J. Agric. Sci. Mansoura Univ., 30 (10): 5885 - 5898, 2005

EFFECT OF SOME FEED ADDITIVES AND DIETARY PROTEIN LEVELS ON LAYING HENS PERFORMACE

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ABSTRACT

The aim of this study was to determine the effect of dietary protein levels and supplementation of amino acids, kemzyme and urea on the performance of local laying hens "Mandarah" fed low protein diet. Hens were fed isocaloric diets varying in dietary crude protein being 14 or 16%, respectively. The lower protein diet was fed to hens alone or supplemented with amino acids (methionine + lysine), kemzyme, urea, amino acids + urea, kemzyme + urea or amino acids + kemzyme + urea, to obtained 8 dietary treatments.

During the experimental period, which lasted 30 weeks, 256 layers were distributed equally into 8 dietary treatments (8 treatments x 8 replicates x 4 hens).

Results could be summarized as follows:

- Under the condition of the present study, layer hens fed diet containing 16% crude protein significantly improved productive performance (body weight gain, egg production, egg mass and feed conversion ratio) comparable with those hens fed low protein diet (14% crude protein).
- Layer hens fed low protein diet supplemented with amino acids (methionine + lysine) gave significantly better performance than that with other treatments and control. While the addition of kemzyme, urea or the combination without or with amino acids gave significantly lower performance compared with other treatments.
- The egg components (yolk % and albumen % in relation to egg weight) and the chemical composition of egg (protein and fat as percentages on dry matter) was not affected by previous dietary treatments.
- Shell % in relation to egg weight significantly affected by dietary treatments while shell weight, shell surface area and shell weight per unit of surface area were not affected.
- The lowest feeding cost/egg value was demonstrated when layers fed the low protein diet supplemented with amino acids and the value was 10% less than that of layer fed the high protein diet.

Keywords: Protein level, amino acids, kemzyme urea, productive performance, egg components.

INTRODUCTION

One of the problems encountered by nutritionists in the feed industry is how to reduce dietary protein content in layers diet and thus minimize feeding costs of layer production. In practical ration formulation, deficit of first-limiting amino acids (methionine and lysine) can be prevented by supplying such amino acids in their free form. However, a progressive reduction of the dietary protein content can lead to a situation where other amino acids, threonine, valine and isoleucine, which are of no special concern in diets with normal protein level, become limiting for performance. The results of previous researches indicated that, in addition to methionine and lysine other amino acids were also limiting in low protein diets.

Morris and Gous (1988) suggested that lysine, methionine and isoleucine were likely to be the first limiting amino acids in layer diets. Harms and Russell (1993) and Sohail *et al.* (2002) reported that supplementing essential amino acids (lysine, methionine, trypotphan, theronine, arginine, isoleucine, valine and total sulpher amino acids) to low protein diets restored performance and egg weight in Hyline W-36 hens. Summers *et al.* (1991) reported that low protein diets supplemented with lysine and methionine improved egg size, while tryptophan supplementation improved feed efficiency of low protein diets equal to the positive control.

In addition, the results of several studies in which amino acids supplemented rations were fed to birds suggested that the proportion of the essential amino acids required for maximum growth decreased as the nitrogen content of the ration increased Stucki and Harper, (1961).

Supplementing diets with urea as a non-protein nitrogen seemed to be an alternative way to increase nitrogen content in poultry diets. Research conducted on supplementing non-protein nitrogen in diets for poultry indicated that, some urea can be utilized by growing chicks, layers and ducks when fed diets low in non-essential amino acids only when supplemented with the essential amino acids needed (Featherston, 1962; Zenisek and Lautner, 1969; Vasilyuk, 1972 and Ahmed, 1978).

On the other hand, there has been increased interest in quantitatively studying, the effect of different enzyme preparations when added to a cereal-based diets, low protein diet or low energy diet on the performance of chickens (Ouhida *et al.*, 2000; Zhang *et al.*, 2001; Greenwood *et al.*, 2002; Mathlouthi *et al.*, 2003 and Ghazalah *et al.*, 2005). The primary objectives of the former studies were to estimate the optimal level of feed enzyme addition required to obtain maximal poultry performance, or to evaluate the efficacy of feed enzymes added to a diet and to evaluate the improving nutrients availability of the diet and estimate the optimal input or most profitable output (economic efficiency).

El-Faham and Manar Ibrahim (2004) reported that adding energetic as enzyme preparation with amino acids methionine and lysine to low protein broiler diets reduced feed intake, improved feed conversion and gave the best economic efficiency. Ghazalah *et al.*, (2005) suggested that supplementing enzymes mixture to low energy broiler diets maintained performance similar to the control (higher energy diets).

Moreover, Attia *et al.*, (2001) reported that, a satisfactory broiler chicks performance and economic efficiency could be achieved by decreasing crude protein level up till 2% and energy level up till 5% when phytase was supplemented at 700 FTU/kg to vegetable diets.

Recently, humerous studies reported in the literature have clearly demonstrated the beneficial effect of added enzymes or enzymes with probiotec products to low protein layer diets. The beneficial effects might be due to the improvement in nutritive value of enzyme supplemented diets coincides with greater digestion and absorption of starch, protein and fat (Yakout *et al.*, 2004; Keshavarz and Austie, 2004; and Xue-Jen and Xue, 1998).

The present study aimed to investigate the adverse effects of low protein diet on performance of Mandarah laying hens. Also, to determine the effects of amino acids (methionine and lysine), natural feed additive (Commercial enzyme preparation, kemzyme) and chemical feed additive (urea) on Mandarah laying hens performance fed low protein diet.

MATERIALS AND METHODS

This experiment was carried out at El-Fayoum Takamoly Poultry Project which belongs to El-Fayoum governorate in upper Egypt.

Experimental diets

During the experimental period, which lasted 32 weeks, hens were fed the experimental diets.

Eight experimental layer diets (Table 1) were formulated as follows:

Diet 1: 16% protein served as a control (T1)
Diet 2: 14% protein served as a basal diet (T2)
Diet 3: 14% protein + amino acids (methionine + lysine) (T3)
Diet 4: 14% protein + kemzyme (T4)
Diet 5: 14% protein + urea (T5)
Diet 6: 14% protein + urea + amino acids (T6)
Diet 7: 14% protein + urea + kemzyme (T7)
Diet 8: 14% protein + urea + amino acids + kemzyme (T8)

As shown in Table (1), the experimental diets were adjusted to be iso-caloric (2800 Kcal ME/Kg) with 2 levels of protein (16 or 14% CP). The main nutrient requirements were based on the recommendation of Takamoly Poultry Project for local egg layers strain.

Layer diet 1 usually used in the Takamoly Poultry Project for commercial production of local strain hen layer and was formulated to supply 2800 Kcal ME/kg diet, 16% CP, 0.60 TSAA and 0.85% lysine. The other diets (2-8) were formulated to supply 2800 Kcal ME/kg diet, 14% CP, 0.55% TSAA and 0.75% lysine (diet 2), while the other remaining diets contained three different supplementations i.e. Amino acids, urea(1) and kemzyme(2) at levels of 0.05% Dl-Methionine + 0.05% L-lysine, 0.7% urea to make it similar to diet 1 (control) with regard to essential amino acids, crude protein contents while the 0.05% kemzyme was the recommended level of manufacturer. The composition and calculated analysis of experimental diets are shown in Table (1).

Layer hens and management

Two hundred and fifty six of Mandarah laying hens (local strain); ageing 22 weeks were reared on a conventional production program to the age of 21 weeks of age. By reaching 22 weeks of age, hens were housed in individual cages and were randomly distributed into 8 groups of dietary treatments (of 32 hens each) with eight replicates (of 4 hens each). All layers were reared under the same environmental and hygienic conditions. Lighting program was

(1) 0.7% urea = 2% crude protein.
(2) Kemzyme is a commercial enzyme preparation which contains Alfa amylase (540 u/g), protease (450 u/g), beta-glucanase (3000 u/g) and cellulose (5000 u/g).

(16L+8D) during the experimental period. Hens were fed *ad-Lilitum* and water was available at all times.

Table (1): Composition and calculated analysis of experimental layer diets.

Ingredients	Experimental diets (%)							
	1	2	3	4	5	6	7	8
Yellow corn	67.0	70.0	70.0	70.0	70.0	70.0	70.0	70.0
Soy bean meal (48%)	18.3	12.75	12.75	12.75	12.75	12.75	12.75	12.75
Wheat bran	3.0	5.50	5.35	5.45	4.80	4.65	4.75	4.60
Meat and bone meal (60%)	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Bone meal	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
Salt	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Limestone	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
Vit & Min. premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DL methionine	0.05	0.05	0.10	0.05	0.05	0.10	0.05	0.10
L-lysine	-	0.05	0.15	0.05	0.05	0.15	0.05	0.15
Urea	-	-	-	-	0.70	0.70	0.70	0.70
Kmzyme	-	-	-	0.05	-	-	0.05	0.05
Total	100	100	100	100	100	100	100	100
Calculated values:								
ME (Kcal/kg)	2800	2800	2803	2800	2791	2792	2791	2791
Crude protein %	16.03	14.03	14.12	14.02	15.93	15.99	15.93	15.98
Methionine %	0.35	0.28	0.37	0.28	0.28	0.37	0.28	0.37
Meth. + Cyst.	0.60	0.55	0.60	0.55	0.54	0.60	0.54	0.60
Lysine %	0.85	0.75	0.85	0.75	0.74	0.85	0.74	0.85
Calcium %	3.27	3.2	3.26	3.26	3.26	3.26	3.26	3.26
Avilable phosphorus %	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40

*** Vitamin and mineral premix supplied per 1 kg of diet: Vit. A, 12000IU; Vit. D3, 2200ICU; Vit. E, 10 mg; Vit. K3, 2 mg; Vit. B1, 1 mg; Vit. B2, 4 mg; Vit. B6, 1.5 mg; Vit. B12, 10µg; Nicotinic acid, 20 mg; Folic acid, 1 mg; pantothenic acid, 10 mg; Biotin 50µg; Choline chloride, 500 mg; Copper, 10 mg; Iron 30 mg; Manganese, 55 mg; Zinc, 50 mg; Iodine, 1mg; Selenium, 0.1mg; and cobalt, 2mg.**

Body weight was recorded at the start as initial weight (at 22 wks of age) and at the end of the experiment (32 weeks of laying or 54 weeks old). Feed intake (FC) and feed conversion (FCR) was calculated as feed/g egg produced. Egg production traits including hen day egg production percent (EP), egg weight (EW), and egg mass (EM) were recorded and calculated on a daily basis.

Egg components (egg weight, yolk %, albumen % and shell %) were performed and calculated during the last period of the experiment using 10 eggs/ treatment. Chemical analysis (protein % and fat %) for inner egg components (yolk + albumen) was determined according to AOAC (1990). Egg shell quality, shell weight (SW), shell surface area (SA) and shell weight per unit surface area (SWUSA) were evaluated using the following equation suggested by Nortstrom and Qusterhout (1982):

$SA\ (cm^2) = 3.9782 \times EW^{0.7056}$

$SWUSA\ (mg/cm) = SW\ (mg)/SA\ (cm^2)$.

where 3.9782 = constant factor, EW = fresh egg weight (g).

Economic efficiency

Economic efficiency for egg production was calculated from the input/output analysis according to the price of experimental diets and egg produced.

Statistical analysis

The statistical analysis was computed using analysis of variance procedure and the significant mean differences between treatments means were separated by Duncan's multiple range test. The procedure described in the SAS, (SAS, 1990).

RESULTS AND DISCUSSION

The effects of nutritional treatments on productive performance of layers can be shown as follows:

Body weight

Data in Table (2) show that the average initial body weight (at 22 wks of age) for groups of layers assigned to dietary treatments were nearly similar. Layer hens fed, high protein diet (control diet T1) during the experimental period reflected the highest significant body weight gain (BWG) compared with those fed low protein diet (T2) and the corresponding values were 390.1 and 135.7 g, respectively. Similar results were reported by El-Faham and Manar Ibrahim (2004) on broilrer chicks, Abdel Azeem *et al.* (2005) on Japanese quails and Keshavarz and Jackson (1992) on growing pullets and laying hens. Layers fed low protein diet supplemented with amino acids (T3) or kemzyme (T4) improved BWG compared with those fed low protein diet (T2) and in most cases differences were insignificant. In addition birds fed low protein diet supplemented with urea alone or with different feed additives showed the lowest BWG (except T6) compared with those fed control diet. The explanation of that could be due to the fact that, diet 1 (control diet) was formulated to meet the optimum nutrient requirements for local strain layer based on the recommendation of Takamoly Poultry Project.

Table (2): Effect of dietary treatments on live body weight gain of layer hens during experimental period.

Treatments	Body weight (g)		
	Initial	Final	Gain
1	1695.0±40.43	2055.1^{a}±76.89	390.1^{a}±94.31
2	1705.3±40.43	1876.2^{ab}±67.12	135.7^{b}±82.32
3	1664.7±40.43	1898.3^{ab}±65.57	157.0^{ab}±80.43
4	1707.5±40.43	1864.8^{ab}±65.57	173.5^{ab}±80.43
5	1757.2±40.43	1865.6^{ab}±61.52	120.8^{b}±78.66
6	1643.1±40.43	1851.7^{ab}±64.13	179.1^{ab}±78.66
7	1755.6±40.43	1891.3^{ab}±61.52	90.1^{b}±75.45
8	1780.9±40.43	1739.7^{b}±64.13	-49.0^{b}±78.66
Significance	NS	*	*

^{ab} values in a column not followed by a common letters are significantly different at (P<0.05).

NS = Non significant

* = P<0.05

Egg production, egg weight, feed consumption and feed conversion ratio:

Egg production (EP%) data are presented in Table (3). The obtained results showed that there were significant differences in EP% among dietary treatments during the studied period. Laying hens fed low protein diet (T2) reflected the lowest egg production (51.8%) compared with those fed high protein diet (T1) being (58.6%), the differences between them were significant.

Table (3): Effect of dietary treatments on productive performance of layers during experimental period.

Treatments	EP (H.D%)	EW (g)	FC (g)	EM (g/d)	FCR (FC/EM)
1	58.6^{b}	46.3	111.5	27.2^{b}	3.69^{d}
2	51.8^{c}	46.8	111.8	24.3^{c}	4.13^{c}
3	61.2^{a}	46.7	113.0	28.6^{a}	3.50^{d}
4	44.6^{e}	46.3	112.5	20.7^{e}	4.86^{a}
5	43.8^{e}	46.3	113.7	20.3^{e}	4.99^{a}
6	48.4^{d}	45.7	112.8	22.2^{d}	4.54^{b}
7	49.3^{d}	46.5	113.6	23.0^{d}	4.37^{b}
8	43.9^{e}	45.9	113.8	20.2^{e}	4.99^{a}
± SE	0.70	0.35	0.62	0.33	0.06
Significance	**	N.S.	N.S.	**	**

EP (H.D%): Hen day egg production %; EW (g): Egg weight; FC (g/d): Daily feed consumption. EM (g/d): Daily egg mass; FCR: Feed conversion ratio (g feed / g egg produced).

a-e Values in a column not followed by a common letter are significantly different at ($P<0.05$)

NS = Non significant; * = $P<0.05$; ** = $P<0.01$.

However, birds fed low protein diet supplemented with amino acids (T3) resulted in the highest EP compared with other treatments and egg production increased by 4.4% (61.2% versus 58.6%) compared with that fed control diet (T1), the differences were significant.

On the other hand, laying hens fed on low protein diets supplemented with natural or chemical feed additives (T4-8) revealed significantly lower EP compared with other treatments (T1-3). Moreover, feeding diets containing 0.7% urea (T5) gave the lowest EP (43.8%) compared with other treatments and the additives failed to improve such depression in EP.

Data in table (3) indicate that there were no significant differences in Egg weight (EW) or in daily feed consumption (FC) values for laying hens fed on different experimental diets.

Decreasing dietary protein level by 2% in experimental diets without or with different feed additives supplementation showed no negative effect in EW or FC and the differences between treatments were not significant (Table 3). On the contrary, egg mas (EM) and feed conversion ratio (FCR) showed the same trend of EP since birds fed low protein diet (T2) were lower in EM and FCR than those fed the control diet (T1). The corresponding figures were

Egg components and egg shell quality

The results in Table (4) show the effect of dietary treatments and egg components on egg shell quality. The percentages of egg yolk and egg albumen in relation to egg weight for layers fed different experimental diets were almost the same and the differences between treatments were not significant. The figures of shell percent showed significant differences between birds fed diets containing low protein supplemented with urea + amino acids (T6) compared with those fed control diet (T1) and the corresponding values were 12.9 and 11.8%, respectively.

Table (4): Effect of dietary treatments on egg components and egg shell quality[(1)].

Treatments	Egg components				Shell quality		
	Egg weight (g)	Yolk (%)	Albumen (%)	Shell (%)	SW (g)	SA (cm^2)	SWUSA (mg/cm^2)
1	44.8	35.9	52.4	11.8^{cb}	5.27	58.1	90.5
2	47.0	35.2	52.2	12.6^{ab}	5.89	60.1	98.0
3	47.2	36.1	51.9	12.7^{ab}	5.99	60.3	98.9
4	49.4	36.8	50.9	11.5^{c}	5.71	62.3	91.4
5	46.7	33.6	53.5	12.8^{ab}	5.96	59.9	99.5
6	46.0	34.9	52.3	12.9^{a}	5.90	59.2	99.6
7	48.2	34.6	53.4	12.1^{abc}	5.81	61.3	95.0
8	48.1	36.1	51.9	12.0^{abc}	5.78	61.2	94.5
± SE	1.11	0.94	0.96	0.34	0.21	1.0	2.70
Significance	N.S.	N.S.	N.S.	*	N.S.	N.S.	N.S.

(1) Shell quality: [shell weight (SW), shell surface area (SA), shell weight per unit of surface area (SWUSA)]

a-c values in a column not followed by a common letter are significantly different at ($P<0.05$).

N.S. = Non signifiant; * = P,0.05.

The lowest shell % was recorded for the birds fed diets of 14% curde protein supplemented with urea (T4). While the best shell % was detected for the birds fed diets based on 14% curde protein without or with different feed additives (except T4), and the differences were significant.

The dietary treatments had no significant effects on most of egg shell quality traits in the study as shown in (table 4). Shell weight (SW) ranged between 5.99 and 5.27 g and layers fed control diet (T1) gave the lowest figures but the differences among treatments were not significant. Shell surface area (SA) and shell weight per unit surface area (SWUSA) values showed the same trend, in which control diet (T1) reflected the lowest figures compared with other treatments and the corresponding figures being 58.1cm^2 and 90.5mg/cm^2 respectively. However, the differences between all treatments were not significant.

Similar results were reported by Abdel-Azeem *et al.* (2005), who concluded that feeding Japanese quails different dietary protein levels up to 20% had no significant effect on percentages of albumin and shell. On the other hand, our findings were in contrast with the results obtained by Yakout *et al.* (2000 and 2004) who found that increasing CP in local layer strain Mandarah diets had no effects on albumen and shell percentages, while yolk % and shell quality increased with increasing CP to reach their highest values.

Chemical composition of egg

The results in Table (5) show the effect of dietary treatments on chemical composition of egg. The chemical composition (protein and fat on dry matter basis) of inner egg components (yolk and albumen) were nearly similar specially for the treatment T1 (control), T2, T3 and T6.

In the same order, the contents of protein and fat percentages of egg were relatively higher when layers fed diets of T4-8 (except T6). However, the values of protein percentages of eggs ranged between 42.70% (T2) and 46.00 (T7) while fat percentages ranged between 23.20% (T1) and 26.20% (T5). However, the differences between all treatments were not significant.

Table (5): **Effect of dietary treatments on chemical composition of egg (yolk and albumen).**

Treatments	Chemical composition*	
	Protein (%)	Fat (%)
1	43.70	23.20
2	42.70	23.55
3	43.45	23.50
4	45.95	25.20
5	44.50	26.20
6	42.85	24.70
7	46.00	25.25
8	45.10	25.10
± SE	1.66	0.95
Significance	NS	NS

*** On dry matter basis.**
NS = Non significant.

Economic evaluation

Data for economical evaluation are presented in Table (6). The obtained data showed that the cost/kg diets were: 1.120 L.E for high protein diet (T1) and 1.018 L.E for low protein diet (T2). Supplementation of amino acids, kemzyme and urea alone or in combinations to low protein diet raised the cost/kg by about 0.024 LE (T3), 0.013 LE (T4), 0.01 LE (T5), 0.034 LE (T6), 0.022 LE (T7) and 0.036 LE (T8) respectively as compared to low protein diet (T2).

The total feed cost/hen using low protein diet without or with different supplementations reduced feed cost/hen compared with those fed on high protein (T1) control diet.

Concerning feeding and relative feeding cost/egg, the obtained data showed that the low protein diet with amino acids (T3) were the best followed by the high protein diet (T1), while low protein diet with combination of urea, amino acids and kemzyme (T8) resulted in the worst value.

Table (6): Economic study for using different dietary treatments on subsequent economic returns.

Item	Experimental treatments							
	1	2	3	4	5	6	7	8
Price/kg feed (L.E)	1.120	1.018	1.042	1.031	1.028	1.052	1.040	1.054
Total feed intake/hen (kg)	23.415	23.478	23.730	23.625	23.877	23.688	23.856	23.898
Total feed cost/hen (L.E)	26.225	23.901	24.727	24.357	25.119	24.920	24.810	25.189
Total number of egg/hen	123	109	129	94	92	102	104	92
Feeding cost/egg*	0.213	0.219	0.192	0.259	0.273	0.244	0.239	0.274
Relative feeding cost/egg**	100	102.8	90.1	121.6	128.2	114.6	112.2	128.6

* Expressed by L.E.
** Relative to control, treatment 1.

CONCLUSION

It could be concluded that Mandarah hens feed low protein diet 14% crude protein + amino acids gave the highest performance compared with control diet (16% CP). While the addition of kemzyme, urea or the combination with or without amino acids gave the lowest performance as compared with unsupplemented group.

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J. Agric. Sci. Mansoura Univ., 30 (10), October, 2005

تأثير بعض إضافات الأعلاف ومستويات بروتين العليقة على الأداء الإنتاجي للدجاج البياض

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أجريت هذه الدراسة بهدف تقييم تأثير مستويين مختلفين في بروتين العليقة مع إضافة الأحماض الأمينية أو الكيمزيم أو اليوريا للمستوى المنخفض في البروتين على الأداء الإنتاجي لدجاج المندرة البياض.

غذيت الدجاجات على علائق تحتوى على ١٦% و ١٤% بروتين خام. غذيت العلائق المنخفضة في محتواها من البروتين الخام بمفردها أو بإضافة الأحماض الأمينية (ميثايونين وليسين) أو كيمزايم أو اليوريا أو أحماض أمينية + يوريا أو كيمزيم + يوريا أو أحماض أمينية + كيمزيم + يوريا وذلك للحصول على ٨ علائق تجريبية.

أهم النتائج المتحصل عليها:

- تغذية الدجاج البياض على علائق تحتوى على ١٦% بروتين خام حسن معنويا من الأداء الإنتاجي لها (زيادة الوزن المكتسب وإنتاج البيض وكتلة البيض ومعامل التحويل الغذائي) مقارنة بتلك المغذاة على علائق تحتوى على ١٤% بروتين خام.
- تغذية الدجاج البياض على عليقة منخفضة المحتوى من البروتين (١٤%) مضاف إليها الأحماض الأمينية. أعطت معنوياً أعلى أداء في معظم الصفات الإنتاجية بالمقارنة بالمعاملات الأخرى ومعاملة الكنترول بينما إضافة الكيمزيم أو اليوريا أو كلاهما مع أو بدون الأحماض الأمينية إلى علائق منخفضة في البروتين أعطت معنوياً أقل أداء في الصفات الإنتاجية مقارنة بالمعاملات الأخرى.
- مكونات البيضة من حيث النسب المئوية لكل من الصفار والبياض وكذلك التحليل الكيميائي للبيض (% بروتين و % للدهن) لم تتأثر بالمعاملات المختلفة.
- النسبة المئوية للقشرة تأثرت معنوياً بالمعاملات المختلفة بينما وزن القشرة وسطح القشرة ووزن القشرة/ وحدة من مسطح القشرة لم تتأثر.
- تغذية الدجاج البياض على عليقة منخفضة في البروتين الخام (١٤%) مضاف إليها الأحماض الأمينية، أعطت أقل تكلفة غذاء للبيضة والقيمة كانت ١٠% أقل من التكلفة للبيض المنتج من الدجاج المغذى على علائق مرتفعة في البروتين.