depth) were recorded in the next step. Villus surface area was calculated as follows (Sakamoto et al., 2000):

$$\text{Villus surface} = 2\pi \times (\text{VW}/2) \times \text{VH}$$

where $\pi = 3.14$, VW = villus width, and VH = villus height.

2.4. Statistical analysis

Data were analyzed in a 2 × 3 factorial arrangement of dietary treatments using analysis of variance and General Linear Model (GLM) procedure of SAS (SAS/STAT Version 9.2, SAS Institute Inc., Cary, NC) to determine the main effects of dietary ME, DAA and their interactions. If a significant effect was detected, the differences between treatments were separated using Duncan's multiple range test. Differences between mean values were considered significant at $P \leq 0.05$. Also, all percentage data were subjected to angular transformation to stabilize variances (arcsine square root percentage transformation) before statistical analysis.

3. Results

3.1. Performance parameters and carcass characteristics

Results showed that there was no significant effect of ME content of prestarter diet on productive performance parameters (Table 3). The BW increased as the DAA density of the prestarter diet increased from 100% to 114%, over the first 10 days ($P < 0.0001$) and the entire period of the study ($P < 0.01$). Interaction of DAA and ME was not significant for the body weight, feed consumption, and feed conversion ratio of the birds during the first 10 days of age and across the 39 days study ($P > 0.05$). When assessed for 39 days, birds fed plenty DAA prestarter diets exhibited the highest body weight, regardless of diet ME content. Feed consumption was not influenced by dietary treatments. Increasing DAA density of prestarter diet improved feed conversion ratio only in the prestarter period ($P < 0.0001$).

As shown in Table 4, carcass weight, breast muscle weight, and legs weight were significantly increased as the DAA density of the prestarter diet increased from 100% to 114% ($P < 0.003$). There was not observed a significant effect of ME or interactions between ME and DAA density of prestarter diets on carcass traits ($P > 0.05$). The main effects of ME, DAA, and their interactions were not significant for the relative weight of carcass, legs, wings and abdominal fat ($P > 0.05$).

3.2. Enzymes activity and serum metabolites

The results of the dietary treatments on the serum metabolites, pancreas weight, and enzymes activity are shown in Table 5. No significant difference ($P > 0.05$) existed among treatments as regard to serum amylase, lipase, and protease levels. Concerning pancreas weight, it was noticed a significant increase ($P < 0.01$) in plenty DAA group (1.16 g) compared to normal and high DAA groups (0.97 and

Table 3
Effect of dietary energy and DAA on the performance of broiler chicks ($n = 4$).

ME, kcal/kg	DAA, %	BWG, g				FI, g				FCR			
		1 to 10 d	11 to 21 d	22 to 39 d	1 to 39 d	1 to 10 d	11 to 21 d	22 to 39 d	1 to 39 d	1 to 10 d	1 to 21 d	22 to 39 d	1 to 39 d
3,000	100	150.06 ± 5.97	512.28 ± 30.89	1,313.55 ± 66.70	1,931.61 ± 102.34	237.60 ± 6.20	788.17 ± 51.40	2,477.99 ± 100.70	3,503.76 ± 152.72	1.58 ± 0.04^{a}	1.54 ± 0.09	1.89 ± 0.08	1.82 ± 0.08
3,000	107	164.57 ± 6.53	518.08 ± 17.27	1,335.89 ± 42.73	1,974.08 ± 49.70	246.64 ± 15.24	781.50 ± 28.50	2,522.87 ± 97.37	3,551.01 ± 100.60	1.50 ± 0.09^{a}	1.51 ± 0.06	1.89 ± 0.12	1.80 ± 0.09
3,000	114	191.72 ± 9.23	546.74 ± 12.02	1,397.77 ± 86.81	2,091.87 ± 102.83	240.49 ± 10.46	807.67 ± 42.64	2,590.05 ± 53.36	3,638.21 ± 71.42	1.26 ± 0.10^{c}	1.48 ± 0.11	1.86 ± 0.11	1.74 ± 0.11
2,900	100	164.46 ± 12.66	514.90 ± 10.76	1,346.00 ± 92.57	1,981.10 ± 105.75	253.39 ± 18.69	780.12 ± 49.92	2,496.66 ± 99.50	3,530.16 ± 161.43	1.54 ± 0.12^{a}	1.52 ± 0.09	1.86 ± 0.14	1.79 ± 0.13
2,900	107	173.01 ± 9.84	526.84 ± 26.04	1,341.65 ± 68.87	1,997.30 ± 90.18	249.28 ± 9.46	800.85 ± 45.54	2,476.50 ± 127.95	3,526.62 ± 140.96	1.45 ± 0.13^{ab}	1.52 ± 0.13	1.85 ± 0.13	1.77 ± 0.13
2,900	114	190.05 ± 9.71	556.06 ± 19.53	1,394.82 ± 47.70	2,096.18 ± 62.76	247.173 ± 8.50	813.68 ± 23.91	2,563.49 ± 132.62	3,624.34 ± 144.20	1.31 ± 0.06^{bc}	1.46 ± 0.06	1.84 ± 0.11	1.73 ± 0.10
SEM		4.46	10.35	35.00	44.14	5.89	20.83	52.57	66.23	0.05	0.05	0.06	0.05
Factorial analysis													
ME, kcal/kg													
3,000		168.78 ± 18.97	525.70 ± 25.07	1,349.07 ± 71.77	1,999.18 ± 106.89	241.57 ± 10.91	792.45 ± 39.65	2,530.30 ± 91.87	3,564.33 ± 117.87	1.45 ± 0.16	1.51 ± 0.08	1.88 ± 0.10	1.79 ± 0.09
2,900		175.84 ± 14.80	532.60 ± 25.47	1,360.82 ± 69.89	2,024.86 ± 95.73	249.94 ± 11.44	798.22 ± 40.12	2,512.22 ± 116.06	3,560.38 ± 142.94	1.43 ± 0.14	1.50 ± 0.09	1.85 ± 0.13	1.76 ± 0.11
P-value		0.07	0.42	0.69	0.49	0.10	0.74	0.68	0.94	0.69	0.79	0.57	0.62
DAA, %													
100		157.26 ± 11.97^{c}	513.59 ± 21.46^{b}	1,329.77 ± 76.68	1,956.35 ± 99.91^{b}	245.49 ± 15.39	784.14 ± 47.10	2,487.32 ± 93.21	3,516.96 ± 146.16	1.56 ± 0.08^{a}	1.53 ± 0.08	1.88 ± 0.10	1.80 ± 0.10
107		168.79 ± 8.03^{b}	522.46 ± 20.99^{b}	1,338.77 ± 513.15	1,985.69 ± 68.55^{b}	247.96 ± 11.83	791.17 ± 36.66	2,499.69 ± 108.13	3,538.81 ± 114.10	1.47 ± 0.11^{a}	1.52 ± 0.09	1.87 ± 0.12	1.79 ± 0.11
114		190.88 ± 8.81^{a}	551.40 ± 15.82^{a}	1,396.30 ± 64.87	2,094.02 ± 78.90^{a}	243.83 ± 10.12	810.68 ± 32.17	2,576.77 ± 94.66	3,631.28 ± 105.61	1.28 ± 0.08^{b}	1.47 ± 0.08	1.85 ± 0.12	1.74 ± 0.10
P-value		<0.0001	0.005	0.15	0.01	0.78	0.44	0.21	0.21	<0.0001	0.45	0.91	0.48

DAA = digestible amino acids; ME = metabolizable energy; FI = feed intake.
[a,b,c] Within a column, means with different superscripts differ significantly ($P < 0.05$).

Table 4
Effect of dietary energy and DAA on the carcass traits ($n = 4$).

ME, kcal/kg	DAA, %	Carcass parameter, g						Carcass parameter, %				
		Body weight	Carcass	Breast	Legs	Wings	Abdominal fat	Carcass	Breast	Legs	Wings	Abdominal fat
3,000	100	1,983.00 ± 75.29	1,353.00 ± 56.60	454.50 ± 37.57	374.25 ± 23.33	144.50 ± 11.59	29.50 ± 6.14	68.24 ± 1.88	33.55 ± 1.38	27.64 ± 0.62	10.68 ± 0.65	2.17 ± 0.37
3,000	107	2,033.00 ± 53.13	1,421.50 ± 42.53	509.00 ± 29.28	389.50 ± 27.92	142.25 ± 10.66	33.25 ± 6.18	69.93 ± 1.58	35.79 ± 1.16	27.40 ± 1.65	10.02 ± 0.89	2.34 ± 0.41
3,000	114	2,145.00 ± 68.63	1,501.50 ± 50.53	538.00 ± 18.83	410.25 ± 20.37	152.25 ± 7.72	33.25 ± 7.18	70.01 ± 1.67	35.83 ± 0.35	27.32 ± 0.93	10.14 ± 0.22	2.21 ± 0.43
2,900	100	2,044.00 ± 83.57	1,411.50 ± 75.43	495.75 ± 29.06	389.25 ± 13.45	147.25 ± 14.57	32.50 ± 7.68	69.03 ± 1.04	35.13 ± 1.27	27.61 ± 0.61	10.43 ± 0.73	2.31 ± 0.49
2,900	107	2,047.50 ± 82.44	1,393.75 ± 71.14	496.50 ± 25.36	376.75 ± 31.45	140.50 ± 13.96	31.75 ± 4.19	68.05 ± 0.87	35.66 ± 1.88	27.02 ± 1.51	10.07 ± 0.77	2.29 ± 0.22
2,900	114	2,151.25 ± 53.86	1,502.00 ± 38.40	532.75 ± 25.43	410.00 ± 27.65	149.75 ± 15.15	40.00 ± 6.73	69.82 ± 0.33	35.47 ± 1.19	27.28 ± 1.30	9.97 ± 0.85	2.66 ± 0.42
SEM		35.28	28.71	14.08	12.37	6.26	3.22	0.67	0.64	0.59	036	0.20
Factorial analysis												
ME, kcal/kg												
3,000		2,053.67 ± 92.76	1,425.33 ± 77.98	500.50 ± 44.97	391.33 ± 26.68	146.33 ± 10.19	32.00 ± 6.18	69.40 ± 1.77	35.06 ± 1.47	27.45 ± 1.05	10.28 ± 0.66	2.24 ± 0.37
2,900		2,080.92 ± 85.15	1,435.75 ± 76.06	508.33 ± 30.12	392.00 ± 27.07	145.83 ± 13.80	34.75 ± 6.96	68.97 ± 1.05	35.42 ± 1.36	27.30 ± 1.11	10.16 ± 0.74	2.42 ± 0.40
P-value		0.36	0.66	0.50	0.95	0.92	0.31	0.44	0.51	0.76	0.69	0.28
DAA, %												
100		2,013.50 ± 80.53^{b}	1,382.25 ± 69.21^{b}	475.13 ± 38.12^{b}	381.75 ± 19.37^{b}	145.88 ± 12.28	31.00 ± 6.63	68.64 ± 1.47	34.34 ± 1.49	27.62 ± 0.57	10.55 ± 0.65	2.24 ± 0.41
107		2,040.25 ± 64.67^{b}	1,407.63 ± 56.25^{b}	502.75 ± 26.22^{b}	383.13 ± 28.36^{b}	141.38 ± 11.54	32.50 ± 4.96	68.99 ± 1.55	35.72 ± 1.45	27.21 ± 1.47	10.04 ± 0.77	2.31 ± 0.30
114		2,148.13 ± 57.21^{a}	1,501.75 ± 41.55^{a}	535.38 ± 20.91^{a}	410.13 ± 22.48^{a}	151.00 ± 11.21	36.63 ± 7.39	69.91 ± 1.12	35.65 ± 0.84	27.30 ± 1.05	10.05 ± 0.58	2.44 ± 0.46
P-value		0.003	0.001	0.002	0.06	0.33	0.22	0.17	0.09	0.76	0.30	0.62

DAA = digestible amino acids; ME = metabolizable energy.
[a,b] Within a column, means with different superscripts differ significantly ($P < 0.05$).

Table 5
Effect of dietary energy and DAA on the enzyme activity ($n = 4$).

ME, kcal/kg	DAA, %	Pancreas weight, g	Pancreatic enzymes			Serum enzymes		
			Amylase, mg/g per min	Lipase, U/L	Protease, mg/g per min	Amylase, U/L	Lipase, U/L	Protease, U/L
3,000	100	0.92 ± 0.09	14,656.67 ± 2,311.32	48,091.00 ± 5,413.00	134.25 ± 9.74	1,270.27 ± 109.84	19.18 ± 1.42	146.40 ± 8.34
3,000	107	1.05 ± 0.14	13,933.33 ± 2,217.11	45,257.33 ± 2,617.50	148.75 ± 15.78	1,394.93 ± 191.26	19.11 ± 0.73	159.65 ± 12.92
3,000	114	1.14 ± 0.13	12,600.00 ± 2,078.46	48,694.00 ± 5,308.52	161.75 ± 9.50	1,354.43 ± 190.71	18.25 ± 2.22	170.40 ± 20.35
2,900	100	1.02 ± 0.11	13,933.33 ± 2,499.78	47,875.00 ± 4,624.91	144.75 ± 23.95	1,490.67 ± 304.06	18.63 ± 2.80	152.18 ± 2.17
2,900	107	0.99 ± 0.14	13,823.33 ± 2,086.73	47,920.50 ± 6,890.03	144.50 ± 11.36	1,347.00 ± 281.63	18.15 ± 0.82	152.80 ± 5.15
2,900	114	1.18 ± 0.08	14,085.00 ± 974.11	47,832.33 ± 3,975.58	157.50 ± 11.56	1,348.67 ± 222.66	19.28 ± 0.92	159.73 ± 47.62
SEM		0.06	1,043.43	2,491.65	7.28	112.98	0.75	11.08
Factorial analysis								
ME, kcal/kg								
3,000		1.04 ± 0.14	13,733.33 ± 2,184.98	47,347.44 ± 4,471.48	148.25 ± 16.00	1,339.88 ± 161.60	18.84 ± 1.49	158.82 ± 16.81
2,900		1.06 ± 0.13	13,947.22 ± 1,778.53	47,875.94 ± 4,805.47	148.92 ± 16.38	1,395.45 ± 255.56	18.69 ± 1.35	154.90 ± 25.29
P-value		0.63	0.80	0.80	0.91	0.55	0.80	0.67
DAA, %								
100		0.97 ± 0.11[b]	14,300.00 ± 2,263.02	47,983.00 ± 4,662.38	139.50 ± 17.83[b]	1,380.47 ± 242.22	18.90 ± 1.68	149.29 ± 6.43
107		1.02 ± 0.13[b]	13,878.33 ± 1,994.07	46,588.92 ± 5,030.71	146.63 ± 12.93[ab]	1,370.97 ± 224.33	18.63 ± 0.89	156.23 ± 9.82
114		1.16 ± 0.10[a]	13,342.50 ± 1,699.46	48,263.17 ± 4,366.13	159.63 ± 10.06[a]	1,351.55 ± 191.95	18.76 ± 1.66	165.07 ± 34.38
P-value		0.01	0.66	0.77	0.04	0.97	0.93	0.38

DAA = digestible amino acids; ME = metabolizable energy.
[a,b] Within a column, means with different superscripts differ significantly ($P < 0.05$).

1.02 g). In addition, no significant effect was observed for ME or the interactions between dietary ME and DAA content on the enzymes activities, serum metabolites, and pancreas weight. Increasing dietary DAA from 100% to 114% significantly increased ($P < 0.04$) pancreatic protease from 139.50 to 159.63 mg/g per min. Besides, pancreatic amylase (mg/g per min) and lipase (U/L) were not influenced by dietary treatments ($P > 0.05$).

3.3. Intestinal morphology

As shown in Table 6, the length of intestine was affected by DAA level of prestarter diets. As compared to normal prestarter diets, the plenty inclusion of DAA increased the total intestine length and small intestine length by 6.63 and 6.25 cm, respectively ($P < 0.05$). The main effect of ME and interaction between DAA and ME was not significant for the whole intestinal length and small intestinal length ($P > 0.05$). Our results showed that villus width and VSA at 10 days of age increased as the DAA level of the prestarter diet increased from 100% to 114% ($P < 0.05$). At 10 days of age, VSA was increased from 121.01 to 146.56 and 138.73 mm^2 as the DAA level increased from 100% to 107% and 114%, respectively. At 10 days of age, crypt depth was the lowest in birds fed plenty DAA diets ($P < 0.09$). Morphological examination showed that dietary ME, DAA, and their interactions did not affect jejunum villus height, width, VSA, V/C ratio at 39 days of age.

4. Discussion

Although we observed that final performance of broiler is influenced by DAA and protein concentration of prestarter diet, no effect of ME levels of the prestarter diet on performance parameters at 10 days of age and across the 39-day study was observed. This observation may in part be due to the fact that decreasing the dietary ME level of prestarter by 100 kcal/kg may not be enough reduction to see differences in growth performance. A previous study (Vieira et al., 2006) in broilers showed that the effect of the level of energy (from 2,870 to 3,100 kcal/kg) in prestarter period was not significant on the BWG of broilers at 7 and 42 days of age whereas 3,000 kcal/kg energy diet improved feed conversion ratio at 7 days of age and 3,100 kcal/kg energy diet significantly decreased feed consumption.

The BWG responses due to increased dietary DAA levels in the prestarter phase reported herein agreed well with findings of Noy and Sklan (2002), and Bahreiny et al. (2013). Noy and Sklan (2002) showed that feed conversion ratio did not change significantly with energy level (from 3,050 to 3,110 kcal/kg) of the diet but decreased with increasing protein level (from 18% to 28%) during the 7 days posthatch. Previous studies showed that increasing essential amino acids in a constant ratio to CP enhanced performance during the 7 days posthatch (Sklan and Noy, 2003). Hargis and Creger (1980) reported that adequate protein availability in the prestarter period seems to be essential to increase muscle development in later phases. The strong effect of DAA and protein on performance of broilers at 10 days of age in the current study can be explained by the high protein and amino acids requirements of newly hatched chicks to meet the needs for rapid growth and the effects of additional supplementation of DAA and protein on the better development of the gastrointestinal tract posthatch. It is well documented that BW enhances fourfold to fivefold during the first 10 days of age, during which considerable changes in gut weight and morphology are observed. The rapid growth of the intestine reaches a maximum between 6 and 10 days and declines thereafter (Sklan, 2001). Uni et al. (1996) reported that in broiler chicks the height and perimeter of villi in all segments of the small intestine increased by 34% to 100% between 4 and 10 days after hatching. The crypt depth and the number of enterocytes per longitudinal section of villi also increased with age. As shown in Table 6, plenty DAA and protein concentration of prestarter diets increased the intestinal length and VSA and decreased crypt depth. It is assumed that an increased villus height is paralleled by an increased digestive and absorptive function of the intestine due to increased absorptive surface area, expression of brushborder enzymes, and nutrient transport systems (Amat et al., 1996). Consequently, these changes might result in improvements in broiler performance. In addition, the higher growth rate of chickens on a plenty DAA and protein diets is most likely the consequence of their increased cumulative DAA and protein consumption. The lack of any consistent interaction between dietary concentration of ME and DAA on BWG, FI, and FCR in the current experiment suggests that the response in performance parameters to increasing concentrations of DAA and CP from 21.4% to 24.6% CP in prestarter period was independent of the dietary ME level over the range from 2,900 to 3,000 kcal/kg. A previous study found a positive correlation between BW of 7 to 10 days of age and final BW on 42 days of age (Vieira and Moran, 1999; Tona et al., 2004; Saki, 2005; Hooshmand, 2006). Li et al. (2007)

Table 6
Effect of dietary energy and DAA on the intestinal histomorphology ($n = 4$).

ME, kcal/kg	DAA, %	10 days						39 days					
		Intestinal length, cm	Small intestine length, cm	Villus height, μm	Villus width, μm	VSA, $\times 10^{-3}$ μm^2	Crypt depth, μm	V/C ratio, μm/μm	Villus height, μm	Villus width, μm	VSA, $\times 10^{-3}$ μm^2	Crypt depth, μm	V/C ratio, μm/μm
3,000	100	104.00 ± 2.83	99.50 ± 3.19	504.25 ± 32.68	77.83 ± 8.66	123.79 ± 21.55	196.00 ± 14.00	2.58 ± 0.09	810.75 ± 34.00	83.93 ± 14.21	213.50 ± 34.84	203.50 ± 14.57	4.00 ± 0.29
3,000	107	107.33 ± 3.30	103.17 ± 3.92	537.50 ± 35.58	86.33 ± 8.41	146.31 ± 24.12	209.75 ± 25.45	2.58 ± 0.30	839.00 ± 74.63	96.00 ± 11.61	251.84 ± 25.14	216.75 ± 14.91	3.87 ± 0.25
3,000	114	113.38 ± 2.87	108.25 ± 1.94	501.75 ± 20.14	84.77 ± 8.25	133.80 ± 17.31	185.50 ± 14.91	2.71 ± 0.12	874.75 ± 74.31	91.30 ± 11.70	249.70 ± 25.75	206.75 ± 25.63	4.27 ± 0.54
2,900	100	107.00 ± 5.72	102.25 ± 5.51	497.00 ± 17.44	74.37 ± 5.90	115.86 ± 5.75	194.00 ± 11.27	2.57 ± 0.14	868.00 ± 71.08	83.77 ± 12.66	226.80 ± 22.27	201.00 ± 24.58	4.35 ± 0.45
2,900	107	107.25 ± 6.08	103.13 ± 6.01	522.33 ± 23.63	88.90 ± 5.03	145.79 ± 10.12	205.00 ± 3.61	2.55 ± 0.11	864.50 ± 55.01	93.13 ± 17.67	251.01 ± 33.25	213.75 ± 26.30	4.07 ± 0.43
2,900	114	110.88 ± 4.70	106.00 ± 4.78	522.75 ± 26.08	87.43 ± 9.21	143.58 ± 17.28	185.75 ± 16.69	2.83 ± 0.18	822.25 ± 74.39	97.73 ± 16.83	249.72 ± 21.12	220.50 ± 30.77	3.77 ± 0.62
SEM		2.22	2.22	15.42	4.46	9.94	9.08	0.10	37.87	8.26	15.91	13.61	0.26
Factorial analysis													
ME, kcal/kg													
3,000		108.24 ± 4.83	103.64 ± 4.69	514.50 ± 31.36	82.98 ± 8.30	134.63 ± 20.78	197.08 ± 19.43	2.62 ± 0.18	841.50 ± 61.83	90.41 ± 12.09	238.35 ± 31.23	209.00 ± 17.57	4.05 ± 0.37
2,900		108.38 ± 5.33	103.79 ± 5.21	514.03 ± 23.42	83.57 ± 9.18	135.08 ± 17.81	194.92 ± 13.21	2.65 ± 0.19	851.58 ± 62.37	91.54 ± 15.06	242.51 ± 25.51	211.75 ± 25.18	4.06 ± 0.56
P-value		0.94	0.93	0.97	0.87	0.96	0.77	0.76	0.75	0.87	0.75	0.81	0.93
DAA, %													
100		105.50 ± 4.47^{b}	100.88 ± 4.42^{b}	500.63 ± 23.76	76.10 ± 6.91^{b}	119.82 ± 14.76^{b}	195.00 ± 11.42^{ab}	2.57 ± 0.11	839.38 ± 58.88	83.85 ± 12.04	220.15 ± 27.15	202.25 ± 18.12	4.17 ± 0.39
107		107.29 ± 4.53^{b}	103.15 ± 4.70^{ab}	530.75 ± 28.26	87.62 ± 6.36^{a}	146.05 ± 16.55^{a}	207.38 ± 16.46^{a}	2.57 ± 0.20	851.75 ± 60.14	94.57 ± 13.46	251.42 ± 26.37	215.25 ± 19.20	3.97 ± 0.33
114		112.13 ± 3.84^{a}	107.13 ± 3.58^{a}	512.25 ± 23.80	86.10 ± 7.96^{a}	138.69 ± 16.37^{ab}	185.63 ± 14.16^{b}	2.77 ± 0.15	848.50 ± 72.45	94.52 ± 13.43	249.71 ± 21.06	213.63 ± 26.42	4.02 ± 0.58
P-value		0.02	0.04	0.20	0.05	0.06	0.09	0.11	0.94	0.36	0.13	0.60	0.73

DAA = digestible amino acids; ME = metabolizable energy; VSA = villus surface area; V/C ratio = villus/crypt ratio.
[a,b] Within a column, means with different superscripts differ significantly ($P < 0.05$).

reported that nutritional deficiency during early posthatch development induced a permanent negative effect on BW at slaughter in broilers.

As shown in Table 4, different levels of DAA in the prestarter period have a significant effect on carcass traits. Total carcass, breast, and legs weight were significantly higher in birds fed the plenty DAA prestarter diets, regardless of diet ME content. Zaboli and Miri (2013) showed that prestarter diets with increased Lys densities resulted in increased breast meat and thigh yield. However, treatments' effect on carcass yield was not significant. The relative weight of carcass, legs, wings, and abdominal fat were not affected by dietary treatments. Thus, the improvement in carcass, breast muscle, and legs weight at the end of experiment could be due to the higher BW of birds fed plenty DAA and protein diets. However, Halevy et al. (2003) emphasized the effect of posthatch feeding on the dynamics of satellite cells during the prestarter phase, which leads to changes in the yield of meat cuts and in the composition of tissues of broilers at slaughter.

Digestion and absorption of nutrients early in life depend primarily on pancreatic enzyme activity (Nitsan et al., 1991). As shown in Table 5, plenty DAA and protein concentration of prestarter diets significantly increased protease activity. This finding was in agreement with Stringhini et al. (2009) wherein the authors studied the effect of protein and amino acid supplementation levels for broilers in the prestarter ration. The conclusion of the authors was that birds fed 20% CP and non-supplemented with amino acids showed higher amylase activities and lower trypsin activities than 22% CP supplemented with amino acids. Zhao et al. (2007) reported that the activities of amylase and protease in the jejunal fluid of ducks are mainly dependent on dietary protein content but not ME content. Dietary protein induces pancreatic proteases via secretion of cholecystokinin, which is a potent pancreatic protease inducer (Green et al., 1992). Moreover, dietary amino acids induce pancreatic protease activity by promoting translation, and transient activation of translation initiation via mammalian target of rapamycin (mTOR) pathway may be associated with this induction (Hashimoto and Hara, 2003). In our experiment, pancreatic lipase was not affected by different ME level. Differences in the effects of ME density on lipase activity observed by other authors (Maiorka et al., 2004) may have been caused by differences in the range of dietary ME used in the respective experiments.

In agreement with our findings, Swatson et al. (2002) and Abbasi et al. (2014) observed significantly heavier pancreas in broilers fed increased levels protein diet. Maiorka et al. (2004) reported that increasing the energy level of prestarter diet did not affect pancreas relative weight. The increase in the pancreas weight in chicks fed plenty DAA and protein diets might be attributed to their higher BW. Our statistical analysis showed a significant positive correlation between BW and weight of pancreas.

In accordance with the present study, Maiorka et al. (2004) stated that dietary energy level (2,900 and 3,200 kcal/kg) had not significant effects on intestine length at 7 days posthatch. A previous study in broilers showed that the increased intestinal length of broilers fed the higher level of dietary Lys might be due to increased nutrient absorption (Jackson and Diamond, 1996). The higher dietary levels of DAA and protein lead to increased nutrients in the small intestine. Such an increase can result in an enhanced protein synthesis in the small intestine and promote the full growth of small intestine, and subsequently, increased small intestinal length. The increase in intestinal length, in turn, can improve digestion by increasing the exposure of nutrients to brushborder hydrolytic enzymes as well as pancreatic and biliary secretions.

There is a lack of report linking the effects of amino acids and ME to the development of the gastrointestinal tract of poultry during the prestarter period. In the current trial, small intestine

lengths, villus width, and VSA increased in broilers fed high or plenty inclusion of DAA diets on day 10. A previous study showed that villus height increased as dietary threonine increased from 0.8% to 0.87% during the first 2 weeks posthatch. However, the threonine supplementation to 0.87% showed no significant effects on crypt depth and VSA (Moghaddam et al., 2011). Laudadio et al. (2012) evaluated the morphometric indices of duodenum, jejunum, and ileum of broilers at 49 days of age and the effect of protein level (from 18.5% to 22.5%) in diet from 14 days of age until slaughter age (49 days) and determined that the VSA of all intestinal segments did not change among groups. Instead, reducing the dietary protein level to 20.5% resulted in a higher villus height and V/C ratio in the duodenum and ileum. The first 2 weeks posthatch represent a period of rapid intestinal development in the broiler chickens (Geyra et al., 2001a). During this period, dramatic morphological changes occur, including increases in the number and proliferation rate of enterocytes, widening and lengthening of the villi, and deepening of crypts (Sklan and Noy, 2000; Moran, 2007). Amino acids maintain intestinal viability and mass, in addition to providing energy for normal intestinal function. As gastrointestinal tissues have relatively high protein turnover rate, high amino acid and protein diets provide nutrients for basal metabolism and cause a developed small intestine. In fact, wider villi in the birds that consumed the high and plenty DAA and protein diets as compared with birds fed the normal DAA and protein diets may increase total luminal villus absorptive area. Subsequently, a satisfactory digestive enzyme action and higher transport of nutrients at the villus surface is reached. The shallower crypt depth in chicks fed plenty DAA prestarter diets at 10 days of age may indicate that more mature enterocytes inhabited the villi of these birds, with less cell recruitment and hence less energy expenditure needed to maintain the absorptive function. More mature enterocytes surrounding the villi may imply that there is a greater enterocyte functionality and, hence, a greater absorptive surface. On the other hand, shorter crypt depths are indicative of a longer time needed for cell regeneration (Xu et al., 2003; Gao et al., 2008; Markovicva et al., 2009). This result, combined with less energy needed to maintain cells on the villi potentially, allows for more nutrients to be allocated to different regions of the body to support growth.

5. Conclusions

Data obtained in the current study demonstrated that increasing the dietary DAA and protein concentration in the prestarter diet by up to 114% of Cobb recommendations significantly improved final BW with a little change in feed intake. Also, increasing DAA density of prestarter diet increased the intestinal length, pancreas weight, villus width, VSA, and pancreatic protease while decreasing crypt depth in the prestarter period. Therefore, plenty DAA and protein levels in the prestarter period can be financially attractive due to the relatively low contribution of the prestarter diet to the total feed costs of a broiler up to market age. Moreover, decreasing the dietary ME level of prestarter by 100 kcal/kg did not negatively affect the BWG and FCR of the birds.

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