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≪**Research Note**≫

The Effect of Supplementation of Enzyme on Laying and Reproductive Performance in Japanese Quail Hens Fed Nigella Seed Meal

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The effect of dietary enzyme (Optizyme) on the performance of Japanese quail hens fed isocaloric and isonitrogenous diets containing 0, 10, 20 and 30% of Nigella seed meal (NSM) as a protein source was studied. A corn-soybean meal diet was used as a control diet. Eight groups of birds having 8 replicates consisting of 2 females and 1 male were used, and productive and reproductive traits and egg quality were measured. Hens fed 10% NSM produced significantly more eggs than those fed 0, 20 and 30% NSM diets. Shell thickness was also significantly higher in the 10% NSM group. Supplementation of enzyme significantly improved egg production and tended to improve shell thickness when a 20% NSM diet was fed, but not when 30% NSM was given. Furthermore, the enzyme significantly improved the fertility rate when fed a 20% NSM diet, but not in the groups fed a 30% NSM diet. From these observations, Japanese quail hens can be fed diet containing 10% NSM. Moreover, this level can be increased to 20% when supplemented with enzyme.

Key words: Japanese quail, laying performance, Nigella meal

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Introduction

Feed resources in developing countries are limited especially after the appearance of Mad cow disease (BSE), thus increasing demand for SBM as a main protein source (Attia *et al.,* 2003a). However, there are other potential feed resources that have not been efficiently utilized as poultry feed due to either their antinutritional factors or lack of adequate information (Attia, 2003b).

Nigella sativa, L. (Kalongi) has been used for centuries, both as a herb or spice and pressed into oil, by people in Asia, Middle East, and Africa for medicinal purposes (Akhtar, 2003). It has been traditionally used for a variety of treatments related to respiratory health, stomach and intestinal health, kidney and liver function, circulatory and immune system support, and for general overall well-being (Zeweil, 1996). In herbal medicine, Nigella sativa has hypertensive, carminative, and anthelminthic properties (Nickavara *et al.,* 2003).

Nigella seed meal (NSM) is a possible source of feed protein as shown in Table 1. The protein content varies from 34.01 to 27.87%, crude fibre from 14.25 to 10.54%, ether extract from 18.72 to 15.20% and ash from 9.54 to 5.52%. Furthermore, NSM production is expected to increase in the near future due to extended use of it as a medical seed (Zeweil, 1996; Akhtar, 2003). Zeweil (1996) reported that NSM could be successfully included as a protein replacement in the diets for growing Japanese quail up to 13.5%. However, higher levels of NSM depressed growth and feed utilization.

Enzymes may be a practical mean to improve performance and permit utilization of higher levels of agro-industry by-product in monogastric animal nutrition (Attia *et al.,* 2003b). Multienzymes containing β-glucanase, α-amylase, cellulase, pectinase, xylanase, hemicellulase without or with protease and phytase could improve feed utilization and overcome the antinutritional factors of feedstuffs, and improve gut health and immune response

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Table 1. Chemical composition and some essential amino acid profiles of Nigella seed meal as reported in the literature

Nutrient	Zeweil (1996)	Youssef *et al.* (1998)
Proximate chemical composition (%):		
CP	34.01	27.87
EE	15.20	18.72
CF	14.25	10.54
ASH	5.52	9.54
NFE	21.90	33.33
Some essential amino acid profiles (%):		
Amino acid	Abdel-All and Attia (1993)	El-Faham (1994)[1]
Methionine	0.43	0.36
Methionine+cystine	0.74	NR
Lysine	1.19	0.45
Arginine	2.16	NR
Threonine	1.44	3.97
Tryptophan	NR	NR

CP=Crude protein. EE=Ether extract. CF=Crude fiber. NFE=Nitrogen free extract. [1]=percent of total amino acid content.

(Jeroch *et al.,* 1995; Saleh *et al.,* 2003; Yonemochi *et al.,* 2003; Meng *et al.,* 2005; Choct, 2006; Yörük *et al.,* 2006).

The purpose of the present work was to determine the effect of an enzyme mixture supplementation on laying and reproductive performance in Japanese quail hens fed diets based on partial or complete replacement of soybean meal by NSM.

Materials and Methods

Screw-pressed NSM derived from Nigella sativa L. was obtained from a commercial oil processing plant (Screw pressed method) in Egypt. A total number of 144 Japanese quail (96 females and 48 males) were randomly distributed into six treatment groups. Each diet was fed to 8 cages each containing 2 females and 1 male. The Japanese quail was fed a diet containing 20.2% crude protein (CP), 2880 kcal ME/kg, 2.78% calcium and 0.41% available phosphorus for 37 d from 42 d of age. Hens were illuminated with 16:8 light-drake cycle during the laying period.

Isocaloric and isonitrogenous diets were formulated (NRC, 1994) using corn-soybean meal and NSM to contain 0, 33, 66 and 100% NSM (Table 2). The replacement was done based on a protein-to-protein basis of soybean meal. In addition to the above-mentioned 4 diets, diets containing either 66% or 100% nigella seed meal supplemented with 0.1% of commercial enzyme mixture (Optizyme) were also formulated. Optizyme is a product of Optivite International LTD, Main Street, Laneham, Retford, Nottinghamshire, DN22 ONA, England. It is a multi-enzyme product, containing proteases, amyloglucosidase, xylanase, glucanase, cellulases and hemicellulases.

Protein concentration used herein is composed mainly of herring fishmeal, meat meal, meat and bone meal, corn gluten meal, dicalcium phosphate, methionine and lysine and vitamin and mineral mixture. Its nutritive value showed ME (kcal/kg) 2400, crude protein (CP) 52%, crude fat 4%, crude fiber 2.3%, calcium 7.4%, phosphorus 3.0%, methionine 1.5%, methionine+cystine 2.5%, lysine 2.9%, threonine 1.87%, and tryptophan 0.46%.

Measurements

Daily egg production, egg weight, egg mass and shell-less eggs were recorded. Feed intake was measured every week on cage basis. Feed conversion was calculated as amount of feed (g) per g egg, after adjusting the feed for female only. Birds were weighed at the beginning and at the end of the experiment. The mortality rate was presented as the percentage of dead birds in each treatment for the whole experimental period.

Eggs laid on two successive days were used to determine egg quality at 110, 140 and 170 d of age as outlined by Attia *et al.* (1995). Fertility and hatchability were measured for eggs collected for 7-day periods and were stored in an egg room at 15.5℃ dry bulb, at 70% relative humidity. They were incubated at 37.6℃, relative humidity was 55–60%, and they hatched at 37.3℃ with relative humidity of 65–70% in automatic incubators. Eggs that had not hatched by d 18 were broken and open to differentiate infertile eggs from those containing dead embryos. Fertility was calculated as number of fertile eggs relative to total number of eggs set. Meanwhile, hatchability was calculated as number of hatched chicks relative to total number of eggs set.

At the end of the experimental period, 5 females were randomly chosen from each treatment, weighed after fasting for 12 h, slaughtered, their feathers were plucked and the total inedible parts (head, legs and inedible viscera) were taken aside. Then, the remaining carcass (dressed weight) was weighed. The liver, spleen, gizzard, heart,

Table 2. Composition of the diets fed to Japanese quail laying hens

	Nigella seed meal protein, %			
Ingredients (%)	0.0	33	66	100
Yellow corn	61.695	57.2	53.0	51.3
Soybean meal, 44% CP	22.1	15.8	9.1	0.0
Nigella seed meal, 33.5% CP	0.00	9.67	19.33	31.64
Protein concentrate*	10.0	10.0	10.0	10.0
Lime stone	5.0	5.0	5.0	5.0
DL-methionine	0.08	0.08	0.08	0.07
L-lysine	0.00	0.07	0.15	0.27
Commercial oil	1.12	1.21	1.21	0.00
Antioxidant	0.005	0.005	0.005	0.005
Sand	0.00	0.965	2.125	1.715
Total	100.0	100.0	100.0	100.0
Calculated values (%)				
ME (kcal/kg)	2901	2914	2921	2933
CP	20.22	20.40	20.41	20.52
Total methionine	0.48	0.48	0.48	0.48
Total methionine+cystine	0.83	0.85	0.87	0.88
Total lysine	1.04	1.03	1.01	0.99
Calcium	2.72	2.73	2.73	2.74
Available Phosphorus	0.41	0.42	0.43	0.44
Crude fat	4.99	5.32	5.74	5.97
Crude fiber	3.02	3.93	4.82	5.99

* Vit+Min mixture provides per kilogram of diet: vitamin A, 12,000 IU; vitamin E, 20 IU; menadione, 1.3 mg; Vit. D_3, 2,500 ICU; riboflavin, 5.5 mg; Ca pantothenate, 12 mg; nicotinic acid, 50 mg; choline chloride, 600 mg; vitamin B_{12}, 10 μg; vitamin B_6, 3 mg; thiamine, 3 mg; folic acid, 1.00 mg; dbiotin, 0.50 mg. Trace mineral (milligrams per kilogram of diet): Mn, 80; Zn, 60; Fe, 35; Cu, 8; Se, 0.60.

pancreas, intestines, and cecum were separated and individually weighed, and intestines and cecum lengths were measured. The percentages of carcass yield and organs to live body weight were calculated.

Statistical analysis

Data were analyzed using one way ANOVA of the General Linear Model (GLM) Procedure of the Statistical Analysis System of SAS Institute (1990) using the following model:

$$Yik = \mu + Di + eik$$

where Y is a single observation; μ is the general mean; D is the effect of the treatment (i=A, B, C, D, E, F); and eik is the random error. Before analysis, all percentages were subjected to logarithmic transformation (log10x+1) to approximate normal distribution. A 0.05 level of significance of Student Newman Keuls test of SAS (1990) was used to test mean differences among the experimental treatments.

Results and Discussion

Chemical composition of NSM

The present NSM sample and the values reported by other researchers are shown in Table 1. There were apparent differences in chemical composition and amino acid profiles (Table 1). This could be attributed to the variations in agronomic conditions and method of oil extraction (screw pressed, hydraulic vs. solvent extraction).

Productive performance

Japanese quail hens fed 33% NSM had significantly better rates of laying and egg mass than the control (0% NSM) and those fed unsupplemented 66 and 100% NSM (Table 3). The significant improvement in laying rate and egg mass by 33% NSM could be attributed to the antimicrobial and antifungal properties of nigellone and thymoquinone of NSM (Rathee *et al.,* 1982; Hanafy and Hatem, 1991; Akhtar *et al.,* 2003; Nickavara *et al.,* 2003).

It is worth noting that laying rate and egg mass declined ($P \leq 0.05$) progressively with increasing levels of NSM above 33%. However, the group fed 66% NSM exhibited a similar rate of laying and egg mass to the control. This shows that NSM up to 66% had no adverse effects on egg production and egg mass. Similarly, Zeweil (1996) indicated that dietary NSM had no adverse effect of productive performance of laying hens. Furthermore, Akhtar *et al.* (2003) found that inclusion of Nigella sativa L. seeds up to 1.5% significantly increased egg production and egg mass in laying hens.

Evidently, complete replacement of SBM by NSM had deleterious influences on the laying rate and egg mass (Table 3). On the other hand, 100% replacement of SBM by NSM had no negative effects on egg weight, body weight changes and livability rate.

Japanese quail hens fed an enzyme-supplemented 66%

Table 3. Effect of enzyme mixture supplementation on productive performance of Japanese quail hens fed diets containing different levels of nigella seed meal

Criteria	Nigella seed meal (%)				Enzyme treatment		SEM	*P* Value
	0.0	33.0	66.0	100.0	66	100		
Egg production (H/D) %	47.0^{b}	56.3^{a}	46.1^{b}	39.3^{c}	56.7^{a}	40.2^{c}	1.59	0.01
Egg weight, g	12.7	12.4	12.2	11.9	11.9	11.7	0.31	NS
Egg mass, g	674.5^{bc}	788.9^{a}	635.5^{cd}	528.5^{d}	762.4^{ab}	531.5^{d}	60.9	0.001
Feed intake, g/bird	2726^{abc}	2718^{abc}	2660^{bcd}	2765^{ab}	2911^{a}	2440^{d}	35.5	0.001
Feed conversion ratio, g/g	4.04	3.44	4.18	5.23	3.82	4.59	0.33	NS
Body weight gain, g	24.2	27.2	22.5	17.9	28.5	31.8	5.08	NS
Mortality, %	4.2	10.8	4.2	8.3	10.8	8.3	4.75	NS

SEM = standard error of means. *P* value = probability value. NS = not significant. NSM = Nigella seed meal. 0% = positive control (diet containing no nigella seed meal protein).
[a, b, c, d] Means within a row followed by a common superscript are not significantly different.

NSM diet laid significantly more eggs and tended to produce more egg mass than those of the control group (0% NSM diet) and the 66% NSM diet, however, the differences were significant for both criteria (Table 3). This reveals an improvement in feed utilization for egg production. On the other hand, addition of enzymes to 100% NSM did not beneficially affect egg production and egg mass compared to the control and 100% NSM diet (Table 3).

Feed intake was not affected by the level of the unsupplemented NSM in male and female diets (Table 3). Enzyme mixture supplementation to the 66% NSM diet increased feed intake significantly compared to the control. In contrast, enzyme supplementation to the 100% NSM diet decreased feed intake significantly (Table 3). This indicates that the effect of enzyme mixture depends on the level of NSM.

There were non-significant differences in feed conversion ratio (FCR) due to dietary NSM and enzyme supplementation to 66 and 100% NSM (Table 3). However, the improvement in FCR (8.6%) due to enzyme supplementation to 66% NSM diet was not statistically significant, but may be of practical importance. These results supported those reported by Attia *et al.* (2001) and Choct (2006), that enzyme improved feed utilization laying hens.

There was no effect in body weight gain in male and female Japanese quail due to different dietary treatments and enzyme supplementation. Livability rate of male and female Japanese quail ranged from 89.2 to 95.8% during the period from 80 to 193 d of age. The postmortem investigation indicated that livability was not affected by NSM. Similar results were reported by Zeweil (1996) and Akhtar *et al.* (2003)

Egg quality traits, fertility and hatchability

Shape index, yolk percentage and color, albumen percentage and Haugh unit score and other shell quality criteria were not significantly affected by the level of NSM and enzyme supplementation (Table 4). Results reported by Zeweil (1996) revealed that egg quality was not affected by NSM in the quail diets. However, Akhtar *et al.* (2003) reported that Nigella sativa seed significantly increased Haugh unit, and had no significant effects on yolk index and blood and meat spots. Similarly, El-Full *et al.* (2000) found that enzyme mixture addition to laying hen diets had no effect on shell quality criteria and Haugh unit score, yolk color percentage and albumen percentage. On the other hand, shell thickness was significantly better in the group fed 33% NSM than groups fed the 0, 66 and 100% NSM diets (Table 4). Also, Zeweil (1996) found that feeding a 37.5% NSM diet yielded significantly higher shell weight, and attributed this to the accompanied increase in egg weight as an indirect response to egg weight. Also, Akhtar *et al.* (2003) reported that Nigella sativa seed significantly increased egg shell-thickness. The present results indicate that enzyme mixture improved shell thickness of groups fed 66 and 100% NSM, thus enzyme supplemented 66% NSM had similar shell thickness to those fed 33% NSM, and were better than that of the control group. Along the same line, Attia *et al.* (2001) revealed that multienzyme supplementation to rice bran containing diet significantly improved shell thickness. The improvement in shell thickness of enzyme supplemented 66 and 100% NSM containing-diet could be due to the improvement in the utilization of mineral complexes in the cell wall (Jeroch *et al.,* 1995; Attia *et al.,* 2001). However, Yörük *et al.* (2006) revealed that interior and exterior egg quality were not affected by multienzyme supplementation to corn-soybean meal.

It was found that fertility of Japanese quail was decreased significantly with increasing levels of NSM above 33% (Table 4). The present results reveal that enzyme mixture improved the percentage fertility of Japanese quail fed 66% NSM compared to its negative control, although complete relief was not achieved. On the other hand, enzyme supplementation to 100% NSM diet had no beneficial effect on percentage fertility of Japanese quail. Hatchability was not significantly affected by NSM and enzyme (Table 4).

Dressed carcasses and body organs

The percentage of dressed carcasses, liver, gizzard, heart, pancreas, and spleen, length of intestines, cecum and ovary were not affected significantly by enzyme

Table 4. Effect of enzyme mixture supplementation on egg and shell quality traits and fertility and hatchability of total eggs set of Japanese quail hens fed diets containing different levels of nigella seed meal

Criteria	Nigella seed meal (%)				Enzyme treatment		SEM	*P* Value
	0.0	33.0	66.0	100.0	66	100		
Egg quality traits:								
Shape index	79.0	78.6	79.1	77.0	78.9	79.1	0.86	NS
Albumen, %	54.1	51.8	52.1	54.6	53.1	53.3	0.63	NS
Yolk, %	33.4	35.6	34.9	33.0	33.7	33.6	0.57	NS
Yolk color	4.4	4.4	4.2	4.4	4.3	4.0	0.29	NS
Haugh unit score	86.8	90.3	91.5	90.1	90.2	87.9	0.95	NS
Shell-less-eggs, %	9.2	9.4	4.5	3.0	3.0	4.3	1.33	NS
Shell weight, g	12.6	12.7	13.0	12.4	13.2	13.1	0.26	NS
Shell thickness, (mm)	0.237^{c}	0.270^{a}	0.244^{bc}	0.236^{c}	0.262^{ab}	0.246^{bc}	0.005	0.02
SWUSA, mg/cm^2	68.6	69.5	69.6	66.5	70.7	69.2	1.22	NS
Reproductive traits (%):								
Fertility	91.1^{a}	92.9^{a}	74.8^{c}	85.9^{b}	85.9^{b}	81.1^{b}	2.46	0.02
Hatchability	51.1	51.5	47.8	42.4	55.3	44.2	3.66	NS

SEM=standard error of means. *P* value=probability value. NS=not significant. NSM=Nigella seed meal. 0%=positive control (diet containing no nigella seed meal protein).
[a, b, c] Means within a row followed by a common superscript are not significantly different.

Table 5. Effect of enzyme mixture supplementation on dressed carcasses and organs of 193 d old Japanese quail hens fed diets containing different levels of nigella seed meal

Criteria	Nigella seed meal (%)				Enzyme treatment		SEM	*P* Value
	0.0	33.0	66.0	100.0	66	100		
Dressed carcasses, %	63.7	64.7	62.9	64.2	60.4	59.0	1.85	NS
Liver, %	2.37	2.55	2.80	2.52	2.34	2.79	0.39	NS
Gizzard, %	2.24	2.75	2.34	2.57	2.39	2.50	0.24	NS
Heart, %	0.851	0.759	0.783	0.858	0.738	1.03	0.06	NS
Pancreas, %	0.309	0.259	0.342	0.289	0.274	0.317	0.03	NS
Spleen, %	0.126	0.159	0.137	0.136	0.124	0.117	0.02	NS
Intestinal length, %	24.5	23.9	23.4	25.0	21.5	23.4	1.34	NS
Cecum length, %	8.06	9.91	9.10	9.26	9.42	10.2	1.43	NS
Ovary, %	0.732	0.492	0.686	0.614	0.757	0.726	0.117	NS

SEM=standard error of means. *P* value=probability value. NS=not significant. NSM=Nigella seed meal. 0%=positive control (diet containing no nigella seed meal).

supplementation in birds fed 66 and 100% NSM diets (Table 5). Zeweil (1996) and Abou-Egla *et al.* (2001) found similar results in broilers and Japanese quail, respectively. However, Abou-Egla *et al.* (2001) reported that heart percentage was significantly increased when Japanese quails were fed a diet containing 40% NSM instead of dietary crude protein. It is concluded that 100% substitution of SBM protein by NSM had no adverse effect on dressed carcasses and organs.

Conclusion: Japanese quail hens can be fed a diet containing 10% NSM. Moreover, this level can be increased to 20% when supplemented with enzyme.

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