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Research Article

Climate Change Mitigation by Dietary Phytogenic Addition in Layer Hens: Some Re-productive Traits and Endocrine Hormones Responses

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Abstract | Hyperthermia is one of the most critical problems in poultry during the laying period. Photogenic is used increasingly alone or in supplement for the treatment of different dysfunctions. The objective of this study was to estimate the impact of Seder (*Ziziphus spina-christi*) leaves aqueous extract (SLAE) on endocrine hormones and reproductive function in laying hens under hyperthermia stress. Two hundred and twenty 25-wks-old-laying hens were randomly split into 4 groups, each is composed of 50 hens and 5 cock; the groups were included control group (received a basal diet without addition), SLAE2.5 group (received basal diet + 2.5 ml SLAE /kg diet), SLAE 5 (basal diet + 5 ml SLAE /kg diet) and SLAE 7.5 (basal diet + 7.5 ml SLAE /kg diet). Averages of ambient temperature, relative humidity, and THI during this study were 32.27±0.44°C, 71.65±0.85%, and 30.54±0.43; respectively. The result revealed that SLAE addition significantly ($P<0.05$) increased T_3 hormone and FSH hormone levels, and T_3/T_4 ratio in compared to control group. The highest concentrations of these values were observed in dose 7.5 ml SLAE/kg diet. Also, SLAE addition result a dose-dependent decrease ($P<0.05$) in ACTH levels in laying hens. The treatment did not affect T4 and LH hormones (p=0.1470 and 0.2764, respectively). Regarding reproductive traits, the treatment increased the hatchability to total and fertilized eggs, maximizing in groups treated with 5 and 7.5 ml SLAE/kg diet. There was a non-significant association between SLAE addition and all reproductive traits, however, SLAE at the dose of 7.5 and 5 ml /kg diet decreased numbers of unfertilized eggs compared to the control treatment, as well increased fertilized eggs, the counts of chicks produced, the percentages of un-hatched eggs and the hatchability to the total. It is concluded that dietary addition of laying hens with SALE, can at least in part alleviate heat stress-induced damage to reproductive physiology. SLAE in 7.5 ml/kg dose could be used as a safe natural antioxidant for improving reproductive status in heat-stressed hens.

Keywords | Phytogenic, Hyperthermia, Endocrine hormones, Productive traits, Layer hens

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INTRODUCTION

One of the most critical problems that may stifle the poultry industry's growth is an increase in environmental temperature, which causes heat stress in birds. By 2100, the global surface temperature is predicted to rise 1.4°C to 5.8°C (IPCC, 2007), which is a serious problem for the poultry business as well. As weather change alleviation policies have been unsuccessful in reaching the IPCC (2014) recommendations, this constraint is expected to be severe, particularly in the tropics and sub-tropic regions. Heat stress has a negative impact on laying hens' reproductive and laying performance, and also their economic features and welfare (Daghir, 2008). High temperature negatively affects homeostasis and consequently the endocrine system. These factors have a negative impact on egg production, ovulation, and oviposition in the ovary and reproductive tract (Oguntunji and Alabi, 2010).

Phytogenic chemicals and botanical materials are gaining popularity since they are regarded as safe, environmentally friendly, and efficient in improving laying performance because of their beneficial bioactive compounds (Lin et al., 2017). The majority of these phytochemicals (plant-derived chemical substances) have strong chemopreventive and antioxidant properties (Surh and Na, 2008). The novel concept was based on the idea that supplying different natural antioxidants to birds could help them cope with stress more successfully (Fotina et al., 2013).

Ziziphus spina-christi is a semitropical plant called in Egypt as 'Nabq' or 'Seder' and in English, as Dom/Christ thorn belongs to the family *Rhamnaceae* and the genus *Ziziphus* (Tackholm and Boulos, 1974). There are about a hundred species of deciduous and evergreen trees and shrubs in this genus, which are found in the worlds' tropical and subtropical regions (Adeli and Samavati, 2015). The local Arabs use this plant components to maintain a healthy lifestyle, hence the species Zizyphus has medicinal significance (Adzu et al., 2001). Phytochemically demonstrated that *Zizyphus spina-christi L.* is famous for its cyclopeptide alkaloids and polysaccharides. However, large flavonoids, tannins, and saponins numbers have been isolated from this valuable plant (Waqar et al., 2015). Recently, many researches were conducted the study on plant extracts of *Ziziphus* species and exhibited as anti-bacterial (Abd-Alrahman et al., 2013) as well as an antifungal (Abu-Taleb et al., 2011), anticancer (Mishra et al., 2011), and antioxidant (Khaleel et al., 2016). It is well known that plant phenolics in general are highly effective free radical scavengers and hence are antioxidants (Khaleel et al., 2016). The in vitro antioxidant properties of *Z. spina-christi* leaves may be possibility attributed to the phytochemicals present. Thus, there is an urgent need to explore promising potential of natural antioxidants as a novel strategy to mitigate climate change. Therefore, this study aimed to assess the *Z. spina-christi* grown in Egypt for its' potential to counteract the adverse effects of heat stress on reproductive indices and endocrine hormones in laying hens.

MATERIALS AND METHODS

Experimental Design and Treatments

The study was a completely randomized design with a 4-levels, using the dietary addition of Seder (*Ziziphus spina-christi*) leaves aqueous extract (SLAE) to the following experimental groups, **Con**: basal diet + 0 ml/kg SLAE, **SLAE2.5**: basal diet + 2.5 ml/kg SLAE, **SLAE5**: basal diet + 5 ml/kg SLAE, and **SLAE7.5**: basal diet + 7.5 ml/kg SLAE. The identified compounds in aqueous extract of *Ziziphus spina-christi* (L.) according to El-Kholy et al. (2021) were: Biotin, 34.66%; β-Eudesmene, 1.43%; D-Glucuronic acid, 1.81%; α-Guaiene, 2.11%; Spathulenol, 1.03%; Isomyristic acid, 2.29%; Globulol, 3.69 %; Farnesol, 1.98 %; Elemene, 0.72%; Hexadecane, 2.2%; Cedren-13-ol,8-,1.12%; Thunbergol, 2.63%; Retinol, 10.69%; Vitexin, 1.74%; Linolenic acid, 2.04%; 4',6-Dimethoxyisoflavone-7-O-β-D-glucopyranoside, 7.08%; Deguelin, 6.71%; Trans-13-Octadecenoic acid, 0.61%; Nobiletin, 0.96%; Phytanic acid , 1.59%; Linalyl acetate, 2.57%; Cis-9-Hexadecenoic acid, 1.14%; Squalene, 2.94%; 4',7-Dimethoxy-8-methylisoflavone, 3.68%; and Campesterol, 2.58%. In addition El-Kholy et al. (2021) showed that phytochemical screening of SLAE were: Total Flavonoids (mg of QE2/100g), 017.92±0.09; Total antioxidant capacity (mg of AC3/100 g), 269.25±4.46; Saponin (%), 00.045±0.01.

Husbandry and Experimental Design

All procedures used in this experiment were in accordance with the Animal Care Committee of the Damietta University, Damietta, Egypt (Approval date: 03/2018/du.edu). During the months of September to November 2019, this research was conducted at the animal research station of the Sakha agricultural research Centre in Egypt. A total of 220, 25-week-old laying hens (Inshas strain; Egyptian developed strain) were allocated randomly to 4 treatments, with 5 replicates of 10 hens and 1 cock each. The experimental period lasted for 12 weeks from 25 to 36 weeks old. All of the birds were housed in separate rooms (260 cm long x 210 cm wide). All of the birds were raised on litter floors under the same management, sanitary, and environmental settings. Feed was available ad libitum and the light program consisted of 16 h light daily throughout the experiment. the layer hens feed as requirements by NRC 1994.

Averages of ambient temperature (AT, oC) and relative humidity (RH, %) of interior building were measured four times each month. Then, the temperature humidity index (THI, units) was calculated using the equation of Zulovich and DeShazer, (1990) as follows:

THItb = 0.60Tdb + 0.40 Twb

Where:

THItb = Temperature Humidity Index for laying hens; Twb = Wet bulb temperature (0C) Tdb = Dry bulb temperature (0C); Where: db oC = dry bulb temperature in Celsius, RH= relative humidity percentage/100.

The values obtained are then 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).

Averages of ambient temperature, relative humidity and THI were 32.27±0.44 ˚C71.65±0.85%, and 30.54±0.43 respectively.

Serum hormones analysis

For each sampling, 3 birds were randomly selected from each replicate. After fasting for 12 hours, blood samples (5 mL/sample) were taken from the wing vein and collected into a tube containing 1% EDTA at the end of the experiment (36 weeks of age). A 3 ml blood sample was centrifuged for 15 minutes at 3,000 g to collect serum and kept at 4 °C. To measure the concentrations of serum endocrine hormones including (luteinizing hormone (LH) , follicular stimulating hormone (FSH), tri-iodothyronine (T3, ng/ml) and thyroxine (T4, ng/ml) and adrenocorticotropic hormone (ACTH) was carried out using commercially available ELISA kits (Jianglai, Biotechnology Co., Ltd., Shanghai, China).

Fertility and hatchability of eggs

For the evaluation of fertility and hatchability, the samples were collected at the age of 34 weeks from hens of both experimental group and control group. From each group, 150 eggs (50 eggs per replicate) were collected. The eggs for incubation were gathered manually for five days in a row and stored at the farm under the prescribed conditions. Eggs were put in setter trays (50 eggs per tray) and incubated. After the eggs were identified, they were transferred to incubator trollies to allow for air circulation and fumigated with formaldehyde gas for 20 minutes. Following that, the eggs were placed in brand PTO incubators (Fathallah Co., Egypt), with special attention paid to avoiding the effects of positioning on incubation. The eggs were then automatically monitored during incubating at the standard temperature and humidity (37.5°C and 65–70%). During the incubation period, the eggs were turned 90 degrees every hour. The eggs were individually candled using a hand candling lamp on day 10 of incubation. Only fertilized eggs with viable embryos were maintained, whereas dead embryos and infertile eggs were discarded. The number of viable, dead embryo, and infertile eggs were counted. The following (Bonnier and Kasper, 1990) formula was used to Calculate fertility for each replication. % Fertility = (Total fertile eggs/Total egg set)×100. Hatched chicks were collected and counted on the 21st and 22nd days of incubation to Calculate hatchability in relation to the number of fertile eggs (North, 1984). The percentage hatchability was calculated using following formulas (Fayeye et al., 2005).

% Hatchability on fertile egg basis = (Number of Chicks hatched/Total Fertile eggs)×100

% Hatchability on total egg basis = (Number of Chicks hatched/Total eggs set) × 100

Statistical Analysis

A MIXED procedure for repeated measurements (SAS, 2012, release 9.2, Cary, NC, USA) was used for assessing endocrine hormones and indicators as dependent variables. Levels of dietary Seder (*Ziziphus spina-christi*) leaves aqueous extract (SLAE) (0, 2.5, 5.0, and 7.5 ml/kg diet), time of sampling and/or data collection weeks 25-28, 29-32, 33-36 and 25-36 for biochemical variables) were introduced as fixed independent variables. A general linear model method (one-way analysis of variance; SAS, 2012) was used to examine meteorological and physiological variables. Duncan's new multiple range post-hoc test was used to find differences between treatment groups, and $p < 0.05$ was considered significant. All results were expressed as least square means ± pooled standard error of the mean (SEM). Variance homogeneity and normality were examined by Shapiro– Wilk and Levene tests. The level of statistical significance was set at p-value < 0.05. The association between dietary addition of Seder leaves aqueous extract and each of fertility and un-fertility eggs as well as chicks produced and Pipped egg were detected by Chi-Square test (X square).

RESULTS

Endocrine hormones

Effects of the dietary addition of Seder aqueous extract (SLAE) on endocrine hormones of heat stressed-layer hens are presented in Table 1 and Figure 1. The treatment did not affect T4 and LH hormones significantly (p=0.1470 and 0.2764, respectively). T3 hormone was affected quadratically (p=0.0008) by the treatment; regression analysis showed that the greatest value was observed at 6mg SLAE /kg diet (Figure 1B). Ascending levels of SLAE decreased ACTH (p=0.0338) and increased both of T3/T4 (p=0.0130), and FSH (p=0.0004) hormones linearly (Figure 1A, C and D), the greatest values of FSH were observed in the groups treated with SLAE at levels of 5 and 7.5 mg /kg diet (p<0.05) compared to the control. However, the differences between the three levels of SLAE (2.5, 5 and 7.5 mg/kg diet) were not significant (p>0.05) for ACTH and T3/T4.

Reproduction performance

Results of incubation performance are shown in Table (2). Despite of there was non-significant association between Seder leaves aqueous extract (SLAE) supplementation and each of fertilized (p=0.9756) and unfertilized eggs (p=0.5422) in addition to the counts of chicks produced (p=0.6912) and Pipped egg (p=0.1520), it is worth noting that the numbers of unfertilized eggs decreased by 29.4 and 41.2% in groups treated with 5 and 7.5 mg SLAE/kg diet, respectively compared to the control treatment, whereas the corresponding values for fertilized eggs increased by 3.76 and 5.26%, respectively. Likewise, the counts of chicks produced increased by 8.55 and 11.97 % in SLAE5 and SLAE7.5 groups compared to the control. In contrast, there were 31.2 and 43.8% increases in the percentages of Pipped egg in SLAE5 and SLAE7.5 groups. The observed non-significant associations between SLAE treatments and incubation performance could be attribut

Table 1: Effect of seder leaves aqueous extract addition on some blood parameters at 36 and 40 weeks of age.

Parameters*	Seder Aqueous Extract Concentrations (ml/kg diet)				SEM	P-value			
	Con	SLAE2.5	SLAE5	SLAE7.5		T	L	Q	C
ACTH	20.84a	16.16ab	14.89ab	14.57b	1.75	0.0143	0.0338	0.2504	0.7623
T3	1.91c	2.72b	2.93ab	2.98a	0.07	<.0001	<.0001	0.0008	0.2142
T4	24.66	18.66	17.66	18.78	2.07	0.1470	0.0790	0.1243	0.7637
T3/T4	0.07b	0.15a	0.17a	0.16a	0.02	0.0336	0.0130	0.0713	0.8983
FSH	3.64c	4.84b	4.01ab	4.11a	0.06	0.0029	0.0004	0.4183	0.8768
LH	13.70	40.33	15.17	16.61	1.01	0.2764	0.0675	0.7010	0.9330

* ACTH: Adrenocorticotropic hormone; T3: Tri-iodothyronine; T4: Thyroxine; FSH: Follicular stimulating hormone; LH: Luteinizing hormone.

T: treatment; L: linear response; Q: quadratic response; C: cubic response; SEM: Standard error of means.

a,b,c Means within a row with different superscripts are significantly different ($p < 0.05$).

Table 2: Effect of seder leaves aqueous extract addition on the hatching traits of broiler breeder hen.

Items	Aqueous Extract Concentrations (mg/kg diet)				χ2	P-value
	Con	SLAE2.5	SLAE5	SLAE7.5		
Total egg	150	150	150	150	--	--
Unfertilized eggs	17	15	12	10	2.1481	0.5422
Fertilized eggs	133	135	138	140	0.2125	0.9756
Chicks produced	117	115	127	131	1.4612	0.6912
Pipped egg	16	20	11	9	5.2857	0.1520
Fertility (%)	88.66	90	92	93.33	--	--
Hatchability to Total eggs (%)	78	76.66	84.66	87.33	--	--
Hatchability to fertilized eggs (%)	87.93	85.19	92.01	93.55	--	--

T: treatment; L: linear response; Q: quadratic response; C: cubic response.

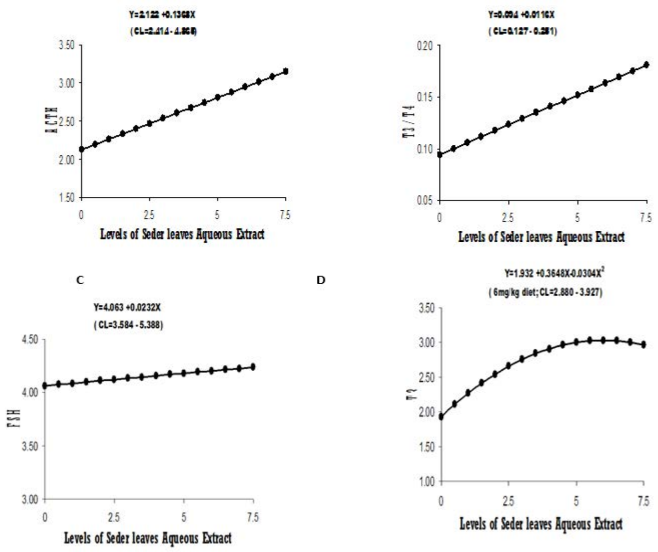

Figure 1: Dose–response curve of ACTH (A) T3/T4 (B), FSH(C),T3 (D) ,for different levels of dietary supplemental, x and y are the dependent (Seder aqueous extract levels) and the independent variables of the regression equation, respectively. The figure only shows significant relationships ($p < 0.05$).

ed to the large sample size and derived values as well. Additionally, the treatment increased the hatchability to total and fertilized eggs, maximizing in groups treated with 5 and 7.5 mg SLAE/kg diet.

DISCUSSION

Reproductive status of laying hens can be influenced by environmental pressures as a result of climate change (such as heat stress), which may be one of the most common challenges in production systems worldwide. The layer hens throughout current experimental periods exposure to severe heat stress (30.54 THI) as evidenced by a drop in endocrine hormones and reproduction performance. This finding is in agreement with the report by Saleh et al. (2020). Layers are more susceptible to heat stress due to their high metabolic heat production caused by increasing egg formation (Blem, 2000). On the other hand, oxidative stress, resulting in heat stress, when reactive species (RS) are produced in excess, and the antioxidant defense systems of the chickens can be overwhelmed as the activity of antioxidant enzymes (superoxide dismutase, catalase, and glutathione peroxidase) decreases (Zhong and Zhou, 2013). Supplementing with medicinal herbs (natural extracts) with antioxidant capacity, can help to reduce the detrimental effects of heat stress (H. Lin et al., 2006). As mentioned in identified compounds of SLAE as mentioned by El-Kholy et al. (2021), the major compounds detected in chemical analysis of an aqueous extract of SLAE were biotin (23.51%) and retinol (10.69), which have been previously associated with antioxidant activity (Elomda et al., 2018) These results were in agreement with previous phytochemical studies on the different species of the genus Ziziphus except present of alkaloids which are absent in current study (aqueous extract) and ethanol extract (Temerk et al., 2017). Herbal flavonoids activate appetite mechanisms in poultry and contribute to balancing the gastrointestinal microflora system, and also have anti-inflammatory and anti-oxidant effects (Iskender et al., 2016). Besides that, phytochemical screening of SLAE, showed that the total antioxidant capacity and flavonoids concentration of SLAE were 17.92±0.09 mg of AC/100 g and 17.92±0.09 mg of QE2/100g (El-Kholy et al., 2021). The presence of these characteristics in SALE have been shown as in vitro antioxidant activities (El Maaiden et al., 2020).

In females, heat stress can disrupt the normal status of reproductive hormones at the hypothalamus, and at the ovary, leading to reduced systemic levels and functions (Lara and Rostagno, 2013). Also, high ambient temperatures change the function of the neuroendocrine system in chickens, causing the hypothalamic-pituitary-adrenal (HPA) axis to activate and plasma corticosterone concentrations to rise (Lara and Rostagno, 2013). Corticosterone, catecholamines, and lipid peroxidation in cell membrane increased due to exposure to heat stress, and animal welfare decreased; SLAE can lower the adverse influences of heat stress on corticosterone secretion as mentioned by Metwally (2003) who used natural antioxidant (vitamin E) on heat-stressed hens. Siegel, (1995) denoted that heat stress caused adrenocortical insufficiency and a significant decline in levels of plasma corticosteroids. Stormer et al. (1993) reported that where stress is a major factor, then supporting the nervous system with SLAE addition may be the best approach. Similar results with our results were obtained by Wan et al. (2017) concluded significant increase in corticosterone, tri-iodothyronine (T3), and triiodothyronine/thyroxine (T3/T4) levels and no significant difference in T4 level as affected by dietary inclusion of herbal to broiler diet under summer heat stress conditions in Egypt. In the same respect, Guo et al. (2021) showed that the addition of *Macleaya cordata* extract (MCE) to the layer hen's diet, levels of the follicular stimulating hormone, estradiol, and serum luteinizing hormone significantly ($P<0.05$) increased.

The results of this study showed no significant differences in hatching traits among the treatment groups. Unfortunately, very limited scientific data were found in the literature relating to the effects of dietary SLAE on the hatching traits of broiler breeders. Due to the lack of information with respect to broiler breeders, the results of this study were compared with research that examined effects of dietary supplementation with some botanical additives on the laying performance of layers. The improvement of fertility and hatchability in the present study due to SLAE dietary addition were in agreement with studies of Ather, (2000) who showed that 48-wk-old broiler breeders were given diets supplemented with a poly herbal additive that consisted of 6 herbs. The author reported that hen-day egg production, settable egg rate, and fertility significantly improved for hens receiving the herbal addition in their diet during the 8-wk trial period. On the other hand the present results are in agreement with findings of Asrat et al. (2018). The increase in fertility and hatchability in treated groups can be attributed mainly to the biotin "the major component in SLAE", hence, biotin is required for normal embryonic development and hatchability (Whitehead et al., 1985). Increased dietary biotin intake or biotin injections into the egg at the start of incubation can help to overcome an egg deficit. The breeder dietary natural antioxidant (SLAE) could be transported to the egg yolk (Surai and Fisinin, 2012), which was beneficial for the egg yolk and embryo to resist against lipid peroxidation. During the embryonic development, especially from d 12 of incubation to d 1 after hatching, the yolk antioxidant concentration gradually decreased. So, SLAE could be counteract the deprivation of that. The hatching process is considered to be a time of oxidative stress. Therefore, improved antioxidant defenses during embryonic development potentially could increase hatchability. It was shown that antioxidants can be transferred from the diet to the egg and consequently to the embryo (Surai, 2000). In addition, low levels of reactive oxygen species have also been shown to be essential for fertilization, acrosome reaction, hyperactivation and motility (Agarwal et al., 2004).

CONCLUSIONS

Based on the results in our study, it is concluded that dietary addition of laying hens with SALE, can at least in part alleviate heat stress induced oxidative damage. This is evidenced by the increase recorded in the endocrine hormones and reproductive status of the treatment groups when compared to the control. Thus, SLAE in 7.5 ml/kg dose could be used as a natural and safe antioxidant for improving reproductive status in heat-stressed hens.

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CONFLICT OF INTEREST

There is no potential for a conflict of interest.

NOVELTY STATEMENT

We found that dietary addition of laying hens with aqueous extract of Z. spina-christi leaves have a potential to counteract the adverse effects of heat stress on reproductive indices and endocrine hormones in laying hens. Also, this aqueous extract in 7.5 ml/kg dose could be used as a safe natural antioxidant for improving reproductive status in heat-stressed hens.

AUTHORS' CONTRIBUTION

KHE: Study supervision, study design and drafting of the manuscript. AR: Statistical analysis. THT: Analysis and interpretation of data. FT: Technical, and material support, and acquisition of data. SHMH: Critical revision of the manuscript for important intellectual content.

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