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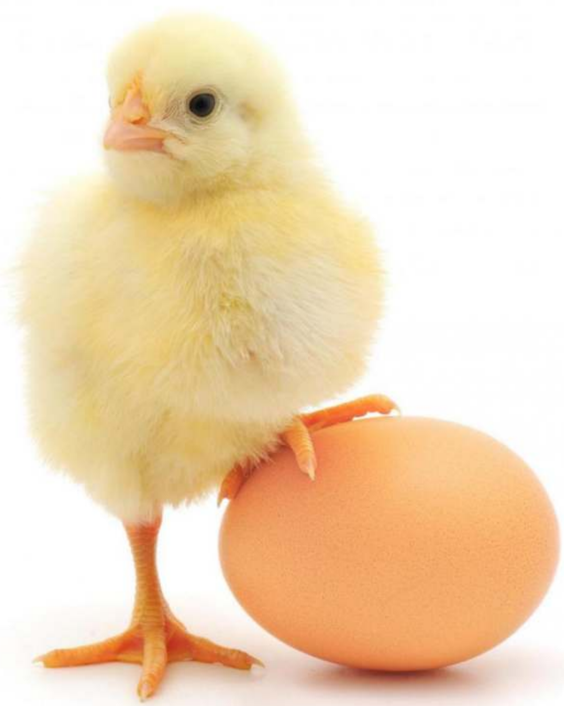

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Immunomodulation of Black Seed in Two Strains of Laying Hens

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Abstract: There are limited studies that investigated the effect of black seed supplementation on the immune status of laying hens. The current study investigated the effect of different levels of dietary black seed on the immune status of different strains of laying hens. A total of 600 pullets were used, 300 from Hy-Line Variety W-98 white and 300 from Hy-Line Variety brown. The white and brown pullets were divided into five groups, 60 pullets each which was divided into three replicate (n = 3), each replicate includes 20 pullets. The first group received a regular diet with no black seed (T1, control group). The second group received a diet containing 1.5% black seed from 28 wk of age until 70 weeks of age (T2). The third group received a diet containing 3.0% black seed from 28 wk of age until 70 weeks of age (T3). The fourth group received a diet containing 1.5% black seed from 16 weeks of age until 70 weeks of age (T4). The fifth group received a diet containing 3.0% black seed from 16 weeks of age until 70 weeks of age (T5). Antibody titer, cell- mediated immune response and White Blood Cell Counts (WBCs) were measured. Results showed that feeding layer chickens on 1.5 or 3.0% black seed increased the total WBCs and the difference was significant for the white hens ($p<0.02$). Black seed generally enhanced cell-mediated immune response and antibody titer, however, this effect was not significant. In conclusion, black seed could be used to enhance the immune status of laying chickens.

Key words: Black seed, antibody titer, immune status, laying chickens

INTRODUCTION

Harsh environmental conditions caused stress that negatively affects physiological functions, feed consumption, body weight gain and feed conversion efficiency of chickens (Korver *et al*., 1998, Takahashi *et al*., 2000). One of the ways for elevating production performance of chickens is to use effective nutritional additives in the feed such as black seed.

Nigella sativa L. (Ranunculaceae family) seeds or what is commonly known as black seeds or black cumin seeds are known since thousands of years ago for their health benefits for both human and animals. The main active ingredient in black seeds is crystalline nigellone. The seeds also contain beta sitosterol, thymoquinone, myristic acid, palmitic acid, stearic acid, palmitoleic acid, oleic acid, linoleic acid, arachidonic acid, proteins and vitamins B1, B2 and B3. They also contain calcium, folic acid, iron, copper, zinc and phosphorous. The black seeds are known for centuries to be anti-hypertensive, anti-parasitic, anti-inflammatory, anti-histamine, immune-stimulator, anti-tumor and anti-bacterial (Ait Mbarek *et al*., 2007; Ali and Blunden, 2003; Worthen *et al*., 1998; Swamy and Tan, 2000; Gilani *et al*., 2004; Ghosheh *et al*., 1999).

Several research studies reported that poultry diets supplemented with black seed have positive benefits in production performance in addition to their ability to modulate the immune function. However, there is inconsistency in the literature because some studies showed no effect.

A number of poultry studies investigating effects of dietary black supplementation in broiler chickens have shown positive effects. For example, Toghyani *et al*. (2010) fed broiler chickens on diet containing 2 and 4 g/kg black seed and showed that black seed supplementation caused a marked ($p<0.05$) increase in the weight of lymphoid organs of broiler chickens at 42 days. This increase may indicate Immunocompetence state. In the same study, antibody titer was not statistically different among treatments. Also, Shewita and Taha (2011) investigated the effect of 2, 4, 6, 8 and 10 g black seed/kg diet of broiler chickens on antibody titer against Newcastle disease, phagocytic activity and phagocytic index and White Blood Cells Count (WBCs). There was no significant difference on antibody titer against Newcastle virus, WBCs count. However, lymphoid organs (Bursa and thymus) improved significantly with increasing black seed level in all supplemented groups. Al-Beitawi (2009) investigated the effect of using crushed Nigella sativa seeds instead of the antibiotic growth promoter bacitracin methylene disalicylate in broilers. Results showed that antibody titer against Newcastle and infectious bursal diseases were significantly improved. In the same study, growth performance was also improved significantly. Co-administration of black seed at a level of 2.5% with chronic administration of oxytetracycline acts as immunoprotective agent because it produces immunostimulant effects in pigeons (Al-Ankari, 2005). In addition to its immune-stimulatory effect, black seed

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supplementation has a significant effect on body weight gain, feed consumption, feed conversion and feed intake of broiler chickens (Durrani *et al*., 2007; Guler *et al*., 2006; Mehmet, 2008; Al-Beitawi *et al*., 2009).

The main objective of the current study is to investigate the effect of feeding different levels of dietary supplementation of black seed on the immune responses in two strains of laying chickens.

MATERIALS AND METHODS

Birds, diet and housing: A total of 600 pullets from two different strains were used in this experiment, 300 pullets from Hy-Line Variety W-98 white and 300 pullets from Hy-Line Variety brown. The pullets were fed pre-lay ration from 16 weeks till 22 weeks of age (17.5% protein, 2750 kcal/kg) and laying ration from 23 weeks of age to the end of the laying period (18.0% protein, 2900 kcal/kg). Food and water were fed *ad libitum*. The hens were provided with 14 h of light and 10 h of darkness. Both the brown and the white pullets were divided into five groups. Every group had 60 pullets which is divided into three replicate (n = 3), each replicate involved 20 pullets. The first group received a regular diet with no black seed added in the feed and served as the control group (T1). The second group received the regular diet until the pullets were 28 wk of age and then received a diet containing 1.5% black seed until the end of the experiments (T2). The third group received the regular diet until the pullets were 28 wk of age and then received a diet containing 3.0% black seed until the end of the experiment (T3). The fourth group received a diet containing 1.5% black seed from 16 weeks of age until the end of the experiment (T4). Finally, the fifth group received a diet containing 3.0% black seed from 16 weeks of age until the end of the experiment (T5).

Measurement of immune response

Humoral immune response: Sheep Red Blood Cells (SRBC) were diluted to a 7% vol/vol suspension in 0.9% NaCl. Chicks were intravenously injected with 1 ml of the 7% washed SRBC and blood samples are collected 7 days post-challenge Then, sera samples were prepared by centrifugation at room temperature (1000 rpm), samples could be stored at-20°C until tested. The test is performed in 96-well U-bottom plates. Sera samples were added to the first and second wells (25 µl). Then, 25 µl of 0.9% NaCl (prepared previously and kept in the fridges, prewarmed before use in the assay) were added to columns 2-11. Serial dilutions (1:2) were made from column 2-11. A negative control of phosphate buffer saline was used in column 12. Then, 25 µl of a 2% solution of sheep red blood cells were added to each well. The plates were vortexed gently for a few seconds and incubated overnight at room temperature. The titers were recorded as the column number of the last plasma dilution showing clear evidence of agglutination (clear ring). In other words, titers were expressed as log of the reciprocal of the highest dilution exhibiting visible agglutination.

Cell-mediated immune response: The PHA-skin test involves subcutaneous injection of a mitogen (phytohemagglutinin, PHA) and measurement of subsequent swelling as a surrogate of T-cell mediated Immunocompetence. The PHA is dissolved in Phosphate-Buffered Saline (PBS) and the resulting concomitant swelling is quantified at the site of injection over time. The resulting swelling, usually measured 24 h post-injection, is interpreted as an index of cell-mediated Immunocompetence. Prior to injection, the injection site (wattle) should be marked. The thickness of the injection site was measure with micrometer. Then birds were injected intradermally in the wattle with 0.5 mg of PHA-P in 0.1 mL of PBS. Post-injection thickness was typically measured 24 h post-injection. Wattle swelling was calculated as the difference between the thickness of the wattle prior to and after injection of PHA-P (Goto *et al*., 1978).

White blood cell counts: Total White Blood Cell (WBC) were counted by a hemocytometer slide and light microscopy using brilliant crystal blue stain.

Statistical analysis: The data were analyzed using a one-way ANOVA utilizing the S-Plus statistical program (Crawley, 2002). Means were separated using Tukey's test and the significance was set at $p<0.05$.

RESULTS AND DISCUSSION

The current study investigated the effect of different levels of black seed as dietary supplementation in white and brown laying hens on different parameters of the immune response. These parameters involved total count of circulating WBCs, antibody titer as humoral immune response and cell-mediated immune response. For cell-mediated immune response, PHA test was used.

Circulating blood leukocytes represent a pivotal measure of the immune system status. They possess an effective influence on foreign particles in immune responses. Consequently, they are considered as a mirror which reflects the immune status strength. Thus, counting the total and differential numbers of leukocytes is a general parameter of the health status. Abnormal numbers of the specific types of leukocytes may indicate immunosuppression or immunocompetence in response to an immunomodulator or an infectious challenge (Brennan *et al*., 2002; Eeva *et al*., 2005; Swamy *et al*., 2004; Mashaly *et al*., 2004).

Total circulating WBC was measured in blood samples collected at 28, 35, 53 and 70 weeks of age for both Hy-Line Brown and White Hens. Table 1 shows the effect of

Table 1: Effect of black seed dietary supplementation on total circulating White Blood Cells (WBCs), antibody response and PHA response in Hy-Line Brown and White Hens

	Hy-Line brown hens					White hens				
Strain	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5
Total WBC (Millions/m)	49.3 ^{a}±7.89	57.0^{a}±5.40	53.1^{a}±6.04	47.9^{a}±2.14	53.9^{a}±9.99	42.5 ^{b}±2.14	50.1^{b}±11.5	56.9^{a}±8.73	59.8^{a}±4.29	42.4^{b}±10.33
Antibody Response	10.18^{ab}±0.75	10.00^{a}±0.77	9.18^{ac}±0.94	10.17^{a}±0.94	9.36^{a}±0.81	7.58^{a}±2.75	9.67^{b}±2.52	8.00^{a}±1.76	7.33^{a}±2.67	7.91^{a}±2.43
PHA Response	133.3^{a}±35.53	199.1^{a}±78.01	187.1^{a}±50.27	193.2^{a}±50.86	167.6^{a}±49.93	61.1^{a}±13.6	112.4^{a}±11.49	105.8^{a}±7.57	96.8^{a}±40.20	105.8^{a}±58.6

T1 = Control group, no black seed added in the feed
T2 = 1.5% black seed added from 28 weeks of age until the end of the experiment
T3 = 3.0% black seed added from 28 weeks of age until the end of the experiment
T4 = 1.5% black seed added from 16 weeks of age until the end of the experiment
T53 = 3.0% black seed added from 16 weeks of age until the end of the experiment
WBC = White blood cells
PHA = Phytohemagglutinin. Means within the same row of the same strain with different superscripts are significantly different (p<0.015)

black Seed dietary supplementation on total circulating WBCs. The black seed dietary supplementation resulted in a significant increase in circulating WBC total count in the white hens. Also, there was increase in the total number of WBC in the case of brown hens but it did not reach statistical significance.

Results of the current study showed that for the control group where no black seed added, the overall total WBC for the brown hens (49.3 million/ml) was higher than that of the white hens (42.5 million/ml). This could indicate a better general immune status of the brown hens than the white hens.

Feeding the layer chickens on dietary treatments using 1.5 or 3.0% black seed for both white and brown Hy-Line hens increased the total WBC and the difference was significant for white hens ($p<0.02$). These results indicate that using black seed in the diet of laying hens could improve their immune status as lymphocytes which are the major part of WBC, are involved in both humoral and cell-mediated immune response.

For antibody titer, chickens were injected intravenously with 10% washed sheep red blood cells and the humoral response was measured a week after. The effect of dietary black seed treatment on overall antibody response against Sheep Red Blood Cells (SRBC) for both Hy-Line Brown and White Hens is shown in Table 1. Results were expressed as log of the reciprocal of the highest dilution exhibiting visible agglutination.

Results showed that regardless of the treatment, the antibody response for the brown hens was better than that of the white hens, emphasizing the fact that the immune status of the brown hens could be genetically better than that of the white hens. In addition, results showed that treatments used in the study somewhat improved the antibody response for white hens and brown hens. In other studies that where birds are challenged with viruses, a significant Immunocompetence effect of the black seed was observed. For example, Durrani *et al.* (2007) investigated the effect of feeding broiler chickens on diet enriched with black seed on antibody titers in challenged birds. The authors of the study showed that mean antibody titers against Newcastle disease virus, Infectious Bronchitis and Infectious bursal disease virus were significantly higher in the group of birds that received the black seed diet than the control group. Also, Yaseen (2003) reported that extract of black seed had antiviral activity against Newcastle disease virus. On the other hand, recent studies showed that black seeds did not have significant effect on antibody titers against Newcastle virus in broiler chickens (Shewita and Taha, 2011; Toghyani *et al.*, 2010).

The PHA test has been used after the pioneering work by Goto *et al.* (1978), who showed a reduction of the skin response in thymectomized chickens (thus being unable to produce circulating T-cells). Histologically, this reaction was characterized by a perivascular accumulation of lymphocytes and macrophage migration in the central layer of the wattles. Heterophilic infiltration was observed mostly at early hours of the reaction. These responses were significantly decreased in cases of neonatal thymectomy. Therefore, the PHA skin test was considered to be a thymus-dependent response (Martin *et al.*, 2006).

The effect of dietary black seed treatment on PHA response of white and brown Hy-Line hens is shown in Table 1. The resulting concomitant swelling was quantified at the site of injection after 24 h.

As to the differences in the cell-mediated immune response measured by the PHA response in the control group between the white and brown hens, results of the current study showed that the PHA response of the brown hens was better than that of the white hens. This is an important observation for the poultry industry planning to use brown hens as their laying stocks.

In addition, results showed that using 1.5 or 3.0% of black seed in the diet of both white and brown Hy-Line hens increased the PHA response indicating that, it enhanced the cell-mediated immune response. However, since the variability within groups was high, the differences were not significant ($p>0.05$). Therefore, our results could indicate that using black seed in the diet of laying hens might improve their general humoral and cellular immune responses. This information could be important for the poultry industry to improve the immune response of their hens by dietary manipulation using effective additives such as the black seed.

In conclusion, dietary supplementation with black seeds could enhance the immune status of laying hens. Also, the brown laying hens may be more immune-competent than the white laying hens. This could have an important application in the poultry industry.

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