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EFFECT OF FEEDING NIGELLA SEED MEAL ON THE IMMUNE RESPONSE AND SOME BLOOD CONSTITUENTS OF GIMIZAH CHICKS.

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SUMMARY

Four hundred , unsexed one day – old Gimizah chicks were used to evaluate the effects of feeding Nigella seed meal (NSM) instead of Soybean meal (SBM) on the performance, some blood constituents and immunity response . The chicks were divided randomly into four equal groups (100 chicks each) . Each group were divided into four replicates (25 chicks each) . The first group was served as control, Nigella seed meal was used instead of SBM on the basis of protein units with the levels of 10 , 30 and 50% for the second , third and fourth group, respectively . Accordingly , eight experimental diets were formulated to contain about 19.2% crude protein (CP) and 3075 Kcal ME / kg feed during the grower period (1– 4 weeks) and 17.2% CP with 3125 Kcal ME/ kg feed during the finisher period (5-12 Weeks) . Feed and water were offered ad Libitum and birds were kept under similar conditions during the whole experimental period . Live body weight, body gain, feed consumption and feed conversion were recorded weekly. At the end of the experimental period (12 weeks) , three birds form each replicate were randomly slaughtered , and blood samples were taken to determine white blood cell number(WBC) , plasma total protein , albumin and globulin . Bursa of fabricia and spleen were also separated and weighted. The results indicated that replacement of NSM instead of SBM by 10 and 30% in Gimizah chick diets improved gross performance , increased WBCs, plasma total protein , albumin and globulin levels and Bursa of fabricia's weight compared to other treatments. In conclusion, feeding growing chickens NSM as a protein source instead of SBM up to 30% improved immunity response and metabolic traits without any detrimental effect on growth performance .

***Keywords**: Nigella seed meal, Gimizah chicks, performance, immunity, blood constituents.*

INTRODUCTION

Recent studies have illustrated that medicinal plants can be used instead of chemical compounds as natural additives in animal and poultry diets to improve quality and quantity of their products. Zeweil (1996), found that Nigella seed meal improved

performance of growing Japanese quail at the level of 6.78 and 13.54% of the diet . The essential oil of Nigella seed was found to contain a crystalline compound named Nigellaone which has protective action against disease (Mahfuoz and El-Dakhakny , 1960) .

Feed represents 60-70% of total poultry production costs. Protein sources are usually most expensive ingredients and sometimes are not available in adequate quantities to support high potential of poultry breeds . Accordingly , there is urgent needs to search for alternative feed stuffs by products which could be used as a cheep sources of plant protein instead of imported soybean meal in formulating poultry rations . Khalifah (1995) reported that NSM contains most of essential amino acids, rich in protein contents and could replace the imported soybean meal to reduce feed cost . Nigella seed meal is rich in oleic and linoleic acids which are essential for poultry (Ustun, et. al., 1990) .

Consequently, conventional ingredients from any available local materials to formulate the least cost rations for poultry seems to be a partial solution for current Egyptian poultry industry problems .

It is well recognized that feeding Nigella sativa (natural biological growth promoters) prevent intestinal infection (Mohan et.al.,1996) , inhibits colon carcinogenesis by modulating the activities of beta- glucurondase and mucinase (El-Ghamry , 2004 and Devasena and Menon, 2003). Therefore,feeding Nigella sativa improve general health status in most species . Therefore, the aim of this study was to investigate the effects of replacing Soybean meal (SBM) by Nigella seed meal (NSM) at the levels of 10,30 and 50 % on performance , some blood constituents and immune response of Gimezah chicks .

MATERIALS AND METHODS

A total number of four hundred one-day old unsexed Gimizah chicks were wing banded individually, weighed to the nearest gram and randomly divided into 4 groups . Each group was divided into 4 replicates with 25 chicks each . The first group served as the control group. Nigella seed meal (NSM) was used instead of Soybean meal (SBM) on the basis of protein units by the levels of were 10,30 and 50% for the second , third and fourth group , respectively . All chicks were fed the starter diets from day 1 to 4 week of age , then the finisher diets from 4 to 12 week of age. The starter diets were formulated to contain 19.2% crude protein (CP) and 3075 kcal ME/kg diet while the finisher diets contain 17.2% cp and 3125 kcal ME/kg as shown in Tables (1 and 2) .

Feed and water were offered *ad lib.* during the whole experimental period which lasted for 12 weeks. Chicks in all treatments were kept under similar managerial and hygienic conditions. Chicks were individually weighed weekly throughout the experimental period. Feed consumption in each replicate was recorded weekly. Cumulative feed conversion (feed / gain) was calculated . At the end of the experiment , three birds from each replicate were randomly chosen and slaughtered . Bursa of fabricia and spleen were separated and weighed . Blood sample were withdrawn at the slaughter time . The blood sample were collected to determine total white blood cell (WBCs') count (10^3 / mm^3) . Plasma was separated by centrifugation at 3000 rpm for 20 minutes . The

plasma was stored in the deep freezer at approximately – 20 ^{0}C till analyzed. Chemical analysis of blood plasma were carried out for quantitative determination of plasma total protein , albumin and globulin.

Table (1): Composition of experimental diets .

Ingredients	% NSM protein to replace SBM							
	Starter				Finisher			
	0%	10%	30%	50%	0%	10%	30%	50%
Yellow corn	65.4	64.65	59.55	55	71.5	69.7	65.0	60.8
Soybean meal (44%)	30.0	27	21	15	24	21.6	16.8	12
Nigella seed meal	0	4.13	12.4	20.7	0	3.31	9.93	16.6
Wheat barn	0	0	2.6	4.8	0	0.9	3.5	5.6
Bone meal	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7
Oil	1.4	1.02	1.25	1.3	1.3	1.29	1.57	1.8
Premix *	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt (NaCl)	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100	100	100	100
Crude protein	19.3	19.3	19.2	19.2	17.2	17.2	17.2	17.2
L-lysine	-	-	-	-	-	-	-	-
DL-Methionine	-	-	-	-	-	-	-	-
ME Keal / Kg diet	3077	3075	3076	3075	3127	3127	3125	3127

**Each 2.5 kg contains Vit.A 12 MIU , Vit.D 34 MIU Vit.E 15 mg , Vit.K 3200 mg , VitB 110mg , Vit.B_2 8 g, pantothenic 10.87 g , Nicotinic acid 30g , Vit.B_6 2 g, Vit.B_{12} 10 mg , folic acid 1 g , Biotin 50 mg , copper 5 g , Iron 15 g , Manganese 70g , Iodine 0.5 g, selenium 0.15 g, Zinc 60g and Antioxidant 10g .*

Table (2): Chemical analysis of Nigella seed meal .

Item	%
Moisture	6.98
Crude protein	31.92
Ether extract	16.31
Crude fiber	14.59
Ash	6.01
NEF	24.19

The statistical analysis was computed using analysis of variance procedure described in the SAS (1988) and the significant mean differences among treatment means were separated by Duncan's Multiple Range test (Duncan , 1955) .

RESULTS AND DISCUSSION

Growth performance :

Body Weight :

From Table (3) it could be noticed that, the highest body weight were recorded in the group fed diet supplemented with 10% NSM, followed by those fed 30% , while the lowest value was observed in the group fed 50%.

Table (3): Average of live body weight (gm) , body gain (gm) , accumulative feed consumption (gm) and accumulative feed conversion (feed / gain) .

Item	4 weeks	8 weeks	12weeks
	Live body weight (gm)		
Nigella meal levels	**	**	**
control	250.1 ^{b}	704.4 ^{b}	1015.7 ^{b}
10%	280.2 ^{a}	802.2 ^{a}	1240.5 ^{a}
30%	278.0 ^{a}	802.1 ^{a}	1231.7 ^{a}
50%	243.4 ^{b}	691.1 ^{b}	974.4 ^{b}
±SE	5.09	17.8	29.6
	Accumulative body gain (gm)		
Nigella meal levels	**	**	**
control	186.4 ^{b}	660.6 ^{b}	971.1 ^{b}
10%	236.9 ^{a}	757.3 ^{a}	1185.2 ^{a}
30%	190.9 ^{b}	758.5 ^{a}	1195.6 ^{a}
50%	174.2 ^{c}	646.9 ^{b}	929.1 ^{b}
±SE	4.2	17.5	28.3
	Accumulative feed consumption (gm)		
Nigella meal levels	**	**	**
control	556.7 ^{b}	1966.5 ^{b}	3615.7 ^{b}
10%	590.9 ^{a}	2083.4 ^{a}	4013.8 ^{a}
30%	564.3 ^{b}	2167.9 ^{a}	3983.4 ^{a}
50%	566.3 ^{b}	1887.7 ^{b}	3647.1 ^{b}
±SE	8.1	33.0	60.9
	Accumulative feed conversion (gm)		
Nigella meal levels	**	**	**
control	2.99 ^{a}	2.98 ^{a}	3.72 ^{b}
10%	2.49 ^{b}	2.75 ^{b}	3.39 ^{c}
30%	2.96 ^{b}	2.86 ^{b}	3.33 ^{c}
50%	3.25 ^{a}	2.92 ^{a}	3.93 ^{a}
±SE	0.11	0.05	0.06

*(**) highly significant at ($P \leq 0.01$).*

a ,b , c Means having different letter (s) within each column are significantly different (P ≤ 0.05) .

From these results , it could be concluded that Nigella seed meal could be used as a protein substitution instead of soybean meal up to 30% without any detrimental effects on body weight . Statistical analysis showed that NSM had highly significant effect ($P \leq 0.01$) on live body weight at 4 , 8 and 12 weeks of age . The result are similar to those reported by Amin (2000) who revealed that , the best results for body weight at marketing age were obtained with 30% substitution of NSOM For Gimizah strain . Zeweil (1996) also reported that the quails given diets containing 38% or 48% NSOM resulted in significantly depressed body weight as compared to control groups . The same author reported that the decrease in body weight of quails when fed on NSM protein at the levels excess of 28% of dietary crude protein may be caused either to the low palatability problems due to its higher fiber content or amino acids imbalance.

Body weight Gain :

Table (3) shows that , the averages of body weight gain at 4 weeks of age were 186.4 , 236.9 .190.9 and 174.2gm in chicks fed on control diet, 10% , 30% and 50% of NSM replacement , respectively . The corresponding results of body gain at 8 weeks of age were 660.6. 757.3, 758.5 and 646.9 gm for control , 10% , 30% and 50% of NSM , levels , respectively . At 12 weeks of age , the body gain was 971.1, 1185.2, 1195.6 and 929.1 gm for control , 10% , 30% , and 50% of NSM level , respectively . It is also noticed that , the supplemented diets with 10% and 30% Nigella seed meal increased body gain by 18.06% and 18.78% , respectively than those fed on un-supplemented diets at 12 weeks of age . From the obtained results , it could be concluded that, addition of Nigella seed meal up to 30% in Gimizh chicks diets significantly improved body weight gain at 12 weeks of age . In agreement , Radwan *et al* (2000) reported that the use of Nagila sativa meal at 8% and 16% significantly ($P \leq 0.01$) improved body weight gain of broiler chicks compared to those fed basal diets

Feed Consumption :

At 4 weeks of age , the averages of feed consumption were 556.7, 590.9 , 564.3 and 566.3 gm for control , 10%, 30% and 50% of NSM substitution instead of SBM , respectively . The corresponding figures at 8 weeks of age were 1966.5 , 2083.4 , 2167.9 and 1887.7 gm for control , 10% , 30% and 50% level of NSM , respectively . Similarly , the corresponding figures at 12 weeks of age were 3615.7 , 4013.8 , 3983.4 and 3647.1 gm in control , 10% , 30% and 50% of NSM level , respectively . From these results, it could be noticed that feeding Gimizah chicks diets containing 10% or 30% Nigella seed meal instead of SBM increased significantly ($P \leq 0.01$) feed consumption by 11.01 and 10.17% , respectively compared with the control . El-Gharmry et al (1997) found that replacing up to 20% of SBM by NSM protein in quail diet did not significantly affect consumption . While , feed consumption was significantly decreased when quail fed diet containing NSOM instead of 40% SBM protein in the period from 0- 3, 3 – 6 and 0-6 weeks of age compared to the control birds . Moreover , Osman and El-Barody (1999) revealed that, the high levels of Nigella sativa seeds (8 % and 10%) had an adverse effect ($P < 0.05$) on feed consumption at 4 and 6 weeks of age . Kalifah (1995) revealed that , replacement of SBM proteins with NSOM did not significantly affect feed consumption at any experimental period or for the whole experimental period when compared to the control one .

d-Feed Conversion :

Table (3) revealed that replacement of soybean meal by Nigella seed meal affected significantly ($P < 0.01$) feed conversion at all tested experimental ages. Feed conversion values at 4 weeks of age were 2.99 , 2.49 , 2.96 and 3.25 for control , 10% , 30% and 50% NSM , respectively . From these results , it could be observed that , the best feed conversion was obtained when 10% of NSM was substituted instead of SBM , followed by 30% NSM and the control , while , the lowest value was recorded by using 50% NSM . At 8 weeks of age , feed conversion values were 2.98, 2.75 , 2.86 and 2.92 for the control , 10% , 30 % and 50% respectively . The best value was observed also at 10% followed by 30% while the lowest value was obtained by the control group , At 12 weeks of age , feed conversion values were 3.72 , 3.39, 3.33 and 3.39 , for control , 10% and 30% and 50% NSM , respectively . feeding Nigella seed meal instead of Soybean meal at levels 10% and 30% improved feed conversion by 8.87% and 10.48% , respectively compared with the control group . This improvement may be attributed to the increased body weight gain . These results are similar to those obtained by Amin (2000) who showed that , the fed conversion of Gimizah chicks throughout the whole experimental period (0-10 weeks) was 3.51 , 3.41 and 3.53 for the control , 30% and 50 % NSM instead of SBM. Khalifah (1995) reported that for the whole experimental period , there was some improvement in feed conversion when feeding on 20% of NSOM instead of SBM , this improvement is dependent of body weight gain .

From the above mentioned results , it could be concluded that , Nigella seed meal could be considered as a rich protein source as well as energy since it contains a considerable amount of ether extract . Also, the Nigella seed meal has a considerable amount of amino acids that could support the growth of chicks . Finally , Nigella seed meal could be used as un- conventional protein instead of soybean meal up to 30% without any detrimental effect on growth performance, of local Gimizah strain chicks .

Blood constituents traits:

The values of total protein and protein fractions in blood plasma of Gimizah chicks receiving different experimental diets at 12weeks of age are presented in Table (4).

Table (4): Effect of Nigella seed meal(NSM) at different levels on some blood constituents of Gimizah chicks.

Treatment	Parameters			
	Total protein g/100ml	Albumin g/100ml	Globulin g/100ml	A l G ratio
NSM levels	**	*	*	NS
Control	3.54 ^{c}	2.57 ^{b}	0.97 ^{c}	2.65
10%	4.32 ^{b}	2.91 ^{b}	1.41 ^{b}	2.06
30%	5.54 ^{a}	3.83 ^{a}	1.71 ^{a}	2.24
50%	4.17 ^{a}	3.23 ^{a}	0.94 ^{c}	3.44
±SE	0.04	0.03	0.05	0.09

*(*) significant at ($P \leq 0.05$) ., (**) highly significant at ($P \leq 0.01$) ., NS = not significant . a ,b , c Means on the same column with different letter are significantly different ($P \leq 0.05$).*

The levels of plasma total protein ranged between 3.54 g / 100 ml for control treatment and 5.54 g/100 ml for 30% level . The results showed that the control treatment recorded the lowest value(3.54 g / 100 ml) , while the highest total protein value was obtained by chicks fed 30% replacement level of SBM from NSM with highly significant ($P \leq 0.01$) when compared to other treatments . The same trend was found regarding the albumin fraction which ranged between 2.57g / 100 ml for control group and 3.83 g / 100 ml for 30% level with significant differences ($P \leq 0.05$) between these treatments . The plasma globulin content ranged between 0.94 and 1.71 m / 100 ml for chicks received diets containing 50 and 30 % replacement of SBM by NSM , respectively with significant differences ($P \leq 0.05$). The A/G ratio varied in moderately wide range between 2.06 (10% level) and 3.44 (50% level) as they are significantly different in respect to the total protein and globulin values.

The results are in agreement with finding of Abd El- Latif et. al.,(2002) , Tolba et . al .,(2005) and Hassan et. al ., (2007) who reported that adding Nigella seed meal to quail diet increased plasma total protein, albumin and globulin .

Most plasma proteins are synthesized from amino acids derived from the food or the tissues catabolism (Sturkie , 1976) . The increase in plasma total protein or albumin in those treatments which containing low levels of NSM (10 and 30%) reflects the ability of the chicks receiving NSM to " store reserve " protein even after the body has reached its maximum capacity for depositing tissues or less" labile protein " .

This reserve of protein is very important in resisting the stresses of either nutrient restriction (Rosenthal and Allison , 1951) or some diseases infection (sleeler and Ott, 1945) . Hassanin and Hassan (1997) and Khodary *et al* (1996) confirmed the immune stimulant effect of Nigella sativa due to its high content of essential amino acids (Khalifah, 1995).

3-Some immune parameters :

The values of white blood cells of Gimizah chicks receiving different experimental diets at 12 weeks of age are presented in Table (5) .

Table (5): Effect of Nigella seed meal at different levels on some immunity parameters of Gimizah chicks

Item	Control	10%	30%	50%	SE
White blood cells (10^3/ml)	15.49 ^{c}	16.47 ^{b}	18.89 ^{a}	14.46 ^{d}	0.55
Spleen (mg)	1470 ^{a}	1260 ^{c}	1260 ^{c}	1370 ^{b}	50.50
Bursa (mg)	630 ^{d}	840 ^{b}	950 ^{a}	740 ^{c}	40.0

a ,b , c Means on the same column with different letter are significantly different ($P \leq 0.05$).

The values of white blood cells ranged from 14.46 to 18.89 (10^3/ ml) . From these data , it was noticed that , WBC count were increased with increasing the level of NSM from 10 up to 30% , while a decreased in WBC was found at 50% level . These results are coincided with those reported by Osman and EL Barody (1999)and Abou El– soud (2000) who found that WBCs increased by increasing the level of Nigella sativa seeds . Also , Abou El-Soud (2000) reported increased immunity and inhabiting non-enzymatic peroxidation by increasing some blood hematological parameters for Japanese quail . El-Feki (1987) indicated that , the increase in lymphocyte , heterophil and eosinophil percentages may be attributed to the production of specific and non-specific antibodies against different antigens , since lymphocyte eosinophil and heterophil are responsible for achieving the defense mechanism and immune response introduced into body . The change in basophil percentage may be due to the engagement of these cells in the phagocytic process against different antigens (harding and Hogland, 1984) . The increase in WBC 's may be due to an increase in the membrane protection from auto oxidation , where numerous of reports indicated that there is a significant role of black seed in increasing immunity and inhibiting non-enzymatic peroxidation (Houghton *et al*, 1995) .

Table (5) shows, a decrease in spleen weights and increase of bursa of fabricia weights due to feeding different levels of NSM instead of soybean meal . Osman and EL-Barody (1999),El-Kaiaty *et al* (2002), Abo El- Soud (2000) and Hassan *et al* (2007) found that spleen and bursa of fabricia weights increased by using black seed. Khalifah (1995) reported that Nigella seed oil meal had a considerable amount of fat before and after extraction (35% vs 17.7%, respectively). It seems that extraction processing did not remove all fats and residual amounts are present . From these results, it could be conduced that, , supplementation of NSM at different levels instead of SBM in Gimizah chicks diets had highly significantly effect ($P \leq 0.01$) on the immune response . Houghton et. al ., (1995) explain these cases of enlargement of spleen and bursa of fabricius which might be related to the improvement in immune response.

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تأثير تغذية كسب حبة البركة على الاستجابة المناعية وبعض صفات الدم لكتاكيت الجميزة

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استخدم فى هذه التجربة عدد ٤٠٠ كتكوت عمر يوم من سلالة الجميزة وذلك لدراسة تأثير إحلال كسب حبة البركة بدلاً من كسب فول الصويا وعلاقة ذلك بالأداء الانتاجى والمناعة وبعض مكونات الدم . قسمت الكتاكيت عشوائياً إلى ٤ معاملات التى قسمت إلى ٤ مكررات بكل منها عدد ٢٥ كتكوت . اعتبرت المجموعة الأولى كنترول وتم إحلال كسب حبة البركة محل بروتين كسب فول الصويا بنسبة ١٠% ، ٣٠% ، ٥٠% للمعاملة الثانية والثالثة والرابعة على التوالى كوحدة وزنية وطبقاً لذلك تم تكوين ٨ علائق تحتوى على ١٩,٢% بروتين خام و٣٠٧٥ طاقة ممثلة لكل كيلو جرام عليقة خلال فترة البادىء(عمر يوم -٤ أسبوع)و١٧,٢١% بروتين خام ٣١٢٥ كيلو كالورى لكل كيلو جرام عليقة أثناء فترة الناهى(٥ – ١٢ أسبوع).

تم تقديم الماء والغذاء للكتاكيت طول فترة التجربة لحد الشبع وربيت الطيور تحت ظروف متشابهة من الإضاءة والحرارة والرعاية الطبية طول فترة التجربة . تم تقدير وزن الجسم الحى والزيادة فى وزن الجسم والغذاء المستهلك والكفاءة التحولية أسبوعياً وفى نهاية التجربة تم اختيار ٣ طيور من كل تكرار عشوائياً وتم ذبحهم وتم أخذ عينات دم أثناء الذبح لتقدير د عدد كرات الدم البيضاء والبروتين الكلى والألبيومين والجليوبيولين للبلازما ووزن الطحال وغدة البرسا .

من الدارسة يتضح أن قيم كرات الدم البيضاء زادت بزيادة إحلال كسب حبة البركة محل كسب فول الصويا عند النسب ١٠% و ٣٠% بينما عند النسبة ٥٠% انخفض عدد كرات الدم البيضاء . كما وجد نقص فى أوزان الطحال وزيادة فى وزن غدة البرسا وقيم البروتين الكلى والألبيومين والجليوبيولين عند التغذية على ١٠% ، ٣٠% كسب حبة البركة . نستخلص من هذه الدراسة أنه يمكن إحلال كسب حبة البركة محل كسب فول الصويا لعليقة كتاكيت الجميزة حتى ٣٠% فقط لزيادة المناعة وتحسن الأداء لدى الطيور .