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EFFECT OF DIETARY INULIN SUPPLEMENTATION ON INTESTINAL CALCIUM AND PHOSPHOROUS ABSORPTION AND EGG SHELL QUALITY IN BANDARAH LAYING HENS.

By

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Abstract: *The objective of this study was to determine the effect of various levels of dietary inulin on intestinal calcium and phosphorous absorption, egg production, egg shell quality and some physiological traits in Bandarah laying hens. One hundred and twenty 54-wks-old of Bandarah laying hens were randomly assigned into 4 groups (30 hens each) and fed on the basal diets supplemented with 0 (control), 0.5, 1.0, or 1.5% inulin for 6 wks. Supplementing 1.0 or 1.5% inulin increased ($P\leq0.05$) live body weight, shell weight percentage, shell thickness, Haugh unit, calcium and phosphorous levels in both of shell and tibiae and the absorption of calcium and phosphorous in different parts of intestinal. Meanwhile, there were no significant ($P\leq0.05$) differences in feed consumption, serum estradiol level, and relative weights of ovary, oviduct and intestine and oviduct length among the treatments during the experimental period. Inulin supplementation increased ($P\leq0.05$) egg weight, egg production, serum calcium and phosphorous and intestinal lengths. Moreover, egg weight, egg production, shell weight percentage and shell thickness increased ($P\leq0.05$) with increase hen age. Serum magnesium level was increased ($P\leq0.05$) as affected by dietary 1.5 % inulin. In conclusion, inulin supplementation in laying hen diets especially at 1.0 or 1.5 % levels can improve egg production, egg shell quality and absorption of calcium and phosphorus in the intestinal of laying hens at late ages.*

INTRODUCTION

It is widely acknowledged that egg shell quality is affected by many factors, such as diseases, nutritional status of the flock, heat stress and age **(Roberts, 2004)**. The greatest amounts of calcium are obtained from blood, bones and the gastrointestinal tract **(Etches, 1995, 1996)**. Intestinal calcium uptake plays an important role in providing the amount of calcium required to perform this task, moreover, the decline in egg shell quality as the hens' age may be attributed to reduced intestinal calcium uptake as well as to increased egg size (**Al-Batshan** ***et al.*****, 1994**). Increasing calcium absorption might be responsible for increased egg shell strength or decreased occurrence of bone fracture (**Chen and Chen, 2004**). Estrogens (E_2) have a complex relationship with calcium metabolism and intestinal absorption of calcium and phosphorus (**Castillo** ***et al.*****, 1977; Bar** ***et al.*****, 1978**). As the hen ages, the number of eggs produced declines along with a reduction in egg shell quality, estrogen levels, and estrogen receptor population (**Hansen** ***et al.*****, 2003**). The increase in egg size that age brings along with insufficient calcium carbonate secretion consequently results in a reduction in thickness of the egg shell (**Etches, 1996**).

Prebiotics are carbohydrates that are not digested by vertebrates and that interact selectively with intestinal fermentation. The non-digestible carbohydrates ("dietary fiber") have been reported to improve the intestinal absorption of mineral presumably because of their binding or sequestering action (**Roberfroid** ***et al.*****, 2002; Coudray** ***et al.*****, 2003**). Inulin is considered the archetypal prebiotics, and it occurs naturally in many food plants. Also, it is extracted from chicory roots with hot water. **Roberfroid (2000)** indicated that a higher concentration of short-chain carboxylic acids resulted from the colonic fermentation of non-digestible carbohydrates, accelerating the colonic absorption of minerals particularly calcium (Ca^{2+}) and magnesium (Mg^{2+}). Increased levels of calcium and phosphorus in the tibia have been observed when feeding White Leghorn hens with inulin (**Chen and Chen 2004**). Recent research has indicated that supplementing oligofructose and inulin increased weekly egg production, cumulative weekly egg weight and elongated small and large intestinal (**Chen** ***et al.*****, 2005**). Numerous studies have been conducted to address poor egg shell quality but few studies have improved egg shell quality by improving calcium and phosphorous absorption by dietary inulin in aged laying hens. Therefore, the objective of this study was to assess the effects of adding inulin to the diet on intestinal calcium and phosphorous absorption, egg production, egg shell quality and some physiological traits in aged local laying hens.

MATERIALS AND METHODS

Husbandry and Experimental Diets

This study was conducted at El-Sabahia Poultry Research Station (Alexandria), Animal production Research Institute, Agricultural Research Center, Ministry of Agriculture. One hundred and twenty 54-wks-old of Bandarah local chickens were randomly assigned into 4 groups (30 hens each) and fed a basal diets supplemented with 0 (control), 0.5, 1, or 1.5% inulin. Each kg of inulin contained inulin 90%, glucose, fructose and sucrose 10% (Beneo™ ST , Orafti, Belgium) for 6 wks. Table 1 presents the composition of the experimental diets.

Water and feed were provided *ad libitum* and a regimen of 16L: 8 D was provided throughout the experimental period. The birds were housed individually in layer cages and body weights were recorded and intervals biweekly. Eggs were collected and weighed daily. Egg production (%) and Feed consumption (g) for each treatment were calculated biweekly for all of the experiment.

Shell quality and Haugh unit

Five eggs from each treatment group randomly collected every two weeks to assess egg quality parameters such as shell %, shell thickness, and albumin height to calculate Haugh unit. Shell thickness (including the membranes) was measured on three pieces from the equator of each egg. The shell thickness was determined after rinsing shells with distilled water and oven-dried at 110°C for 5 hours. Egg shells were ground prior to ashing (600°C for 6 h). Calcium and phosphorus were determined according to the methods of the **Association of Official Analytical Chemists (1990).**

Blood samples

Blood samples were collected from wing vein after oviposition directly from 6 hens in each group and centrifuged for 15 minutes at 3000 rpm at the last week of the experiment (60 wks) to obtain clear serum. Serum calcium, phosphorus and magnesium were determined by spectrophotometer using available commercial Kits produced by Bio-diagnostic, Egypt. Serum estradiol was determined by using radioimmunoassay Kit.

Calcium and phosphorus absorption

At 60 weeks of age, thirty two birds from groups were randomly chosen and weighed; these birds were used to estimate the calcium and phosphorus absorption in the small intestine (in vivo) according to **Khalil *et al.*,** (1987). Three ligated loops of the small intestine of each bird were performed duodenum, jejunum and ileum (3 loops X 4 birds X 4 treatment) = 48 intestine ligated loops were offered for each absorption). Calcium or phosphorus was determined from each isolated intestinal part by EDTA titration as stated by **Hawk's (1965)** and **Volasy and Szalio (1971)** respectively. Calcium or phosphorus absorption was measured as total calcium or phosphorus absorption (mg/hr).

Also, some measurements were recorded such as relative weight of ovary, oviduct, intestine and intestinal and oviduct length (cm).

Analytical Evaluation of Tibiae

The left tibiae were excised, cleaned of connective tissue, weigh and stored in individual plastic bags at −20°C. Before analysis, the frozen tibiae were thawed inside the plastic bags at room temperature. Afterward, the tibiae were ashed at 600°C for 6 h without previous fat extraction as indicated by **Yan *et al.*, (2005)**. The ash weight and the percentages of ash were recorded. Ca and P were determined by atomic absorption spectrophotometry (Solaar, Model AA) according to the methods of the **Association of Official Analytical Chemists (1990).**

Statistical Analysis

The statistical analysis was computed using the General Linear Model for analysis of variance through the SAS programe **(SAS Institute 1996)**. Means were compared for significant differences using Duncan's multiple range test **(Duncan, 1955)**.

RESULTS AND DISCUSSION

Body weight and Feed consumption

Inclusion of inulin at the level of 1 or 1.5% in the diet significantly ($P \leq 0.05$) increased live body weight compared with the control goup (Table 2). Several studies have reported that prebiotics can improve the body weight in broilers **(Ammerman *et al.*, 1989; Yusrizal and Chen, 2003)**. **Chen *et al.*, (2005)** indicated that no differences in the percentages of changes in live body weight (%) in White Leghorn hens. However, regardless of treatment, live body weight was insignificant decreased between 56 and 60 weeks of age. This result is in agreement with those reported by **Chen *et al.*, (2005)** who indicated that a decrease in live body weight (%) was recorded between 57 and 61 wks. The improvement of body weight attributed to prebiotics could be due to decreasing colonization of bacteria such as *E. coli* and *Salmonella*, while increasing the growth of non-pathogenic microorganisms **(Yusrizal and Chen, 2003)**. Furthermore, prebiotics improve nutrient availability and absorption, produce digestive enzymes and improve intestinal microbial balance **(Choudhari *et al.*, 2008)**.

No differences (P≤0.05) of feed consumption among treatments with respect to the whole period of the experiment were found (Table 2). **Chen *et al.*, (2005)** indicated that no differences in feed consumption in White Leghorn hens were recorded after 4-wks of feeding Oligofructose or inulin. However, little reference about the effect of prebiotic on layer's body weight was available. No mortality was recorded during the experimental period.

Egg weight and Egg production

Supplementing layer diets with inulin for 6 wks significantly (P≤0.05) increased egg weight and egg production when compared with the control group (Table 2). Regardless of inulin, egg weight and egg production were significantly (P≤0.05) increased with increasing of hen age. Also, there are significant (P ≤ 0.05) effect in the interaction between age of hens and inulin supplementation during the experimental period. Supplementing layers with oligofructose and inulin has been reported to improve layer performance. **Chen *et al.*, (2005)** reported that an improvement in weekly egg production and increment in cumulative weekly egg weight per bird were observed when fed the laying hens with 1.0% inulin compared with control group. Prebiotics, such as inulin or oligofructose, have been shown to stimulate mineral absorption, mainly calcium and magnesium **(Scholz-Ahrens *et al.*, 2002)**. **Gibson and Roberfroid (1995)** indicated that prebiotics stimulate the growth of healthy bacteria such as *bifidobacteria* and *lactobacilli* in the gut and increase resistance to invading pathogens. This effect is inducing metabolic activity and leading to health improvements. However, there is a few of available literature on the effect of inulin for older laying hens concerning the improving in egg production. On the basis of our results, significant improvements in egg weight and egg production might be due to healthier birds whose mineral absorption and serum estradiol have been improved by inulin supplementation.

Shell quality and Haugh unit

Supplementing layers with inulin significantly (P≤0.05) improved egg shell weight percentages, shell thickness, Haugh unit, calcium and phosphorous levels in shell especially with higher levels (1 and 1.5%) (Table 3). Increasing calcium absorption (Table 4) is thought to be the reason of increasing egg shell weight percentage, egg shell thickness and the content of shell from calcium and phosphorous. **Chen and Chen (2004)** reported that oligofructose and inulin increased (P≤0.05) egg shell weight percentages and egg shell strength after 1 wk of feeding.

Regardless of the treatments, there are significant ($P \leq 0.05$) improvement in egg shell weight percentages and shell thickness with increasing hen ages. These results disagree with those reported with **Hansen *et al*., (2003)** who showed that egg shell quality was reduced with the hen age.

From my opinion, I think that the improvement in egg shell weight percentages and shell thickness with hen ages in this experiment could be due to the cumulative effect of inulin on egg shell weight percentage and shell thickness.

Blood parameters, Calcium and phosphorus absorption and tibia mineral analysis:

Supplementing layer's diets with inulin for 6 wks showed higher ($P \leq 0.05$) serum calcium and phosphorous levels (Table 4). While the highest level of inulin significantly ($P \leq 0.05$) increased serum magnesium level as compared with the other groups. Insignificant improvement was shown in serum estradiol as affected of dietary inulin. An increase ($P \leq 0.05$) of the absorption of calcium and phosphorous in different parts of intestinal, tibiae weight percentage and calcium and phosphorus levels in tibiae was observed when adding 1 or 1.5 inulin (Table 4). Higher ($P \leq 0.05$) calcium and phosphorus levels in tibiae might be due to the higher ($P \leq 0.05$) serum calcium and phosphorus and the improvement of mineral absorption with the highest two levels of inulin supplementation. Apparently, prebiotic supplementation improved calcium and phosphorus absorption levels. As stated by **Chen and Chen (2004)** supplementing oligofructose and inulin increased ($P \leq 0.05$) the layer's serum calcium, total ash, calcium and phosphorus levels in the tibia. An improvement of mineral absorption by prebiotics in rats and humans has been reported **(Delzenne *et al*., 1995; Scholz-Ahrens *et al*., 2002; Kruger *et al*., 2003)**. **Roberfroid (2000) and Coudray *et al*., (2003)** reported that a higher concentration of short-chain carboxylic acids resulting from the colonic fermentation of non- digestible carbohydrates accelerates the colonic absorption of minerals, particularly calcium and magnesium. **Kruger *et al*., (2003)** indicated that improved calcium absorption could decrease the occurrences of bone fracture and osteoporosis.

Slaughter traits

No difference ($P \leq 0.05$) of relative weights of ovary, oviduct, intestinal weights and oviduct length among treatments was found (Table 5). However, no reference to an effect of inulin on layer's organs was available. Inulin significantly elongated ($P \leq 0.05$) intestinal lengths compared with the control (Table 5). Recently, as stated by **Chen *et al.*, (2005)** both oligofructose and inulin supplementations elongated ($P \leq 0.05$) both small and large intestinal lengths in White Leghorn hens. Moreover, **Yusrizal and Chen (2003)** showed that increased gut length was recorded only in female broilers with oligofructose supplementation.

Conclusion

Addition of inulin to the laying hens diet at 1 or 1.5 % increased absorption of calcium and phosphorus in the intestinal especially with birds of relatively high age and improved egg shell quality, which may result in reduced breaking of the egg shells. Therefore, this study could be beneficial to the egg industry and to the marketing of eggs.

Table 1: Composition and calculated analysis of basal diet.

Ingredients	%
Yellow corn	64.00
Soybean meal 44%	24.78
Wheat bran	1.00
Di-calcium phosphate	1.61
Limestone	7.91
DL-Methionine	0.10
Sodium chloride	0.30
Vit. & Min. Mixture*	0.30
Total	100.00
Calculated analysis:	
Metabolizable energy (Kcal/Kg)	2718.00
Crude protein %	16.02
Crude fiber %	3.46
Crude fat %	2.96
Calcium %	3.34
Available phosphorous %	0.42
Lysine %	0.89
Methionine %	0.39
Met+cystine %	0.66

*Supplied per kg diet: Vit A, 10000IU; Vit D_3, 2000 IU; Vit E, 10 mg; Vit K_3, 1 mg; Vit B_1, 1 mg; Vit B_2. 5mg; Vit B_6, 1.5 mg; Vit B_{12}, 10 mcg; Niacin, 30 mg; Pantothenic acid, 10 mg; Folic acid, 1 mg; Biotin, 50mcg; Choline, 260 mg; Copper,4 mg; Iron, 30 mg; manganese, 60 mg;Zinc, 50 mg; Iodine, 1.3 mg; Selenium, 0.15mg;Cobalt,0.1mg.

Table 2: Body weight (kg), feed consumption (g/hen/day), egg weight (g) and egg production (%) as affected by dietary inulin of Bandarah laying hens during different ages (Means ±S.E).

Age (wks)	control	Inulin			Overall mean
		0.5%	1%	1.5%	
Body weight (kg)					
56	1766.1±58.5	1804.2±56.4	1848.2±77.8	1867.7±62.4	1821.53±50.8
58	1717.3±43.3	1703.1±61.9	1787.5±67.3	1844.3±67.2	1763.0±39.1
60	1720.7±53.9	1733.4±71.7	1794.7±69.0	1786.4±56.4	1758.8±62.1
Overall mean	$1734.7±35.4^{b}$	$1746.9±45.7^{b}$	$1810.2±48.9^{a}$	$1832.7±50.9^{a}$	
Feed consumption (g/hen/day)					
56	101.98±3.82	92.83±6.94	97.51±2.55	100.94±3.69	98.31±4.70
58	113.47±2.27	104.12±1.79	102.99±2.48	105.09±2.28	106.42±1.80
60	106.63±1.78	107.05±1.85	102.79±2.54	100.54±2.47	104.25±1.11
Overall mean	107.36±2.01	101.33±3.46	101.09±1.15	102.19±1.95	
Egg weight (g)					
56	50.95±0.61	52.62±0.59	53.07±0.60	53.96±0.58	$52.65±0.40^{B}$
58	51.58±0.91	53.55±0.57	57.59±0.52	55.18±0.54	$53.73±0.54^{A}$
60	51.59±0.87	53.29±0.63	55.62±0.53	54.89±0.66	$53.85±0.49^{A}$
Overall mean	$51.38±0.59^{c}$	$53.15±0.45^{b}$	$54.43±0.39^{a}$	$54.68±0.50^{a}$	
Egg production (%)					
56	$27.14±2.47^{h}$	$45.24±3.96^{def}$	$42.86±3.87^{ef}$	$49.05±2.28^{de}$	$41.07±2.30^{B}$
58	$32.38±3.11^{gh}$	$46.19±2.92^{def}$	$45.24±3.06^{def}$	$50.48±3.04^{de}$	$43.57±2.89^{AB}$
60	$37.50±2.80^{fg}$	$56.19±4.88^{d}$	$53.33±3.95^{de}$	$50.95±3.99^{de}$	$49.49±3.01^{A}$
Overall mean	$32.34±1.25^{b}$	$49.21±3.35^{a}$	$47.14±2.18^{a}$	$50.16±2.33^{a}$	

a,b,c = Means having different letters exponents within each row are significantly different at $P \leq 0.05$.

A,B = Means having different letters exponents within each column are significantly different at $P \leq 0.05$.

d,e,f,g,h= Means within age of hens by inulin supplementation interaction effect within no common superscript differ significantly ($P \leq 0.05$).

Table 3: Egg shell weight (%), shell thickness (mm), Haugh unit, shell calcium (%) and shell phosphorous (ppm) as affected by dietary inulin of Bandarah laying hens during different ages (Means ±S.E).

Age (wks)	control	Inulin			Overall mean
		0.5%	1%	1.5%	
Egg shell %					
56	12.35±0.45	12.57±0.65	12.61±0.39	13.70±0.43	12.81±0.35^{C}
58	12.19±0.69	12.75±0.80	13.80±0.97	15.40±0.39	13.53±0.56^{B}
60	13.73±0.27	14.09±0.67	14.23±0.89	14.86±0.62	14.23±0.43^{A}
Overall mean	12.76±0.31^{c}	13.13±0.45^{bc}	13.55±0.60^{b}	14.65±0.25^{a}	
Shell thickness (mm)					
56	0.358±0.013	0.368±0.021	0.374±0.009	0.388±0.014	0.372±0.007^{B}
58	0.370±0.007	0.390±0.018	0.420±0.016	0.412±0.017	0.398±0.013^{A}
60	0.376±0.021	0.388±0.012	0.412±0.024	0.446±0.010	0.406±0.018^{A}
Overall mean	0.368±0.019^{b}	0.382±0.015^{b}	0.402±0.011^{a}	0.415±0.008^{a}	
Haugh unit					
56	79.52±2.60	81.38±1.67	81.95±1.51	84.25±1.52	81.78±1.05
58	78.39±3.20	83.48±2.79	84.32±1.00	84.85±0.94	82.76±1.25
60	79.52±2.60	81.38±1.67	81.95±1.51	84.22±1.54	81.77±0.95
Overall mean	79.14±2.20^{b}	82.08±1.45^{ab}	82.74±0.92^{a}	84.44±0.86^{a}	
Shell calcium (%)					
56	32.5±0.52	33.09±0.52	35.47±0.65	37.02±0.41	34.52±0.30
58	32.73±0.60	33.12±0.53	35.78±0.47	37.48±0.34	34.78±0.41
60	32.75±0.73	33.27±0.58	35.95±0.51	37.75±0.25	34.93±0.35
Overall mean	32.66±0.45^{b}	33.16±0.25^{b}	35.73±0.39^{a}	37.42±0.19^{a}	
Shell phosphorous (ppm)					
56	1199±58	1239±30	1482±30	1860±60	1445±48
58	1203±42	1246±29	1552±55	1899±36	1475±25
60	1216±53	1260±59	1598±54	1925±49	1500±45
Overall mean	1206±35^{c}	1248±40^{c}	1544±28^{b}	1894±52^{a}	

a,b,c = Means having different letters exponents within each row are significantly different at $P \leq 0.05$.

A,B,C = Means having different letters exponents within each column are significantly different at $P \leq 0.05$.

Table 4: Some blood parameters, calcium and phosphorus absorption and tibia mineral analyses as affected by dietary inulin of Bandarah laying hens at 60 weeks of ages (Means ±S.E).

Traits	control	Inulin		
		0.5%	1%	1.5%
Calcium (mg/dl)	8.87±0.18^{c}	10.20±0.20^{b}	11.07±0.22^{a}	11.12±0.35^{a}
Phosphorous (mg/dl)	4.44±0.26^{c}	5.46±0.24^{b}	5.60±0.19^{b}	6.53±0.37^{a}
Magnesium (mg/dl)	3.02±0.17^{b}	3..49±0.24^{b}	3.51±0.11^{b}	3.90±0.24^{a}
Estradiol (pg/ml)	246.59±26.41	282.38±28.70	312.35±24.24	346.59±26.41
Calcium absorption (mg Ca/hr)				
Duodenum	8.08±0.33^{b}	8.75±0.47^{b}	10.23±0.64^{a}	11.37±0.70^{a}
Jejunum	12.18±0.89^{b}	13.63±0.30^{b}	15.98±0.28^{a}	15.88±0.50^{a}
Ileum	19.38±0.54^{b}	20.85±0.60^{b}	23.28±0.59^{a}	23.38±0.81^{a}
phosphorous absorption (mg Ca/hr)				
Duodenum	4.20±0.57^{b}	4.45±0.39^{b}	5.83±0.42^{a}	6.10±0.43^{a}
Jejunum	4.43±0.37^{b}	4.93±0.24^{b}	6.00±0.55^{a}	6.23±0.22^{a}
Ileum	4.85±0.36^{b}	5.15±0.37^{b}	6.78±0.58^{a}	7.18±0.53^{a}
Tibia traits				
Weight %	1.00±0.03^{b}	1.10±0.05^{b}	1.43±0.08^{a}	1.48±0.07^{a}
Ca (% of ash)	13.52±1.44^{b}	15.96±0.56^{ab}	17.66±0.66^{a}	18.43±0.78^{a}
P (% of ash)	5.63±0.59^{c}	6.04±0.49^{bc}	7.34±0.40^{ab}	9.69±0.47^{a}

a,b,c = Means having different letters exponents within each row are significantly different at P ≤0.05.

Table 5: Some organs weight and length as affected by dietary inulin of Bandarah laying hens at 60 weeks of ages (Means ±S.E).

organs	control	Inulin		
		0.5%	1%	1.5%
Ovary (%)	0.33±0.04	0.29±0.03	0.38±0.05	0.39±0.04
Oviduct%	2.29±0.17	2.36±0.13	2.32±0.08	2.36±0.18
Oviduct length (cm)	73.25±2.91	74.00±3.22	74.38±3.26	77.63±0.18
Intestinal (%)	5.00±0.28	5.07±0.23	5.23±0.43	5.54±0.20
Intestinal length(cm)	108.08±5.51^{b}	163.31±6.25^{a}	167.81±5.20^{a}	171.64±6.79^{a}

a,b = Means having different letters exponents within each row are significantly different at P ≤0.05.

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الملخص العربى

تأثير أضافة الأنيولين للغذاء على أمتصاص الكالسيوم و الفوسفور المعوى و جودة قشرة البيضة فى دجاج البندرة البياض.

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أجريت هذه التجربة لدراسة تأثير أضافة المستويات المختلفة من الأنيولين على الأمتصاص المعوى للكالسيوم و الفوسفور و انتاج البيض و جودة قشرة البيض و بعض الصفات الفسيولوجية لدجاجات البندرة البياضة. حيث أستخدم ١٢٠دجاجة عمر ٥٤ أسبوع قسمت عشوائيا إلى أربعة مجموعات متساوية (٣٠ دجاجة لكل مجموعة) و غذيت على الغذاء الاساسى مضاف اليه صفر، ٠.٥ ، ١، ١.٥ % أنيولين لمدة ٦ أسابيع. وجد أن أضافة المستوى ١ ، ١.٥ % أنيولين يزيد من وزن الجسم الحى ، النسبة المئوية للقشرة ، سمك القشرة ، وحدات هيو ، مستوى الكالسيوم و الفوسفور فى كل من القشرة و عظمة الساق و معدل أمتصاص الكالسيوم و الفوسفورفى الاجزاء المختلفة من الامعاء. بينما لم يلاحظ أختلافات معنوية فى الغذاء المستهلك ومستوى هرمون الاستراديول فى السيرم و الوزن النسبى للمبيض و قناة البيض و الامعاء و كذلك طول قناة البيض بين المعاملات. كما أدى أضافة الأنيولين الى زيادة معنوية لوزن البيضة و انتاج البيض و مستوى الكالسيوم والفوسفور فى السيرم و طول الامعاء. بالاضافة الى ذلك وجد أن كل من وزن البيضة و انتاج البيض و الوزن النسبى لقشرة البيضة و سمك القشرة ازداد معنوياً بتقدم الدجاجات فى العمر. كما لوحظ زيادة مستوى الماغنسيوم فى السيرم زيادة معنوية نتيجة لاضافة الأنيولين بالمستوى ١.٥ %. وتوضح نتائج هذه الدراسة أن أضافة الأنيولين فى غذاء الدجاج البياض بالمستويين ١ ، ١.٥ % أديا الى تحسين انتاج البيض و جودة قشرة البيضة و أمتصاص الكالسيوم والفوسفورفى الأمعاء للدجاج البياض خاصة فى الأعمار المتأخرة.