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PRODUCTIVE PERFORMANCE AND HEMATOLOGICAL PARAMETERS OF LAYING HENS FED *NIGELLA SATIVA* MEAL UNDER HOT CONDITIONS

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ABSTRACT: This experiment was conducted to evaluate the effect of different levels of *Nigella Sativa* meal (NSM) on productive performance and some hematological parameters of laying hens under hot conditions. A total number of 120 laying hens (34 weeks old – Commercial Browne Lohmann LSL) and body weight of 1996.90±18.75 g were used in this study. Chickens were randomly divided into four equal treatments (30 chickens of each). Each treatment was randomly divided into 3 equal replicates (10 chickens of each).The 1st treatment, chickens fed basal diet and considered as control group (C). The 2nd, 3rd and 4th treatments, chickens were fed diets supplemented with 5, 10 and 15 % NSM, respectively). The results showed that egg weight, egg number and feed conversion were improved (P<0.05) in the laying hens fed 10 and 15 % NSM compared with other groups. However, egg shell thickness was increased (P<0.05) in the hens fed 5, 10 and 15 % NSM compared to control group. Red blood cells and hemoglobin concentration were increased (P<0.05) in the hens fed high level of NSM (15 %) compared to hens in control group. Adding NSM at level 15 % led to significantly decreased in serum total protein, albumin, globulin, glucose and cholesterol when compared with control group. While, creatinine level was significantly increased at level 5 % of NSM compared with other levels of NSM and control group. Using NSM by rate 5, 10 and 15 % in the chicken diets led to a decrease in the cost of feed and this in turn contributed to the improvement of net income/hen. In conclusion, under hot conditions, use *Nigella Sativa* meal up to level 15 % as a source of alternative feed resources enhancement the productive performance and physiological responses of laying hens.

Key words: laying hens, *Nigella Sativa*, heat stress, productive performance, hematological

INTRODUCTION

Climate changes, in particular global warming, will affect the health and welfare of farm animals, especially in country located in tropical and sub-tropical regions (IPCC, 2007). Egypt suffered from heat stress conditions where the peak of outdoor ambient temperature reached to 45 °C and the temperature higher than 35 °C was recorded more regulatory during summer months (Faisal et al., 2008 and Morsy, 2018).

The optimum environmental temperature for performance of adult laying hens were ranged between 19 and 22°C, if the temperatures were above or below, this range requiring thermoregulation (Lin et al., 2006). Researchers have been reported that high environmental temperature led to harmful effects on the performance of laying hens through altered blood chemistry, decreasing productive performance and increased mortality (Barrett et al., 2019; Attia et al. 2016; Kilic and Simsek, 2013; Song et al., 2012; Khan et al., 2011 and Oguntunji and Alabi 2010).

The natural feed additives or resources like herbs and edible plants have some properties as growth enhancers, to replace synthetic drugs. These natural feed additives, *Nigella Sativa* seed, meal or oil are given to birds to improve their physiological and productive performance under normal or stress conditions. (Attia et al., 2008).

Nigella Sativa meal (NSM) is the residual by-product of pressed *Nigella Sativa* seed that is believed to contain nutrients, which are not completely obtained from the whole seed (Silvia et al. 2012).

Numerous studies have been carried out in relation to the utilization of *Nigella Sativa* seed as a feed supplement. However, its residual seeds meal as a supplemental source of polyunsaturated fatty acid in the diet. Owing to their high nutritional properties, a number of studies have highlighted the effect of NSM supplementation in poultry such as broiler (Tollba and Hassan 2003; Abdo 2004; Hassan et al. 2007; Al-Muffarrej 2014; Ghasemi et al. 2014), quail (Hassan and Alaqil 2014) and laying hens (Akhtar et al., 2003). The NSM is a rich source of fatty acid that able to control the antimicrobial effects, so, the addition of these supplements will enable regulation of bio-hydrogenation process in order to improve the feed digestibility (Jenkins, 1993). In addition, NSM can be used as a protein-rich meal because it contains most of essential amino acids, with crude protein 33 % (EL-Nattat and ELKady, 2007) 33.13 % (Mahmoud and Bendary, 2014) that may help chickens to tolerate hot climate conditions. El-kashef (2020) showed that addition of 3 and 6% of NSM to broiler diet seem to have a positive influence on growth performance, blood biochemical, immune-responsiveness and economic efficiency, Furthermore, it could be considered growth promoter for broiler chicks. The addition of NSM in the broilers diet led to improved growth performance (Guler et al., 2006; Abu-Dieyeh and Abu-Darwish, 2008; Islam et al., 2016) feed conversion and decreasing feed cost (Abdel-Magid et al., 2007).

Therefore, this experiment was conducted to evaluate the effect of using different levels of *Nigella Sativa* meal (NSM) in laying hens' diets on productive performance and some hemato-biochemical parameters under hot conditions.

MATERIALS AND METHODS

The present study was conducted in the South Sinai Research Station (Ras Suder City) that belongs to the Desert Research Center, Ministry of Agriculture, Egypt. The experiment started from June to August (experiment period 100 days). This experiment was conducted to evaluate the effect of using different levels of *Nigella Sativa* meal (NSM) in laying hens' diets on productive performance and some hemato-biochemical parameters under hot conditions. A total number of 120 laying hens (34 weeks old – Commercial Browne Lohmann LSL) were obtained from a commercial farms and body weight of 1996.9±18.75 g was randomly divided into four equal treatments (30 chickens of each), which in turn divided into 3 equal replicates (10 chickens of each). The 1st treatment, chickens fed on basal diet and considered as control group(C). The 2nd, 3rd and 4th treatments, chickens were fed on diets supplemented with 5, 10 and 15 % NSM, respectively which presented in Table (1). Indoor climatic conditions (ambient temperature, AT, °C and relative humidity, RH, %) which recorded during the experimental period by electronic digital thermo-hygrometer were showed in Table (2). The relationship between ambient temperature and relative humidity was termed as temperature-humidity index (THI) and calculated according to Marai *et al*. (2001). THI = db°C – [(0.31 – 0.31 × RH) × (db°C – 14.4)]. Where, db°C = dry bulb temperature in centigrade and RH = relative humidity %. The THI values were classified as absence of heat stress (<27.8), moderate heat stress (27.8-28.8), severe heat stress (28.9-29.9) and very severe heat stress (>30.0).

Chickens were housed in wire cages, exposed to a specific lighting program consisted of a period of (16 h light and 8 h of darkness), Chickens were kept under the same managerial and hygienic conditions, all chickens in current experiment were good healthy and treated with vaccines.

Live body weight was individually recorded for each chicken and the average live body weights were calculated for each treatment during the experimental period (one hundred days). Total feed intake (TFI) was recorded on weekly by calculated the difference between the amount of feed provided for each treatment and the residual quantity for the same treatment. Feed conversion ratio was calculated for each treatment by divided TFI / egg mass. At the end of experimental period, 3 ml of blood was collected from wing vein from 25 birds of each treatment, 10 samples into anticoagulant EDTA treated for determination of red blood cells (RBC's), hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were determined by the counter (HA-VET, Clinding – Belgium). Another 15 blood samples were collected in non-EDTA tubes. Serum was collected by centrifugation for 20 minutes at 3000 rpm and it stored at – 20 ^{0} C until determination of blood metabolites (total protein, albumen, glucose, cholesterol, creatinine, alanine and aspartic transaminase) by using commercial kits. Globulin was calculated by the difference between total protein and albumin.

Data were analyzed by the least square analysis of variance using the General Linear Model Procedure (SAS, 2004) according to following model:

$Y_{ij}=\mu + Tr_i + e_{ij}$

Where,

Y_{ij}= Observations.

μ = Overall mean.

Tr_i = Effect of i^{th} treatment (i: 1-4).

e_{ij}= Experimental error.

Duncan's New Multiple Range Test (Duncan, 1955) separated differences among treatment means.

RESULTS AND DISCUSSION

1. Productive performance

Egg weight was increased (P<0.05) in the laying hens fed 5, 10 and 15 % *Nigella sativa* meal (NSM) by 3.61, 7.22 and 6.62 %, respectively as compared with control group (Table 3).

Medium and high Levels (10 and 15 % NSM) in the diet of laying hens led to significantly increased in egg number by 5.05 and 8.28 %, respectively when compared to control group (diet without NSM). In the same trend, egg mass was increased (P<0.05) in the hens fed 10 and 15 % NSM by 12.64 and 15.45 %, respectively as compared with control group. Indeed, feed conversion was significantly improved in the hens fed 10 and 15 % NSM by 10.41 and 12.08 %, respectively as compared with control group (Table 3).

However, egg shell thickness (ShTh) was increased (P<0.05) in the hens fed diets supplemented with different levels of NSM (5, 10 and 15 %) by 6.26, 7.01 and 7.26 % when compared with control group (Figure 1).

On the other hand, there are insignificant differences among hens in control group and hens fed *Nigella sativa* meal treatments in the final body weight and total feed intake (Table 3). These enhancements in egg production, egg weight, egg mass and feed conversion under heat stress may be due to nigellone, thymoquinone and phenolic compounds of NSM, these substances of MSN act as anti-stress (Tollba and Hassan, 2003) antioxidant (Mariod et al. 2009) antimicrobial and antifungal activity (Rathee et al., 1982; Hanafy and Hatem, 1991; Akhtar et al., 2003; Nickavara et al., 2003)

The previous results agreement with Saleh *et al*., 2020; Aydin et al., 2006 and Yalcın et al., 2009 who shown that using different levels of *Nigella sativa* seed or cumin meal supplementation significantly improved egg production, egg shell thickness of laying hens. Additionally, Zeweil (1996) found that the performance of growing Japanese quail fed diets containing 6.25 or 13.50% NSM were significantly improved as compared to the control one.

2. Blood picture

Table (4) shown that red blood cells (RBC's), hemoglobin (Hb) and mean corpuscular hemoglobin concentration (MCHC) were increased (P<0.05) in the hens fed 15 % NSM by 5.85, 6.41 and 5.70 % as compared to hens in control group. These results may be due to the hematopoietic effect of *Nigella sativa* meal (Akon et al., 2019). Meanwhile, mean corpuscular volume (MCV) was significantly decreased in the hens fed 5, 10 and 15 % NSM by 5.45, 4.50 and 4.59

%, respectively compared with control group. These results agreement with El-kashef (2020), who showed that addition of 3 and 6% NSM to broiler chicks diet seem to have a positive influence on blood parameters such as albumin level, Akon et al. (2019) reported that RBC's, PCV and Hb concentrations were significantly increased in the treated groups with *Nigella sativa* in broilers as compared to control. In addition, Hermes et al. (2010) found that adding 10 % of *Nigella sativa* meal to broilers diets under heat stress improved RBC's, Hb and PCV as compared with control group.

3. Biochemical parameters

Serum biochemistry reference values might provide valuable information about physiological status and provide an indication of health status and disease resistance in birds. Changes in internal milieu can be caused by various factors, one of which involves diet and feed (Capcarová and Kolesárová, 2010). Table (5) showed that *Nigella sativa* meal (NSM) treatment at level 15 % significantly decreased in total protein, albumin, globulin, glucose and cholesterol when compared with control group While, creatinine level was significantly increased at level 5 % of NSM compared with other levels of NSM and control group. On the other hand, there are insignificant differences between control and the *Nigella sativa* meal treatments in the level of aspartic transaminase. These results agreement with Hermes et al. (2009) who found that *Nigella sativa* oil at 0.5 and 1% significantly decreased alanine aminotransferase, total protein, albumin, globulin and cholesterol concentrations compared to the control group. These decreases in total proteins, albumin, globulin, glucose and cholesterol may be due to the increased in egg number and egg mass (Table 3). While, Zanouny et al. (2013); Mohammed and Al-Suwaiegh (2016) and Retnani, et al. (2019) found that supplementation of *Nigella sativa* meal to lamb diet decreased plasma glucose and plasma cholesterol concentration and they attributed this decreased to the increase of thyroid gland activity. Also, Omar (2003) attributed the reduced cholesterol in diets of growing rabbits containing high levels of *Nigella sativa* meal to unsaturated fatty acids, which may encourage the cholesterol secretion into the intestine and the oxidation of cholesterol to bile acids. On the other hand, Retnani, et al. (2019) reported that transaminases enzymes (AST) concentrations were not significantly affected by *Nigella sativa* meal.

4. Economic indicator

Results in Table (6) showed that the highest value for economic indicator, net income was achieved with the diets contain NSM when compared to control group. The economic indicator showed that the use of *Nigella Sativa* meal by rate 5, 10 and 15 % in the laying hen diets decrease in the cost of feed and this in turn might contribute to the improvement of net income/hen.

CONCLUSION

The use of *Nigella Sativa* meal up to level 15 % as a source of alternative feed resources enhancement the productive performance and physiological responses of laying hens under hot conditions.

Table (1): Composition and calculated analysis of the experimental diets.

Ingredients	Control	levels of *Nigella Sativa* meal		
		5%	**10%**	**15%**
Yellow corn	57.00	56.00	53.10	50.90
Soybean meal (44 %)	29.00	26.00	22.80	20.00
Wheat bran	2.84	2.14	3.44	3.74
DL-Methionine	0.06	0.06	0.06	0.06
Salt	0.30	0.30	0.30	0.30
Vit and Min. Premix*	0.30	0.30	0.30	0.30
Oil	1.30	1.00	0.80	0.50
Di-calcium phosphate	1.50	1.50	1.50	1.50
Limestone	7.60	7.60	7.60	7.60
Anti-toxins	0.10	0.10	0.10	0.10
Nigella Sativa meal	0.00	5.00	10.00	15.00
Total	100	100	100	100
Calculated value**				
Crude protein (%)	18.05	18.11	18.20	18.39
Crude fiber (%)	3.32	3.47	3.60	3.75
ME (Kcal/Kg)	2763.60	2770.50	2778.20	2779.40
Calcium (%)	3.31	3.30	3.29	3.28
Available phosphorus (%)	0.40	0.39	0.39	0.38

* Each 2.5 kg Vitamins and minerals premix comprises (per ton of feed), Vit. A 10000000 IU, Vit. D_3 2000000 IU, Vit. E I0g, Vit.K_3 1000 mg, Vit. B_1 1000 mg, Vit. B_2 5000 mg, Vit. B_6 1.5g, Vit. B_{12} 10 mg, Pantothenic acid 10g, Niacin 30g, Folic acid 1g, Biotin 50 mg, Iron 30g, Manganese 70g, Choline chlorite 10g, Iodine 300 mg, Copper 4g, Zinc 50g and Selenium 100 mg.

** According to NRC (1994).

Table (2): Indoor ambient temperature (AT, ^{o} C), relative humidity (RH, %) and temperature-humidity index (THI) throughout experimental period

Months	Minimum AT	Maximum AT	Minimum RH	Maximum RH	Minimum THI	Maximum THI
June	24.1	33.9	25.9	41.6	21.9	30.4
July	23.8	35.6	25.3	42.1	21.6	31.8
August	25.1	35.9	29.8	49.6	22.8	32.5

Table (3): Effect of different levels of *Nigella Sativa* meal on productive performance of laying hens

Traits	Control	levels of *Nigella Sativa* meal			±SE
		5%	10%	15%	
IBW (g)	1779.55	1777.11	1761.88	1785.33	30.33
FBW (g)	1901.40	1932.50	1944.20	1958.10	52.50
Egg weight (g)	61.15^{c}	63.36^{b}	65.57^{a}	65.20^{a}	0.48
Egg number	68.45^{c}	69.19^{bc}	71.91^{ab}	74.12^{a}	1.44
Egg mass (g)	4185.71^{b}	4383.87^{b}	4715.13^{a}	4832.62^{a}	98.60
TFI (g)	10069.95	10132.42	10181.17	10238.63	89.82
Feed comversion	2.40^{b}	2.31^{b}	2.15^{a}	2.11^{a}	0.067

IBW = initial body weight; FBW = final body weight; TFI = feed intake.

$^{a,\ b,\ c}$ Means bearing different superscripts within the same row are significantly different (P<0.05).

Table (4): Effect of different levels of *Nigella Sativa* meal on blood picture of laying hens

Traits	Control	levels of *Nigella Sativa* meal			±SE
		5%	10%	15%	
Red blood cells ($\times 10^{6}$)	2.39^{b}	2.45^{ab}	2.47^{ab}	2.53^{a}	0.03
Hemoglobin (g/dl)	9.04^{c}	9.32^{b}	9.26^{bc}	9.62^{a}	0.08
Packed cell volume (%)	27.14^{a}	26.21^{b}	26.75^{ab}	27.30^{a}	0.27
MCV (fl)	113.20^{a}	107.03^{b}	108.10^{b}	108.00^{b}	1.67
MCH (pg)	37.74	38.06	37.47	38.04	0.84
MCHC (%)	33.33^{b}	35.56^{a}	34.63^{a}	35.23^{a}	0.55

MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration.

$^{a,\ b,\ c}$ Means bearing different superscripts within the same row are significantly different (P<0.05).

Table (5): Effect of different levels of *Nigella Sativa* meal on biochemical parameters of laying hens

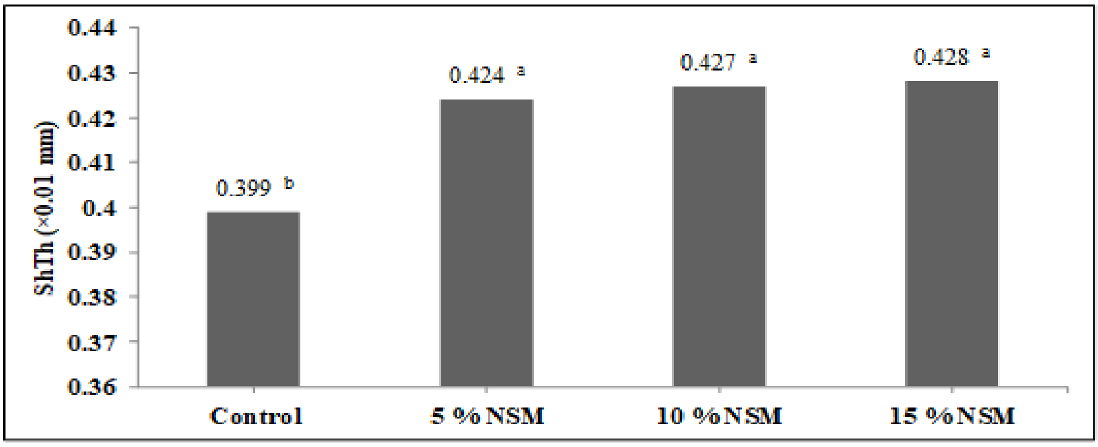
Traits	Control	levels of *Nigella Sativa* meal			±SE
		5%	10%	15%	
Total protein (g/dl)	8.93[a]	7.54[bc]	7.89[ab]	6.44[c]	0.43
Albumin (g/dl)	4.75[a]	4.74[a]	4.21[ab]	3.89[b]	0.20
Globulin (g/dl)	4.18[a]	2.79[b]	3.68[ab]	2.54[b]	0.44
Glucose (mg/dl)	165.64[a]	156.02[a]	140.50[b]	142.01[b]	1.98
Cholesterol (mg/dl)	155.90[a]	137.42[b]	122.57[b]	136.02[c]	3.70
Alanine transaminase (i.u./l)	10.13[ab]	10.97[a]	11.45[a]	8.90[b]	0.58
Aspartic transaminase (i.u./l)	30.22	29.76	28.18	29.37	0.87
Creatinine (mg/dl)	0.75[b]	1.58[a]	0.52[b]	0.76[b]	0.18

[a, b, c] Means bearing different superscripts within the same row are significantly different (P<0.05).

Table (6): Economic indicator of using *Nigella Sativa* meal in laying hens diets.

Traits	Control	levels of *Nigella Sativa* meal		
		5%	10%	15%
Egg production /hen /100 days	68.45	69.19	71.91	74.12
Total income / pounds	68.45	69.19	71.91	74.12
Total feed intake / kg	10.06	10.13	10.18	10.23
Price / kg feed	6.49	6.36	6.22	6.08
Total costs / feeds	65.28	64.42	63.31	62.19
Net income / hen	3.17	4.77	8.60	11.93

Figure (1): Effect of different levels of *Nigella Sativa* meal on shell thickness of laying hens



ShTh= shell thickness, NSM = *Nigella Sativa* meal
[a, b] Means bearing different superscripts are significantly different (P<0.05).

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الملخص العربى

الأداء الإنتاجى وصفات الدم للدجاج البياض المُغذى على كسب حبة البركة تحت الظروف الحارة

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تهدف هذه التجربة إلى تقييم تأثير المستويات المختلفة من كسب حبة البركة على الأداء الإنتاجى وصفات الدم للدجاج البياض تحت الظروف الحارة. إستخدم عدد ١٢٠ دجاجة بياضة عمر ٣٤ أسبوع من سلالة دجاج اللوهمان البنى بمتوسط وزن ١٩٩٦,٩٠± ١٨.٧٥ جرام. تم تقسيم الدجاج عشوائياً إلى أربعة معاملات (٣٠ دجاجة / معاملة). كل معاملة قُسمت عشوائياً إلى ثلاثة مكررات متساوية (١٠ دجاجة / مكررة). المعاملة الأولى تم تغذية الدجاج على عليقة أساسية واعتبرت مجموعة الكنترول ، بينما غُذيت المعاملات الثانية والثالثة والرابعة على علائق تحتوى على ٥ ، ١٠، ١٥ % كسب حبة البركة من العليقة الكلية، على التوالى.
أظهرت النتائج زيادة معنوية فى كلا من وزن وعدد البيض والكفاءة الغذائية فى المعاملات المُغذاه على ١٠، ١٥ % كسب حبة البركة مقارنة بالمعاملات الأخرى. على الرغم من الزيادة المعنوية فى سمك قشرة البيضة فى المعاملات المُغذاه على ٥ ، ١٠، ١٥ % كسب حبة البركة مقارنة بالكنترول. زاد معنوياً عدد كرات الدم الحمراء وتركيز الهيموجلوبين فى المعاملات المُغذاه على ١٥ % كسب حبة البركة مقارنة بمجموعة الكنترول. إضافة كسب حبة البركة بنسبة ١٥ % أدى إلى إنخفاض معنوى فى تركيزات سيرم البروتين الكلى والالبيومين والجلوبيولين والجلوكوز والكولسترول مقارنة بالمعاملات الأخرى. بينما زاد معنوياً مستوى الكرياتينين فى معاملة الـ ٥ % مقارنة بمعاملات كسب حبة البركة ومجموعة الكنترول. إستخدام كسب حبة البركة بمستوى ٥ ، ١٠ ، ١٥ % فى علائق الدجاج البياض أدى إلى إنخفاض تكاليف التغذية مما انعكس على تحسن الدخل النقدى لكل دجاجة.
تخلص الدراسة إلى أن إستخدام كسب حبة البركة فى علائق الدجاج البياض تحت الظروف الحارة بمستوى ١٥ % كمصدر غير تقليدى حسّن من الأداء الإنتاجى والإستجابات الفسيولوجية للدجاج البياض.
الكلمات الدالة : الدجاج البياض ، حبة البركة ، الإجهاد الحرارى ، الأداء الإنتاجى، صفات الدم