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EFFECT OF DIETARY SUPPLEMENTATION OF SPIRULINA PLATENSIS AND ORGANIC SELENIUM ON SOME BIOLOGICAL TRAITS OF LOCAL LAYING HEN UNDER HEAT STRESS CONDITIONS

Enass Abd El-khalek[1]; Abaza, I. M[2]; Asmaa, A. Saad[3]
1 Poult Prod. Dept., Fac. of Agric., Alexandria Univ., Egypt
2fac.Des.And Enviro.Agric.Matrouh Univ.Egypt
3 Poult.Nut. Dept, Anim. Prod. Res. Institute, A.R.C., Dokki, Egypt
***Corresponding author:** e-mail: Enastalaat77@gmail.com

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ABSTRACT: The current experiment was conducted to determine the effect of dietary supplementation of Spirulina platinsis and organic selenium on productive and reproductive performance, egg quality, and blood traits of laying hen under heat stress conditions. A total of 90 Gimmizah developed local strain laying hen and 18 cocks (54 weeks of age) with an average initial weight 1724 g were used for three months during late production period. Birds were randomly distributed into three treatment groups with three replicates, each contained 10 hens and 2 cocks. The first treatment was fed the basal diet only without any supplementation and the other two treatments were fed the basal diet supplemented with Spirulina platinsis powder and seleno-methionine at levels (0.15 and 0.10 mg/kg diet), respectively. All treatment groups were exposed to chronic hot ambient temperature (38 °C±1; 55-65 % RH) for three successive days a week from 11.00 am until 15.00 pm. The results indicated that dietary Spirulina platinsis and organic selenium improved productive and reproductive performance, total antioxidant capacity, glutathione peroxidase, superoxide dismutase and immunoglobulins whereas decreased malondialdehyde level in blood, and can decrease the adverse effect of stress on laying hen under heat stress condition. It can be concluded that Spirulina platinsis and organic selenium supplementations can improve productive, reproductive performance, antioxidant and immunity status under heat stress conditions.

Key words: spirulina algae- organic selenium-egg production-heat stress- Antioxidant

INTRODUCTION

Spirulina platinsis (blue-green alga) is one of the high-quality natural feed additives which highly in essential nutrients that can be used in animal and poultry nutrition, as it has strong antioxidant, inflammatory properties and immune-modulating (Jamil et al., 2015). The phytochemical screening of the ethanolic extract of algae, Spirulina platensis, shows the presence of flavonoids, glycosides, tannins, phenolic and alkaloids compounds, steroids and saponins (Anbarasan et al., 2011). Productive and reproductive performances were superior when hens fed Spirulina diet compared to the control birds (Nikodémusz et al.,2010). Abd El-hady (2018) noticed that Spirulina platensis Algae (SPA) supplementation can improve productive performance, serum lipid profile, and Ca-IP metabolism of broiler chickens.

Selenium is one of the vital trace elements required for the normal function of the body, plays an important role in the maintaining health, and play an antioxidant defence role (Wassem., et al 2016). Selenium has an important role improving the productive and reproductive performance of both male and female birds (Attia et al., 2010).

Organic selenium from selenium yeast was used more efficiently for performance in fast growing, high yielding broiler chickens (Upton et al., 2008).

The ideal temperature for poultry is about 20-25 °C (Daghir, 2008;Tumová and Gous, 2012). Heat stress is induced at temperatures above 27 °C and can be easily observed at 30 °C or higher (Attia et al., 2006). High environmental temperature during the summer months is a major challenge for the breeder and layer industry in Egypt; it negatively affects productive and physiological traits (Mashaly et al., 2004; Daghir, 2008; Yoshida et al., 2011). Hence, feed addititives like spirulina and Ose play an important role to decrease heat stress (Zewil et al.,2016).

Therefore, the aim of the current study was to investigate the effects of Spirulina platinsis and organic selenium on productive and reproductive performance, egg quality and blood parameters of the Gimmizah laying hen during late production period under heat stress conditions.

MATERIALS AND METHODS

The present study was carried out at El-Sabahia Poultry Research Station, Animal Production Research Institute, Agricultural Research Center, Alexandria, Egypt. Spirulina platinsis (SP) powder was prepared in the National Institute of Oceanography and Fisheries (NIOF), Egypt, and Alkosel, a product of Lallemand Animal Nutrition Co. (France), was used as the source of organic selenium (OSe).

Experimental birds, treatments and Parameters:

A total of 90 Gimmizah developed local strain laying hen and 18 cocks (54 weeks of age) were used for three months during late production period. The Birds were randomly distributed according to a random design considering three treatment groups with three replicates (each contained 10 hen and 2 cocks) in environmental controlled house. The birds were housed in 9 floor pens, and the dimensions of each pen is 2.0 m × 1.2 m × 2.0 m. Pens were furnished with wheat straw. Each pen was supplied with laying nest of 6 holes each. The composition of the basal diet (without any supplementation) is given in Table (1) and served as a control treatment. The other two treatments were the basal diet supplemented with Spirulina platinsis (SP) powder at 0.15 mg/kg diet and seleno-methionine (OSe) at 0.1 mg/kg diet. The all treatment groups were kept under

chronic heat stress conditions (CHS) (38 °C ± 1; 55-65 % RH) for three consecutive days a week from 11:00 am until 15:00 pm. The experimental diets were similar in their nutritive value and formulated to meet the requirements of the Agriculture Ministry Decree. Feed and water were supplied ad-libitum throughout the tested period. Also, the birds were exposed to 16 hours of continuous lighting. Hens were weighed individually at the beginning and at the end of the studied period (54 and 66 weeks of age) getting between 1640 and 1700 g, while feed intake (FI), egg number (EN) and egg weight (EW) were recorded throughout experimental period. Feed conversion ratio (FCR, g feed/g egg), egg production percentage (EP), egg mass (EM, g egg/day), were estimated from the previous data. Eggs were collected for a 7-day period at 62 weeks of age and were incubated in an automatic incubator. Fertility and hatchability were calculated. Eggs laid on two successive days, from each treatment at 59 and 60 weeks of age, were used for egg quality traits. Eggshell, yolk and albumen were weighed to nearest 0.1 g (egg shells were washed and inner eggshell membrane separated and air-dried for 72 h before weighing) and their relative weights were calculated.

At the end of the experiment, four birds from each treatment were randomly chosen for collection of blood samples, about 3 ml blood, at 08:00 – 09:00 am taken from branchial wing vein in tubes. The protocol for the present study was carried out meeting the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes.

Blood samples were collected immediately in heparinized tubes and coagulated blood samples were centrifuged at 4000 rpm for 15 minutes and the clear plasma was separated and stored in a deep freezer at -20ºC until biochemical analysis. Plasma total protein (g/dl) and albumin(g/dl) were measured by using special kits delivered from sentinel CH. Milano, Italy by means of spectrophotometer according to guidelines and recommendation of Bogin and Keller (1987). Globulin level(g/dl) value was obtained by subtracting the values of albumin from the corresponding values of total protein. Malondialdehyde (MDA) (µmol/L), total antioxidant capacity (TAC), glutathione peroxidase (GPx) and superoxide dismutase (SOD) were determined in blood plasma using (Reactivos GPL, Barcelona, Spain) as a commercial medical kit. The level of immunoglobulin (IgG) in the blood serum was determined with enzyme immunoassay using commercial ELISA kits (Kamiya Biomedical Company, USA) according to Mancini et al., (1965). Computerized one-way analysis of variance and Duncan's multiple range test procedures using (SAS software, 2001) were run to compare between treatment groups.

RESULTS AND DISCUSSION

Productive performance

The effects of different supplements on productive performance of laying hen during late production period are shown in Table (2). The initial BW (54wk old) and change BW at (66wk old) were not significantly different among the experimental groups. The results of body weight change during this experiment proved that no adverse effect of SP. and OSe on final body weight and this result is in harmony with those showed by Ross et al. (1994). Whereas, other researchers reported that dietary SP. significantly improved weight gain of chickens compared with the control group (Shanmugapriya and Saravanababu, 2014). Data showed that egg production %, egg weight, egg mass and feed

conversion ratio were affected by dietary treatments. The results indicated that group of birds fed OSe recorded the highest significant egg production % followed by birds fed SP compared with control group. These results are in agreement with those reported by several authors who found that laying hens fed SP containing diets, recorded the best means of egg production and feed conversion compared with those of control group (Nikodémusz et al., 2010,Mariey et al. 2012 and Kaoud, 2013).The increase in egg weight for hens fed SP diet may be due to heavier egg yolks (Ross et al., 1994) who found that egg weight was significantly higher for hens fed diets containing different levels of SP. Addition of OSe or SP numerically decreased feed intake compared with control group. These results are in accordance with Mariey et al. (2012) who reported that no differences were observed in feed intake of hens fed the experimental diets containing SP. The best feed conversion had been recorded by birds fed OSe followed by birds group fed SP compared with control group. Similar results are consistent with several investigators, as they reported that addition of SP to diet improved feed conversion of laying hens (Nikodémusz et al., 2010). Moreover, Gjorgovska et al. (2012) and Attia et al. (2010) indicated that egg weight and egg mass increased and feed conversion ratio was improved by Se supplementation compared with hens fed control diet. Results of egg quality presented in Table (3) clear that OSe increased Shell (%) and Shell thickness compared to other treatments. Whereas, different supplements had insignificant effect on haugh unit, shape index, yolk index and yolk %. Addition of SP significantly increased Albumen % in egg. These results are in agreement with findings of Attia et al. (2010) who found that supplementation of organic selenium in the diets, had no significant effect on any traits of egg quality. However, Halle et al. (2009) observed positive effect of the microalgae on the egg quality. Also, others indicated that diets had no significant effect on egg quality parameters (Mariey et al., 2012 and Zahroojian et al., 2013).

Reproductive performance

Data presented in Table (2) showed that addition of OSe significantly increased egg Fertility followed by SP. supplementation compared to control group. Different supplementations significantly increased commercial or scientific hatchability % compared to control group. The current results revealed an agreement with those obtained by Ross and Dominy (1990), who mentioned that SP inclusion in hen diets resulted in an improvement in egg fertility, from 87% to over 96%. Also, Inborr (1998) found that the addition of SP to the diets improved the egg fertility, which increased the hatchability by 5% rates. Generally, supplementation of either SP or OSe decreased adverse effect of heat stress on productive and reproductive performance during late production period. The results of the present study are in line with some other researchers who demonstrated that selenium sources help in increasing egg production traits as well as fertility and hatchability (Attia et al., 2010; Canogullari et al., 2010; Waseem et al., 2017). Also, selenium source supplementation (Nano Se or organic Se) at 0.3 mg/ kg under heat stress conditions improved the productive and reproductive performance of Sinai laying hens, (Rizk et al. 2017)

Blood parameters

Results of blood parameters presented in Table 4 showed that OSe significantly decreased total protein and albumin, however, SP decreased only total protein and had insignificant effect on albumin

compared with control group. Different supplementations had insignificant effect on globulin values. Addition of OSe and SP significantly decreased MDA compared to control group. The decreases of MDA concentration protect the body from the harmful effects of free radicals and reflected in the improvements of the general health and performance of chicken (Attia et al., 2015). The highest significant TAC, GPx and IgG values were recorded with birds fed 0.15 g SP/kg diet followed by those fed OSe compared with control group. These results are in agreement with those reported by Zeweil et al. (2016) who reported that addition of SP improved immunity and can decrease the adverse effect of stress on chickens under heat stress conditions. Also, Saad (2016) reported that addition of SP significantly increased SOD compared with control group. Also, Wang and Xu (2008) detected an increase of TAC and GPx in plasma of broilers fed diet supplemented with Nano-Se at levels between 0.15 and 1.2 mg/kg diet, while MDA values were not affected.

IN CONCLUSION,

addition of either SP or OSe improved productive and reproductive performance, total antioxidant capacity, glutathione peroxidase, superoxide dismutase and immunoglobulins and can be used to decrease the adverse effect of stress on laying hen under heat stress conditions.

Table (1): Ingredients and calculated analysis of the experimental basal diet for laying hens.

Ingredients	**%**
Yellow corn	66.33
Soybean meal 44%	24.20
Limestone	7.50
Dicalcium phosphate	1.32
Vit+Min Premix[1]	0.25
NaCl	0.25
DL-methionine	0.15
Total	100
Calculated analysis[2]	
ME kcal/Kg	2767.2
CP %	15.84
Calcium %	3.18
Av. Phos %	0.368
Methionine %	0.436
Meth.+Cys %	0.703
Lysine %	0.872

[1]Vit+Min mixture provides per kilogram of diet: vitamin A, 12000 IU; vitamin E, 10 IU; menadione,
3 mg; Vit. D_3, 2200 ICU; riboflavin, 10 mg; Ca pantothenate, 10 mg; nicotinic acid, 20 mg; choline
chloride, 500 mg; vitamin B_{12}, 10 μg; vitamin B_6, 1.5 mg; vitamin B_1, 2.2 mg; folic acid, 1 mg; biotin, 50 μg. Trace mineral (milligrams per kilogram of diet): Mn, 55; Zn, 50; Fe, 30; Cu, 10; Se, 0.10;Anti oxidant, 3 mg.
[2]Calculated analysis was done according to Feed Composition Tables for Animal and Poultry Feedstuffs used in Egypt (2001).

Table (2): Effect of Spirulina platinsis (SP) and organic selenium (Ose) supplementation on productive and reproductive performance of laying hens under heat stress of 38° C±1.

Parameters	Treatments		
	Control	0.15 g P/Kg	0.1 g OSe/Kg
Egg Production %	38.61^{c}± 0.31	45.46^{b}± 0.09	56.76^{a} ± 0.08
Egg Weight, g	49.90±1.23	52.74±2.72	51.61±0.62
Egg mass(g/hen/day)	21.44±2.73	26.02±7.08	32.58±4.50
Feed intake (g/hen/day)	126.48±2.30	125.6±1.71	121.19±0.84
Feed conversion ratio	4.90±0.41	4.83±0.68	3.72±0.54
Initial body weight, g	1716.62±25.49	1739.28±33.01	1717.0±44.22
Final body weight, g	1733.24±10.19	1748.62±44.19	1731.4±42.13
Body weight change, g	16.62±11.03	9.34±25.03	14.4±18.18
Egg Fertility%	80.1^{a}±1.02	88.2^{b}±1.06	89.62^{a}±21
Commercial hatchability %	75.0^{b}±0.12	85.0^{a}±0.25	85.0^{a}±1.05
Scientific hatchability %	94.0^{c}±1.15	97.0^{a}±0.59	96.0^{b}±2.01

a, b,c…. Row means with different superscripts are differ significantly ($p < 0.05$).

Table (3): Effect of Spirulina platinsis (SP) and organic selenium (OSe) supplementation on some egg quality of laying hens under heat stress of 38° C±1**.**

Parameters	Treatments		
	Control	0.15 g SP/Kg	0.1g OSe/Kg
Shell (%)	13.26^{ab}±1.18	11.53^{b}±0.39	14.2^{a}±0.53
Shell thickness	0.34^{b}±0.01	0.36^{ab}±0.01	0.39^{a} ±0.02
Haugh unit score	82.26±2.28	84.94±1.43	76.90±3.97
Shape index (%)	75.52±20.57	74.88±12.82	76.05±11.26
Yolk index (%)	479.08±6.29	463.0±10.20	462.8±11.92
Yolk (%)	35.04±1.14	33.31±0.92	36.46±2.06
Albumen (%)	51.03^{ab}±1.69	55.76^{a}±1.05	49.61^{b}±2.09

a,b,c; Row means with different superscripts are differ significantly ($p < 0.05$).

Table (4): Effect of Spirulina platinsis (SP) and organic selenium (OSe) supplementation on blood parameters of laying hens under heat stress of 38° C±1.

Parameters	**Treatments**		
	Control	**0.15 g SP/Kg**	**0.1g OSe/Kg**
Total protein (g/dI)	4.30^{a}±0.06	3.93^{b}±0.30	3.80^{b}±0.25
Albumin (g/dl)	2.20^{a}±0.13	2.20^{a}±0.04	1.83^{b}±0.14
Globulin(g/dl)	2.10±0.71	1.73±0.24	1.97±0.34
Immunoglobulin (mgl/ml)	134.0^{b}±6.93	228.5^{a}±9.53	165.5^{b}±8.76
Malondialdehyde (µmol/L)	2.55^{a}±0.09	1.15^{b}±0.03	0.94^{c}±0.02
Total antioxidant Capacity(µmol/L)	1.31^{b}±0.14	1.69^{a}±0.05	1.61^{ab}±0.02
Glutathione peroxidase (µmol/L)	1.93^{b}±0.07	2.78^{a}±0.03	2.08^{b}±0.03
Superoxide dismutase (IU)	1.10^{c}±0.01	1.66^{b}±0.07	1.89^{a}±0.06

a,b,c; Row means with different superscripts are differ significantly ($p < 0.05$).

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الملخص العربى

تأثير إضافة طحلب الإسبيرولينا والسيلينيوم العضوى على بعض الصفات البيولوجية للدجاج البياض المحلى تحت ظروف الإجهاد الحرارى

إيناس عبد الخالق محمود[1]، إبراهيم أباظة[2]،أسماء سعد[3]

[1] قسم إنتاج الدواجن- كلية الزراعة(الشاطبى)- جامعة الأسكندرية

[2]كليه الزراعه الصحراويه والبيئيه –فوكه-جامعه مطروح-مصر

[3] قسم تغذية الدواجن- معهد بحوث الإنتاج الحيوانى- الدقى، مصر

أجريت هذه التجربة لدراسة تأثير طحلب الاسبيرولينا والسيلينيوم العضوى فى العليقة على بعض الصفات البيولوجية للدجاج البياض المحلى تحت ظروف الإجهاد الحرارى. أستخدم فى هذا البحث 90 طائر فى عمر 54 أسبوع فى اخر ثلاث شهور من فترة إنتاج البيض.

تم وزن الطيور وتوزيعهم عشوائيا إلى ثلاث مجموعات فى كل منهما ثلاث مكررات متساوية.

المعاملة الأولى:- الكنترول (بدون أى إضافات)

المعاملة الثانية:- إضافة طحلب الاسبيرولينا بمستوى 0.15 مليجرام/كجم علف

المعاملة الثالثة:- إضافة السيلينيوم العضوى بمستوى 0.1 ملجرام/كجم علف

كل المعاملات تمت تحت ظروف الإجهاد الحرارى (38±1°، 55-65%رطوبة) لمدة ثلاثة أيام متتالية فى الأسبوع من الساعة الحادية عشر صباحا وحتى الساعة الثالثة مساءا .

تم تسجيل وزن الجسم- إستهلاك العلف- وزن البيض- الكفاءة التحويلية- معدل إنتاج البيض- نسبة الخصوبة - التفريخ وقد تم تقييم جودة البيض وقد تم أخذ عينات دم لتقدير خصائص الدم الهيماتولوجية

ويمكن تلخيص أهم النتائج التى تم التوصل إليها فيما يلى:-

1- لا توجد إختلافات معنوية على وزن الجسم والزيادة فى وزن الجسم بين المعاملات المختلفة.

2- إنتاج البيض- وزن البيض- كتلة البيض- تأثرت بالمعاملات المختلفة، السيلينيوم العضوى سجل أعلى إنتاج بيض يليه الطيور اللتى تم تغذيتها على الإسبيرولينا مقارنة بالكنترول.

3- المعاملات المختلفة أدت إلى زيادة نسبة التفريخ العلمية والمعملية مقارنة بالكنترول.

4- إضافة الاسبيرولينا والسيلينيوم العضوى(Ose) أدى إلى إنخفاض MDH مقارنة بالكنترول وإلى تحسين معنوى .Superoxide dismutase and immunoglobulin – TAC- Gpx and IgG

لذا توصى الدراسة بإضافة الاسبيرولينا والسيلينيوم العضوى إلى أعلاف البياض مما يؤدى إلى تحسين الأداء الإنتاجى وصفات المناعة تحت ظروف الإجهاد الحرارى.