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Effect of hen diet supplemented with 25-OH-D_3 on the development of small intestinal morphology of chick

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ABSTRACT

The effect of hen dietary 25-OH-D_3 (added 3000 IU to control diet with 1000 IU) on the morphological changes of the small intestine of Ovambo chicks during prehatch and posthatch period was investigated. After 2 week feeding hens with the experimental diets, eggs were collected and incubated. Results show that the weight, length, thickness of small intestine and the height, perimeter and surface area of villi in small intestine increased with age, and they increased faster in jejunum and duodenum ($P<0.05$) than in ileum. Comparison with the control diet, 25-OH-D_3 supplement in maternal diet caused the lower weight and shorter length of the small intestine of offspring after hatch ($P<0.05$). Meanwhile, 25-OH-D_3 of maternal diet induced the increase of the villi height and the crypt depth in the three segments after hatch, in particular in the jejunum and duodenum ($P<0.05$). We suggest that dietary 25-OH-D_3 can be used as a feed additive in maternal diet to stimulate morphological maturation and in consequence intestinal function in chicks during later incubation and the first week of life.

KEY WORDS: 25-OH-D_3, small intestine, morphology, breeder hen, chick

INTRODUCTION

The growth of the chick is due to the function of small intestine, which depends

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on the maturity of intestine during final incubation and first week of posthatch. Morphological studies point out that at the moment of hatching, the weight of the small intestine presents 1.2 to 2.6% of the body weight of the bird and 6.2 to 6.6% at the maximum development. The peak of the small intestine development is found to be between d 5 and 7 posthatch (Sell, 1996).

Research has suggested that different substrates can accelerate intestinal development soon after hatching. It is well known that calcitriol plays an important role in the intestine, where it promotes Ca^{2+} resorption *via* vitamin D_3 receptor-mediated genomic mechanisms. Vitamin D_3 may have a role in regulating the morphological and functional development of intestinal villus mucosa (Shinki et al., 1991). It is absorbed through the small intestine and transported in the blood to the liver where it is converted into 25-hydroxycholecalciferol (25-OH-D_3), the major circulating form of vitamin D_3. 25-OH-D_3 is then transported to the kidneys where it is converted into lα,25-dihydroxycholecalciferol, which is the most biologically active, hormonal metabolite of the vitamin (McDonald and Edwards, 1995). 25-OH-D_3 was generally recognized as safe source of vitamin in poultry (Ward, 1995). Compared with corresponding levels of vitamin D_3, under certain conditions, it improves body weight gain, feed efficiency, bone ash and breast meat yield (Yarger et al., 1995a). Moreover, Soto-Solanova and Hernandez (2004) demonstrated that replacing vitamin D_3 partly with 25-OH-D_3 improved laying performance, decreased the number of broken eggs and increased egg weight. A single injection of 1α,25-$(OH)_2D_3$ into chicks deficient in vitamin D_3 produced a marked increase in putrescine accumulation from ornithine and spermidine in the duodenum (Shinki et al., 1985). Tabor (1984) reported that polyamines (putrescine, spermidine and spermine) play an essential role in cell proliferation and differentiation in the intestine.

Materials passed by hens to their offspring are either used during embryonic growth and development or stored in the absorbed yolk sac for after hatching use (Vleck and Vleck, 1996). Recent research into maternal nutrients affecting the development of embryo and poult has refocused on the role of vitamin, fatty acid and microelements (An et al., 2010). Amounts, but also forms, of nutrients deposited in the egg determine success of intestine development and hatching of a healthy chick.

The transfer of nutrients from a hen to the egg follows two pathways: *via* the ovary to the yolk or *via* the oviduct to the albumen, eggshell and membrane. Inside the egg, the embryo develops specific mechanisms to mobilize previously stored vitamins and minerals by means of transport proteins (Lillie et al., 1951). Vitamin D is preferably deposited in the yolk in the form of vitamin D_3 as compared to D_2. The efficiency of vitamin D_3 deposition in the yolk is around 2.2 times higher than D_2 (Mattila et al., 2004).

The effect of vitamin D_3 on the intestinal morphology of adult fowls have been reported (Shinki et al., 1991; Chou et al., 2009), however, the references to the development of the small intestinal morphology of poult or chick from maternal fed with 25-OH-D_3, are scarce.

The aim of this study was to investigate the effect of fed diet supplemented with 25-OH-D_3 on the morphological changes of the duodenum, jejunum and ileum of chicks during prehatch and posthatch period.

MATERIAL AND METHODS

Birds management and treatments

A total of 60 Ovambo (South African laying breed) laying breeders (45 week old) were reared in 6 open air pens (6 m × 3 m, 1.8 m^2/hen) of 10 birds each, with a ratio of 1 male to 10 females. There were 3 replicates of 2 diet treatments. One group (D3g) was fed diet supplemented with 4000 IU 25-OH-D_3 while the control group (Cg) with the same diet containing 1000 IU 25-OH-D_3. The composition of diets was listed in Table 1. Feed and water were provided *ad libitum*. Eggs from both groups, after feeding with the experimental diets for 2 weeks, were collected and incubated. The experiment was lasted six weeks (from 45 to 51 weeks). Thirty samples per dietary group (2 samples per replicate) were taken from embryo on 15 day (15E), 19 day (19E), at hatch (0), and from chicks on 2 day (2d) and 7 day of life (7d).

Table 1. Composition of experimental diets

Ingredients, %	D3g	Cg	Composition, analysed except for DE	D3g	Cg
Maize	62.6	62.6	DE, MJ/kg	12.3	12.3
Soyabean meal	25	25	Crude protein, %	12.8	13.0
Dry yeast	3.5	3.5	Lipid, %	3.2	3.1
Soyabean oil	0.5	0.5	Ash, %	19.8	19.7
Dicalcium phosphate	1.8	1.8	Fibre, %	2.5	2.4
Limestone	0.7	0.7			
Salt	2.5	2.5			
DL-methionine	3	3			
Mineral-vitamin premix1a	0.4	0.4			
25-OH-D_3	3000 IU	-			

Supplied per kg of diets: IU: vit. A 5000; vit. D3 1000; mg: Fe 72; Cu 7; Zn 72; Mn 90; I 0.9; Se 0.1; vit. E 50; vit. K3 1.4; vit. B1 1.8; vit. B2 8; vit B6 4.1; vit. B12 0.01; nicotinic 32; calcium pantothenate 11; folic acid 1.08; biotin 0.18

Chemical analysis

Digestible energy (DE), crude protein (CP), fibre, lipid and ash contents of feed and digesta were measured according to AOAC (1990).

Sample processing

For each embryo and chick, samples of approximately 2 cm were cut from each segment of the proximal duodenum, the proximal jejunum and the middle ileum. The samples were gently flushed with phosphate buffer 0.1 M PH 7.1 (PB) to washout the intestinal contents and fixed in PB with 4% formaldehyde. After one day fixing, each sample was divided into 2 parts, routinely dehydrated in alcohol (70, 80, 95 and 100%), and embedded in resin (JB-4, Polyscience). Sections of 4 µm were cut with a microtome (Reichert-Jung. Mod. 1140/Autocut) and collected onto gelatin coated slides. For morphologic measurements, sections were stained with haematoxylin and eosin according to Mayer (Luna, 1968). The sections were examined by a light microscope (Leitz, Diaplan) connected to a PC *via* a Nikon digital system (Digital Sight DS-U1). Images were acquired using the NIS-Elements F version 2.10 software.

Staining procedure

Parameters of morphometry were done by staining 4-µm sections with haematoxylin and eosin according to the following protocol. Slides were briefly washed in distilled water, stained in Mayer haematoxylin solution for 5-10 min, washed in running tap water for 10 min, stained in eosin solution (1% in distilled water) for 30 sec to 1 min, washed in distilled water, dehydrated through 95% alcohol and twice in absolute alcohol 5 min each, cleared in 2 changes of xylene 5 min each and mounted with xylene based mounting medium.

Measurements and analysis

The intestinal weight, length, villi height, villi width, villi area, crypt depth, crypt width and crypt number in each intestinal segment of these birds were estimated and measured using a light microscope (Leitz, Diaplan).

Measurements were taken using ImageJ 1.37V software. Ten well-oriented and intact crypt-villi units of each slide were measured in triplicate. The villi height was defined as the distance from villi tip to the crypt junction. The villi width was measured from the outside epithelial edge to the outside of the opposite epithelial fringe at the half-height of the villi. The perimeter of the villi was measured all the edge of villi. Villi surface area was calculated from villi height and width at the

half-height. The crypt depth was defined as the depth of the invagination between adjacent villi. The muscle thickness was measured from the junction between the submucosal and muscular layers to that between the muscular layer and the tunica serosa.

Mean values generated from all individual measurements were statistically analysed by a one factor variance analysis using the GLM procedure (SAS, 1999). Once main effects were significant with $P<0.05$, means were compared by Duncan's multiple range test using a significance level of $P<0.05$.

RESULTS

The effect of 25-OH-D_3, added to the diet of hens, on the egg weight, production, feed conversion rate (FCR) and hatchability were shown in Table 2. The average egg weight and FCR were not affected by 25-OH-D_3. However, the addition of 25-OH-D_3 resulted in higher production and hatchability ($P<0.05$).

Table 2. Egg weight, production, feed conversion ratio and hatchability

	Egg weight, g	Production, %	FCR	Hatchability, %
D3g	65.81 ± 8.34^{A}	81.76 ± 8.76^{A}	3.13 ± 0.08^{A}	89.34 ± 9.32^{A}
Cg	66.24 ± 9.52^{A}	83.95 ± 9.12^{B}	3.06 ± 0.12^{A}	91.65 ± 8.28^{B}

means within columns with different superscript capital letters are different ($P<0.05$)
FCR - feed conversion ratio

Body weight, intestinal weight and intestinal length of embryos and chicks from the D3g and the Cg were presented in the Table 3. Body weight decreased from E15 to hatch in both groups ($P<0.05$), however, in D3g was higher 7.1 g than that in the Cg at hatch ($P<0.05$). Body weight increased with age after posthatch in both groups ($P<0.05$). After hatch there were not differences in the body weight between the two groups. The small intestine grew persistently in both groups from E15 to 7 day posthatch. The intestinal weight increased 25 times in D3g and 30 times in Cg from 15 day embryo to 7 day of life. Although

Table 3. Body weight, intestinal weight and intestinal length

Age	Body weight, g		Intestinal weight, g		Intestinal length, cm	
	D3g	Cg	D3g	Cg	D3g	Cg
E15	48.48 ± 2.5^{Ba}	51.5 ± 3.5^{Ba}	0.21 ± 0.04^{Da}	0.22 ± 0.01^{Da}	15.16 ± 2.41^{Da}	14.8 ± 1.91^{Da}
E19	45.4 ± 3.0^{Ba}	42.1 ± 2.4^{Ca}	0.67 ± 0.12^{Ca}	0.51 ± 0.13^{Ca}	20.03 ± 3.91^{Da}	20 ± 4.97^{Da}
0	42.4 ± 3.7^{Ba}	35.3 ± 1.2^{Db}	1.58 ± 0.05^{Ba}	1.68 ± 0.23^{Ba}	29.03 ± 0.25^{Ca}	32.53 ± 1.34^{Ca}
2d	43.4 ± 2.0^{Ba}	43.3 ± 2.4^{Ca}	2.08 ± 0.04^{Ba}	2.27 ± 0.44^{Ba}	40.81 ± 3.26^{Ba}	45.8 ± 2.69^{Ba}
7d	59.3 ± 1.1^{Aa}	60.4 ± 9.8^{Aa}	5.47 ± 0.31^{Ab}	6.92 ± 1.99^{Aa}	57.53 ± 3.54^{Aa}	62.46 ± 3.85^{Aa}

means within columns with different superscript capital letters are different ($P<0.05$). Means within rows (same section) with different superscript lower case letters are different ($P<0.05$)

there were not significant differences between the two groups from E15 to 2 day posthatch, the intestinal weight in the D3g was lower 1.45 mg than that in the Cg at 7 day posthatch ($P<0.05$). Moreover, there was a tendency of lower weight of the D3g intestine after hatch. The intestinal length increased constantly from E15 to 7 d in the two groups. Although there was no difference between both groups, we observed that the intestinal length in the D3g tended to be shorter.

The weight of the duodenum, jejunum and ileum increased continuously from E15 to 7d in both groups (Table 4). In the two groups, the weight of jejunum increased more rapidly than both duodenum and ileum. Although there were not significant differences in the weight of the three intestinal sections between the groups from E15 to 2d, the weight of the duodenum and jejunum in the Cg was higher than that in the D3g on 7 d ($P<0.05$).

Table 4. Weight of intestinal section

Age	Weight of intestinal sections, mg					
	duodenum		jejunum		ileum	
	D3g	Cg	D3g	Cg	D3g	Cg
E15	0.04 ± 0.01^{Ca}	0.05 ± 0.01^{Da}	0.09 ± 0.02^{Ca}	0.07 ± 0.02^{Ca}	0.03 ± 0.01^{Da}	0.03 ± 0.01^{Ca}
E19	0.11 ± 0.02^{Ca}	0.11 ± 0.03^{Da}	0.25 ± 0.08^{Ca}	0.21 ± 0.07^{Ca}	0.06 ± 0.01^{Da}	0.08 ± 0.01^{Ca}
0	0.44 ± 0.11^{Ba}	0.35 ± 0.05^{Ca}	0.58 ± 0.06^{BCa}	0.51 ± 0.09^{BCa}	0.09 ± 0.01^{CDa}	0.09 ± 0.0^{BCa}
2d	0.71 ± 0.02^{Ba}	0.80 ± 0.11^{Ba}	1.16 ± 0.06^{Ba}	1.2 ± 0.26^{Ba}	0.18 ± 0.08^{Ba}	0.17 ± 0.02^{Ba}
7d	1.55 ± 0.20^{Ab}	1.95 ± 0.44^{Aa}	2.84 ± 0.15^{Ab}	3.76 ± 1.77^{Aa}	0.39 ± 0.02^{Aa}	0.40 ± 0.13^{Aa}

means within columns with different superscript capital letters are different ($P<0.05$). Means within rows (same section) with different superscript lower case letters are different ($P<0.05$)

The length of the duodenum grew 2.78 times in the D3 group and 2.16 times in the C group (Table 5), however, the length of the jejunum and ileum increased 3.43 times and 1 time in the D3g and 4.18 times and 1.45 times in the Cg from E15 to 7 d, respectively. No significant differences regarding the length were noted between the two groups, in the same section and at the same period of time. However, the ileum in Cg was longer than that in the D3g on 2 d ($P<0.05$).

Table 5. Length of intestinal section

Age	Length of intestinal section, cm					
	duodenum		jejunum		ileum	
	D3g	Cg	D3g	Cg	D3g	Cg
E15	3.26 ± 0.47^{Da}	3.83 ± 0.45^{Da}	8.81 ± 1.75^{Da}	8.53 ± 2.44^{Da}	3.1 ± 0.45^{Ca}	2.46 ± 0.21^{Da}
E19	4.56 ± 0.81^{Da}	4.3 ± 0.88^{Da}	12.23 ± 3.51^{Da}	12.1 ± 4.41^{Da}	3.23 ± 0.45^{BCa}	3.63 ± 0.15^{Ca}
0	6.44 ± 0.11^{Ca}	6.86 ± 0.83^{Ca}	19.0 ± 0.51^{Ca}	22.0 ± 1.0^{Ca}	3.73 ± 0.21^{BCa}	3.66 ± 0.15^{Ca}
2d	7.98 ± 0.16^{Ba}	8.9 ± 0.52^{Ba}	28.83 ± 3.32^{Ba}	32.01 ± 2.26^{Ba}	4.01 ± 0.2^{Bb}	4.9 ± 0.4^{Ba}
7d	12.33 ± 0.76^{Aa}	12.1 ± 2.30^{Aa}	39 ± 2.78^{Aa}	44.26 ± 3.02^{Aa}	6.2 ± 0.36^{Aa}	6.03 ± 1.09^{Aa}

means within columns with different superscript capital letters are different ($P<0.05$). Means within rows (same section) with different superscript lower case letters are different ($P<0.05$)

In both groups thickness of the small intestine wall was the biggest in the duodenum and ileum at hatch and in the jejunum on 2 d ($P<0.05$; Table 6). Although there were no significant differences between the D3g and the Cg, the thickness of small intestine wall in the duodenum and ileum increased 21 and 14.2 μm in the D3g and 26 and 57.8 μm in the Cg from E15 to hatch, respectively. Moreover, in the jejunum it increased 19.1 μm in D3g and 28 μm in the Cg from E19 to 2d. The thickness of the small intestine wall in the C group was higher than that in the D3 group in the jejunum on 2 d and in the ileum at hatch and on 2d ($P<0.05$).

Table 6. The thickness of small intestinal wall

Age	Thickness of intestinal wall, μm					
	duodenum		jejunum		ileum	
	D3g	Cg	D3g	Cg	D3g	Cg
E15	149.2 ± 13.7^{ABa}	125.69 ± 14.0^{Ba}	128.4 ± 35.1^{Aa}	136.5 ± 30.1^{ABa}	166.6 ± 8.8^{BCa}	160.2 ± 15.1^{Ba}
E19	134.6 ± 44.4^{Ba}	149.9 ± 13.8^{Aa}	122.6 ± 16.6^{Aa}	136.9 ± 35.7^{ABa}	167.4 ± 8.5^{ABa}	161.0 ± 51.7^{Aa}
0	155.6 ± 7.1^{Aa}	151.2 ± 27.8^{Aa}	137.3 ± 18.6^{Aa}	142.4 ± 34.7^{ABa}	180.8 ± 7.8^{ABa}	218.3 ± 24.7^{Ba}
2d	112.9 ± 2.8^{Cb}	142.8 ± 18.4^{Aa}	141.7 ± 25.8^{Ab}	165.5 ± 54.9^{ABa}	149.2 ± 61.6^{Ca}	162.8 ± 13.1^{Ba}
7d	142.9 ± 1.6^{ABa}	145.3 ± 19.2^{Aa}	125.2 ± 17.4^{Aa}	127.3 ± 17.5^{ABa}	176.9 ± 52.5^{Aa}	159.1 ± 2.7^{Ba}

[a,b] means within rows (same section) with different superscript capital letters are different ($P<0.05$)

The muscle thickness of intestine was presented in Table 7. It decreased in the duodenum and jejunum in the D3g from E15 to hatch ($P<0.05$), while it increased in Cg group from E15 to hatch ($P<0.05$). It increased in the ileum in both groups from E15 to hatch ($P<0.05$). In the D3g it was thicker than that in the Cg on E15, however in D3g it was thinner than that in the Cg on 2 d and 7 d in the duodenum and jejunum ($P<0.05$). Besides, the muscle thickness of the ileum in Cg was thicker than that in D3g from E15 to 2 d, while that in Cg was thinner than that in D3g on 7 d ($P<0.05$).

Table 7. The thickness of small intestinal muscle

Age	Thickness of muszle, μm					
	duodenum		jejunum		ileum	
	D3g	Cg	D3g	Cg	D3g	Cg
E15	112.4 ± 26.6^{Aa}	68.6 ± 13.2^{Cb}	91.2 ± 28.8^{Aa}	63.8 ± 8.9^{Cb}	56.8 ± 14.0^{Cb}	78.2 ± 15.2^{Ca}
E19	79.8 ± 27.1^{Ba}	63.1 ± 10.9^{Ca}	75.3 ± 3.8^{Ba}	70.0 ± 20.9^{BCa}	69.3 ± 13.9^{Cb}	84.1 ± 16.0^{Ca}
0	70.1 ± 7.9^{BCa}	91.4 ± 15.1^{ABa}	62.7 ± 15.6^{Ca}	75.8 ± 22.8^{BCa}	90.0 ± 11.6^{Bb}	111.6 ± 19.9^{Aa}
2d	60.8 ± 11.1^{Cb}	77.9 ± 8.7^{BCa}	70.6 ± 18.1^{Ab}	95.8 ± 36.3^{Aa}	70.6 ± 23.5^{Cb}	104.9 ± 8.5^{ABa}
7d	71.5 ± 5.4^{BCb}	95.9 ± 95.9^{Aa}	64.5 ± 20.0^{Cb}	83.0 ± 13.1^{ABa}	134.0 ± 36.1^{Aa}	97.2 ± 13.7^{Bb}

means within columns with different superscript capital letters are different ($P<0.05$). Means within rows (same section) with different superscript lower case letters are different ($P<0.05$)

The height of villi grew constantly from E15 to 7 d in the whole small intestine of both groups ($P<0.05$; Table 8). Moreover, in both groups the increasing rate of villi height was higher from 2 to 7 d in the three segments ($P<0.05$). Between two

groups, villi height was obviously higher in D3 group in the duodenum on E15, hatch, 2 and 7 d, in the jejunum on E15, hatch and 2 d and in the ileum at hatch, 2 d and 7 d ($P<0.05$).

Table 8. The height of small intestinal villi

Age	Height of villi, μm					
	duodenum		jejunum		ileum	
	D3g	Cg	D3g	Cg	D3g	Cg
E15	120.8 ± 15.2^{Da}	93.6 ± 9.7^{Db}	102.8 ± 15.6^{Ca}	85.8 ± 8.6^{Eb}	68.8 ± 9.3^{Da}	71.7 ± 6.6^{Ea}
E19	199.3 ± 31.1^{Ca}	175.2 ± 29.4^{Ca}	146.5 ± 34.2^{Ca}	138.4 ± 19.3^{Da}	122.9 ± 32.1^{Ca}	132.2 ± 21.0^{Da}
0	447.0 ± 107.5^{Ba}	333.2 ± 115.5^{Bb}	326.8 ± 82.3^{Ba}	248.0 ± 48.4^{Cb}	272.8 ± 64.8^{Ba}	203.2 ± 29.0^{Cb}
2d	492.8 ± 92.7^{Ba}	417.5 ± 131.3^{Bb}	364.8 ± 96.5^{Ba}	302.8 ± 86.2^{Bb}	320.2 ± 60.1^{Ba}	271.0 ± 21.6^{Bb}
7d	706.4 ± 109.5^{Aa}	645.4 ± 133.0^{Ab}	650.1 ± 87.3^{Aa}	639.1 ± 64.1^{Aa}	461.8 ± 56.0^{Aa}	349.4 ± 45.0^{Ab}

means within columns with different superscript capital letters are different ($P<0.05$). Means within rows (same section) with different superscript lower case letters are different ($P<0.05$)

The perimeter of villi grew continuously with age in the whole small intestine in both groups ($P<0.05$; Table 9). Villi perimeter of D3g was higher than that of the Cg in the duodenum on E15 and E19, in the jejunum at hatch and in the ileum on E15, at hatch and on 7 d ($P<0.05$). In the three segments the villi perimeter in D3g tended to be higher than that in C group from E15 to 7 d.

Table 9. The perimeter of small intestinal villi

Age	Perimeter of villi, μm					
	duodenum		jejunum		ileum	
	D3g	Cg	D3g	Cg	D3g	Cg
E15	354.3 ± 44.2^{Ca}	274.5 ± 26.4^{Cb}	303.9 ± 54.8^{Da}	281.8 ± 66.0^{Da}	264.8 ± 34.7^{Da}	234.7 ± 33.4^{Db}
E19	503.3 ± 45.1^{Ca}	438.9 ± 65.3^{Cb}	363.4 ± 77.4^{Da}	344.6 ± 59.5^{Da}	347.0 ± 87.0^{Da}	340.1 ± 55.4^{Ca}
0	1053.2 ± 304.8^{Ba}	984.7 ± 239.1^{Ba}	757.3 ± 199.5^{Ca}	599.3 ± 129.2^{Cb}	657.9 ± 151.6^{Ca}	517.2 ± 83.2^{Bb}
2d	1125.8 ± 304.8^{Ba}	1011.3 ± 289.4^{Ba}	1036.5 ± 266.9^{Ba}	1005.2 ± 180.9^{Ba}	763.1 ± 139.2^{Ba}	759.7 ± 74.4^{Aa}
7d	1533 ± 370.2^{Aa}	1351.7 ± 253.5^{Aa}	1435.3 ± 223.9^{Aa}	1406.8 ± 122.9^{Aa}	930.6 ± 160.7^{Aa}	823.9 ± 148.5^{Ab}

means within columns with different superscript capital letters are different ($P<0.05$). Means within rows (same section) with different superscript lower case letters are different ($P<0.05$).

In both groups the surface area of villi increased constantly in the three segments ($P<0.05$; Table 10). The villi surface area of D3g was higher than that of Cg in the duodenum from E15 to 2 d, in the jejunum at hatch and in the ileum on E15 and at hatch ($P<0.05$).

Crypts were detected in the small intestine at hatch in both groups (Table 11). The depth of crypt increased with age in both groups in the three segments ($P<0.05$; Table. 9). Crypt depth was higher in D3 group in the duodenum on 2 and 7 d, in the jejunum at hatch, on 2 and 7 d and in the ileum on 7 d ($P<0.05$).

Table 10. The surface area of villi in small intestine

Age	Surface area of villi, μm ×10^3					
	duodenum		jejunum		ileum	
	D3g	Cg	D3g	Cg	D3g	Cg
E15	8.8 ± 3.2^{Ca}	5.0 ± 1.5^{Db}	6.2 ± 1.9^{Da}	5.4 ± 3.0^{Da}	5.6 ± 1.1^{Ca}	3.6 ± 0.7^{Db}
E19	11.3 ± 3.7^{Ca}	9.6 ± 3.3^{Db}	6.6 ± 3.4^{Da}	7.1 ± 2.8^{CDa}	7.6 ± 5.1^{Ca}	7.0 ± 2.4^{CDa}
0	40.7 ± 17.2^{Ba}	36.1 ± 4.9^{Cb}	25.7 ± 14.7^{Ca}	15.7 ± 6.1^{Cb}	21.4 ± 9.3^{Ba}	14.1 ± 5.7^{Cb}
2d	73.8 ± 40.5^{Aa}	55.3 ± 28.7^{Bb}	49.2 ± 2.6^{Ba}	49.5 ± 15.4^{Ba}	38.9 ± 10.3^{Ba}	39.6 ± 14.1^{Ba}
7d	87.6 ± 15.9^{Aa}	83.2 ± 24.6^{Aa}	81.7 ± 22.3^{Aa}	84.1 ± 16.3^{Aa}	52.2 ± 27.3^{Aa}	49.4 ± 13.5^{Aa}

means within columns with different superscript capital letters are different ($P<0.05$). Means within rows (same section) with different superscript lower case letters are different ($P<0.05$)

Table 11. Depth of crypt in the small intestine

Age	Depth of krypt, μm					
	duodenum		jejunum		ileum	
	D3g	Cg	D3g	Cg	D3g	Cg
0	48.3 ± 23.1^{Ba}	50.1 ± 5.4^{Ca}	50.1 ± 3.9^{Ca}	40.2 ± 4.3^{Cb}	48.4 ± 5.2^{Ca}	43.1 ± 9.2^{Ba}
2d	83.9 ± 25.5^{Aa}	62.8 ± 11.9^{Bb}	88.6 ± 30.5^{Ba}	60.7 ± 60.7^{Bb}	69.4 ± 20.7^{Ba}	79.5 ± 21.5^{Aa}
7d	96 ± 13.5^{Aa}	74.2 ± 30.0^{Ab}	116.3 ± 28.2^{Aa}	92.6 ± 31.2^{Ab}	92.7 ± 11.9^{Aa}	78.6 ± 13.1^{Ab}

means within columns with different superscript capital letters are different ($P<0.05$). Means within rows (same section) with different superscript lower case letters are different ($P<0.05$)

Although the ratio of villi height to crypt depth increased with age in the duodenum and the jejunum, it decreased in the ileum from hatch to 2 d ($P<0.05$; Table 12). There were no significant differences between the two groups.

Table 12. The ratio of villi height to crypt depth

Age	The ratio of villi height to crypt depth					
	duodenum		jejunum		ileum	
	D3g	Cg	D3g	Cg	D3g	Cg
0	5.94 ± 2.99^{Ca}	5.67 ± 2.35^{Ca}	6.53 ± 1.64^{Ba}	6.19 ± 1.13^{Ba}	5.63 ± 1.15^{Aa}	4.92 ± 1.27^{Aa}
2d	7.22 ± 2.24^{Ba}	6.85 ± 2.62^{Ba}	6.43 ± 1.55^{Ba}	6.88 ± 1.70^{ABa}	4.05 ± 1.01^{Ba}	3.65 ± 1.02^{Ba}
7d	8.39 ± 1.91^{Aa}	8.47 ± 3.46^{Aa}	7.49 ± 1.71^{Aa}	7.54 ± 2.21^{Aa}	3.96 ± 0.78^{Ba}	4.52 ± 0.79^{Aa}

means within columns with different superscript capital letters are different ($P<0.05$). Means within rows (same section) with different superscript lower case letters are different ($P<0.05$)

DISCUSSION

The results of this work indicate that the 25-OH-D_3 in the hen's diet was effective in improving egg production and hatchability. These findings are in agreement with the results of Soto-Solanova and Hernandez (2004) and Mohammad et al. (2010). Moreover, they reported that the 25-OH-D_3 in the maternal diet was effective in improving body weight and the intestinal weight and length of the progeny. We observed that maternal 25-OH-D_3 induced a lower weight and a short length of the offspring intestine after hatch. These results were consistent with those of

Chou et al. (2009) who suggested that supplemental 25-OH-D_3 resulted in lighter and shorter small intestines.

The small intestine is one of the most important sites of nutrient absorption. The age and the nutrients are critical factors for the morphological development of the small intestine. In our study, the weight, length and thickness of the small intestine increased with age. In particular, they increased quickly in the jejunum during the later incubation and the first week of life. This was in line with what was previously described by Uni et al. (1999) in posthatch chick. Moreover, the jejunum increased in weight and length more rapidly than both duodenum and ileum. These findings indicate, as formerly suggested by Soriano et al. (1993), that intestinal growth is directly proportional to the age-related increase.

In the present study, the height, perimeter and surface area of villi increased with age in the later incubation and first week of life, in particular, at hatch in the duodenum and jejunum. It has been suggested that longer villi result in an increased surface area and a greater absorption of available nutrients (Caspary, 1992) as well as an increased body weight gain (Samanya and Yamauchi, 2002; Maneewan and Yamauchi, 2004). Our results showed that longer villi caused an increase of the surface area of villi but not an increase of body weight. We may speculate that this difference could result from the different age of the chicks analysed by the former authors.

Nutrition plays a key role in the modulation of the developing intestine in early life. The importance of nutrition on morphologic changes in gastrointestinal tract was adequately demonstrated by several studies (Yu et al., 1998; Samanya and Yamauchi, 2002; Shirpoor et al., 2006). Vitamin D_3 not only plays a critical role in the absorption of calcium to calcify bone (Fritts and Waldroup, 2003) but also mediates the intestinal function and morphology (Yu et al., 1998). Chou et al. (2009) reported that the small intestine of 1 week birds fed with 25-OH-D_3 weighed numerically less than that of the control birds. Besides, this author noted a numerical trend for birds supplemented with 25-OH-D_3 to have lighter small intestines at 2, 3, 4, and 5 weeks of age.

Numerous researchers demonstrated that the supplement with 25-OH-D_3 resulted in significantly higher villi height of the small intestine (Yarger et al., 1995b; Chou et al., 2009). We found that 25-OH-D_3 in the maternal diet induced the increase of the villi height in the three segments at hatch. This was in line with previous researchers who reported that 25-OH-D_3 caused longer villi in the small intestine (McCarthy et al., 1984; Shinki et al., 1991). However, Chou et al. (2009) showed that 25-OH-D_3 increased the height of villi in the duodenum and jejunum and also the ratio of villi length to crypt depth, while decreased the height of villi in the ileum. In our study 25-OH-D_3 provoked the increase in the crypt depth in the three sections after hatch, in particular in the jejunum. However, the ratio of villi height to crypt depth was not significantly different in the presence or absence

of vitamin D_3. Our results agree with the conception that 25-OH-D_3 positively influenced villi length which may suggest an enhanced rate of nutrient absorption. Furthermore, it has been indicated that 25-OH-D_3 could stimulate an increase in the enterocyte migration rate from the crypt to the villi (Berlanga et al., 2001). We can hypothesize that crypt depth increase in the jejunum of chick from hen fed with 25-OH-D_3 resulted from the absorption of 25-OH-D_3 in the small intestine. This hypothesis is supported by some studies on mammals and on broiler chicks where 25-OH-D_3 is absorbed more rapidly from the proximal jejunum into the portal vein (Bar et al., 1980; Sitrin et al., 1982).

CONCLUSIONS

In conclusion, it can be stated that dietary 25-OH-D_3 supplement of hen diet positively influenced the morphological development of the small intestine, in particular of the duodenum and jejunum of chick during the later incubation and first week of life, and, in consequence, improves nutrient absorption facilitating the maturation and the integrity of the intestinal tract. Therefore, we suggest that 25-OH-D_3 can be used as a feed additive in maternal diet to stimulate morphological maturation and in consequence intestinal function in chicks during later incubation and first week of life.

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