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Effect of Supplementation of *Withania somnifera* L. Roots on Some Blood Physiological Parameters of Japanese Quail Hens Reared Under High Environmental Temperature

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Abstract:

An experiment was conducted to assess the antistress efficacy of *Withania somnifera* roots ethanolic on some blood physiological traits of Japanese quail hens reared under high environmental temperature (27-37-27ºC).

Three hundred Japanese quail hens of six weeks old were randomly distributed into five treatments T_0 untreated group (control), T_1, T_2 quails supplemented orally with 50, 100 mg/kg/day root ethanolic extract(WSRE) respectively,T_3, T_4 received 1,2 g/kg diet root powder(WSRP) respectively.

Results revealed significant increase in Hb and PCV % in quails supplemented with T_4 at 10 and 15weeks of age, and in T_3 and T_4 groups at 12 weeks of age in comparison with T_0 and WSRE groups. At 10 and 15 weeks old, T_2 resulted in significant increasing in Heterophil/Lymphocyte ratio in comparison with$T_0$and other treatments. At 15 weeks of age,$T_1$and T_2 significantly increased Heterophil% in comparison with T_0 and WSRP groups, while, quails in T_4 showed significant reduction in Heterophil% and significant increasing in Lymphocyte% compared with T_1, also results showed that supplementing quails with T_4 significantly increased serum iron .Thyroid hormones didn't affected significantly due to treatments while T_3 significantly increased TSH.

Keywords: *Heat stress; Withania somnifera roots; Physiological parameters; Japanese quail.*

الخلاصة:

أجريت الدراسة بهدف اختبار الفعالية المضادة للاجهاد لجذور نبات*Withania somnifera*من خلال تأثيرها في بعض صفات الدم الفسلجية لطائر السلوى الياباني المربى تحت درجات حرارة بيئية مرتفعة(27-37-27°م).

استخدم في هذه الدراسة 300 طائر انثى وزعت عشوائيا على خمس معاملات وكما يلي: T_0مجموعة غير معاملة(سيطرة) وT_1وT_2مجموعتي المعاملة بالمستخلص الكحولي للنبات وبجرعتي 50و100ملغم/كغم/يوم وعلى التوالي وT_3و T_4 مجموعتي المعاملة اليومية بالمسحوق وبمستوى اضافة 1 و2 غم/كغم علف على التوالي.

أظهرت النتائج حصول زيادة معنوية في كل من(Hb) و (PCV%) لدى الطيور المعاملةT_4 في الاسبوعين 10و 15من العمر ولدى طيور المعاملة T_3 وT_4 عند عمر 12 أسبوع بالمقارنة مع السيطرة ومجموعتي المستخلص. بينت نتائج الاسبوعين 10و15 ايضا بأن المعاملة T_2 قد ادت الى زيادة معنوية في نسبة الهتروفيل/اللمفوسايت بالمقارنة مع السيطرة وبقية المعاملات كما أدت المعاملة بالمستخلص الكحولي الى حصول زيادة معنوية في النسبة المئوية لخلايا الهتروفيل عند عمر 15 أسبوع بالمقارنة مع السيطرة ومجموعتي المسحوق، في حين ادت المعاملة T_4 الى حصول انخفاض معنوي في الهتروفيل% وزيادة معنوية في اللمفوسايت% بالمقارنة مع T_1وبينت النتائج ايضا ان المستوى الاعلى من مسحوق جذور النبات(T_4) قد ادى الى زيادة معنوية في حديد مصل الدم ولم تتأثر معنويا مستوى هرمونات الدرقية نتيجة المعاملات المختلفة في حين ان T_3 قد ادت الى حصول زيادة معنوية في TSH.

Introduction:

Heat stress is one of the most important stresses associated with compromised performance and productivity in poultry which leads to increased economic losses due to mortality of birds[11]. Dietary modifications are among the most preferred and practical ways to alleviate the effect of high environmental temperature in poultry. Natural medicinal plant-derived products from herbs and

spices, has proven to be natural, less toxic, residue free and is thought to be ideal feed additives in animal production food [26].

A few researches have been done to investigate the role of some medicinal plant in alleviating heat stress in poultry. *Withania somnifera* (*W.somnifera*) belongs to the family solanaceae, also known as Ashwagandha and winter cherry, has been an important traditional herbal medicine for over 3000 years [12]. This plant were found to offer protection against physical, chemical and biological stressors [18], stabilized and revitalize systemic functions by potentiating the immune system, arresting homeostasis and increasing resistance to adverse environmental factors[13]. Studies indicated that the root represents the premier herbal adaptogens. This plant has haemopoetic effect and resulted in significant increase in white and red blood cell count in various kinds of organisms [21].

The hematological results of PCV%, Hb, WBC count, neutrophil and lymphocyte of mice supplemented with 500 mg/kg b.wt leaves ethanolic extract of WS showed significant increase as compared with unsupplemented group[8]. Administration aqueous extract of *W.somnifera* at concentration of 20g/L, increased hemoglobin and packed cell volume in broiler whereas a non-significant differences was noted in neutrophils, lymphocytes, eosinophils and monocytes[14].

Studies refered that using adaptogenic herbs, like *W. somnifera* could help to increase nonspecific response to stress and returning bodies physiological balance, such as, stimuli thyroid gland activity. Preliminary work in mice shows that *W. somnifera* increased the concentrations of T3 and T4 hormones perhaps by stimulating thyroidal activity and protecting hepatic tissue (involved in T4 to T3 conversion) from per oxidation[16].

Recent study try to testing the antistress - adaptogenic efficacy of *W. somnifera* grown in Iraq in alleviating heat stress in Japanese quail hens through studying its effect on some blood physiological traits.

Material and methods:

Plant Materials:

A- Plant collection and extraction:

Fresh healthy plants of *W. somnifera*, 2–3 years old were collected from several places in Baghdad during April 2011. The herb was identified and authenticated at the Iraqi National Herbarium, Abu Ghariab. The roots were separated, cleaned, washed, air dried in shades, and then crushed by an electric grinder. The fresh powder was extracted with 70% ethyl alcohol [7], suspension was left stirring for 72 hours at room temperature and then sieved by sterile gauze to get rid of coarse particulars, filtered through Whiteman filter paper. The filtrate was evaporated to dryness in a vacuum oven. The sticky brownish extract was placed in sterile tube and kept in freezer until use.

B- Specific chemical detection of active compounds:

Tests was carried out on the plant powder using standard procedures to identified tannins, saponins, flavonoids, alkaloids, glycosides and resins according to[8].

Animal husbandry and Experimental treatments:

A total of three hundred, 6 weeks (wks) old Japanese quail hens, proximate in weight, were randomly allocated (20 hens per pen) to floor pens during the study period. Birds were fed *ad libitum* with standard basal diet of production period containing 20%crude protein and 2903Kcal/Kg ME (Table-1). Environmental temperatures were recorded along the experiment period and the average cyclic house temperature was 27-37-27°C. The hens were randomly distributed into five equal groups as follows:

(T_0): control group without any addition.

(T_1): Supplemented orally with 50 mg/ kgb.w.t. roots ethanolic extract (WSRE)
(T_2): Supplemented orally with 100 mg/kg b. wt. roots ethanolic extract (WSRE)
(T_3): Supplemented with 1g/kg diet roots powder (WSRP)
(T_4): Supplemented with 2g/kg diet roots powder (WSRP)

For ethanolic extrac teatments (T_1 and T_2), doses were administrated daily at 12PM for every bird during entire experiment period, by using stomach tube which inserts the substance into the crop. For crude powder treatments, 1 and 2g of fresh powder were mixed for every kg of T_3 and T_4 diet respectively and presented to hens daily, until the end of experiment.
For blood sampling, six quails from each experimental group were taken and slaughtered at 1200 PM.

Blood were immediately collected in two types of sterile tubes; one for fresh blood tests and the other to obtain serum for biochemical tests by centrifuged blood sample at 4000 rpm for 10 minutes. Hemoglobin was measured according to[25], PCV was estimated according to[1]. Heterophil %, Lymphocyte% and their Ratio (H/L) was measured according to [5], serum Iron according to [4])using Linear Kit (Spain), triiodothyronine (T3), thyroxine (T4) and Thyroid Stimulating Hormone (TSH) were measured by enzyme linked immune sorbent assay using Human Kit (Germany) according to [3].

Statistical Analysis:

A completely randomized design– CRD within the Statistical Analysis System [19] was used to analysis the data for the effect of difference factors in the studied parameters.

Table- 1: Composition and Calculated analysis of the Experimental Diet [1].

Ingredient	%in diet
Yellow corn	56.1
Soybean meal	31.1
Protein concentrate	5.0
Vegetable oil	2.0
Limestone	4.9
Dicalcium phosphate	0.6
food salt	0.3
Calculated composition*	
%crude protein	20.0
ME(Kcal /Kg)	2903
%Lysine	1.11
%Mehionine	0.77
%Calcium	2.54
%Available phosphorus	0.35

***Calculated composition according to[15].**

Results and Discussion:

Specific chemical detection of active compounds:

Specific chemical analysis demonstrated the detection of glycosides, alkaloids, flavonoids, resins and saponin while tannins were not found in the tested roots (Table-2).

Table -2: The specific chemical detection of some phytochemical compounds in Iraqi *Withania somnifera* roots.

compound	Detection used	Detection Index	Detection result
Alkaloids	Dragendroff reagent	Appearance of orange sediment	+
Flavonoides	Ethyl alcohol 95%+KOH	Appearance of yellow color	+
Glycosides	Fehling reagent	Appearance of red sediment	+
Tannins	Ferric chloride	Appearance of greenish–blue color	–
Saponins	mercury chloride	Appearance of white sediment	+
Resins	Ethyl alcohol 95% $\xrightarrow{\text{Boiling}}$ Acidic distilled water	Appearance of muddiness	+

Hemoglobin and Packed Cell Volume:

Statistical analysis (Table-3) showed that at 10 and 15 wks of age, Hb and PCV% were significantly higher ($p \leq 0.01$) in blood of quails supplemented with 2g/kg diet WSRP (T_4) than control and other treatments (T_0,T_1,T_2 and T_3).

At 12 wks of age, quails supplemented with 1 or 2g/kg diet WSRP (T_3 and T_4) had significantly ($p \leq 0.01$) higher Hb and PCV% parameters than control and ethanolic extract group. The significant increasing in Hb and PCV% found in groups supplemented with roots as powder may be linked to the increased in erythrocytes count [21, 20] as a result of stimulatory effect of roots on bone marrow cells [6] and the enhancement of stem cell differentiation [14],in addition to the rich content of iron in the root. The obtained results are an indicator to the hematopoitic stimulatory effects of *W. somnifera* which in-turn increased these parameters. These results agreed with those reported by [2] when they mentioned that supplementing 1 percent of *W.somnifera* roots powder significantly increased Hb and PVC% in Japanese quails.

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Table- 3: Effect of supplementing *Withania somnifera* roots as ethanolic extract or crude powder on hemoglobin concentration (g/dl) and PCV% of Japanese quails reared under high environmental temperatures.

Treatments	Test	Age(wke)		
		10	12	15
T_0	Hb PVC	b 13.33±0.57 c 42.00±1.73	b 13.41±0.05 bc 42.50±0.28	b 14.16±0.28 b44.50±0.85
T_1	Hb PVC	b 14.49±0.19 bc 45.00±0.57	b 13.26±0.433 c 42.00±1.57	b 13.91±0.14 b 43.50±0.28
T_2	Hb PCV	b 14.08±0.57 bc 44.50±1.44	ab 14.16±0.28 b 44.50±0.86	b 14.00±0.28 b 44.50±0.86
T_3	Hb PCV	b 14.33±0.09 b47.50±1.44	a 15.58±0.43 a 45.50±0.28	b 14.33±0.09 b 45.50±0.28
T_4	Hb PCV	a 16.58±0.43 a 52.00±1.15	a 15.00±0.00 a 47.00±0.00	a 15.66±0.38 a49.50±0.86
Significant levle		**	**	**

**** Significant differences at ($p \leq 0.01$) in the same column, Values are mean ± SE**

Heterophil%, Lymphocyte% and their Ratio:

As shown in table-4, at 10 wks of age, s quails supplemented with 1 or 2g /kg diet WSRP (T_3 and T_4) resulted in significant ($p\leq0.01$) reduction in H% and significant ($p\leq0.01$) increasing in L% in comparison with T_2.On the other hand, T_3, T_4 and T_1 didn't differ significantly from control group in H and L% . At 15 wks of age, quails treated with 100mg/kg WSRE showed significant increased ($p\leq0.01$) in H% and significant ($p\leq0.01$) decreased in L% compared with control and with T_1, T_3 andT_4. The supplementation of quails with roots as ethanolic extract or powder had significantly ($p\leq0.01$) lowered L% at 15 wks of age compared with control. Concerning H/L ration, it was found that T_0, had significantly ($p\leq0.01$) lower ratio than T_2value at 10 and 15 wks of age, besides, no significant differences were found between T_0,T_1,T_3 and T_4 at 10 wk of age.

It was widely known, treated with *W.somnifera* would significantly increased the number of white blood cells (WBC)in the body[9;20],yet, in this study, it was found that under heat stress conditions, Japanese quails supplemented with *W.somnifera* roots didn't had significant effect in improving blood picture as compared to unsupplemented group. Furthermore, it seems that daily oral administration of 100mg/kg b.wt ethanolic extract conduced increase in stress on quails and this effect was well-defined from the significant increasing in H% and significant reduction in L%, in-turn, significant increasing in H/L ratio in this treatment compared to others. The possible explanation for that could be as follows: heat stress resulted in increasing the formation of H% without affecting other kinds of WBC [17] , this increment may be due to bone marrow stimulation as a response to stress[23],while, the reduction in L percentage perhaps belong to lymphopenia which happenes as a result of the rapid migration of these cells from circulatory system to the areas of tissue injury especially liver and kidney in which they adhere to the endothelium and emigrate to the perivascular areas[9], another reason for L% reduction could be lympholysis that happened under the effect of increasing in corticoid secretion[10].

Serum Iron:

As shown in Table (4) ,the addition of 2g/kg diet WSRP (T_4) caused in highly significant increased($p\leq0.01$) in serum iron at 10 wks of age, in comparison to control and other supplemented groups(T_1,T_2andT_3).As well as ,at 15 wk of age, it was found that T_4 had significantly ($p\leq0.01$) higher serum iron than control and extract groups (T_1andT_2) .The significant increasing in serum iron in WSRP groups were inharmonic with their increasing in blood Hb and PVC% which has been found in present study.

From the above results, one may conclude that, adding *W.smnifera* roots to diet as powder (especially 2g/kg diet) found to be more useful in increasing serum iron than supplementing it as ethanolic extract, this increasing could be attributed to the rich content of iron in *W.somnifera* roots powder, whereas the resinous contents of its ethanolic extract may reduced the concentration of iron in it.

Serum TSH, T3 and T4:

Results revealed the superiority ($p\leq0.05$) of 1g/kg diet WSRP (T_3) on serum TSH, compared to control and WSRE group's (T_1andT_2) .No significant differences were found among treatments inT3and T4 hormones (Table-5).

Increasing the level of serum TSH in blood of heat stressed Japanese quail supplemented with 1g/kg diet WSRP is may be related to some chemical compounds of *W.somnifera* roots which, under heat stress condition, alleviate TSH concentration thru effects on Hypothalamic Pituitary Thyroid (HPT) or Hypothalamic PituitaryAdrenal (HPA) axis function. Our result disagreed with [24] who found that supplementing heat stressed broilers with *W.somnifera* increased T_4 due to reducing corticosterone and the variation in results

could be ascribed to the geographic location of the animals, the feed used, or the species.

Table-4: Effect of supplementing *Withania somnifera* roots as ethanolic extract or crude powder on%Heterophil, %Lymphocyte, Heterophil/ Lymphocyte ratio and serum Iron of Japanese quails reared under high environmental temperature.

Items	Age (wks)	T_0	T_1	T_2	T_3	T_4	Signi-ficant level
Heterophils %	10	bc 32.50±4.33	b 41.50±2.59	a 53.50±3.17	b 42.00±4.04	c 30.00±2.08	**
	15	d 24.50±0.86	b 35.50±0.00	a 49.00±1.44	c 31.00±0.05	c 31.00±0.05	**
Lymphocyte %	10	a 62.50±4.33	a 53.50±4.33	b 35.83±4.83	a 50.00±4.04	a 63.50±2.59	**
	15	a 67.50±2.02	b 62.50±0.28	d 43.50±0.28	c 56.00±1.15	b 62.50±0.28	**
Heterophil/ Lymphocyte ratio	10	b 0.52±0.86	a 0.77±0.11	a 1.49±0.27	ab 0.84±0.15	b 0.47±0.05	**
	15	c 0.36±0.03	b 0.56±0.00	a 1.12±0.00	bc 0.55±0.05	bc 0.49 ±0.04	**
Iron g/dl	10	b 132.50±1.44	b 157.50±10.10	b 138.50±15.2	b 136.25±6.49	a 248.75±22.37	**
	15	bc 264.00±16.16	c 236.25±3.60	c 246.25±9.38	ab 296.25±7.93	a 316.25±19.4	**

****Significant differences at (p≤0.01) in the same raw, Values are mean ± SE**

Table-5: Effect of supplementing *Withania Somnifera* roots as ethanolic extract or crude powder on serum TSH, T4 and T3 of Japanese quails reared under high environmental temperature.

Treatment	Hormone concentration		
	TSH(mlu/ml)	T4 (mg/dl)	T3(µ g/dl)
T0	b 0.64±0.08	3.38±0.52	2.16± 0.54
T1	b 0.60±0.18	4.02±2.12	1.04±0.56
T2	b 0.65±0.13	5.81±2.80	3.77±1.53
T3	a 1.18±0.02	4.08±2.35	2.23±0.95
T4	ab 0.93±0.15	3.36±0.32	1.90±0.89
Significant level	*	NS	NS

****Significant differences at (p≤0.01) in the same column, Values are mean ± SE**

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