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Effect of gradual substitution of soyabean meal by *Nigella sativa* meal on growth performance, carcass traits and blood lipid profile of growing Japanese quail

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ABSTRACT. The aim of the study was to evaluate the effect of different inclusion levels of *Nigella sativa* meal (NSM) on growth performance, carcass yield and blood lipid profile of 300 unsexed one-week old Japanese quails. The experimental diets contained four levels of NSM replacing 0 (control), 20, 30 and 40% of soyabean meal (SBM) in growing Japanese quail diets. Live body weight was linearly (P = 0.017) and quadratically (P = 0.024) increased at week 3 of age and only quadratically (P > 0.001) at week 6 of age due to 30% SBM substitution. Birds fed diets with 20 and 30% SBM replaced by NSM consumed more feed in comparison with other animals during 3-6 and 1-6 week of age. The substitution of 20% SBM for the same percent of NSM resulted in a significant (P < 0.001) linear increase in both dressing and carcass percentages in comparison with the control and other treatment groups, giblets yield was also significantly (linearly P = 0.011 and quadratically P = 0.021) affected. A gradual reduction in serum total lipids, total cholesterol and HDL-cholesterol was observed along with the increasing level of NSM in the diet, except HDL which was elevated when 40% of SBM was replaced. It could be concluded that NSM could partially replace SBM up to 30% in growing Japanese quail diets without any harmful hazards regarding performance, feed utilization and carcass traits. Moreover, the blood lipid parameters are decreased with increasing NSM content in the diet.

Introduction

It is well known that prices of maize and soyabean, which are mainly used in poultry diet, have reached the highest values in years. Thus, there is an urgent need for the alternative protein sources which would be affordable and nutritious. The soyabean meal (SBM) is the most commonly used protein source in poultry feeding which is known for its high quality. Due to the fact that SBM is the major by-product of oil extraction from soyabeans, costs and availability of SBM are strongly correlated with the price of agricultural commodities on the world market (Laudadio and Tufarelli, 2011). The prices are influenced by variations in economic growth, changes in consumer product preferences and weather conditions (Jezierny et al., 2010). Therefore, the price and availability of SBM on the global market may change rapidly; thereby increasing interest in maximizing the use of locally produced feed ingredients including grain legumes (Laudadio et al., 2011). The best reducing costs strategy is the development of diet formulation based on alternative, locally available ingredients, so the nutritionists proposed many alternatives for poultry diets such as raw faba bean or sunflower meal (Abd El-Hack and Alagawany, 2015; Alagawany et al., 2015).

Medicinal plants and their extracts are presently gaining the great importance in livestock production as well as health care systems because of their broad beneficial effects – promoting growth and production, immune enhancement and safeguarding health (Alagawany et al., 2016). The recent studies have described various biological and protective impacts of black cumin (*Nigella sativa*), including antioxidant, anti-inflammatory, anticancer, antibacterial, immunomodulatory and health-promoting activities (Abd El-Hack et al., 2016).

N. sativa meal (NSM) contains most essential amino acids together with about 33% of crude protein (El-Nattat and El-Kady, 2007). Furthermore, NSM production is expected to increase in the near future due to its extended use as a medical seed (Zeweil, 1996). Zeweil (1996) also reported that NSM could be successfully included as a protein replacer (up to 13.5%) in the growing Japanese quail diets. However, too high levels of NSM depressed growth and feed utilization of these animals. Therefore, this study aimed to examine the effects of the inclusion of NSM as a substitute for soyabean meal in growing Japanese quail diets on growth performance, carcass yield and some blood metabolites.

Material and methods

Experimental design and husbandry

The study was conducted at Poultry Research Farm, Poultry Department, Faculty of Agriculture, Zagazig University, Zagazig (Egypt). All procedures were reviewed by the Local Experimental Animal Care Committee, and approved by the institutional ethics committee of Poultry Department, Faculty of Agriculture, Zagazig University, Zagazig (Egypt) in December 2014.

Table 1. Composition and chemical analysis of experimental diets

Indices	SBM % replaced by NSM[1], %			
	0	20	30	40
Ingredients,%				
maize	52.01	50.40	49.51	48.45
SBM 44%	40.00	32.00	28.00	24.00
maize gluten meal	2.50	4.30	5.20	6.30
NSM	0.00	8.00	12.00	16.00
Di-calcium phosphate	1.67	1.37	1.24	1.10
limestone	0.81	1.01	1.10	1.19
vitamin-mineral premix[2]	0.30	0.30	0.30	0.30
NaCl	0.30	0.30	0.30	0.30
DL-methionine	0.12	0.06	0.04	0.00
L-lysine	0.37	0.27	0.21	0.16
soyabean oil	1.92	1.99	2.10	2.20
Calculated analysis, %[3]				
crude protein	24.08	24.04	24.00	24.08
crude fibre	3.98	4.28	4.43	4.58
Ca	0.85	0.85	0.85	0.85
P (available)	0.45	0.45	0.45	0.45
lysine	1.61	1.61	1.61	1.61
methionine + cysteine	0.88	0.88	0.88	0.88
ME[4], kcal · kg^{-1} diet	2906	2900	2900	2900

[1] SBM – soyabean meal, NSM – *Nigella sativa* meal; [2] each 2.5 kg consists of: IU: vit A 12 000 000, vit D_3 2000 000; g: vit. E 10, vit. K_3 2, vit. B_2 49, vit. B_6 105, pantothenic acid 10, niacin 20, biotin 50, Fe 30, Mn 40, Cu 3, Zn 45; mg: vit. B_1 1000, vit. B_{12} 10, folic acid 1000, choline chloride 500, Co 200, Si 100;[3] calculated according to NRC (1994); [4] ME – metabolizable energy

A total of 300 unsexed one-week old Japanese quails were randomly assigned into 4 treatment groups, of 75 chickens each. Each group was sub-divided into 5 replicates, of 15 chickens each. Each replicate was housed in a cage (90×40×40 cm). Four experimental isonitrogenous and isocaloric diets were formulated: a basal diet and three diets with graded substitution levels (0, 20, 30 and 40%) of soyabean meal (SBM) with *N. sativa* meal (NSM), which represent 8, 12 and 16% NSM addition to the diet (Table 1).

Chickens were grown in brooders with raised wire floors and were reared under the same conditions with 23 h light : 1 h dark. Feed and water were available *ad libitum* throughout the whole experimental period (1 - 6 weeks of age). All chickens received feeds in mash form until 42 day of age, according to their treatment.

Growth performance

Chickens were weighed individually at one week intervals to obtain live body weight (LBW). Average daily feed intake (FI), body weight gain (BWG) and feed efficiency (FE) were calculated at the end of each period (after week 1, 3, 6) and during each period (1-3, 3-6, 1-6). Feed waste was recorded daily and on this basis the feed consumption was calculated. Protein efficiency ratio (PER) was calculated from BWG divided by protein intake (Kamran et al., 2008). Also, energy utilization (EU) was calculated as follows: EU = metabolizable energy (ME) consumed (kcal) during the studied period/ BWG (g) during the same period.

Carcass characteristics

At the end of the experiment, 20 birds (five from each group) were randomly chosen, weighed and manually slaughtered at 6 week of age. The carcasses were weighed and the weights of the liver, gizzard and heart were recorded and expressed as $g \cdot kg^{-1}$ of slaughter weight (SW). Carcass and dressed weights were studied (dressed % = (carcass weight + giblets weight) / live body weight).

Blood sampling and laboratory analyses

Six birds per treatment were randomly used to collect blood samples after slaughtering into sterilized tubes. Samples were left to coagulate and centrifuged at 3500 rpm for 15 min to obtain serum. The serum samples were kept at -20 °C until further analyses. The following serum biochemical parameters: $mmol \cdot l^{-1}$: total lipids, total cholesterol, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol levels were measured using a Shimadzu 1200 spectrophotometer (Shimadzu Scientific Instruments; Kyoto, Japan), and kits and calibrators from Biodiagnostic Co. (Giza, Egypt), according to Akiba et al. (1982).

Statistical analysis

Data were subjected to ANOVA procedure using a completely randomized design using the GLM procedure of SAS (version 9.2, 2008). The differences among means were determined using the post-hoc Tukey's test. The statements of statistical significance are based on $P < 0.05$ unless otherwise stated.

Results and discussion

Growth performance and feed utilization

There was observed a significant effect of SBM substitution for NSM levels on both LBW and BWG at all experimental periods (Table 2). It was noticeable that LBW was linearly ($P = 0.017$) and quadratically ($P = 0.024$) increased at week 3 of age and only quadratically ($P < 0.001$) at week 6 of age due o SBM substitution levels up to 30%. The results obtained for BWG had the same trend. The heaviest birds were observed in the experimental group fed diet with 20% SBM substituted for NSM in different phases studied. Our results are in line with those reported by Zeweil (1996) who observed that LBW and BWG of growing Japanese quails fed diet including 9 or 18% NSM instead of SBM protein (6.78 or 13.54% in the diet) were significantly ($P < 0.01$) higher in comparison with control group. Tekeli (2014) postulated that feeding Japanese quail chickens diets with 4 or 8% NSM resulted in the best LBW in comparison with the control. Jamroz and Kamel (2002) found a stimulating impact of black cumin seed on the digestive system, which reflects positively in increased absorption and growth performance. The supplementation of *N. sativa* in the diet increases the bile flow rate which consequently increases emulsification simulating the pancreatic lipase activity (helps in the fat digestion and fat-soluble vitamins absorption) as theorized by Crossland (1980). The improved body weight could be also due to antimicrobial activity of the black cumin seed active components (Gilani et al., 2004). Also the same authors found that *N. sativa* has many components such as thymohydroquinone and thymoquinone which possess antimicrobial properties and are known for their pharmacological impacts. Furthermore, black cumin seed acts as antifungal and antibacterial agent and so exhibits protective action vs hepatotoxicity; all these actions can result in improved nutrient utilization (Rathee et al., 1982). It has been reported that *N. sativa* can directly or indirectly activate thyroid gland through the pituitary gland. Thyroid hormones are essential for the metabolism because they increase the rate of metabolism which can lead to improved amino acid utilization (by fastening their metabolism) (More et al., 1980).

Table 2. Effect of dietary *Nigella sativa* meal (NSM) replacing soyabean meal (SBM) on growth performance of Japanese quail from 1 to 6 week of age

Indices	SBM % substituted by NSM, %				SEM[1]	*P*-value[2]	
	0	20	30	40		linear	quadratic
Live body weight, g							
week 1	19.99	19.98	19.91	20.16	0.03	0.204	0.053
week 3	87.74[a]	87.97[a]	87.16[a]	75.74[b]	1.86	0.017	0.024
week 6	222.28[c]	249.87[a]	241.16[b]	211.49[d]	4.63	0.079	<0.001
Body gain, g							
week 1-3	4.84[a]	4.86[a]	4.80[a]	3.96[b]	0.13	0.016	0.022
week 3-6	6.40[b]	7.71[a]	7.33[a]	6.46[b]	0.18	0.339	<0.001
week 1-6	5.77[c]	6.56[a]	6.32[b]	5.46[d]	0.12	0.008	<0.001

[1] SEM – standard error means; [2] linear and quadratic effect of NSM meal; [a-d] – means with different superscripts within a row are significantly different

In our study both LBW and BWG were negatively influenced when the substitution level of

Table 3. Effect of dietary *Nigella sativa* meal (NSM) replacing soyabean meal (SBM) on feed intake and feed efficiency of Japanese quail from 1 to 6 week of age

Indices	SBM % replaced by NSM, %				SEM[1]	*P*-value[2]	
	0	20	30	40		linear	quadratic
Feed intake, g							
week 1-3	13.78[a]	13.30[a]	12.86[a]	10.86[b]	0.35	<0.001	0.008
week 3-6	22.33[b]	26.89[a]	25.80[a]	21.00[b]	0.75	0.501	<0.001
week 1-6	18.06[b]	20.09[a]	19.33[a]	15.93[c]	0.49	0.011	<0.001
Feed efficiency, g gain · g^{-1} feed							
week 1-3	0.350	0.366	0.376	0.366	0.007	0.405	0.582
week 3-6	0.283	0.286	0.283	0.310	0.005	0.128	0.196
week 1-6	0.313	0.313	0.316	0.326	0.003	0.128	0.362

[1,2] – see Table 2; [ab] – means with different superscripts within a row are significantly different

Table 4. Effect of dietary *Nigella sativa* meal (NSM) replacing soyabean meal (SBM) on energy and protein efficiency of Japanese quail from 1 to 6 week of age

Indices	SBM % replaced by NSM, %				SEM[1]	*P*-value[2]	
	0	20	30	40		linear	quadratic
Energy utilization, ME intake/BWG[3]							
week 1-3	8.27	7.96	7.78	8.02	0.18	0.564	0.614
week 3-6	10.13	10.13	10.21	9.44	0.14	0.172	0.150
week 1-6	9.32	9.28	9.25	8.86	0.09	0.151	0.275
Protein efficiency, g gain per protein intake							
week 1-3	1.46	1.52	1.55	1.52	0.03	0.468	0.687
week 3-6	1.19	1.19	1.18	1.28	0.01	0.172	0.161
week 1-6	1.29	1.30	1.31	1.36	0.01	0.175	0.329

[1,2] – see Table 2; [3] ME – metabolizable energy, BWG – body weight gain

SBM reached 40% (corresponding to 16% of NSM). The declined LBW and BWG in birds fed diets supplemented with 16% NSM instead of 40% SBM could be due to the high content of crude fibre in NSM and consequently deterioration of digestibility and depressed appetite of birds as reported by Azeem et al. (2014). Zeweil (1996) observed that body weight was declined with increased substitution of SBM protein with NSM protein at 28 or 38% (20.32 or 27.58% in the diet, respectively).

There was observed the linear and quadratic ($P < 0.05$) increase in feed consumption within weeks 1 - 3 and 1 - 6 in groups fed diets with replacement level of SBM for NSM up to 30% (Table 3). At the middle phase (weeks 3 - 6 of age), feed intake quadratically ($P < 0.001$) varied due to the NSM levels in the diet. Birds fed diets with 20 or 30% SBM replaced by NSM (8 and 12% of NSM, respectively) consumed more feed in comparison with the control and 40% SBM substituted group. Conversely, the worst palatability and consequently the lowest feed consumption were observed in the 40% replacement group. This reduction in feed intake may be due to the high content of fibre in NSM which increased as the level of NSM in the diet increased (Table 1).The improved feed intake due to NSM up to 12% (30% SBM substituted by NSM) may be caused by improved quails' appetite as cited by Abdel-Hady et al. (2009) and Tekeli (2014). In this respect, Gilani et al. (2004) and Amad and Radman (2013) suggested that *N. sativa* as feed supplement acts as stimulator for the digestive system activity and promotes the appetite and palatability. Similar results were obtained by Al-Homidan et al. (2002) who reported improved feed intake by incorporating black cumin seeds in broiler rations. Moreover, Azeem et al. (2014) demonstrated a significant increase in feed consumption in broilers fed NSM up to 10% which could be due to the different ways of affecting body metabolism by NSM. The contradictive results were reported by Sogut et al. (2012) who postulated that 7% addition of ground black cumin seeds to the broiler diets caused a significant decrease ($P < 0.01$) in feed consumption as compared with the control group.

No linear or quadratic differences ($P < 0.05$) were noticed in feed efficiency, energy utilization or protein efficiency due to SBM substitution for NSM (Table 3 and 4). In line with our results, Amad and Radman (2013) claimed that feed conversion ratio was not significantly affected by *N. sativa* seeds addition to the broiler diet. Further, Saeid et al. (2013) observed that rations containing black cumin seeds at various levels had no statistical impact on feed efficiency during all experimental periods. In contrast, several researchers, such as Abdel-Hady et al. (2009), confirmed that *N. sativa* seed had a significant influence on feed conversion ratio, energy utilization and protein efficiency. Obtained results showed good concentration of protein, fat, fibre, N-free extractives, vitamins, variety of minerals and moisture content on the basis of dry matter analysis in *N. sativa* seed (Ali and Blunden, 2003). Also, based on dry matter content analysis the components levels were: g · kg^{-1}: crude protein 216, N-free extractives 249, fat 406, crude fibre 84; mg · kg^{-1}: iron 105, copper 18, zinc 60, phosphorus 527, calcium 1860, thiamine 15.4, niacin 57, pyridoxine 5.0; μg · kg^{-1}: folic acid 160 as reported and documented by Ali and Blunden (2003) and Sultan et al. (2009).

Carcass characteristics

The substitution of 20% SBM for 8% of NSM resulted in a significant ($P < 0.001$) linear increase in both dressing and carcass percentages in comparison with the control and other treatment groups. Giblets yield was also significantly (P = 0.11 and P = 0.21 for linear and quadratic analysis, respectively) affected as a result of the substitution levels. A significant increases in liver percentage (P = 0.002 and P < 0.001 for linear and quadratic analysis, respectively), heart (P < 0.001 for both linear and quadratic analysis) and gizzard (quadratic; P < 0.001) were also associated with replacing 20% SBM by 8% of NSM in growing Japanese quail diet. This increase in the dressing, carcass and edible organs percentages indicate that *N. sativa* may have a good impact on the protein metabolism (Azeem et al., 2014). Our results are in accordance with those reported by Zeweil (1996) who demonstrated that the inclusion of NSM in quail diets positively influenced carcass percentage, heart, gizzard and spleen weights. Moreover, Toghyani et al. (2010) observed an increase in carcass, breast, thigh, wing, liver, neck and abdominal fat weights in broilers fed diet containing 1% black cumin seeds. Otherwise, AL-Beitawi et al. (2009) observed no improvement in carcass traits by adding crushed as well as uncrushed *N. sativa* seeds into broiler diets. In addition, Amad and Radman (2013) reported no significant impact on the slaughter weight, the dressing percentage and edible inner organs weight due to addition of *N. sativa* oil seeds into diets.

Table 5. Effects of dietary *Nigella sativa* meal (NSM) replacing soyabean meal (SBM) on carcass traits of Japanese quail at 6 week of age

Indices, %	SBM % replaced by NSM, %				SEM[1]	*P*-value[2]	
	0	20	30	40		linear	quadratic
Dressing	62.48[bc]	65.26[a]	63.33[b]	61.78[c]	0.41	0.172	<0.001
Carcass	57.48[c]	60.44[a]	58.94[b]	56.96[c]	0.43	0.613	<0.001
Giblets	5.00[a]	4.81[a]	4.39[b]	4.82[a]	0.25	0.011	0.021
Liver	10.18[c]	12.71[a]	10.81[b]	9.04[d]	0.40	0.002	<0.001
Gizzard	9.91[b]	12.84[a]	12.62[a]	8.86[b]	0.54	0.686	<0.001
Heart	5.57[b]	6.66[a]	4.29[c]	4.35[c]	0.30	<0.001	0.001

[1,2] – see Table 2; [a-d] – means with different superscripts within a row are significantly different

Blood lipid profile

The blood lipid parameters are vital signs of the physiological and nutritional condition of animals (Alagawany and Abd El-Hack, 2015). All studied lipid profile components were significantly (linear; P < 0.05) affected by substitution levels of SBM with NSM (Table 6). A gradual reduction in serum total lipids, total cholesterol and HDL-cholesterol content was observed along with an increasing level of NSM in the diet, except the HDL-cholesterol level (elevated in group with 40% SBM replaced by NSM). In the same time, LDL-cholesterol level was the lowest in the group fed diet with 20% SBM substituted for NSM, while the highest value was observed in the 40% SBM substituted group. The decrease in serum total lipids and total cholesterol content could be attributed to the high content of unsaturated fatty acids by feeding on diets containing *N. sativa* seeds which may stimulate the cholesterol oxidation to bile acids as explained by Tollba and Hassan (2003). Another reason for the decrease in serum lipids is the 'Nigellone' – active substance present in NSM which is mainly responsible for 3-hydroxy-3-methyl-CoA reductase (HMG-CoA) activity reduction. Furthermore, in several cases, a reduction in secretion of some hormones from the cortex of the adrenal glands, like glucocorticoids and androgens, could be the reason for the observed serum cholesterol decrease. Reducing the secretion of these hormones causes a run-down in fatty acids secretion from the adipose tissue which leads to a depression in fatty acids and cholesterol blood levels as well (Laudadio et al., 2015). Similarly to our results, Abdel-Hady et al. (2009) suggested that feeding growing Japanese quails diets with NSM addition significantly decreased total lipids and cholesterol concentration in serum in comparison with the control group. Similarly Amad and Radman (2013) showed that the inclusion of *N. sativa* seeds in broilers diet decreased serum cholesterol level comparing with the control group. Also, Ali et al. (2014) confirmed the ability of *N. sativa* seeds in combination with vitamin C to reduce blood content of total cholesterol in broiler chickens. Contrarily, Toghyani et al. (2010) observed that blood triglyceride, cholesterol and LDL levels were not statistically altered by the addition of *N. sativa* seeds to broiler diets.

Table 6. Effect of dietary *Nigella sativa* meal (NSM) replacing soyabean meal (SBM) on lipid profile of Japanese quail at 6 week of age

Lipid profile, mmol · l^{-1}	SBM % replaced by NSM, %				SEM[1]	*P*-value[2]	
	0	20	30	40		linear	quadratic
Total lipids	27.06[a]	28.08[a]	21.34[b]	20.24[b]	0.88	<0.001	0.444
Total cholesterol	5.79[a]	5.51[a]	5.07[b]	4.73[c]	0.13	<0.001	0.103
HDL cholesterol	2.43[a]	2.28[b]	2.13[b]	2.23[b]	0.03	0.003	0.120
LDL cholesterol	0.675[ab]	0.585[b]	0.724[ab]	0.809[a]	0.02	0.022	0.010

[1,2] – see Table 2; [abc] – means with different superscripts within a row are significantly different

Conclusions

Partial replacement of soyabean meal (SBM) by *Nigella sativa* meal (NSM) up to 20 and 30% (8 and 12% NSM addition, respectively) improves growth performance of growing Japanese quails, increases carcass and giblets yield and decreases blood total lipids and cholesterol levels. Therefore, NSM as an alternative feedstuff can partially replace SBM up to 30% in growing Japanese quail diets without any harmful effect on performance, feed utilization, carcass traits as well as blood lipid parameters.

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