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Vitamin E Improves Morphology and Absorptive Surface of Small Intestine in Broiler Chickens Reared at High Altitude

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Abstract
Under high altitude conditions, the effects of vitamin E (α-tocopherol) on growth performance, intestinal morphology (villus size and type), and absorptive surface area of broiler chickens were evaluated. Chickens were fed diets supplemented with 0, 100, 200 or 400 IU/kg vitamin E for 42 days. On days 14, 28 and 42, birds were sacrificed and three segments of small intestine were dissected. The feed conversion ratio at day 42 significantly decreased when supplementing the diet with 400 IU/kg vitamin E ($P < 0.05$). The duodenal and jejunal villus height, width, and lamina propria at both 28 and 42 days significantly increased when supplementing the diet with all concentrations of vitamin E, but the ileal villus height was only higher with 400 IU/kg vitamin E compared to the control diet at 42 days ($P < 0.05$). The proportion of jejunal leaf + tongue-like villi increased while convoluted + ridge-like villi decreased with 200 and 400 IU/kg vitamin E supplementation at 42 days compared to the control ($P < 0.05$). The sum of measured villus surface area in three intestinal parts increased due to vitamin E supplementation at days 28 and 42 compared to control ($P < 0.05$). It is concluded that vitamin E (especially 400 IU/kg) had beneficial effects on feed efficiency, intestinal morphology and absorptive area.

Introduction

Vitamin E (α-tocopherol) is a powerful lipid-soluble antioxidant that scavenges lipid radicals and acts as a biologic antioxidant in the membranes of cells and sub-cellular organelles. It reacts with primary products of lipid peroxidation (fatty acid peroxyl radicals) and intercepts the chain reaction preventing further radical reactions (Carreras *et al.*, 2004; Rebolé *et al.*, 2006). During the antioxidant reaction, vitamin E appears as a stable radical. In this way, vitamin E prevents oxidative stress and cellular damage (Brenes et al., 2008; Delles *et al.*, 2014). In chickens, it has been shown that high levels of vitamin E reduces oxidative deterioration by protecting polyunsaturated fatty acids (Li *et al.*, 2009). Vitamin E also regulates gene expression via interaction with transcription factors (Azzi *et al., 2003;* Gohil *et al.*, 2003). For example, gene expression of cytosolic phospholipase A2, an important enzyme involved in phospholipid oxidation, is regulated by vitamin E (Azzi *et al.*, 2004). Vitamin E may be involved in the regulation of immune system by stabilizing fatty acids which act as immunoregulatory molecules, mediate cellular communication, membrane fluidity, and second messenger elaboration. Moreover, vitamin E modulates arachidonic acid metabolism which

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leads to the synthesis of prostaglandins and leukotrienes (Blumberg, 1994; Leshchinsky and Klasing, 2001).

Modern broiler chickens (Gallus gallus domesticus) are prone to pulmonary hypertension and ascites. This is probably due to extreme selection for either growth rate or feed conversion ratio, which puts high demands on metabolic processes and oxygen demand. It has been confirmed that high oxygen demand due to rapid growth in broiler chickens could not be fully compensated by changes in the cardio-pulmonary system. Therefore, modern broiler chickens face an even greater challenge when faced with environmental stresses such as high altitude. This situation results in tissue hypoxia which could increase generation of reactive oxygen species and ultimately damage heart, lungs, kidneys, and intestine of growing chickens (Balog, 2003; Solis de los Santos *et al.*, 2005). Cellular hypoxia causes membrane protein aggregation, protein degradation, and changes in molecular chaperones or growth factors. Hypoxic mitochondria produce excess superoxide that can activate signaling pathways or react vicinally with proteins and lipid membranes (Clanton, 2007).

In chickens, most of the production cost (70 to 80%) is related to feed (Murakami *et al*,. 2007). Therefore, the function of the digestive system and mucosal epithelial cells could considerably influence chicken performance (Murakami *et al.*, 2007). This experiment was done to evaluate the influence of three concentrations of vitamin E supplementation on the morphology of intestinal mucosa (villus sizes and types) and status of the intestinal absorptive area in broiler chickens reared at high altitude.

Materials and Methods

Birds, experimental facility, and treatments

180 one-day-old, fast-growing broiler chickens (Ross 308) were assigned to four treatments containing three replicates of 15 chicks each. Birds were housed in pens of identical size (2 × 1 m) in a deep litter system with wood shaving. Chickens were reared at an altitude of 2100 m above sea level (Shahrekord, Iran) under standard conditions for 6 weeks and had ad libitum access to water and feed. A corn-soybean meal basal diet was formulated for starter (1–14 days), grower (15–29 days), and finisher (30–42 days) periods (Table 1; NRC, 1994). The basal diet had 2822, 2950, 3050 Kcal/kg ME and 21, 19, 18% CP for starter, grower and finisher periods respectively, and did not have coccidiostat nor growth promoting additives. To prepare experimental treatments, vitamin E was added to the basal diet to create four concentrations: 0 (as control), 100, 200 and 400 IU/kg diet.

Table 1. Composition of basal diet

Feedstuff (g/kg)	Starter (1-14 d)	Grower (15-29 d)	Finisher (30-42 d)
Corn	556.9	592.4	647.6
Soybean meal (440 g/kg CP)	389.3	345.8	287.2
Soybean oil	10	22.1	27.3
Limestone	12.2	11.2	11.0
Dicalcium phosphate	19.3	16.9	15.8
Vitamin premix [1]	3.0	2.5	2.5
Mineral premix [2]	3.0	2.5	2.5
Salt	3.1	3.1	3.1
DL-Methionine	2.2	2.5	2.0
L-Lysine	1.0	1.0	1.0
Chemical composition			
ME (Kcal/kg)	2822	2950	3050
Crude protein (%)	21.5	20.0	18.0
Calcium (%)	1.0	0.9	0.85
Available P (%)	0.5	0.45	0.42
Sodium (%)	0.15	0.15	0.15
Methionine (%)	0.58	0.58	0.51
Lysine (%)	1.37	1.27	1.1
Methionine + Cystine (%)	0.93	0.91	0.81
Threonine (%)	0.92	0.85	0.77

[1] Supplied per kg diet: vitamin A, 9000 IU; cholecalciferal, 1500 IU; vitamin E, 18 IU; vitamin K, 2 mg; cobalamin, 0.015 mg; thiamin 1.8 mg; riboflavin, 6.6 mg; folic acid, 1mg; biotin, 0.1 mg; pantothenic acid 3 mg; niacin, 30 mg; pyridoxine, 3 mg; choline chloride, 500 mg.
[2] Supplied per kg diet: Mn, 100 mg; Cu, 10 mg; Zn, 85 mg; I, 1 mg; Se, 0.2mg; Fe, 50 mg.

Measurements

Body weight and feed consumption were measured on pen basis and feed conversion ratio was calculated and corrected for mortality. The percentage of mortality recorded during the entire experimental period was 1.7%.

Four birds from each pen were killed at 14, 28, and 42 days. Intestinal morphometrics and villus types were assessed in different parts of the small intestine according to procedures from Zamani Moghadam *et al*. (2009) and Hassanpour *et al*. (2013). Briefly, 2-cm segments of the midpoint of the duodenum, jejunum (between the bile duct entry and Meckel's diverticulum), and ileum (distal end) were dissected. After washing with phosphate buffered saline, the segments were fixed in Clark fixative for 45 min and then were maintained in ethyl alcohol. Each segment was divided into two sections. One section was stained with a periodic acid-Schiff (PAS) reagent for 2 min, then, using an ordinary dissecting microscope with magnifying power (object lens) of 10X, types and numbers of different villi in four randomly chosen fields were recorded. Another section was stained with PAS and muscle layers were removed from mucosa, rows of villi were cut, transferred to glass slides, and covered with a cover-slip. These samples were evaluated by a microscope with eyepiece graticules. The villus height was measured from the top of the villus to top of the lamina propria. Villus surface area was calculated using the formula = (π) × (VW) × (VL), where VW = villus width and VL = villus length. The lamina propria thickness was measured at the space between the base of the villus and top of the muscularis mucosa.

Statistical analysis

Data were analyzed using one-way ANOVA on SPSS software (SPSS, 1996). Mean treatments were compared with Tukey's post-hoc test. A *P*-value < 0.05 was considered statistically significant.

Results

Growth performance

Initial body weight was similar between treatments (data not shown). Vitamin E – at all concentrations measured – did not impact body weight and feed consumption of chickens at days 14, 28, and 42 ($P > 0.05$). However, the feedconversion ratio of chickens was significantly lower in the Vit E-400 group compared to the control group only at day 42 ($P < 0.05$) (Table 2).

Table 2. Effect of vitamin E on broiler growth performance at different growth periods

Item	BW (g)	FC (g)	FCR
Day 14			
Control	330	341	1.13
Vit E-100	331	342	1.15
Vit E-200	342	341	1.14
Vit E-400	343	340	1.12
SEM	8.35	9.96	0.30
P-value	0.09	0.18	0.11
Day 28			
Control	1112	1811	1.69
Vit E-100	1114	1731	1.63
Vit E-200	1131	1670	1.54
Vit E-400	1130	1652	1.51
SEM	25.21	17.22	0.31
P-value	0.12	0.10	0.09
Day 42			
Control	2081	4070	1.99[a]
Vit E-100	2090	3873	1.89[ab]
Vit E-200	2191	3931	1.82[ab]
Vit E-400	2200	3830	1.77[b]
SEM	24.01	27.91	0.16
P-value	0.08	0.07	0.04

[a,b]Means with different superscripts within the same column differ ($P < 0.05$).
Vit E, vitamin E in concentrations of 100, 200 and 400 IU/kg diet; BW: body weight; FC: feed consumption; FCR: feed conversion ratio.

Intestinal morphometric assessment

We found four different shapes of chicken intestinal villi: convoluted-like villus, leaf-like villus, tongue-like villus, and ridge-like villus (Figure 1).

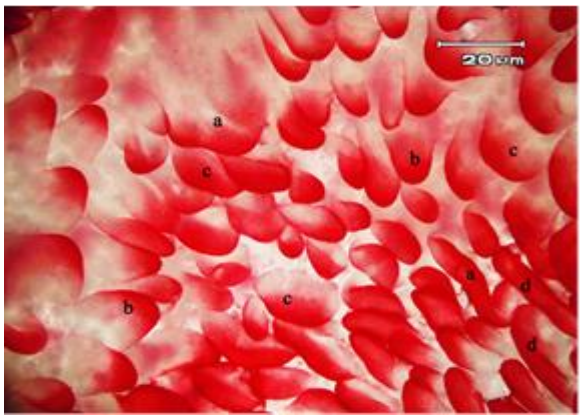

Figure 1. Different shapes of chicken intestinal villi: (a) convoluted-like villus; (b) leaf-like villus; (c) tongue-like villus; (d) ridge-like villus (Periodic acid-Schiff stain, 10X).

Duodenal villus height, width, and lamina propria were greater by day 42 in chickens fed

diets supplemented with all concentrations of vitamin E, but this was observed sooner (by day 28) only in chickens supplemented with the highest concentration, 400 IU/kg ($P < 0.05$, Table3). Duodenal surface area also increased by day 28 or 42 in chickens fed all concentrations of vitamin E, but this pattern was observed by day 14 with 400 IU/kg supplements ($P < 0.05$). The proportion of villus types in the duodenum was similar between experimental groups (Table 3).

Table 3. Effect of vitamin E on morphologic parameters in duodenum section of broiler chickens at different ages

Item	N	Height (mm)	Width (mm)	Laminae propria (mm)	Surface area (mm^2)	Ridge + convoluted (%) / leaf + tongue (%)
Day 14						
Control	12	0.91	0.65	0.39	1.84^{b}	21.6/78.4
Vit E-100	12	0.99	0.69	0.38	2.23^{ab}	19.7/80.3
Vit E-200	12	0.94	0.70	0.41	2.06^{ab}	19.3/80.3
Vit E-400	12	1.10	0.82	0.42	2.98^{a}	16.6/83.4
SEM	-	0.05	0.04	0.03	0.18	2.12
P-value	-	0.10	0.09	0.19	0.03	0.12
Day 28						
Control	12	1.28^{b}	0.86^{b}	0.37^{b}	3.47^{b}	25.4/74.6
Vit E-100	12	1.49^{ab}	1.06^{ab}	0.46^{ab}	4.99^{a}	24.7/75.3
Vit E-200	12	1.47^{ab}	1.04^{ab}	0.50^{ab}	4.87^{a}	25.8/74.2
Vit E-400	12	1.57^{a}	1.10^{a}	0.51^{a}	5.46^{a}	22.8/77.3
SEM	-	0.06	0.05	0.03	0.36	3.45
P-value	-	0.01	0.03	0.01	0.01	0.11
Day 42						
Control	12	0.93^{b}	0.92^{b}	0.38^{b}	2.71^{b}	18.7/81.3
Vit E-100	12	1.29^{a}	1.21^{a}	0.57^{a}	5.09^{a}	22.0/78.0
Vit E-200	12	1.35^{a}	1.16^{a}	0.57^{a}	5.13^{a}	24.7/75.3
Vit E-400	12	1.58^{a}	1.33^{a}	0.55^{a}	6.62^{a}	19.6/80.4
SEM	-	0.07	0.06	0.04	0.44	3.98
P-value	-	0.01	0.01	0.02	0.01	0.14

[a, b]Means with different superscripts within the same column differ ($P < 0.05$).
Vit E, vitamin E in concentrations of 100, 200 and 400 IU/kg diet; N, the total number of chickens, and 4 chickens/pen at each time.

Jejunal villus height was greater in chickens fed the diets supplemented with vitamin E compared to control at days 28 and 42 ($P < 0.05$, Table 4). Jejunal villus width and lamina propria also increased in these chickens by day 28, but this change was significantly higher than control values in chickens fed 400 IU/kg viamin E ($P < 0.05$). Nonetheless, jejunal surface area increased in all treatments compared to control at both 28 and 42 days of the experiment ($P < 0.05$). The proportion of jejunal leaf + tongue-like villi increased while convoluted + ridge-like villi decreased in chickens fed 200 and 400 IU/kg vitamin E supplement on day 42 compared to control ($P < 0.05$).

Ileal villus height was only higher in chickens fed 400 IU/kg vitamin E supplement than control on day 42 (Table 5). Ileal surface area was greater by day 28 in chickens fed 200 and 400 IU/kg vitamin E, but this was only sustained with 400 IU/kg to day 42 ($P < 0.05$). The proportion of the villus types, villus width and lamina propria in the ileum were similar among the experimental groups (Table 5).

The sum of measured villus surface area in the three intestinal parts was greater in chickens fed vitamin E supplement on days 28 and 42 compared to control ($P < 0.05$; Figure 2).

Discussion

Vitamin E as a dietary antioxidant is important in poultry nutrition (Özkan *et al.*, 2007) and maintains and enhances performance in chickens and turkeys (Panda and Cherian, 2014). In the present study, broiler chickens were fed one of three vitamin E-supplemented diets and their performance and intestinal morphology were evaluated. Previous studies have shown that vitamin E improves FCR in broiler chickens (Swain *et al.*, 2000; Brenes *et al.*, 2008; Panda *et al.*, 2009; Biswas *et al.*, 2012; Habibian *et al.*, 2015), possibly through its antioxidant effects on the digestive system and by improving intestinal nutrient absorption. On the other hand, other reports (Murakami *et al.*, 2007; Ghazi Harsini *et*

al., 2012) found that vitamin E had no positive effect on the performance characteristics of broilers. Conflicting data may be due to the time- and dose-dependency of vitamin E. We found the greatest beneficial feed efficiency in chickens only with 400 IU/kg of vitamin E on day 42 of the rearing period. It has been also noted that the effects of vitamin E are also influenced by genetic stocks, age of the poultry, assessment criteria, stress conditions, and management aspects (reviewed by Panda and Cherian, 2014).

Table 4. Effect of vitamin E on morphologic parameters in jejunum section of broiler chickens at different ages

Item	N	Height (mm)	Width (mm)	Laminae propria (mm)	Surface area (mm^2)	Ridge + convoluted (%) / leaf + tongue (%)
Day 14						
Control	12	0.67	0.67	0.34	1.38	17.4/82.6
Vit E-100	12	0.81	0.79	0.37	1.79	21.7/78.3
Vit E-200	12	0.74	0.75	0.39	1.46	20.4/79.6
Vit E-400	12	0.68	0.67	0.43	1.44	24.8/75.2
SEM	-	0.05	0.06	0.02	0.15	2.12
P-value	-	0.19	0.09	0.17	0.11	0.18
Day 28						
Control	12	0.81^{b}	0.67^{c}	0.30^{b}	1.71^{d}	23.3/76.7
Vit E-100	12	1.01^{a}	0.86^{b}	0.40^{a}	2.70^{c}	21.7/78.3
Vit E-200	12	1.03^{a}	0.98^{ab}	0.46^{a}	3.15^{b}	24.8/75.2
Vit E-400	12	1.12^{a}	1.07^{a}	0.42^{a}	3.73^{a}	26.3/73.7
SEM	-	0.04	0.03	0.02	0.14	1.23
P-value	-	0.02	0.01	0.02	0.01	0.16
Day 42						
Control	12	0.92^{c}	1.09^{b}	0.37^{b}	3.20^{d}	$26.6/73.4^{a}$
Vit E-100	12	1.19^{b}	1.22^{b}	0.46^{ab}	4.26^{c}	$24.6/75.4^{a}$
Vit E-200	12	1.40^{a}	1.15^{b}	0.48^{ab}	5.13^{b}	$17.4/82.6^{b}$
Vit E-400	12	1.34^{a}	1.48^{a}	0.56^{a}	6.29^{a}	$18.0/82.0^{b}$
SEM	-	0.05	0.05	0.02	0.23	1.98
P-value	-	0.01	0.04	0.02	0.02	0.04

[a,b]Means with different superscripts within the same column differ ($P < 0.05$).
Vit E, vitamin E in concentrations of 100, 200 and 400 IU/kg diet; N, the total number of chickens, and 4 chickens/pen at each time.

In this experiment, oral supplementation of vitamin E had positive effects on intestinal parameters such as villus height, width, lamina propria thickness, and surface area. These effects were predominant in the duodenum and jejunum with a high concentration of vitamin E (400 IU/kg) after 28 days of life. Dietary antioxidants protect enterocytes from pro-apoptotic oxidant stress and improve their growth and development (Miller *et al.,* 2001; Ahmadipour *et al.,* 2015). It has been also reported that vitamins E and C supplementation improve development of the intestinal mucosa in broiler chickens: intestinal crypt cell proliferation and villus height/width and surface area increase (Murakami *et al.,* 2007; Zamani Moghaddam *et al.,* 2009).

The small intestine has a critical function in digestion and nutrient absorption. The structure and the function of the intestinal mucosa, which has the high turnover rate, depend on the balance between proliferation and apoptosis of enterocytes (Macari, 1998; Miller *et al.,* 2001).

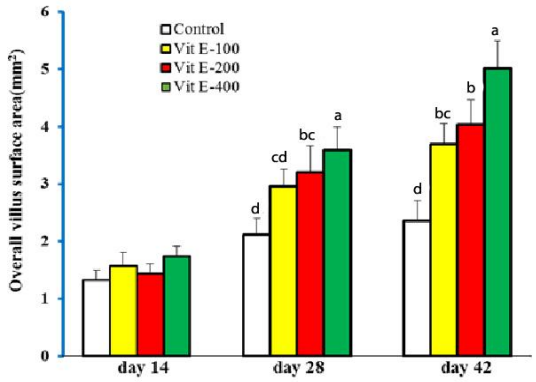

Figure 2. The sum of measured villus surface area in three intestinal parts in broilers supplemented vitamin E with 100, 200 and 400 IU/kg diet and control at different times.
All data are presented as means (total of 12 chickens/ treatment at each time period).
[a-d]Means with different indices within same times differ ($P < 0.05$).

Table 5. Effect of vitamin E on morphologic parameters in ileal section of broiler chickens at different ages

Item	N	Height (mm)	Width (mm)	Laminae propria (mm)	Surface area (mm^2)	Ridge + convoluted (%) / leaf + tongue (%)
Day 14						
Control	12	0.48	0.49	0.24	0.75	30.1/69.9
Vit E-100	12	0.47	0.47	0.24	0.70	23.0/77.0
Vit E-200	12	0.53	0.45	0.23	0.77	25.4/74.6
Vit E-400	12	0.49	0.51	0.26	0.80	24.8/75.2
SEM	-	0.03	0.06	0.01	0.09	2.11
P-value	-	0.21	0.11	0.07	0.14	0.12
Day 28						
Control	12	0.56	0.67	0.24	1.19^{b}	21.5/78.5
Vit E-100	12	0.51	0.76	0.27	1.18^{b}	25.9/74.1
Vit E-200	12	0.61	0.86	0.27	1.68^{a}	20.0/80.0
Vit E-400	12	0.64	0.80	0.30	1.63^{a}	22.5/77.5
SEM	-	0.04	0.06	0.02	0.10	1.23
P-value	-	0.07	0.09	0.17	0.04	0.19
Day 42						
Control	12	0.53^{b}	0.70	0.47	1.17^{b}	16.9/83.1
Vit E-100	12	0.66^{b}	0.83	0.61	1.73^{a}	19.8/80.2
Vit E-200	12	0.67^{b}	0.90	0.54	1.86^{a}	15.3/84.7
Vit E-400	12	0.83^{a}	0.84	0.56	2.11^{a}	17.9/82.1
SEM	-	0.03	0.05	0.06	0.12	1.90
P-value	-	0.03	0.08	0.15	0.01	0.18

[a,b]Means with different superscripts within the same column differ ($P < 0.05$).
Vit E, vitamin E in concentrations of 100, 200 and 400 IU/kg diet; N, the total number of chickens, and 4 chickens/pen at each time.

The increased villus height/width observed in the present study could be due to the antioxidant effect of vitamin E which may delay apoptosis and increase enterocyte viability (Miller *et al.,* 2001). As the present study was performed at high altitude (2100 m), broiler chickens were exposed to hypoxia. Since the gastrointestinal tract has a high oxygen demand, hypoxia inhibits gut development and reduces overall gut architecture in commercial broilers (Solis de los Santos *et al*., 2005). Vitamin E improved villus dimensions and increased surface area which could be evidence of improved nutrient absorption in the intestine. Vitamin E also ameliorated lamina propria thickness in duodenum and jejunum which show developed Lieberkühn's glands in these segments of intestine. Thus, vitamin E may influence intestinal secretions. Further studies are needed to clarify this effect of vitamin E in broiler chickens. Our data indicated that villus shapes changed from convoluted and ridge to leaf and tongue in the jejunum. Many studies suggested that these changes may have positive effects on intestinal function (Teshfam *et al.* 2006; Hassanpour *et al.* 2013).

We conclude that oral supplementation of vitamin E (especially 400 IU/kg) has a beneficial effect on growth performance and improves gut morphology in growing broiler chickens under hypoxic conditions.

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ویتامین E ریخت شناسی و سطح جذبی روده‌ی باریک در جوجه‌های گوشتی پرورش یافته در ارتفاع بالا را اصلاح می‌کند

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چکیده

تحت شرایط ارتفاع بالا، اثرات ویتامین E (α- توکوفرول) بر بازده رشد، ریخت‌شناسی روده (اندازه و نوع پرز) و سطح جذبی جوجه‌های گوشتی ارزیابی شدند. جوجه‌ها با جیره‌های حاوی صفر، ۱۰۰، ۲۰۰ و ۴۰۰ واحد در کیلوگرم مکمل ویتامین E به‌مدت ۴۲ روز تغذیه شدند. در روزهای ۱۴، ۲۸ و ۴۲ پرندگان ذبح شده و سه قسمت از روده باریک جدا گردید. ضریب تبدیل غذایی به‌طور معنی‌داری ($P < ۰/۰۵$) با جیره حاوی ۴۰۰ واحد در کیلوگرم مکمل ویتامین E کاهش یافت. ارتفاع، عرض و آستر مخاطی پرزهای دوازده و تهی روده با مکمل کردن تمامی سطوح ویتامین E به‌طور معنی‌داری در روزهای ۲۸ و ۴۲ افزایش یافت، اما ارتفاع پرزهای ایلئوم فقط با ۴۰۰ واحد در کیلوگرم ویتامین E در روز ۴۲ بیشتر از شاهد بود ($P < ۰/۰۵$). مکمل کردن جیره غذایی با ۲۰۰ و ۴۰۰ واحد در کیلوگرم ویتامین E، میزان نسبی پرزهای زبانی+ برگی شکل نسبت به شاهد افزایش و پرزهای رشته ای+ مارپیچی شکل در روز ۴۲ کاهش یافت ($P < ۰/۰۵$). در اثر ویتامین E، مجموع ناحیه سطحی پرزهای اندازه‌گیری شده در سه قسمت روده در روزهای ۲۸ و ۴۲ نسبت به شاهد افزایش یافت ($P < ۰/۰۵$). نتیجه آنکه، ویتامین E (بویژه ۴۰۰ واحد در کیلوگرم) اثرات مفیدی بر بازده غذائی، ریخت شناسی و سطح جذبی روده دارد.

کلمات کلیدی
ویتامین E
توکوفرول
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