EFFECTS OF USING BLACK SEEDS ON EGG PRODUCTION, EGG QUALITY AND IMMUNE RESPONSE IN LAYING DIETS VARYING IN THEIR PROTEIN CONTENT

Hanan A. Hassan and Mona S. Ragab
Poultry Dept., Faculty of Agriculture, Fayoum Univ., Fayoum, Egypt.

ABSTRACT:

The experimental work of the present study was carried out at the Poultry Research Station, Poultry Department, Faculty of Agriculture, Fayoum University. This experiment was conducted for 12 weeks to evaluate black seeds (*Nigella sativa*) (BS) as natural feed additives for laying hens. A total number of 180 Hy- Line W- 36 laying hens 49 weeks old were used. The hens were randomly distributed into 15 groups of 12 birds each. Each group was subdivided into 12 replicates (one hen/replicate) and assigned randomly for one of the experimental diets.

The experimental treatments were as follows:-

1. Hens were fed 14.75 % crude protein (CP) as a control diet (D1).
2. Hens were fed 13.25 % CP (adjusted methionine & lysine) (D2).
3. Hens were fed 13.25 % CP (non adjusted methionine & lysine) (D3).
4. Hens were fed 11.75 % CP (adjusted methionine & lysine) (D4).
5. Hens were fed 11.75 % CP (non adjusted methionine & lysine) (D5).
6. Hens were fed D1 + 1% black seeds.
7. Hens were fed D2 + 1% black seeds.
8. Hens were fed D3 + 1% black seeds.
9. Hens were fed D4 + 1% black seeds.
10. Hens were fed D5 + 1% black seeds.
11. Hens were fed D1 + 2% black seeds.
12. Hens were fed D2 + 2% black seeds.
13. Hens were fed D3 + 2% black seeds.
14. Hens were fed D4 + 2% black seeds.
15. Hens were fed D5 + 2% black seeds.

Results obtained could be summarized in the following:

1-There were significant differences among treatments in productive performance except, egg weight (EW), crude protein conversion (CPC) and live body weight gain (LBWG). Higher, dietary protein levels had a positive effect on average egg production (EP) and egg mass (EM) of layers. Average feed conversion (FC) and caloric conversion ratio (CCR) improved significantly as dietary protein levels increased. BS level had insignificant effects on productive performance.

2-There were significant differences in egg quality among all dietary treatments, except, yolk color and shell %. Yolk index% and Haugh unit values significantly increased as dietary protein levels decreased. Hens fed diet containing 2% BS had higher shell thickness. Laying hens fed diet containing 1% BS had higher yolk index. Hens fed diet containing 0.0% BS had higher shape index.

3-Black seeds supplementation had significant effects on serum calcium, triglycerides, AST, total protein and phosphorus values. Calcium and phosphorus values of serum significantly reduced as dietary protein levels decreased.. Hens fed diet containing 1% BS had higher ALT level.

4- No significant effects on immune response as a result to different treatments supplementation was found in laying hen diets throughout the whole experimental period except hemoglobin.

Level of CP% insignificantly affected all immune response except, secondary immunity response, hematocrit% and white blood cells values. Regarding to secondary immunity response, values significantly increased as dietary protein levels decreased. There were insignificant effects on immunity response during all experimental period except, hemoglobin%. It is clear that hemoglobin value was significantly decreased as black seed levels increased.

5- Hens fed diet 3 gave the best economical and relative efficiency values being 1.324 and 101.5%, respectively. The rate of change in the relative efficiency varied between –50.87 to +1.50%.

Key words: Black seeds, crude protein, productive performance, serum constituents, immune response, laying hens.

INTRODUCTION

In this century the medical properties of black seed (BS) have some consideration. Nowadays, there is an increase demand for using the natural biological feed additives which is produced from fermented extracting of some herbs and edible plants instead of using synthetic drugs. Although, good results were obtained with synthetic drugs for production and physiological studies, it has some adverse effects such as their residual problems in tissues and eggs of birds. While, using natural biological feed additives is not accompanied by these problems. Black seed (*Nigella sativa*) is becoming commonly used for medical purposes. Many workers have isolated and identified some active materials known as nigellone (**Mahfouz and El-Dakhakhny, 1960**); thymoquinone (**El-Dakhakhny, 1963**) and thymohydroqinone (**El-Alfy *et al.*, 1975**). These compounds are well known for their antibacterial, antifungal, antihelminthic, antineoplastic, antidiabetic, bronchodilator, immune enhancing and antispasmotic effects (**Khodary *et al.*, 1996 and El-Ghamry *et al.*, 1997**).

Gad *et al.* (1963) studied the chemical composition of BS They found that BS contained 26.6% oil of which the major fatty acids were linoleic (64.0%) and palmitic (20.4%) acids. **Babayan, *et al.* (1978)** reported that BS have 21.0% protein, 35% fat and 5.5% nitrogen free extract, whereas **Abdel-All and Attia (1993)** found that BS have 38.7% crude fat, 21% crude protein, 13.9% crude fiber, 14.9% starch, 6.0% soluble sugars and 4.9% ash, and it was considered as a good source of protein, phosphorus, calcium, potassium, magnesium and sodium.

Adding black seeds to poultry diets resulted in improving body weight in laying hens (**El-Kaiaty *et al.*, 2002**), in growing and laying Japanese quail (**Zeweil, 1996**) improved feed conversion (**Abdo, 1998 and Tollba *et al.*, 2005**). **Khodary *et al.* (1996)** found that feeding Balady hens a diet containing 1% of freshly crushed BS for 65 successive days resulted in significant increase of egg production meanwhile 3% of BS in the diet elicited a significant decrease in egg production in hens. **Soltan (1999)** concluded that the addition of 1% BS to the diet of quail improved egg production percentage (EP%), egg mass (EM) and feed conversion (FC). However, dietary BS had no effect on the average egg quality parameters (yolk and albumen weight, yolk/albumen ratio and yolk index) as compared with control group. Moreover, BS addition had reduced the concentration of serum cholesterol and triglycerides in Pekin ducklings

(**Mandour *et al.*, 1995 and El-Bagir, *et al.*, 2006**).

For greatest economy, dietary formulation should attempt to combine protein sources that will be complete as possible in amino acids (AA) at a minimum total percentage of crude protein (CP) in the diet to promote optimal energy and protein intake or to supplement low protein diets with synthetic AA **(Cable and Waldroup, 1991)**. Also, supplementing low protein diets with natural feed additives may be an alternative way to cut feed cost down to the minimal levels. In practical poultry diets; methionine is the first limiting AA followed by lysine. Therefore, supplementation of methionine and lysine to practical poultry diets should increase the efficiency of protein utilization and result in a reduction of nitrogen excretion.

Lowering the crude protein of the laying hen diets not only reduces nitrogen consumption but also means that less unutilized nitrogen is excreted. Matching dietary protein and amino acids levels to the production requirement of laying hens is another important mean of reducing nitrogen emissions and excretion. The only practical way of reducing the crude protein of layer diets is by supplementation with specific essential AA, which are available from industrial production. Closely, matching the dietary protein and AA supply to the animals needs will result in more efficient utilization of the amino nitrogen. Also, lowering nitrogen losses via excreta will be less stressful for the birds metabolism. Finally, a marked reduction in the environmental pollution through nitrogen emissions will be realized **Yakout (2000).**

The response by the laying hens to dietary protein levels has been controversial for many years. Several workers reported that dietary protein levels have the greatest effect on laying hen performance, dietary protein content has a much consideration due to its high cost and its great effect on the production parameters of laying hens. **Fernandez *et al.* (1973)**, reported that increasing dietary protein level to an increase in egg production %. Also, average egg weight of layers increased as dietary protein level increased **(Summers, 1993)**. Moreover, **Calderon and Jensen (1990)** observed an improvement in FC due to increasing dietary protein level. In this respect, **Angelovicova (1994)** found that a low-protein diet containing14.1 and 14.7% CP reduced average daily feed intake (FI) and improved FC. Also, **Abd–Elsamee (2005)** showed no significant differences in FI values due to the use of different levels of methionine in laying hen diets.

Glick *et al.* (1983) showed that diet deficient in protein (33% of requirement) could reduce numbers of lymphocytes in the thymus of chickens. However, the responses are varied by strain **(Manzoor *et al.*, 2003)**, environment, stress, production state and health status. Thus, protective immune responses require a supply of nutrients at the appropriate times and amounts **(Humphrey *et al.*, 2002)**.

The present study aimed to evaluate the effectiveness of dietary supplementation of black seeds (*Nigella sativa*) with different levels of crude protein on some productive performance, egg quality, physiological parameters and economical efficiency in Hy-Line W36 layers at the last stage of production.

MATERIALS AND METHODS

The experimental work of the present study was carried out at the Poultry Research Station, Poultry Department, Faculty of Agriculture, Fayoum University from April to July 2003.

A total number of 180 Hy- Line W- 36 laying hens 49 weeks old were reared under the same management conditions in egg production batteries. The hens were randomly distributed into 15 groups of 12 birds each. Each group was subdivided into 12 replicates (one hen / replicate) and assigned randomly for one of the experimental diets. The basal diets were formulated to satisfy nutrient requirements of laying hens according to the strain catalog recommendations (14.7 CP% and 2770 ME, K cal / Kg). This experiment was conducted for 12 weeks to evaluate of black seeds (*Nigella sativa*) as natural feed additives for laying hens.

The experimental treatments were as follows:-

1. Hens were fed 14.75 % crude protein (CP) as a control diet (D1).
2. Hens were fed 13.25 % CP (adjusted methionine & lysine) (D2).
3. Hens were fed 13.25 % CP (non adjusted methionine & lysine) (D3).
4. Hens were fed 11.75 % CP (adjusted methionine & lysine) (D4).
5. Hens were fed 11.75 % CP (non adjusted methionine & lysine) (D5).
6. Hens were fed D1 + 1% black seeds.
7. Hens were fed D2 + 1% black seeds.
8. Hens were fed D3 + 1% black seeds.
9. Hens were fed D4 + 1% black seeds.
10. Hens were fed D5 + 1% black seeds.
11. Hens were fed D1 + 2% black seeds.
12. Hens were fed D2 + 2% black seeds.
13. Hens were fed D3 + 2% black seeds.
14. Hens were fed D4 + 2% black seeds.
15. Hens were fed D5 + 2% black seeds.

The composition and chemical analyses of the experimental diets are shown in Table 1. Artificial light was used beside the normal day light to provide 16-hour day photoperiod. Feed and water were provided *ad libitum*. Individual body weights were recorded at the beginning and at the end of the study to calculate body weight changes. Egg shape index % **(Carter, 1968)** and yolk index % **(Well, 1968)** were calculated. Data on egg production (EP), egg weight (EW) and feed intake (FI) were recorded weekly and feed conversion (FC) was calculated. Mortality was recorded daily. No mortality of birds were recorded during the study period. Egg quality measurements were determined monthly on eggs of the last three days. Representative egg samples from each treatment were collected monthly throughout the experimental period in order to determine egg and shell quality.

Egg shell thickness, including shell membranes, was measured using a micrometer at three locations on the egg (air cell, equator, and sharp end). Haugh unit score was applied from a special chart using egg weight and albumen height which was measured by using a micrometer according to **Haugh (1937).** The egg yolk visual color score was determined by matching the yolk with one of the 15 bands of the "1961, Roche Improved Yolk Color Fan".

Four hens of each group at 54 weeks of age were injected in wing vein by 0.2 ml of sheep red blood cells solution (SRBCs 9% suspension), and the blood samples were collected from the wing vein of these birds after one week to determine SRBCs primary immune response. The same birds were reinjected at 60 weeks of age and the blood samples were collected from these birds after 5 days to determine SRBCs secondary immune response in serum and determine the serum constituents. From these birds, blood sample were put in tubes containing heparin to determine the hematological parameters. Packed cell volume, PCV and red and white blood cells counts (WBCs and (RBCs), according to **Bauer (1970).** Serum constituents were determined commercially using kits, total protein **(Weichselbaum, 1946)**; albumin **(Dumas and Biggs, 1972)**; globulin concentration was calculated as the difference between total protein and albumin;

Table 1 : Composition and calculated analyses of the experimental diets.

Item,%	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Yellow corn, ground	69.30	71.42	71.40	73.47	73.50	68.35	70.45	70.45	72.45	72.55	67.40	69.50	69.50	71.50	71.54
Wheat bran	0.00	2.36	2.25	5.06	4.68	0.37	2.77	2.75	5.67	5.20	0.74	3.13	3.13	6.03	5.63
Soybean meal (44%CP)	20.00	15.34	15.65	10.44	11.12	19.58	14.90	15.20	9.95	10.65	19.17	14.50	14.77	9.56	10.23
Black seeds	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	2.00	2.00	2.00	2.00	2.00
Sodium chloride	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Calcium carbonate	8.00	8.10	8.10	8.10	8.10	8.00	8.10	8.00	8.00	8.00	8.00	8.10	8.00	8.00	8.00
Di calcium phosphate	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Vit. and Min. premix *	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
DL – methionine	0.10	0.12	0.00	0.15	0.00	0.10	0.12	0.00	0.15	0.00	0.09	0.11	0.00	0.13	0.00
L – lysine	0.00	0.06	0.00	0.18	0.00	0.00	0.06	0.00	0.18	0.00	0.00	0.06	0.00	0.18	0.00
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Calculated analysis %** :															
CP	14.75	13.25	13.25	11.75	11.75	14.75	13.25	13.25	11.75	11.75	14.75	13.25	13.25	11.75	11.75
EE	2.83	2.94	2.94	3.05	3.05	3.14	3.25	3.25	3.36	3.36	3.46	3.56	3.56	3.67	3.67
CF	2.30	2.43	2.43	2.58	2.57	2.37	2.49	2.51	2.67	2.65	2.44	2.57	2.58	2.74	2.72
Ca	3.59	3.62	3.62	3.61	3.61	3.60	3.63	3.59	3.58	3.58	3.61	3.64	3.60	3.59	3.59
Available P	0.46	0.46	0.46	0.45	0.45	0.46	0.46	0.46	0.45	0.45	0.46	0.46	0.46	0.45	0.45
Methionine	0.36	0.45	0.24	0.36	0.22	0.37	0.36	0.25	0.37	0.23	0.36	0.36	0.26	0.36	0.24
Methionine+Cystine	0.63	0.60	0.49	0.58	0.44	0.64	0.61	0.49	0.59	0.45	0.61	0.60	0.50	0.57	0.45
Lysine	0.77	0.71	0.66	0.71	0.55	0.78	0.72	0.67	0.71	0.55	0.78	0.72	0.67	0.71	0.55
ME, K cal./Kg	2772	2772	2770	2772	2771	2771	2771	2771	2771	2772	2771	2771	2771	2771	2771
Cost (L.E./ton) ***	816.3	793.0	767.7	779.4	732.3	885.2	861.7	836.6	847.6	801.0	952.7	929.3	905.4	913.8	869.6
Relative cost ****%	100.00	97.15	94.05	95.48	89.71	108.44	105.56	102.49	103.83	98.13	116.71	113.84	110.92	111.94	106.53

*Each 3.0 Kg of the Vit. and Min. premix manufactured by Agri-Vet Company, Egypt contains : Vit. A, 10000000 IU ; Vit. D_3 2000000 IU ; Vit. E, 10.0 g ; Vit. K_3, 1.0 g ; Vit. B1, 1.0 g ; Vit. B2, 5.0 g ; Vit. B6, 1.5 g; Vit. B12, 10.0 mg ; choline chloride, 250.0 g ; biotin, 50.0 mg ; folic acid, 1.0 g ; nicotinic acid , 30.0 g ; Ca pantothenate, 10.0 g ; Zn, 50.0 g ; Cu, 4.0 g ; Fe, 30.0 g ; Co, 100.0 mg ; Se, 100.0 mg ; I, 300.0 mg ; Mn, 60.0 g, and completed to 3.0 Kg by calcium carbonate.

** According to NRC, 1994.

*** According to market prices of 2003.

**** Assuming that the control equals 100.

hemoglobin (**Wintrobe, 1965**); cholesterol (**Allain, 1974**); triglycerides (**Wahlefeld, 1974**); aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (**Reitman and Frankel, 1957**); calcium (**Lehman and Henry, 1984**); glucose (**Howanitz and Howantitz, 1984**); phosphorus (**Goodwin, 1970**).

Antibody response against SRBCs were measured in serum using micro haemagglutination technique as described by **Yamamoto and Glick (1982) and Dix and Taylor (1996)**. The titers were expressed as the log 2 of the reciprocal of the highest dilution giving visible agglutination (**Atta *et al.*, 1998**).

To determine cutaneous basophil hypersensitivity (CBH) response, three hens from each group were randomly selected at 61 weeks of age and injected with 0.1 ml of phytohaemagglutinin –P (PHA-P) (100 µg/ml) subcutaneously in the right toe web, whereas, 0.1 ml saline was injected subcutaneously in the left toe web which served as the control. The thickness of both toe webs were measured in mm using a micrometer at 24 hr after injection. The CBH response was calculated as described by **Atta *et al.* (1998)** as follows: Thickness of right toe web (PHA-P response) / Thickness of left toe web (saline response).

Economical efficiency of egg production was calculated from the input-output analysis which was calculated according to the price of the experimental diets and eggs produced. The values of economical efficiency were calculated as the net revenue per unit of total cost. Analysis of variance was conducted according to **Steel and Torrie (1980)**. Significant differences among treatment means were separated using Duncan's multiple range test (**Duncan, 1955**).

RESULTS AND DISCUSSION

Laying hens productive performance:

The effect of treatments on egg production (EP%), egg mass (EM), egg weight (EW), daily feed intake (FI), feed conversion (FC), crude protein conversion (CPC), caloric conversion ratio (CCR) and live body weight gain (LBWG) are shown in Table 2. There were significant differences ($P \leq 0.01$) among treatments in productive performance except, EW, CPC and LBWG. It is clear that laying hens fed control diet had higher EP and EM, whereas, those fed diet 14 (11.75 CP%+ AA +2% BS) had lower EP and EM during the experimental period. Laying hens fed diet 8 (13.25 CP%- AA +1% BS) had lower FI, whereas, those fed diet 13 (13.25 CP%- AA + 2% BS) had higher FI during the experimental period. Laying hens fed diet 11 (14.75 CP% +2% BS) had better FC and CCR values whereas, those fed diet 14 (11.75 CP% + AA +2% BS) had worst FC and CCR values.

Concerning level of CP% (Table 2), there were significant effects ($P \leq 0.01$) on EP, EM, FC and CCR during all experimental periods. Higher dietary protein levels have a significant and positive effect on average EP% and EM of layers. In this connection, **Doran *et al.* (1980), Summers (1993) and Bunchasak, *et al.* (2005)** reported that, EP increased as dietary protein levels increased. However, **Ibrahim *et al.* (2007)** noted that EP percentage was not affected significantly by feeding different protein levels.

There were no significant differences among treatments in EW and daily FI. Similar results were obtained by **Leeson and Caston (1997) and Ibrahim *et al.* (2007)** who noted that dietary protein levels had no significant effect on FI values. However, **Doran *et al.* (1980), Summers (1993), Bunchasak, *et al.* (2005)** and **Ibrahim *et al.* (2007)** reported that, EW increased as dietary protein levels increased.

Table 2 : Effects of using black seeds in Hy- Line W- 36 laying hen diets varying in their protein content on productive performance.

Items	Egg production (EP) %	Total egg mass (EM,g)	Average egg weight (EW,g)	Daily feed intake (FI,g)	Feed conversion (FC)	Crude protein conversation (CPC)	Caloric conversation ratio (CCR)	Live body weight gain (LBWG,g)
1	70.83±1.88^{1A}	3118±116^{A}	52.59±1.0	94.12±1.9^{AB}	2.58±0.2^{D}	0.380±0.02	7.15±0.44^{D}	136.7±30.3
2	67.21±1.97^{ABCD}	2932±116^{ABC}	53.25±1.0	93.35±1.9^{AB}	2.74±0.2^{CD}	0.363±0.02	7.59±0.44^{CD}	80.96±30.3
3	66.79±2.06^{ABCD}	2711±116^{BCDE}	51.88±1.0	93.58±1.9^{AB}	3.04±0.2^{ABC}	0.402±0.02	8.42±0.44^{ABCD}	185.4±30.3
4	58.93±2.30^{EF}	2380±116^{EF}	52.47±1.0	88.39±1.9^{ABC}	3.28±0.2^{AB}	0.385±0.02	9.08±0.44^{AB}	107.4±30.3
5	57.14±1.97^{EF}	2510±116^{DEF}	53.00±1.0	91.38±1.9^{AB}	3.23±0.2^{ABC}	0.380±0.02	8.96±0.44^{ABC}	164.0±30.3
6	69.25±1.88^{AB}	3002±116^{AB}	51.44±1.0	92.53±1.9^{AB}	2.64±0.2^{D}	0.390±0.02	7.32±0.44^{D}	126.7±30.3
7	61.11±1.88^{DE}	2756±116^{ABC}	53.63±1.0	94.29±1.9^{AB}	2.99±0.2^{ABC}	0.396±0.02	8.28±0.44^{ABCD}	152.0±30.3
8	60.71±2.06^{DE}	2590±116^{CDEF}	53.51±1.0	84.82±1.9^{C}	2.85±0.2^{BC}	0.377±0.02	7.89±0.44^{BCD}	78.88±30.3
9	57.54±2.17^{EF}	2331±116^{F}	50.76±1.0	91.05±1.9^{AB}	3.20±0.2^{ABC}	0.376±0.02	8.86±0.44^{ABC}	100.3±30.3
10	62.04±2.17^{CDE}	2379±116^{EF}	52.94±1.0	92.83±1.9^{AB}	3.44±0.2^{A}	0.404±0.02	9.53±0.44^{A}	160.6±30.3
11	67.76±1.88^{ABC}	3030±116^{AB}	53.28±1.0	91.16±1.9^{AB}	2.56±0.2^{D}	0.378±0.02	7.10±0.44^{D}	148.6±30.3
12	63.53±1.97^{BCDE}	2777±116^{ABC}	53.40±1.0	87.99±1.9^{BC}	2.80±0.2^{BC}	0.370±0.02	7.74±0.44^{BCD}	160.9±30.3
13	61.80±1.97^{CDE}	2770±116^{ABC}	54.17±1.0	94.54±1.9^{A}	3.00±0.2^{ABC}	0.398±0.02	8.32±0.44^{ABCD}	76.73±30.3
14	53.91±2.46^{F}	2254±116^{F}	53.31±1.0	88.81±1.9^{ABC}	3.46±0.2^{A}	0.406±0.02	9.58±0.44^{A}	101.8±30.3
15	58.33±2.46^{EF}	2382±116^{EF}	54.37±1.0	92.54±1.9^{AB}	3.45±0.2^{A}	0.405±0.02	9.56±0.44^{A}	108.4±30.3
Over all mean	62.46±0.54^{EF}	2662±30	52.93±0.3	91.42±0.49	3.02±0.04	0.387±0.01	8.36±0.11	125.9±7.8
Level of CP % :								
14.75	69.28±1.11^{A}	3050±66^{A}	52.54±0.6	92.60±1.14	2.59±0.1^{C}	0.383±0.01	7.19±0.25^{C}	137.3±17.7
13.25	63.48±0.83^{B}	2756±47^{B}	53.31±0.4	91.43±0.81	2.90±0.1^{B}	0.384±0.01	8.04±0.18^{B}	122.5±12.6
11.75	58.08±0.93^{C}	2373±47^{C}	52.81±0.4	90.83±0.81	3.34±0.1^{A}	0.393±0.01	9.26±0.18^{A}	123.8±12.6
Black seeds Level % :								
0.00	64.56±1.08	2730±60	52.64±0.4	92.16±0.88	2.97±0.08	0.382±0.01	8.24±0.22	134.9±13.8
1.00	62.45±1.08	2612±60	52.45±0.4	91.10±0.88	3.02±0.08	0.389±0.01	8.38±0.22	123.7±13.8
2.00	62.03±1.13	2643±60	53.71±0.4	91.01±0.88	3.05±0.08	0.392±0.01	8.46±0.22	119.3±13.8
Level of amino acid :								
Requirement	64.17±0.80^{a}	2731±45^{a}	52.68±0.3	91.30±0.66	2.92±0.06^{B}	0.383±0.01	8.08±0.16^{B}	123.9±10.3
Low	61.21±1.01^{b}	2557±55^{b}	53.31±0.4	91.61±0.81	3.17±0.07^{A}	0.395±0.01	8.78±0.20^{A}	129.0±12.6

[1] Mean ± Standard error of the mean.

a,....b, and A,... F, values in the same column within the same item followed by different superscripts are significantly different (at $P \le 0.05$ for a to b ; $P \le 0.01$ for A to F).

Average FC and CCR improved significantly as dietary protein levels increased. These results disagreed with **Ibrahim *et al.* (2007)** who noted that dietary protein levels had no effect on efficiency of feed utilization.

The results indicated that BS level had insignificantly affected productive performance (Table 2). Results of LBWG agree with those of **El-Kaiaty *et al.* (2002), Radwan (2004) and Moustafa (2006)** who indicated that average LBWG was not significantly affected by any level of the BS. Results of EP was agree with **Khodary *et al.* (1996)** in Balady ckickens, **Soltan (1999)** in quails and **El-Kaiaty *et al.*(2002)** who found that using BS in laying hen diets at 2% level had no effect on EP, while, **Moustafa (2006)** showed that addition of BS to laying hens diets significantly increased EP.

Results of EW and EM disagree with **El-Kaiaty *et al.* (2002)** who reported that inclusion of 2% BS in laying hens diets insignificantly improved both of EW and EM. Also, **Khodary *et al.* (1996)** showed that addition of BS to laying hens diets significantly increased EW. The present results agree with those of **El-Kaiaty *et al.* (2002) and Moustafa (2006)** who reported that there were no effect of supplemented BS on FI, however, **Nofal *et al.* (2006)** noted that FI was significantly decreased by dietary supplementation of 0.75 and 1.5% crushed BS either in continuous or intermittent groups compared with control group. **Soltan (1999)** and **Sedaros (2000)** in Japanese quail, **El-Kaiaty *et al.* (2002)** in Balady chickens and **Moustafa (2006)** observed that addition of 2% BS in layer diets improved the FC.

Concerning level of AA% (Table 2), there were significant effects on EP, EM, FC and CCR during all experimental periods studied. Adjusted AA levels have a positive effect on average EP and EM as well as on average FC and CCR of layers. These results agree with **Harms *et al.* (1990), Schutte *et al.* (1994), Liu *et al.* (2005), Narvaez-Solarte, *et al.* (2005) and Wu *et al.* (2005)** who reported that methionine supplementation in laying hen diets increased EP.

External and internal egg quality:

Results presented in Table (3) indicated significant differences in egg quality among all dietary treatments, except, yolk color and shell %. It is clear that laying hens fed diet 13 (13.25 CP%- AA +2% BS) had higher shell thickness and albumen%, laying hens fed diets 3 (13.25 CP%- AA) and 9 (11.75 CP%+ AA +1% BS) had higher yolk% and yolk index%, whereas, those fed diets 10 (11.75 CP%- AA +1% BS), 3 (13.25 CP% - AA), 13(13.25 CP%- AA+2% BS) and 2 (13.25 CP%+ AA) had lower values of shell thickness, albumen%, yolk% and yolk index%, respectively. Hens fed control diet had higher shape index%, while significant lower values ($P\leq0.01$) were observed for hens fed diet 8 (13.25 CP%- AA + 1% BS). Laying hens fed diet 14 (11.75 CP% + AA + 2% BS) had higher Haugh unit values while significant lower values ($P\leq0.01$) were observed for hens fed diet 8 (13.25 CP% – AA +1% BS).

Concerning level of CP% (Table 3), there were significant ($P\leq0.05$ and $P\leq0.01$) effects only on yolk index% and Haugh unit values. Yolk index% and Haugh unit values significantly increased as dietary protein levels decreased. Similar trend was detected for yolk color, but the difference did not reach significance.

As for the effect of level of BS (Table 3), there were insignificant effects on egg quality during all experimental period except, shell thickness, yolk index and shape index. Hens fed diet containing 2% BS had higher shell thickness, while significant lower value ($P\leq0.01$) was observed for hens fed diet containing 1% black seed. Laying hens fed diet containing 1% BS had higher

Table 3 : Effects of using black seeds in Hy- Line W- 36 laying hen diets varying in their protein content on external and internal egg quality.

Items	Yolk color	Shell thickness, mm	Albumen%	Yolk%	Shell%	Yolk index%	Shape index	Haugh unit
Treatments :								
1	9.52±0.23[1]	0.357±0.01^{BC}	61.20±0.59^{ab}	28.61±0.51^{BCD}	10.2±0.2	54.73±1.4^{BC}	77.1±0.50^{A}	81.89±1.99^{ABCD}
2	10.0±0.23	0.355±0.01^{BCD}	60.53±0.59^{abc}	29.08±0.51^{ABC}	10.4±0.2	50.49±1.4^{C}	77.0±0.50^{A}	77.39±1.88^{CDE}
3	9.00±0.23	0.352±0.01^{BCD}	58.93±0.59^{c}	30.52±0.51^{A}	10.6±0.2	51.07±1.4^{C}	76.6±0.50^{AB}	76.43±1.88^{DE}
4	9.48±0.23	0.352±0.01^{BCD}	61.78±0.59^{a}	28.03±0.51^{CD}	10.2±0.2	53.76±1.4^{BC}	76.7±0.50^{AB}	79.26±1.88^{BCDE}
5	9.59±0.23	0.344±0.01^{CD}	60.80±0.59^{abc}	28.69±0.51^{BCD}	10.5±0.2	54.91±1.4^{BC}	76.9±0.50^{A}	79.33±1.88^{BCDE}
6	9.67±0.23	0.351±0.01^{BCD}	60.47±0.59^{abc}	29.26±0.51^{ABC}	10.3±0.2	55.76±1.4^{B}	75.8±0.50^{ABCD}	76.45±1.88^{DE}
7	9.19±0.23	0.356±0.01^{BCD}	61.25±0.59^{ab}	28.43±0.51^{BCD}	10.3±0.2	56.79±1.4^{AB}	77.0±0.50^{A}	77.92±1.88^{CDE}
8	9.56±0.23	0.346±0.01^{CD}	61.51±0.59^{ab}	28.47±0.51^{BCD}	10.0±0.2	56.84±1.4^{AB}	74.8±0.50^{D}	75.49±1.88^{E}
9	9.96±0.23	0.355±0.01^{BCD}	60.01±0.59^{abc}	29.29±0.51^{ABC}	10.7±0.2	60.63±1.4^{A}	76.4±0.50^{ABCD}	84.77±1.88^{AB}
10	9.52±0.23	0.340±0.01^{D}	59.75±0.59^{bc}	29.80±0.51^{AB}	10.5±0.2	57.55±1.4^{AB}	75.2±0.50^{BCD}	85.67±1.88^{A}
11	9.04±0.23	0.363±0.01^{AB}	60.81±0.59^{abc}	28.60±0.51^{BCD}	10.6±0.2	53.73±1.4^{BC}	76.7±0.50^{AB}	76.83±1.88^{CDE}
12	9.78±0.23	0.362±0.01^{AB}	60.39±0.59^{abc}	29.60±0.51^{ABC}	10.0±0.2	57.62±1.4^{AB}	76.3±0.50^{ABCD}	81.36±1.88^{ABCDE}
13	9.33±0.23	0.376±0.01^{A}	61.83±0.59^{a}	27.41±0.51^{D}	10.8±0.2	58.30±1.4^{AB}	74.9±0.50^{CD}	82.83±1.88^{ABC}
14	9.33±0.23	0.358±0.01^{BC}	60.36±0.59^{abc}	29.40±0.51^{ABC}	10.3±0.2	58.44±1.4^{AB}	75.7±0.50^{ABCD}	85.72±1.88^{A}
15	9.44±0.23	0.374±0.01^{A}	61.22±0.59^{ab}	27.95±0.51^{D}	10.8±0.2	58.21±1.4^{AB}	76.5±0.50^{ABC}	79.38±1.88^{BCDE}
Over all mean	9.50±0.06	0.356±0.001	60.72±0.15	28.88±0.13	10.4±0.1	55.92±0.4	76.2±0.10	80.05±0.49
Level of CP % :								
14.75	9.41±0.14	0.357±0.003	60.83±0.36	28.82±0.32	10.4±0.1	54.74±0.91^{b}	76.5±0.30	78.25±1.19^{B}
13.25	9.48±0.10	0.358±0.002	60.74±0.25	28.92±0.22	10.4±0.1	55.18±0.65^{ab}	76.1±0.20	78.57±0.83^{B}
11.75	9.56±0.10	0.354±0.002	60.65±0.25	28.86±0.22	10.5±0.1	57.25±0.65^{a}	76.2±0.20	82.35±0.83^{A}
Black seeds Level % :								
0.00	9.53±0.11	0.352±0.002^{B}	60.65±0.27	28.98±0.24	10.4±0.1	52.99±0.66^{B}	76.8±0.20^{A}	78.79±0.95
1.00	9.58±0.11	0.350±0.002^{B}	60.60±0.27	29.05±0.24	10.4±0.1	57.51±0.66^{A}	75.8±0.20^{B}	80.06±0.94
2.00	9.39±0.11	0.367±0.002^{A}	60.92±0.27	28.59±0.24	10.5±0.1	57.26±0.66^{A}	76.0±0.20^{B}	81.22±0.94
Level of amino acid :								
Requirement	9.56±0.08	0.357±0.002	60.76±0.20	28.92±0.18	10.3±0.1	55.77±0.54	76.5±0.20^{A}	80.16±0.71
Low	9.41±0.10	0.355±0.002	60.67±0.25	28.81±0.22	10.5±0.1	56.15±0.66	75.8±0.20^{B}	79.85±0.86

[1] Mean ± Standard error of the mean.

a,....c, and A,... E, values in the same column within the same item followed by different superscripts are significantly different (at $P \leq 0.05$ for a to c ; $P \leq 0.01$ for A to E).

yolk index, while significant lower value ($P \leq 0.01$) was observed for hens fed the diet containing 0.0% BS. Hens fed diet containing 0.0% BS had higher shape index, while significant lower value ($P \leq 0.01$) was observed for hens fed diet containing 1% black seed.

It is clear that Haugh unit value was insignificantly increased as black seed levels increased. In this respect, **Tollba *et al.* (2005)** and **Moustafa (2006)** revealed no significant effect on yolk index when BS used at 2% level in laying hens. **Moustafa (2006)** reported that BS at levels 0.05, 0.10 and 0.15% had significant increase on yolk color. **Nofal *et al.* (2006)** reported that BS supplementation had no effect on egg components (albumen and yolk percent) and shell thickness, while yolk index was significantly increased by addition of BS to the diets, **El-Bagir, *et al.* (2006)** reported that BS caused insignificant decrease in shape index of egg.

Concerning level of AA% (Table 3), there were insignificant effects on egg quality during all experimental period except, shape index. The adjusted dietary AA levels have a positive effect on average shape index of layers.

Serum constituents: Data of serum constituents are summarized in Table 4. The results of serum constituents indicated that dietary treatments had significant ($P \leq 0.01$ or $P \leq 0.05$) effect on calcium, triglycerides, AST, total protein and phosphorus values. It can be seen that hens fed diets, 1(control), 4 (11.75 CP% + AA), 7 (13.25 CP% + AA +1% BS), 7 (13.25 CP% + AA +1% BS) and 11 (14.75 CP% +2% BS) had higher serum calcium, triglycerides, AST, total protein and phosphorus values, respectively. However, no significant differences were found among dietary treatments for the other serum constituents.

There were significant ($P \leq 0.01$) effects on calcium and phosphorus values as related to dietary CP level (Table 4). Calcium and phosphorus values of serum significantly reduced as dietary protein levels decreased. Similar trend was found in the results for globulin, but the difference did not reach significance. In this respect, **Eggum (1989) and Tewe (1985)** stated that total serum protein, globulin and albumin were directly responsive to both protein quantity and quality. Albumin is serves as the major reservoir of protein and involved in colloidal osmotic pressure, acid-base balance, and it acts as a transport carrier for small molecules such as vitamins, minerals, hormones and fatty acids **(Margaret, 2001)**.

Concerning BS level effect (Table 4), there were insignificant effects on serum constituents during all experimental period except, AST, ALT, total protein, albumin and phosphorus. Hens fed diet containing 1% BS had higher ALT, while significant lower values were observed for hens fed diets containing 0.0% BS. Laying hens fed diet containing 0.0% BS had higher AST, while significantly lower value ($P \leq 0.01$) was observed for hens fed diet containing 2% BS. Hens fed diet containing 1% BS had higher total protein and albumin (lower phosphorus) while significant lower values were observed for hens fed diets containing 2.0 and 1.0% BS for total protein and albumin, respectively. These results disagree with those of **Nofal *et al.* (2006)** who reported that there was a significant decrease in the serum cholesterol levels due to supplementation of BS to Mamourah laying hens diets. Results of glucose agree with those of **Al- Awadi and Gumaa (1987) and El-Naggar and El-Deib (1992)** who reported that no significant change in fasting blood glucose level when BS (40 mg/day and 36 mg/day, respectively) was administered to normal and streptozotocin-induced diabetic rats.

Table 4 : Effects of using black seeds in Hy- Line W- 36 laying hen diets varying in their protein content on serum constituents.

Items	Calcium mg/dL	Cholesterol mg/dL	Triglycerides mg/dL	AST U/ml	ALT U/ml	Total Protein g/dL	Albumin (A) g/dL	Globulin (G) g/dL	A / G ratio	Glucose mg/dL	Phosphorus mg/dL
Treatments :											
1	20.94±1.3^{1A}	276.45±37.4	415.2±37.59^{A}	39.85±1.51^{A}	29.10±1.07	9.23±0.51^{ab}	5.59±0.6	3.73±0.8	1.53±0.81	192.8±34.1	10.2±0.86^{AB}
2	12.47±1.3^{DEF}	209.30±37.4	376.5±37.59^{A}	40.40±1.51^{A}	27.28±1.07	9.94±0.51^{b}	5.36±0.6	3.57±0.8	2.02±0.81	129.9±34.1	8.85±0.86^{BCDE}
3	12.50±1.3^{DEF}	197.67±37.4	225.3±37.59^{B}	36.73±1.51^{ABCD}	26.98±1.07	8.70±0.51^{b}	4.30±0.6	4.40±0.8	1.24±0.81	126.3±34.1	9.09±0.86^{BCD}
4	15.12±1.3^{BCDE}	226.45±37.4	454.0±37.59^{A}	40.03±1.51^{A}	27.25±1.07	8.68±0.51^{b}	4.51±0.6	4.17±0.8	1.13±0.81	165.5±34.1	9.46±0.86^{BC}
5	13.68±1.3^{CDEF}	213.37±37.4	417.4±37.59^{A}	36.95±1.51^{ABCD}	27.05±1.07	9.98±0.51^{ab}	4.94±0.6	5.05±0.8	1.07±0.81	180.9±34.1	9.82±0.86^{ABC}
6	17.24±1.3^{AB}	268.31±37.4	399.4±37.59^{A}	34.15±1.51^{BCD}	29.85±1.07	10.8±0.51^{a}	7.16±0.6	3.68±0.8	2.74±0.81	233.6±34.1	8.00±0.86^{BCDE}
7	16.14±1.3^{BCD}	272.09±33.4	401.0±33.62^{A}	40.58±1.35^{A}	31.36±0.96	11.0±0.46^{a}	5.61±0.5	5.36±0.7	1.22±0.72	205.9±30.5	7.78±0.86^{BCDEF}
8	12.71±1.3^{DEF}	241.57±37.4	249.7±37.59^{B}	32.65±1.51^{D}	28.35±1.07	9.86±0.51^{ab}	5.15±0.6	4.72±0.8	1.36±0.81	194.6±34.1	6.27±0.86^{DEF}
9	16.49±1.3^{BCD}	273.74±37.4	389.3±37.59^{A}	38.95±1.51^{AB}	31.15±1.07	9.34±0.51^{ab}	5.56±0.6	3.79±0.8	1.78±0.81	224.3±34.1	7.27±0.86^{CDEF}
10	14.62±1.3^{BCDEF}	235.17±37.4	362.5±37.59^{A}	37.25±1.51^{ABCD}	30.25±1.07	9.27±0.51^{ab}	6.20±0.6	2.97±0.8	2.45±0.81	194.8±34.1	5.09±0.86^{D}
11	18.65±1.3^{AB}	207.85±37.4	411.3±37.59^{A}	33.30±1.51^{CD}	29.80±1.07	9.51±0.51^{ab}	6.42±0.6	3.08±0.8	3.71±0.81	220.1±34.1	12.2±0.86^{A}
12	15.35±1.3^{BCDE}	210.85±43.1	373.8±43.41^{A}	38.10±1.74^{ABC}	28.60±1.24	9.46±0.59^{ab}	4.71±0.7	4.75±0.9	1.07±0.93	235.0±39.4	8.73±0.86^{BCD}
13	10.59±1.3^{F}	244.88±37.4	400.6±33.62^{A}	32.50±1.35^{D}	28.58±0.96	9.13±0.46^{b}	5.52±0.5	3.60±0.7	2.60±0.72	172.2±30.5	7.07±0.86^{BCDE}
14	11.50±1.3^{EF}	252.04±37.4	336.3±37.59^{AB}	34.75±1.51^{BCD}	28.70±1.07	9.95±0.51^{b}	4.83±0.6	4.12±0.8	1.65±0.81	168.3±34.1	5.82±0.86^{EF}
15	12.15±1.3^{DEF}	294.17±37.4	239.0±37.59^{B}	34.00±1.51^{CD}	29.80±1.07	8.65±0.51^{b}	4.92±0.6	3.74±0.8	1.35±0.81	186.3±34.1	5.73±0.86^{EF}
Over all mean	14.68±0.35	241.60±9.63	363.4±9.68	36.68±0.39	28.94±0.28	9.44±0.13	5.39±0.2	4.05±0.2	1.80±0.21	188.7±8.80	8.14±0.22
Level of CP % :											
14.75	18.94±0.87^{A}	250.9±20.9	408.6±26.89	35.77±1.15	29.58±0.68	9.89±0.23	6.39±0.35	3.50±0.45	2.66±0.45	215.35±19.4	10.1±0.63^{A}
13.25	13.22±0.61^{B}	232.5±14.5	341.4±18.63	36.75±0.80	28.64±0.47	9.55±0.23	5.16±0.24	4.39±0.41	1.63±0.31	175.94±13.4	8.04±0.43^{B}
11.75	13.92±0.62^{B}	249.2±14.8	366.4±19.01	36.99±0.81	29.03±0.48	9.15±0.33	5.18±0.25	3.97±0.32	1.57±0.32	186.69±13.7	7.20±0.44^{B}
Black seeds Level % :											
0.00	14.94±0.82	224.6±16.0	377.7±21.46	38.79±0.79^{A}	27.5±0.47^{B}	9.13±0.24^{B}	4.94±0.3^{b}	4.18±0.36	1.40±0.36	159.06±14.59	9.42±0.49^{A}
1.00	15.47±0.79	258.8±15.6	362.3±20.94	36.90±0.77^{A}	30.2±0.46^{A}	10.1±0.23^{A}	5.94±0.3^{a}	4.16±0.35	1.88±0.35	210.42±14.24	6.43±0.48^{B}
2.00	13.41±0.81	243.7±16.0	353.5±21.46	34.25±0.79^{B}	29.1±0.47^{A}	9.12±0.24^{B}	5.32±0.3^{ab}	3.80±0.36	2.15±0.36	193.27±14.59	8.07±0.49^{AB}
Level of amino acid :											
Requirement	16.01±0.55^{A}	245.8±12.0	396.0±14.60^{A}	37.86±0.61^{A}	29.31±0.39	9.60±0.19	5.55±0.21	4.04±0.27	1.88±0.27	196.46±11.2	8.64±0.39
Low	12.62±0.66^{B}	238.1±14.4	319.2±17.52^{B}	34.91±0.74^{B}	28.50±0.47	9.26±0.23	5.20±0.26	4.06±0.32	1.72±0.32	175.71±13.5	7.38±0.46

[1] Mean ± Standard error of the mean.

a,....b, and A,... F, values in the same column within the same item followed by different superscripts are significantly different (at $P \leq 0.05$ for a to b ; $P \leq 0.01$ for A to F).

Concerning the level of AA% (Table 4), there were insignificant effects on serum constituents during all experimental period studied except, calcium, triglycerides and AST. The adjusted dietary AA levels have a positive effect on average calcium, triglycerides and AST values of serum. Increasing these parameters in the groups feed the requirement of AA related to the increasing of egg production and EM of these groups.

Immune responses: Values of total immune response are listed in Table (5). No significant effects on immune response as a result to BS supplementation was found in laying hen diets throughout the whole experimental period except hemoglobin. It can be observed that hens fed diet 2 (13.25 CP% + AA) had significantly higher hemoglobin level, where those fed diet 9 (11.75 CP% + AA +1% BS) had significantly lower hemoglobin.

The results indicated that level of CP% insignificantly affected all immune response except, secondary immunity response, hematocrit % and white blood cells values (Table 5). The secondary immunity response values significant increased in its values as dietary protein levels decreased. Red blood cells values were insignificantly reduced as dietary protein levels decreased.

Concerning BS level effect (Table 5), there were insignificant effects on immunity response during all experimental periods except, hemoglobin%. It is clear that hemoglobin value was significantly decreased as black seed levels increased. Regarding to white blood cells values were insignificantly increased as dietary BS levels increased. Similar findings were obtained by **Khodary *et al* (1996)** in Balady laying hens fed diets supplemented with BS at levels ranging from 1 up to 3 % and the values of WBSc count increased as the level of BS increased. This may be due to the direct effect of BS on the haemopoietic tissues.

Concerning level of AA% (Table 5), there were insignificant effects on immunity response during all experimental periods.

Economical efficiency (EEf): Table 6 show the economical efficiency (EEf) and the relative economical efficiency (relative EEf) values of dietary treatments. Hens fed diet 3 (13.25 CP% - AA) gave the best economical and relative efficiency values being 1.324 and 101.5 %, respectively. Whereas, those fed diet 14 (11.75 CP% + AA + 2% BS) had the worst corresponding values, being 0.641 and 49.13%, respectively. The rate of change in relative efficiency varied between –50.87 to +1.50 % which is of minor importance relative to the other factors affecting egg production.

In conclusion, the average values of net revenue and economic efficiency were lower with feeding laying hens on black seeds as well with and medium or low protein diets either with or without supplemental methionine and lysine. This may be due to the high price of BS, besides it had no improvement on the performance of Hy- Line W- 36. A similar conclusion was reached by **Nofal *et al.* (2006)** who reported a decrease in relative economic efficiency percentage was evident for groups fed diets supplemented with crushed NS seeds when compared with the control group. However, **Moustafa (2006)** indicated that the incorporation of BS in laying hen diets decreased total feeding cost.

Table 5 : Effects of using black seeds in Hy- Line W- 36 hen laying diets varying in their protein content on immune response.

Items	Primary immunity	Secondary immunity	Cellular immunity	Hemoglobin g/dL	Hematocrit %	Red blood cells 10^6xmm^3	White blood cells 10^3xmm^3
Treatments :							
1	4.50±1.74[1]	6.50±1.03	1.08±0.11	9.85±0.83^{ab}	35.26±2.39	3.36±0.37	68.75±9.20
2	9.00±1.74	8.00±1.03	1.07±0.11	10.4±0.76^{a}	37.77±2.39	3.06±0.46	47.04±9.20
3	5.00±1.74	7.50±1.03	1.08±0.11	9.73±0.76^{abc}	37.88±2.76	3.16±0.41	52.25±9.20
4	6.50±1.74	10.5±1.03	1.12±0.11	9.15±0.83^{abcd}	33.44±2.39	2.22±0.41	54.33±9.20
5	6.00±1.74	9.00±1.03	0.91±0.11	8.43±0.65^{abcd}	30.41±2.76	2.61±0.35	50.17±9.20
6	9.00±1.74	7.00±1.03	1.00±0.11	8.83±0.83^{abcd}	36.70±3.38	2.97±0.46	67.70±10.1
7	8.00±1.74	9.50±1.03	1.23±0.11	9.26±0.76^{abcd}	35.48±2.14	3.33±0.37	60.50±10.1
8	7.50±1.74	9.00±1.03	1.16±0.11	7.29±0.83^{bcd}	36.36±2.39	2.30±0.46	42.67±9.20
9	5.50±1.74	10.0±1.03	1.12±0.11	6.99±0.76^{d}	34.98±2.76	2.56±0.41	53.29±9.20
10	7.50±1.74	9.00±1.03	1.30±0.11	9.18±0.83^{abcd}	32.15±2.39	2.40±0.65	57.75±10.1
11	8.00±1.74	8.00±1.03	1.25±0.11	8.00±0.65^{abcd}	36.36±2.39	3.15±0.41	70.93±8.52
12	8.00±1.74	7.00±1.03	1.19±0.11	7.75±0.76^{abcd}	36.30±2.39	3.05±0.53	52.63±9.20
13	6.50±1.74	8.00±1.03	1.19±0.11	7.22±1.07^{bcd}	37.77±2.76	2.80±0.65	56.42±13.0
14	10.0±1.74	9.00±1.03	1.12±0.11	7.13±0.76^{cd}	32.95±3.38	3.17±0.46	54.51±10.0
15	6.00±1.74	8.00±1.03	1.15±0.11	7.39±0.93^{bcd}	35.29±2.39	3.01±0.53	47.87±11.3
Over all mean	7.13±0.45	8.40±027	1.13±0.03	8.44±0.21	35.27±0.68	2.88±0.12	55.99±2.54
Level of CP % :							
14.75	7.17±0.99	7.17±0.56^{b}	1.11±0.06	8.75±0.47	35.98±1.38^{ab}	3.18±0.23	69.31±4.99^{a}
13.25	7.33±0.70	8.17±0.40^{ab}	1.15±0.04	8.77±0.35	36.79±0.91^{a}	3.00±0.18	51.23±3.74^{b}
11.75	6.92±0.70	9.25±0.40^{a}	1.12±0.04	8.04±0.34	33.28±0.97^{b}	2.64±0.17	53.03±3.74^{b}
Black seeds Level % :							
0.00	6.20±0.74	8.30±0.49	1.05±0.05	9.43±0.34^{A}	35.04±1.09	2.87±0.17	54.51±4.08
1.00	7.50±0.74	8.90±0.49	1.16±0.05	8.30±0.36^{B}	34.99±1.09	2.79±0.20	55.67±4.30
2.00	7.70±0.74	8.00±0.49	1.18±0.05	7.57±0.36^{B}	35.94±1.13	3.07±0.22	57.82±4.47
Level of amino acid :							
Requirement	7.61±0.55	8.39±0.37	1.130±0.04	8.53±0.28	35.57±0.81	2.99±0.14	58.97±3.03
Low	6.42±0.67	8.42±0.45	1.131±0.04	8.37±0.36	34.92±1.00	2.73±0.19	50.58±3.99

[1] Mean ± Standard error of the mean.

a,....d, and A,... B, values in the same column within the same item followed by different superscripts are significantly different (at $P \leq 0.05$ for a to d ; $P \leq 0.01$ for A to B).

Table 6: Effects of using black seeds in Hy- Line W- 36 laying diets varying in their protein content on economical efficiency

Items		1	2	3	4	5	6	7	8
Price/ k feed (L.E.)	a	0.816	0.793	0.768	0.779	0.732	0.885	0.862	0.837
Total feed intake/hen (kg)	b	7.906	7.841	7.861	7.425	7.676	7.773	7.92	7.125
Total feed cost/hen (L.E.)	a x b = c	6.454	6.218	6.035	5.787	5.621	6.88	6.825	5.961
Total number of eggs/hen	d	59.5	56.45	56.10	48.56	47.42	58.17	51.33	51.00
Price/ egg (L.E.)	e	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total price of eggs /hen (L.E.)	d x e = f	14.88	14.11	14.03	12.14	11.86	14.54	12.83	12.75
Net revenue / hen (L.E.)	f – c = g	8.421	7.894	7.99	6.353	6.234	7.662	6.007	6.789
Economical efficiency (E.Ef.)	g / c = h	1.305	1.27	1.324	1.098	1.109	1.114	0.88	1.139
Relative E.Ef.	r	100	97.29	101.5	84.14	84.99	85.35	67.46	87.29
Items		9	10	11	12	13	14	15	
Price/ k feed (L.E.)	a	0.848	0.801	0.953	0.929	0.905	0.914	0.87	
Total feed intake/hen (kg)	b	7.648	7.798	7.657	7.391	7.941	7.46	7.773	
Total feed cost/hen (L.E.)	a x b = c	6.483	6.246	7.295	6.869	7.19	6.817	6.76	
Total number of eggs/hen	d	46.9	52.11	56.92	53.36	51	44.75	46.5	
Price/ egg (L.E.)	e	0.25	0.25	0.25	0.25	0.25	0.25	0.25	
Total price of eggs /hen (L.E.)	d x e = f	11.73	13.03	14.23	13.34	12.75	11.19	11.63	
Net revenue / hen (L.E.)	f – c = g	5.242	6.782	6.935	6.471	5.56	4.371	4.865	
Economical efficiency (E.Ef.)	g / c = h	0.809	1.086	0.951	0.942	0.773	0.641	0.72	
Relative E.Ef.	r	61.97	83.21	72.85	72.2	59.26	49.13	55.16	

a.................................. (based on average price of diets during the experimental time).
e.................................(according to the local market price at the experimental time).
g /c ....(net revenue per unit feed cost).
r..................................(assuming that economical efficiency of the control group (1) equals 100).

REFERENCES

Abdel-All, E.S.M. and Attia, R.S. (1993). Characterization of black cumin (*Nigella sativa*) seeds. 1. Chemical composition and lipids. *Alex. Sci. Exch.*, 14: 467- 482.

Abd–Elsamee, M.O. (2005). Effect of different levels of methionine and vitamin E on laying hen performance under heat stress conditions. *3rd International Poult. Conf. Hurghada. Egypt,* 726 - 741.

Abdo, Z.M.A. (1998). The effect of using some natural growth promoters and fats on broiler performance and immunity. *Ph. D. Thesis, Fac. Agric., Cairo, Univ., Giza, Egypt.*

Al-Awadi, F.M. and Gumaa, K.A. (1987). Studies on the activity of individual plants of an antidiabetic plant mixture. Acta Diabetol. 24:7-41.

Allain, C.C. (1974). *Clin. Chem.,* 20: 470.

Angelovicova, M. (1994). Economic use of a low-protein feed mixture in layer diet. *Zivocisna-Vyroba,* 39: 1049 - 1062.

Atta, A.M.; Abdou, A.M.; Mohamed, F.R. and Goher, N.E. (1998). Immunological variation among commercial broiler strains. *Egypt. J. Anim. Prod.,* 35: 113-124.

Babayan, V.K.; Koottungal, D. and Halaby, G. A. (1978). Proximate analysis, fatty acid and amino acid composition of *Nigella sativa* seeds. *J. Food Sci.,* 43: 1314-1315.

Bauer, J.D. (1970). Numerical evaluation of red blood, white blood cells and platelets, part III, Haematology in; Gradwoll, Clinical Laboratory Methods and Diagnoiss, Ed Frankel, S.; Reitman, S. and Somen Wirth, A. C. 7th Ed. C. V. Mosby Co., Saint Louis, USA.

Bunchasak, C.; Poosuwan, K.; Nukrawe, R.; Markvichitr, K. and Chwthesam, A. (2005). Effect of dietary protein on egg production and immunity response of laying hens during peak production period. *Int. J. Poult. Sci.,* 4: 701-708.

Cable, M.C. and Waldroup, P.W. (1991). Effect of dietary protein level and length of feeding on performance and abdominal fat content of broiler chickens. *Poult. Sci.,* 70: 1550-1558.

Calderon, V. and Jensen, L.S. (1990). The requirement for sulfur amino acid by laying hens as influenced by the protein concentration. *Poult. Sci.,* 69: 934-944.

Carter, T.C. (1968). The hen egg. A mathematical model with three parameters. *Br. Poult. Sci.,* 9: 165 - 171.

Dix, M.C. and Taylor, R.L.J. (1996). Differential antibody responses in 6.B maior histocompatbility (B) complex congenic chickens. *Poult. Sci.,* 75: 203 - 207.

Doran, B.H.; Quisenberry, J.H.; Krueger, W.F. and Bradley, J.W. (1980). Response of thirty egg type stocks to four layer diets differing in protein and caloric levels. *Poult. Sci.,* 59: 1082-1089.

Dumas, B.T. and Biggs, H.G. (1972). In Standard Methods of Clinical Chemistry. *Vol 7, Academic Press New York, USA.*

Duncan, D.B. (1955). Multiple range and multiple F Test, *Biometrics,* 11:1-42.

Eggum, B.O. (1989). Biochemical and methodological principles. In: H.D. Bock, B. Eggum, A.G. Low, O. Simon and T. Zebrowska (eds), Protein metabolism in farm animals. Evaluation, digestion, absorption and metabolism, (Oxford Science Publication‹ Deutscher Handwirtschafts Verlag, Berlin), 1-52.

El-Alfy, T.S.; El-Fatatry, H.M. and Toama, M.A. (1975). Isolation and structure assignment of an antimicrobial principle from the volatile oil of *Nigella sativa L.* seeds. *Pharmazia,* 30: 109-111.
El-Bagir, N.M.; Hama, A.Y.; Hamed, R.M.; Abd El-Rahim, A.G. and Beynen, A.C.(2006). Lipid composition of egg yolk and serum in laying hens fed diets containing black cumin (*Nigella sativa*). *Int. J. Poult. Sci.,* 5: 574-578.
El-Dakhakhny, M. (1963). Studies on the chemical constitution of Egyptian *Nigella sativa L.* seeds II. The essential oil. *Plant Med.* 11, 465-470.
El-Ghamry, A.A.; Abdel-Samee, L.D. and Ibrahim, M.R.M. (1997). Effects of feeding Black cumin (*Nigella sativa*) meal and rice polishing at different levels on the laying performance and some blood plasma constituents. *Egypt. J. Nutr. and feeds,* 1: 311-320.
El-Kaiaty, A.M.; Soliman, A.Z.M. and Hassan, M.S.H. (2002). The physiological and immunological effects of some natural feed additives in layer hen diets. *Egypt. Poult. Sci.,* 22: 175-203.
El-Naggar, A.M. and El-Deib, A.M. (1992). A study of some biological activities of *Nigella sativa* (black seeds). "Habat El Baraka". J. Egypt Soc Pharmacol Exp. Ther. 11: 781-799.
Fernandez, R.; Salman, A.J. and Ginnis, J.M. (1973). Effect feeding different protein levels and of changing protein level on egg production. *Poult. Sci.,* 52: 64-69.
Gad, A.M.; El-Dakhakhny, M. and Hassan, M.M. (1963). Studies on the chemical constitution of Egyptian *Nigella sativa L.* oil. *Planta Medica,* 11: 134-138.
Glick, B.; Taylor, R.L. J.; Martin, D.E.; Watabe, M.; Day, E.J. and Thompson, D. (1983). Caloric-protein deficiencies and immune response of the chicken. II. Cell mediated immunity. *Poult. Sci.,* 62: 1889 - 1893
Goodwin, J.F. (1970). *Clin. Chem.*, 16: 776-780.
Harms, R.H.; Ruiz, N. and Mils, R.D. (1990). Research note; Conditions necessary for a response commercial laying hen to supplemental choline and sulfate. *Poult. Sci.,* 69: 1226-1229.
Haugh, R.R. (1937). The Haugh unit for measuring egg quality. *US Egg Poult. Mag.*, 43: 552-555.
Howanitz, P.J. and Howantitz, J.H. (1984). In Clinical Diagnosis and Management by laboratory Methods. 168.
Humphrey, B.D.; Koutsos, E.A. and Klasing, K.C. (2002). Requirements and priorities of the immune system for nutrients Nutrition biotechnology in the feed and food industries. Proceedings of Alltech's 18th annual symposium Pages 69 – 77 Lyons T.P. Jasques K.A. Nottingham, UK Nottingham University Press.
Ibrahim, S.A.; EL-Alaily, H.A.; EL-Faham, A.I. and Thabet, H.A. (2007). Effect of different dietary protein and energy levels during the late stage of the egg production cycle on hy-line w36 performance and egg shell quality. *4th World Poultry Conference 27- 30 March, Sharm El- Sheikh, Egypt,* 269-280.
Khodary, R.M.; El-Azzawy, M.H. and Hamdy, I.R. (1996). Effect of *Nigella sativa* on egg production, hatchability percentage and some biochemical values in laying hens with reference to fertility in cockerels. *7th Sci. cong., Fac. Vet. Med., Assuit. Univ.,* 17-19 Nov. Ass. Egypt, 91-106.

Leeson, S. and Caston, L.J. (1997). A problem with characteristics of the thin albumin in laying hens. *Poult. Sci.,* 76: 1332 - 1336.
Lehman, H.P. and Henry, J.B. (1984). In Clinical Diagnosis and Management by laboratory methods. 1431-1438.
Liu, Z.; Wu, G.; Bryant, M.M.; Roland, D.A.S. (2005). Influence of added synthetic lysine in low-protein diets with the methionine plus cysteine to lysine ratio maintained at 0.75. *J. Appl. Poult. Res.,* 174-182.
Mahfouz, M. and El-Dakhakhny, M. (1960). Some chemical and pharmacological properties of the new anti-asthmatic drug "Nigellone" *Alex. Med., J.* 6: 357-366.
Mandour, A.A.; Mahmoud, K.; Abou El-Wafa, A.; El-Agamy, E. and Ragab, O. (1995). Effect of aflatoxin and *Nigella sativa* seeds on serum protein and its electrophoretic patterns in White Pekin ducklings. *1st Egyptian Hungarian Poultry Conf., 17-19Sep., Alexandria Egypt.*
Manzoor, A.C.; Qureshi, M.A. and Havenstein, G.B. (2003). A comparison of the immune profile of commercial broiler strains when raised on marginal and high protein diets. *Int. J. Poult. Sci.,* 5: 300-312.
Margaret, A.W. (2001). Avian Plasma Proteins. http:// www.exoticpetvet.net
Moustafa, K.M.E. (2006). Effect of using commercial and natural Growth promoters on the performance of Commercial laying hens. *Egypt. Poult. Sci.,* 26: 941-965.
Narvaez-Solarte, W.; Rostagno, H.S.; Soares, P.R. and Silva, M.A. (2005). Nutritional requirements in methionine + cystine for white-egg laying hens during the first cycle of production. *Int. J. Poult. Sci.,* 4: 965-968, 2005
National Research Council, NRC (1994). Nutrient Requirements of Poultry. 9th revised edition. National Academy Press. Washington, D.C., USA.
Nofal, M.E.; Abo-Etta, E.M. and Salam, A.A. (2006). Some productive and physiological responses to dietary *Nigella sativa* seeds supplementation of Mamourah laying hens. *Egypt. Poult. Sci.,* 26: 455 – 476.
Radwan, N.L. (2004). Effect of using some medicinal plants on performance and immunity of broiler chicks. *Ph. D. Thesis, Fac. Agric. Cairo, Univ., Giza, Egypt.*
Reitman, S. and Frankel, S. (1957). *Amer. J. Clin. Path.,* 28: 56.
Schutte, J.B.; Dejong, J. and Bertram, H.L. (1994). Requirement of the laying for sulfur amino acids. *Poult. Sci.,* 73 : 274 - 280.
Sedaros, A.F.W. (2000). Study on the effect of supplementing fenugreek, black seed and guar in the diet on the performance of Japanese quail. *M. Sc. Thesis, Fac. of Agric., Tanta Univ. Kafr El-Sheikh, Egypt.*
Soltan, M.A. (1999). Effect of diets containing *Nigella sativa* (black seeds) and /or Ox bile on growth and reproductive performance of Japanese quail. *Alex. Vet. Sci.,*15: 655-669.
Steel, R.G.D. and Torrie, J.H. (1980). Principles and Procedures of Statistics: A Biometrical Approach 2nd ed. McGraw-Hill Book Co., Inc., New York, USA.
Summers, J.D. (1993). Reducing excretion of the laying hen by feeding lower crude protein diets. *Poult. Sci.,* 72: 1473 - 1478.
Tewe, O.O. (1985). Protein metabolism in growing pigs fed corn or cassava peel based diets containing graded protein levels. *Res. Vet. Sci.,* 29: 259-263.

Tollba, A.A.H.; Abd El-Galyl, M.A. and Abd El-Samad, M.H. (2005). The effect of using some herbal additives on physiological and productive performance of two Egyptian chicken strains during winter and summer seasons. *Egypt. Poult. Sci.*, 25: 107 - 123.

Wahlefeld, A.W. (1974). In Methods of Enzymatic Analysis. Vol 5, HU Bergmever, Ed. Academic Press, New York, 1831-1835.

Weichselbaum, P.E. (1946). *Am.J.Path.*, 16: 40.

Well, R.J. (1968). The measurement of certain egg quality: A study of the hens egg. Ed. By T.C. Carter Pub. Oliver and Boy Edinbrugh pp. 220-226 and 235-236.

Wintrobe, M.M. (1965). *Clinical Haematology*, 4th ed Philadelphia.

Wu, G.; Bryant, M.M. and Roland, D.A.S. (2005). Effect of synthetic lysine on performance of commercial Leghorns in Phase II and III (second cycle) while maintaining the methionine + cysteine /lysine ratio at 0.75. *Poult. Sci.*, 84 (Suppl.1):43. (Abstr.)

Yakout, H.M. (2000). Response of laying hens to practical and low protein diets with ideal TSAA: laying rations:- effects on egg production, components, nitrogen and nitrogen excretion. *Ph. D. Thesis, Fac. Agric. Alexandria Univ., Alexandria Egypt.*

Yamamoto, Y.; and Glick, B. (1982). A comparison of the immune response between two lines of chickens selected for differences in the weight of the bursa of fabricius. *Poult. Sci.*, 61: 2129-2132.

Zeweil, H.S. (1996). Evaluation of substituting Nigella seed oil meal for soybean meal on the performance of growing and laying Japanese quails. *Egypt. Poult. Sci.*, 16: 451-477.

تأثير استخدام بذور حبة البركة علي إنتاج البيض و جودة البيض والاستجابة المناعية في علائق الدجاج البياض المختلفة في محتواها من البروتين

حنان عبد الله حسن و مني سيد رجب

قسم الدواجن - كلية الزراعة - جامعة الفيوم - مصر

أجريــت هــذه التجربة في المزرعة التجريبية الخاصة بقسم الدواجن - كلية الزراعة - جامعة الفيوم. بغرض تقييم استخدام بذور حبة البركة كإضافات غذائية طبيعية في علائق الدجاج البياض. كان العدد المستخدم 180 دجاجة بياضة عمر 49 أسبوع من سلالة الهاي لين دبليو 36 قسمت الطيور عشوائياً إلي 15 معاملات (12 طائر/معاملة) واحتوت كل معاملة علي 12 مكرر (1 دجاجة/مكرر) كما يلي:

1- كنترول (14.75 %بروتين خام).

2-تغذية الطيور علي 13.25 % بروتين خام +مع ضبط مستوي المثيونين والليسين.

3- تغذية الطيور علي 13.25 % بروتين خام +مع عدم ضبط مستوي المثيونين والليسين.

4- تغذية الطيور علي 11.75 % بروتين خام +مع ضبط مستوي المثيونين والليسين.

5- تغذية الطيور علي 11.75 % بروتين خام +مع عدم ضبط مستوي المثيونين والليسين.

6- تغذية الطيور علي عليقة 1+ 1% بذور حبة البركة.

7- تغذية الطيور علي عليقة 2+ 1% بذور حبة البركة.

8- تغذية الطيور علي عليقة 3+ 1% بذور حبة البركة.

9 - تغذية الطيور علي عليقة 4+ 1% بذور حبة البركة.

10- تغذية الطيور علي عليقة 5+ 1% بذور حبة البركة.

11- تغذية الطيور علي عليقة 1+ 2% بذور حبة البركة.
12- تغذية الطيور علي عليقة 2+ 2% بذور حبة البركة.
13- تغذية الطيور علي عليقة 3+ 2% بذور حبة البركة.
14- تغذية الطيور علي عليقة 4+ 2% بذور حبة البركة.
15- تغذية الطيور علي عليقة 5+ 2% بذور حبة البركة.

وتتلخص أهم النتائج المتحصل عليها فيما يلي:

1- كان هناك تأثير معنوي بين المعاملات بالنسبة للأداء الإنتاجي فيما عدا وزن البيضة و كفاءة تحويل البروتين و معدل الزيادة في وزن الجسم. كان هناك تأثير موجب بالنسبة لإنتاج البيض وكتلة البيض خلال الفترة التجريبية (49-60 أسبوع من العمر) كنتيجة لزيادة مستوي البروتين. وكان متوسط تحويل الغذاء والطاقة يزداد بخفض مستوي البروتين في العليقة. لم يكن لمستوي حبة البركة أي تأثير معنوي بين المعاملات بالنسبة للأداء الإنتاجي.

2- كان هناك تأثير معنوي بين المعاملات بالنسبة لجودة البيض فيما عدا لون الصفار ووزن القشرة%. أظهر دليل الصفار ووحدات هاو زيادة معنوية كنتيجة لخفض مستوي البروتين. تغذية الطيور علي 2% حبة بركة أدي إلي زيادة سمك القشرة، بينما التغذية علي 1% حبة بركة أدي إلي زيادة دليل الصفار. تغذية الدجاج علي صفر% حبة بركة أدي إلي زيادة دليل الشكل.

3- كان هناك تأثير معنوي نتيجة إضافة حبة البركة علي محتوي السيرم من الكالسيوم، الجلسريدات الثلاثية، AST ،البروتين الكلي و الفسفور. أدي خفض مستوي البروتين إلي خفض مستوي الكالسيوم والفسفور معنوياً. أدي تغذية الدجاج علي 1% حبة بركة إلي رفع مستوي ALT.

4- لم يكن هناك أي تأثير معنوي بالنسبة للاستجابة المناعية كنتيجة للمعاملات التجريبية في علائق الدجاج البياض خلال الفترة التجريبية فيما عدا الهيموجلوبين%. لم يكن هناك أي تأثير معنوي لمستوي البروتين بالنسبة للاستجابة المناعية فيما عدا المناعة الثانوية ونسبة الهيماتوكريت وعدد كرات الدم البيضاء. كان هناك زيادة في قيم المناعة الثانوية نتيجة لخفض مستوي البروتين. لم يكن هناك أي تأثير معنوي لمستوي إضافة حبة البركة علي الاستجابة المناعية خلال الفترة التجريبية فيما عدا الهيموجلوبين%. حيث كان هناك نقص في نسبة الهيموجلوبين بزيادة مستوي حبة البركة.

5- أعطت الدجاجات المغذاة علي عليقة 3 (13.25% بروتين خام + مع عدم ضبط مستوي المثيونين والليسين) أحسن كفاءة اقتصادية واعلي كفاءة اقتصادية ونسبية (1.324 و 101.5%) وتراوحت قيمة الكفاءة النسبية بين -50.87 إلي +1.5%.