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

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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 ; 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≤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≤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.

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≤0.01) 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≤0.01) were observed for hens fed diet 8 (13.25 CP% – AA +1% BS).

Concerning level of CP% (Table 3), there were significant (P≤0.05 and P≤0.01) 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≤0.01) was observed for hens fed diet containing 1% black seed. Laying hens fed diet containing 1% BS had higher yolk index, while significant lower value (P≤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≤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≤0.01 or P≤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≤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.

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.

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