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The Effect of Feeding Black Cumin (*Nigella Sativa*) and Vitamin C on Blood Lipid Profiles and Growth Performance of Broilers

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Abstract:- The objective of the present study was to determine the effect of different levels of *Nigella sativa* and vitamin C on blood lipid profiles and performance in broilers. Feeding black cumin combined with vitamin C is expected to be an alternative to minimize broiler from suffering to hot tropical climate. A total of 150 birds of Cobs strain broiler on 8 days old average body weight 187.14 ± 3.64 g . were randomly transferred into 25 experimental units and assigned in a completely randomized design with 5 treatments and 5 replications (6 birds each). Dietary treatments were the levels of black cumin with additional vitamin C, namely, T1: control diet with 500 ppm vitamin C, T2: diet with 0.25% black cumin + 500 ppm vitamin C, T3: diet with 0.5% black cumin + 500 ppm vitamin C, T4: diet with 0.75% black cumin + 500 ppm vitamin C, and T5: diet with 1% black cumin + 500 ppm vitamin C. Diets were provided during 27 days from 8 until 35 days old. Parameters measured were low density lipoprotein/LDL, high density lipoprotein/HDL, cholesterol, heterophyl/lymphocyte ratio, body protein mass, and body weight gain. Analysis of variance with f test was applied, and continued to Duncan test to differentiate the treatment mean values at 5% probability. The results indicated that feeding black cumin significantly (P<0.05) affected HDL and H/L ratio. Feeding 0.5% black cumin added with vitamin C significantly (P<0.05) decreased HDL, and in contrast, increased H/L ratio. It can be concluded that feeding black cumin at higher levels (0.75 and 1%) combined with additional vitamin C 500ppm indicates similar impact on blood lipids as well as growth performances as compared to those of control in broiler.

Keywords:- black cumin (*Nigella sativa*), vitamin C, blood lipids, growth performance, broiler.

I. INTRODUCTION

Chickens belong to a class of animals that regulate body temperature within a narrow range. This is only possible as long as they are maintained within their thermal comfort zone in ambient temperature. When the ambient temperature overshoot the thermal comfort zone, as the case commonly occurred in the tropical environment, the birds basically depends on panting and other behavioural responses to dissipate body heat. The high temperature in the tropics area can reach 34°C during the day and this can lead to the accumulation of heat in the body, thus the animal can suffers heat stress.

Stress responses are characterized as primary, secondary and tertiary. The primary stress response is a neuron endocrine response leading to corticosteroid and catecholamine release. The secondary stress response includes changes in plasma and tissue ion and metabolite levels induced by neuron hormones. The changes in disease resistance, growth, and behaviours at a whole body level are tertiary responses (Wedemeyer *et al*., 1990) and finally, a decline in productivity and resistance to diseases may occur. Animals under stress become sensitive to disease, and excess medicine may be necessary to maintain health. As a result, drug residues increase in animal products and threaten public health directly.

For this reason, stress conditions in animals need to be examined carefully (Seven, 2008). The high temperature environment can also lead to oxidative stress, causing excessive level of free radicals (Miller *et al*., 1993). Free radicals can cause membrane lipid peroxidation, thereby, these free radicals can attack DNA and protein (Rahman, 2003). Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen during stress (e.g. ultraviolet or heat exposure, environmental pollutant), Reactive oxygen species (ROS) levels can increase dramatically. This may result significant damage to cell structures. This cumulates into a situation known as oxidative stress.

The natural supplement can be used as an antioxidant and reduce heat stress is a combination of black cumin and vitamin C. Dietary supplementation with vitamin C has been reported to be efficient in reducing effects of heat stress in chickens (Sahin *et al*., 2003).Vitamin C plays a major role in the biosynthesis of corticosterone (Bain, 1996), a primary glucocorticoid hormone involved in gluconeogenesis to enhance energy supply during stress (Frandson *et al*., 1986). However, under critically high ambient temperatures, the production of vitamin C in broilers is inadequate for optimum performance (Daghir, 1995). Black cumin (*Nigella sativa* oils / NSO), one of the most important medicinal plants belonging to the family *Ranumculaceae*, containing active substance functions as antioxidant and also anti-cestode and anti-nematode actions.

Many effects have been described for the seeds of black cumin (*Nigella sativa*) and their constituents including its antioxidant role. Various beneficial properties have been attributed to *Nigella sativa*, including its antioxidant potential. It was reported previously that fluid extraction could be used to obtain *Nigella sativa* extract rich in antioxidants. *Nigella sativa* extracts were found to prevent protein carbonyl formation as well as depletion of intracellular glutathione (GSH) in fibroblasts exposed to toluene. Although fractions rich in thymoquinone were found to be most potent in terms of antioxidant capacity, the data indicates that the protective effects of *Nigella sativa* may not only be due to thymoquinone (Ashraf *et al*., 2011)

Research concerning the effect of dietary black cumin combined with vitamin C on blood constituents and performance of broilers in the tropical region has not been investigated enough. Therefore, the current study was conducted to determine the usefulness of active compound of black cumin combined with vitamin C as a promoting substance to reduce the unfavorable effect of environmentally hot temperature.

II. MATERIALS AND METHODS

Experimental Animal and Diet

Experimental animal used in the present study were 150 birds of one week old unsex broilers of Cobs strain, with initial body weight was 187.14 ± 3.64 g. Experimental diets were formulated using rice bran, yellow corn, poultry meat meal, meat bone meal, tapioca flour, fish meal, pollard and soybean meal. Diets and drinking water were given *ad libitum*.

Dietary protein and energy was approximately 22% and 3100 kcal/kg, respectively, for starter period, and was 21% and 3000 kcal/kg for finisher period, respectively (Table 1). Basal diets (both starter and finisher) were added with 500 ppm vitamin C, and in combination with black cumin, as describe in detail in the experimental design sub section

Table 1. Composition and Nutritional Content of Experimental Diets

Feed Ingredients	Starter (8 – 21 days)	Finisher (22 – 35 days)
	------------------------ (%)	--------------------
Yellow Corn	50.00	53.00
Rice Brand	10.00	11.00
Soybean Meal	16.00	15.00
Fish Meal	5.00	5.00
Meat Bone Meal	6.00	4.00
Poultry Meat Meal	5.00	4.00
Pollard	6.00	6.00
Tapioca Flour	2.00	2.00
Nutritional Content		
Metabolism Energy (kcal/kg)	3165.95	3095.12
Crude Protein (%)	22.89	21.12
Crude Fat (%)	7.12	7.02
Crude Fiber (%)	6.02	6.04
Calcium (%)	1.07	0.95
Phosporus (%)	0.54	0.46
Methionine (%)	0.34	0.33
Methionine + Cystine (%)	0.72	0.71
Lysine	1.18	1.12

Description:

Nutritional contents were calculated based on the results of laboratory analysis of each feed stuff used in the diet Metabolisable energy was calculated according to Balton formula (Siswohardjono, 1982).

Experimental Procedure

Experimental diets were provided during 27 days from 8 until 35 days old. Vaccine of ND_1 was given eye drops on day 3, and of ND_2 through drinking water on day 21. Rodalon disinfectant was diluted in 1 liter of water for cleaning animal house and equipment for bio security. Parameters observed were blood lipids (low density lipoprotein/LDL, high density lipoprotein/HDL, cholesterol), heterophyl/lymphocyte ratio, body protein mass, and body weight gain.

Experimental Design and Statistical Analysis

The study was arranged in a completely randomized design (CRD) with 5 treatments and 5 replications (6 birds each). There were 25 experimental units totally. The treatments applied in the study were as follows: T1 = 500 ppm vitamin C (control)

T2 = Diet with 500 ppm vitamin C add with 0.25% black cumin . T3
= Diet with 500 ppm vitamin C add with 0.5% black cumin . T4 =
Diet with 500 ppm vitamin C add with 0.75% black cumin . T5 =
Diet with 500 ppm vitamin C add with 1% black cumin .

The data was subjected to analysis of variance (ANOVA) at 5% probability level using f test to clarify the effect of treatment. If there were significantly effect of treatment, it was continued to Duncan multiple range test according to Duwi (2010).

III. RESULTS AND DISCUSSION

Effect of Vitamin C and Black Cumin on Blood Profiles Low Density Lipoprotein (LDL)

Effect of feeding black cumin combined with vitamin C was summarized in Table 2. Addition of vitamin C combined with black cumin at whatever levels did not affect blood LDL as compared to the diet with vitamin C alone. Feeding black cumin at 0.5 to 0.75% tended to results lower blood LDL, although statistically it did not significantly different. The results suggest that active substance especially phytosterol of black cumin has the minor ability to lowering LDL (bad lipoprotein) in the blood of the chicken. The chicken with slightly low blood lipoprotein indicated that those birds were able to produce healthy productivity.

Table 2. Effect of Treatment on Blood Lipids Profile

Blood Profile	Treatments				
	T_1	T_2	T_3	T_4	T_5
	--------------------------		(μl/dl) ----------------------------		
LDL	**96.41^{a}**	**93.96^{a}**	**87.57^{a}**	**84.30^{a}**	**90.71^{a}**
HDL	**24.64^{ab}**	**22.88^{ab}**	**19.80^{b}**	**27.28^{a}**	**25.08^{ab}**
Cholesterol	**121.05^{a}**	**116.84^{a}**	**107.37^{a}**	**111.58^{a}**	**115.79^{a}**
H/L Ratio	**0.28^{c}**	**0.93^{ab}**	**1.23^{a}**	**0.64^{bc}**	**0.64^{bc}**

Values within raw followed by different superscript showed significant difference (P<0.05)

No significant effect of feeding black cumin in reducing LDL can be caused by difference effectiveness of active substance which is also closely related to the condition of the individual broiler. This is in accordance with the report of Al - Jawfi (2008) that the difference levels of cumin origin and condition of the broiler brought about the difference effectiveness of active substances of cumin. In addition to differences of active substances content of black cumin, can be possibly caused by the lower use of black cumin with the level not more than 1%. This result agreed with Sohail (2012) who reported that supplementation of 4% or 5% black cumin significantly decreased serum LDL cholesterol.

In contrast to the present results, previous research has shown that black cumin can reduce cholesterol, as black cumin contain oleic acid (Omega 9), which is an important substance of the omega family in a form of monounsaturated fatty acids or mono unsaturated fatty acid (MUFA), it also has an efficacy to reduce LDL cholesterol (bad lipoprotein). An active substance of black cumin content phytosterol, have the ability to compete with cholesterol absorption in the intestine and finally resulted in reducing the absorption of cholesterol (Richard, 2011).

High Density Lipoprotein (HDL)

It was known that the treatment of black cumin significantly (P<0.05) affect blood HDL of broilers. HDL decreased by feeding 0.50% black cumin, this result agreed with Mandour *et al*. (1998) that reported a decrease in plasma HDL concentration resulted from feeding chicken with 0.50% of black cumin. Phytosterol compound found in black cumin can inhibit the formation of micelles due to the absorption of bile acids into the intestine. This is in accordance with the finding of Bonsdorff (2005) that the mechanism of phytosterols is believed to inhibit cholesterol absorption and reabsorption of endogenous cholesterol derived from the ration in the digestive tract. Therefore, phytosterols increase the spending time of the excess cholesterol absorption, and causes a decrease in serum cholesterol levels. It is assumed that with cholesterol reduction would be offset by the decreased levels of HDL in the blood serum. However, when feeding black cumin was increased to a level of 0.75% the blood concentration of HDL enhanced, it was significantly different. The increased levels of HDL in the blood with the feeding level of 0.75% black cumin is possibly due to ideal level of active substance of black cumin in relation to addition of 500 ppm vitamin C.

Kilic (1993) reported that most of the components of atherosclerotic plaques is cholesterol. Furthermore, vitamin C is required in the hydroxylation of carbon number 7 of cholesterol to a 7-α-hidroksikolesterol, the first intermediate compound which will be bile acids. In addition, vitamin C is also necessary for maintaining the elasticity of the aortic wall via hydroxylation of lysine and proline. Therefore, vitamin C in plasma has correlated negatively with plasma total cholesterol levels, but positively correlated with HDL (Maeda *et al*., 2000). However, in this study, feeding a combination of vitamin C 500 ppm and black cumin until 1% has not been able to increase significantly blood HDL, although in numeric value it tended to be slightly elevated. It is possible that feeding vitamin C 500 ppm and 0.75% black cumin could be categorized as an ideal combination to improve levels of blood HDL.

Cholesterol

There was no feeding effect of vitamin C and black cumin until 1% on blood cholesterol. Numerical value indicated that combination of vitamin C and 0.5 to 1% black cumin was found to slightly reduce blood cholesterol in chickens although statistically was not significant. These results suggest that the slightly increased in HDL level was in accordance with the little reduce in blood cholesterol. The decrease in plasma cholesterol level may be attributed to the high content unsaturated fatty acids of black cumin which may stimulate the cholesterol excretion into the intestine and the oxidation (Khodary *et al*., 1996).

The slightly decrease in blood cholesterol was probably due to active substance of black cumin, especially phytosterol, and vitamin C which is exerting its effect indirectly and slowly on the activity of HMG-CoA reductase, a key enzyme in cholesterol biosynthesis. Al-Beitawi *et al.* (2009) reported that replacing the bacitracin with crushed black cumin at the level of 2% in broiler diets significantly reduced serum cholesterol. Brunton (1999) suggested that reduction in serum cholesterol may be attributed to the lowering effect of thymoquinone and monosaturated fatty acids on the synthesis of cholesterol by hepatocytes or the fractional reabsorption from the small intestine. Moreover, black cumin also contain an appreciable amount of sterols, especially β-sitosterol, that has the ability to inhibit the absorption of dietary cholesterol. The present results were consistent with that obtained by Khalaji *et al.* (2011), who reported that 1% black cumin in broiler diets did not decrease significantly the total serum cholesterol. Black cumin reduced cholesterol only when provided at higher doses.

Heterophyl/Lymphocyte Ratio (H/L Ratio)

There was significant effect ($P < 0.05$) of feeding vitamin C and black cumin on H/L in broilers. Feeding black cumin of 0.25 (T2) and 0.5% (T3) significantly ($P < 0.05$) increased H/L ratio compared to T1 (vitamin C alone without black cumin). However, increasing dietary level of black cumin until 0.75 (T4) and 1% (T5) did not significantly different when compared to T1. The increased H/L ratio in T2 and T3 treated broiler did not imply that the birds suffered stress, but the feeding effect of black cumin at the lower level combined with vitamin C brought about the adaptive process much more intense than that of vitamin C alone. This is in accordance with the repport of Mahmood (2006) that black cumin has an anti stress activity in chicken and demonstrated negative correlation between H/L ratio and feed intake.

Occasionally, the increase in heterophil to lymphocyte (H/L) ratio in the peripheral blood found in the present study was the opposite phenomenon that being recognized as a reliable indicator of stress in birds.

However, the broiler needed the process of adaptation known as “self phagocytosis” due to the low active substance of black cumin which interacts with vitamin C. When feeding black cumin were increased to 0.75 (T4) and 1% (T5), H/L ratio decreased into the levels similar to that of T1 (vitamin C alone). These results indicated that the higher active substance derived from black cumin fed together with vitamin C could improve its interactive balance, and further decreased H/L ratio. The stress may be pathological, nutritional or environmental as stated Al-Murrani *et al*. (1997). The effect of stress in chickens was characterized by elevation of hetrophil and reduction of lymphocyte due to the increased corticosteron level in serum (Mahmood, 2006). Further, Ather (2009) showed that the lowest H/L ratio was observed in the 1% black cumin treated group, this suggests that black cumin is possible to be used as an anti stress factors when added to the feed.

Effect of Black Cumin and Vitamin C on Broiler Performance Body Protein Mass

Broiler performances covering protein body mass and body weight gain was indicated in Table 3. Feeding black cumin at whatever levels combined with vitamin C did not affect body protein mass as compared to T1 (vitamin C alone). Feeding black cumin until the level of 1% with addition of vitamin C did not affect body protein mass. It suggest that active substance of black cumin have no enough promoting effect on the acceleration protein synthesis to be higher rate compared to protein degradation. As it has been reported by Suthama (2010) that the increase in protein deposition in terms of body protein mass was due to the much faster rate of protein synthesis than that protein degradation. The difference between protein synthesis and degradation rates can be caused by the state of animal health. The health status of the birds in the present study was indicated by H/L ratio. Previous workers reported that the hetrophil/lymphocyte (H/L) ratio was an excellent indicator of stresses in chickens (Kassab *et al*., 2000).

Table 3. Effect of Treatment on Broiler Performance

Replication	Treatments				
	T_1	T_2	T_3	T_4	T_5
	----------------------------		(g/bird) ----------------------------		
Protein Body Mass	**155.72^{a}**	**150.71^{a}**	**149.45^{a}**	**147.68^{a}**	**154.39^{a}**
Body Weight Gain	**1137.11^{a}**	**1083.78^{a}**	**1084.79^{a}**	**1161.91^{a}**	**1165.74^{a}**

Values within raw followed by different superscript showed significant difference (P<0.05)

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Body Weight Gain

It was observed that no significant effect of feeding black cumin added with vitamin C on body weight gain of broilers. The pattern of body weight gain due to the feeding effect of black cumin added with vitamin C or vitamin C alone was similar to the data of body protein mass (Table 3). The rate of protein deposition, in term of body protein mass, is the indication of growth capacity. When body protein increase should be followed by the improved body weight gain, and vice versa. Protein deposition is an important factor for the determination of carcass quality. Carcass with high protein deposition deliver on quality traits and carcass composition. Protein and fat are the two types of nutrients that have a direct connection with the metabolic body weight to achieve a certain body weight in broilers (Suthama, 2010).

The present study indicated that the level of active substance of black cumin at 1% was possibly still low and could not accelerate the rate of protein accretion, finally, resulted similar body weight gain to that fed with vitamin C alone. In contrast with El-Bagir *et al.* (2006) reported that dietary black cumin at the level of 1 or 3% significantly increased the final body weight of early laying hens. Similarly, Sogut *et al.* (2008) reported that a low level of black cumin (3%) tended to improve broilers' performance compared to a high level (7%).

However Aydin *et al.* (2008) showed the different phenomenons in laying hens that supplementing layer hen diets with 1, 2, and 3% black cumin had no effects on body weight gain and feed consumption ratio. Ziad (2008), found that supplementation of black cumin with the levels of 1-1.5% in the diet increased weight gain but with increasing the level of black cumin resulted in significantly decreased in weight gain.

IV. CONCLUSION

Feeding black cumin until 1% combined with vitamin C (500 ppm) is able to maintain the health status and productivity of broiler reared in the hot climate. Indeed, the combination of feeding 0.75% black cumin and vitamin C (500 ppm) or vitamin C alone (500 ppm) can be categorized as sufficient level for the protection of unfavorable effect of high environmental temperature, supported by low level of H/L ratio.

REFERENCES

[1]. Al-Beitawi, N. A., S. S. El-Ghousein, and A. H. Nofal. 2009. Replacing bacitracin methylene disalicylate by crushed Nigella sativa seeds in broiler diets and its effects on growth, blood constituents and immunity. Livest. Sci. 125:304–307.

[2]. AL-Jawfi, K.A.M., M.M.A. Hassan, A.M. El-Gohary. 2008. Effect of Nigella sativa oil on the Hamster lymphocytes secondary to DMBA-induced carcinogenesis. Suez Canal Univ. Med. J., 11 (1), pp. 75–80.

[3]. Al-Murrani, W.G., A. Kassab and H. Al-Sam (1997). Heterophil/lymphocyte ratio as a selection criterion for heat resistance in domestic fowls. British Poultry Science 38: 159-163.

[4]. Aydin, R., M. Karaman, T. Cicek and H. Yardibi, 2008. Black cumin (Nigella sativa L.) supplementation into the diet of the laying hen positively influences egg yield parameters, shell quality and decreases egg cholesterol. Poult. Sci., 87: 2590-2595.

[5]. Brunton, L. L. 1999. Agents affecting gastrointestinal water fluxand motility, digestants and bile acids. In: The Pharmacological Basis of Therapeutics. 8th Ed. Pregman Press, Oxford. Pp. 914–932.

[6]. Bain, B. S. 1996. The role of vitamin C in stress management. World Poult. 12(4): 34-41

[7]. Daghir, N. J. 1995. Nutrient requirements of poultry at high temperature, in Poultry production in hot climate, CAB International. Wallingford, UK. Pp. 101-125.

[8]. El Bagir, N.M., A.Y. Hama, R.M. Hamed, A.G. Abd El Rahim and A.C. Beynen, 2006. Lipid composition of egg yolk and serum in laying hens fed diets containing black cumin (Nigella sativa). Int. J. Poult. Sci., 5: 574-578.

[9]. Frandson, J. C., H. C. Murray dan C. Bunck. 1986. Enzyme Activities in plasma, kidney, liver, and muscle of five avian species. J. Wild Dis. 21(1): 33-39.

[10]. Kassab, A.K., H.H. Elaiwi, W.K. Al-Murrani, (2000). Hetropil/ Lymphocyte ration under higher and low tempretuer against Newcastle antigen in chicken. IPA Agre. Res. 10: 184-192.

[11]. Khalaji, S., M. Zaghari, K. H. Hatami, S. Hedari-Dastjerdi, L. Lotfi, and H. Nazarian, 2011. Black cumin seeds, Artemisia leaves (Artemisia sieberi), and Camellia L. plant extract as phytogenic products in broiler diets and their effects on performans, blood constituents, immunity, and cecal microbial population. Poult. Sci. 90: 2500-2510.

[12]. Khodary, R.M., El-Ezzawy, M.H. Andhamdy, I.R. 1996. Effect of Nigella sativa on egg production, hatch ability percentage and some biochemical values in laying hen with references to fertility in cockerels. Procof 7th Sci. Cong., Fac. Vet. Med., Assuit Univ., 17-19. Nov. Ass. Egypt, pp. 91-106.

[13]. Rahman, I. 2003. Oxidative stress, chromatin remodeling and gene transcription in inflammation and chronic lung disease. J. Biochem. Mol. Biol. 36: 95-109.

[14]. Suthama, N. 2010. Pakan Spesifik Lokal dan Kualitas Pertumbuhan untuk Produk Ayam Lokal Organik. Pidato Pengukuhan Guru Besar Universitas Diponegoro, Semarang (in Indonesian language).

[15]. Maeda, K., Arita, Y., S. Kihara, N. Ouchi, M. Takahashi, J. Miyagawa, K. Hotta, I. Shimomura, T. Nakamura, K. Miyaoka, et al. 2000. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem. Biophys. Res. Commun. 257: 79–83.

[16]. Mahmood, S.K. 2006. Immune improvement and production performance of broilers for diseases ND and IBD by employment Nigella, Fenugreek and Garlic. PhD. thesis. University of Baghdad, collage of Veterinary Medicine, Public Health Department.

[17]. Mandour, A.A., K.M. Ashry and S.A. Hedaya, 1998. Biochemical profile of serum constituents of broiler chickens supplemented with different levels of Nigella sativa with special references to its effect on hormonal and mineral concentrations. Egypt. Poult. Sci., 18: 429-439.

[18]. Miller, J. K., E. B. Slebodzunska and F. C. Madsen. 1993. Oxidative stress, antioxidant, and animal function. J. Dairy Sci. 76: 2812-2823.

[19]. Sahin, K., O. Kucuk, N. Sahin, and M. Sari. 2003. Effects of vitamin C and vitamin E on lipid peroxidation status, serum hormone, metabolite, and mineral concentrations of Japanese quails reared under heat stress (340C). Int. J. Vit. Nutr. Res. 72(2):91-100.

[20]. Seven, Tatli P. 2008. The effects of dietary Turkish propolis and vitamin C on performance, digestibility, egg production and egg quality in laying hens under different environmental temperatures. AJAS, 21, 1164-1170.

[21]. Sogut, B., I. Celik and Y. Tuluce. 2008. The effects of diet supplemented with black cumin (Nigella sativa L.) upon immune potential and antioxidant marker enzymes and lipid peroxidation in broiler chicks. J. Anim. Vet. Adv. 7(10):1196-1199.

[22]. Sohail H. Khan, Jahanzeb Ansari, Ahsan u. Haq, Ghulam Abbas. 2012. Black cumin seeds as phytogenic product in broiler diets and its effects on performance, blood constituents, immunity and caecal microbial population. JAS. 22(6):467-78

[23]. Wedemeyer, G.A.; Barton, B.A. & McLeay, D.J. 1990. Stress and acclimation. In: Methods for fish biology, eds. C.B. Schreck and P.B. Moyle, American Fisheries Society, Bethesda, Maryland, USA, 451–489.

[24]. Ziad, H.M.Abu-Dieyeh., S.Abu-Darwish. Mohammad. 2008. Effect of feeding powdered black cumin seeds (Nigella sativa L) on growth performance of 4–8 week-old broilers. J. Anim. Vet. Adv., 7 (3), pp. 86–290