

EFFECTS OF SUPPLEMENTATION WITH *WITHANIA SOMNIFERA* L. ROOTS ON SOME EGG PRODUCTION AND QUALITY TRAITS OF HEAT STRESSED JAPANESE QUAILS

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Abstract

This study was carried out to investigate the anti-stress properties of local *Withania somnifera* roots, (WSR) in alleviating heat stress and improve egg production and quality in Iraq during summer season. The roots were used as ethanolic extract or crude powder. Ethanolic extract was done by using 70% ethanol. Three hundred Quail hens, 6 weeks (wks) old were used which were fed with standard basal diet containing 20% crude protein and 2903Kcal/Kg metabolizable energy and reared under high environmental temperature (27-37-27°C) and relative humidity (40-50%) and were randomly allocated to five groups as follows: Treatment (T₀): control group without any supplementation; Treatment 1 and 2 (T₁, T₂) quails supplemented orally with a dose of 50 or 100 mg/kg body weight (b.wt)/day ethanolic extract of *Withania somnifera* roots (WSRE; Treatment 3, 4 (T₃, T₄) quails received *Withania somnifera* roots as powder (WSRP) mixed with the diet at the rate of 1 or 2g/kg diet respectively. At 7, 9, 11, 13 weeks of age and the total average of these weeks egg production and quality traits were calculated. Quails supplemented with roots powder at the rate of 1g/kg diet. (T₃) were significantly higher ($p \leq 0.05$) in total average of egg production (%) than those received ethanolic extract (T₁ and T₂). Also T₃ was the best treatment in egg weight, feed conversion ratio, egg mass and albumin height. The supplemented groups T₁, T₂, T₃ and T₄ did not differ significantly from control in yolk index and Haugh unit. Egg tests during experimental period showed that there were no appearances of blood spot and meat segments, and no significant differences between treatments concerning with egg flavour test. We can conclude that little benefit in using (WSR) under heat stress to improve egg production and quality and the result favourite T₃.

Key words: *Withania somnifera* roots, egg production, quails, ethanolic extract and powder, Heat stress.

INTRODUCTION

In many countries including, Iraq poultry production suffers from high environmental temperatures in summer for more than six months. High ambient temperature is one of the major factors affecting poultry industry (Al-Hassani and Al-Jebouri, 1988). Thus, several researches have been done to investigate the role of supplementing certain medicinal plant in improving birds performance because, its cheap and safety instead of using chemical drug. *Withania somnifera* (WS), also known as Ashwahandha, and Indian ginseng, is mentioned in the ancient Ayurvedic literature (Ghadha, 1976).

The plant grows widely in all dry parts of subtropical India. It is also found in the Mediterranean region, the Middle East and South Africa; and in Iraq. WS is an important medicinal plant widely used as a home remedy for several diseases in India as well as other parts of the world (Owais et al., 2005). The chemistry of WS, as a rich source of bioactive

compounds (Padmavathi et al., 2005) has been extensively studied. Twelve alkaloids, 35 with anolides and several sitoindosides from WS have been isolated (Matsuda et al., 2001; Ganzera et al., 2003; Jayaprakasam et al., 2003; Kaure et al., 2003).

The pharmacological and therapeutic efficacy of this plant was well established (Dhuley, 2000). It has multifaceted medicinal properties-inducing antioxidant, adaptogen, aphrodisiac, liver tonic, anti-inflammatory, antibacterial (Sandaram et al., 2011)

There were no previous studies that examine the antistress and adaptogenic efficacy of indigenous *Withania somnifera* roots (WSR) as ethanolic extract, or powder to alleviate heat stress on egg production and quality of heat stressed Japanese quail.

MATERIALS AND METHODS

Experiments were carried out at Poultry Farm, Department of Animal Resource, College of Agriculture, University of Baghdad. The

experimental quails were brought from the Poultry Farm of Agriculture Foundation Research, Ministry of Agriculture, Baghdad, Iraq. Three hundred Hens quails 6 weeks (wks) old, 190-200g body weight (b.wt.) were fed with standard basal diet containing 20% crude protein and 2903Kcal/Kg ME (Table 1) All birds were in healthy conditions. The birds reared under environmental temperature (27-37-27°C) and relative humidity (40-50%). Diet and water were supplemented *ad libitum*. All birds were acclimatized to experimental condition for 14 day.

Table 1. Composition and calculated analysis of experimental diet

Ingredient	%
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
% Methionine	0.77
%Calcium	2.54
%Available phosphorus	0.35

*Type "Holdmix" manufactured in Jordan, every 1 Kg contain 40% crude protein, 7.5% fat, 2.5% crude fiber, 8% calcium, 2100 kcal ME, 2.30% phosphorus, 2.60% salt, 2.4% lysine, 1.7% methionine and cystine, 2500 IU vitamin D3, 300 mg vitamin B3, 10 mg vitamin B6, 200 mg vitamin E, 200 mg niacin, 500 mg iron, 600 mg zinc, 10 mg cobalt, 100000 IU vitamin A, 10 mg vitamin B1, 100 mg vitamin B12, 20 mg vitamin K3, 0.5 mg Biotin, 80 mg pantothenic acid, 50 mg copper, 700 mg manganese, 2 mg Selenium and 5 mg folic acid.

**calculated composition according to NRC, (1994).

Fresh healthy plants of WS, 2-3 years old were collected from several places in Baghdad. The herb was identified and authenticated at the Iraqi National Herbarium, Abu Ghariab. The roots were separated, cleaned, washed, air dried in shades, crushed and became a powder by an electric grinder.

The fresh powdered root of WS was extracted with 70% ethanol according to (Harborne et al., 1975). Quails were randomly distributed into five equal groups with three replicates for each group. The experimental treatments were as follows:

Treatment (T₀): control group without any additions to diet;

Treatments 1,2 (T₁,T₂): quails supplemented orally with a dose of 50 or 100 mg/kg b.wt. of ethanolic extract *Withania somnifera* roots (WSRE);

Treatment 3,4 (T₃,T₄): quails supplemented with *Withania somnifera* roots as powder WSRP mixed with the diet at the rate of 1 or 2 g/kg diet. For ethanolic extract treatments (T₁ and T₂), a certain weight of the extract was suspended in a convenience amount of distilled water in order to prepare the dose of 50 mg/kg b.wt. for T₁ and 100mg/kg b.wt for T₂. These doses were administrated daily at 12.00 PM for every bird during entire experiment period, by using stomach tube which inserts the substance into the crop. For crude powder treatments, 1 and 2 g of fresh powder were mixed for every kg of T₃ and T₄ diet respectively. These diets were presented to hens daily, until the end of experiment.

Eggs were collected daily at 9.00AM. Egg production (EP) was determined on daily- basis as the number of eggs laid by birds as hen's day (HD) production according to Naji and Hanna (1999).

Ten freshly laid eggs were collected once weekly from each replicate. The eggs were weighed for average egg weight (EW) by using electronic scale with high precision strain gauge sensor system (400-SF). Egg mass (EM) was calculated according to Al-Zubaidy (1986)

The egg shell was broken at the middle portion with the help of blunt end of knife; its contents were poured on perfectly levelled glass plate. The height of thick albumin was taken between the yolk and the outer border of thick albumen. Albumin height (AH) was measured with the help of micrometer with a least count of 0.001 mm after adjusting for the zero error on the plain glass plate. Yolk height (YH) was measured with the help of micrometer.

Yolk diameter (YD) was measured with the help of digital Verne caliber (0.01 - 150 mm).

Yolk index (YI) was calculated according to Card and Nesheim (1973).

Haugh unit (HU) was measured according to the equation mentioned by Card and Nesheim (1973).

The eggs were tested to determine the presence or absence of blood spot and meat segments.

Egg flavor test (FT) was evaluated by presenting boiled eggs randomly to eleven judges that had high qualification in evaluating the flavor of alimentary product and answering the questions presented in special application which include five grades for flavor test (Table 2) according to Ibrahim and Hill (1980).

Table 2. Grade of egg flavor test

Grade	Flavor Test
1	No flavor
2	Few flavor
3	Medium flavor
4	Strong flavor
5	Very strong flavor

According to Ibrahim and Hill (1980).

A completely randomized design -CRD within the Statistical Analysis System- SAS 2010 was used to analysis the data for the effect of difference factors in the studied parameters. Duncans (1955) multiple range tests was used to significant comparison between means.

RESULTS AND DISCUSSIONS

The mean values of egg production (EP) percentage (Hen Day (H.D %) are shown in Table 3. There were no significant differences between treatments at 9 and 11 wks of age on EP. At 7 wks of ages, EP was significantly decreased ($p \leq 0.05$) in T_1 and T_4 group as compared with T_0 , while T_2 and T_3 didn't differ significantly from T_0 . At 13 wks of age, T_3 and T_4 had significance increased ($p \leq 0.01$) in (EP) compared to T_1 , T_2 and T_0 , which did not differ significantly among each other. Quails supplemented with root powder at the rate of 1g/kg diet (T_3) were significantly higher ($p \leq 0.05$) in total average of (EP) than those received ethanolic extract (T_1 and T_2).

Statistical analyses of Egg weight (EW) are presented in Table 3. No significant differences were found between treatments at 7 wk of age and the total average of EW. The values of EW was reduced significantly ($p \leq 0.01$) in T_1 , T_2 and T_3 as compared with control group at 9 wks of age.

At 11 wk of age there were a significant increased T_3, T_4 as compared with T_0 and T_1 . At 13 wk of age treating quails with root ethanolic extract (T_1 and T_2) and with crude powder (T_3) at the rate of 1g/kg diet significantly ($p \leq 0.05$)

increased EW as compared to control group, which did not differ significantly from T_4 .

Table 3. Effect of supplementing *Withania somnifera* roots as ethanolic extract or crude powder on egg production and egg weight of Japanese quail reared under high environmental temperature

Age (wks)	Treatment					Significant Level
	T_0	T_1	T_2	T_3	T_4	
7	32.144±4.382	22.400±6.00	25.400±1.37	28.010±1.870	21.333±0.49	*
9	34.033±5.095	32.680±1.33	33.333±5.49	36.383±1.971	34.713±2.16	NS
11	61.570±7.892	58.940±4.91	68.373±5.89	61.903±5.499	73.230±4.20	NS
13	69.130±3.389	69.800±1.48	64.753±1.09	82.513±1.157	76.423±2.06	**
Total average	49.219±2.473	45.955±2.24	47.964±3.92	52.202±2.449	51.424±0.13	*
Egg Weight (g)	Treatment					Significant Level
	T_0	T_1	T_2	T_3	T_4	
7	11.00±0.00	10.30±0.17	10.33±0.00	11.33±0.18	10.98±0.76	NS
9	11.49±0.09	10.83±0.28	10.66±0.19	10.49±0.09	11.00±0.00	**
11	10.83±0.09	10.83±0.09	10.99±0.19	11.49±0.09	11.33±0.19	*
13	11.49±0.00	12.00±0.00	12.16±0.09	12.16±0.288	11.83±0.09	*
Total average	11.13±0.10	10.99±0.06	11.035±0.03	11.367±0.05	11.280±0.24	NS

T_0 = control, T_1 = 50 mg/Kg b.wt WSRE, T_2 = 100 mg/Kg b.wt WSRE, T_3 = 1g/Kg diet WSRP, T_4 = 2g/Kg diet WSRP, *Significant differences between treatments at ($p \leq 0.05$) in the same raw, **Significant differences between treatments at ($p \leq 0.01$) in the same raw, NS= No Significant differences between treatments, Values are expressed as mean ± SE.

Table 4 shows that the ratio of feed conversion to egg (FCE) which did not differ significantly due to experimental treatments at 9 wk of age, while, at the rest of experimental periods (7,11,13 wks), there were an improvement in groups supplemented with roots as crude powder in comparison with ethanolic extract and with control. At 7 and 13 wks of age, T_3 improved significantly ($p \leq 0.01$) in (FCE) comparison with T_1 . Treatments T_3 and T_4 were significantly ($p \leq 0.01$) better than T_1 at 7, 11 and 13 wks of age and in total average, there were significant improvement ($p \leq 0.05$) in T_3 compared to T_1 and T_2 .

As shown in Table 4, there were significant differences in egg mass (EM) between treatments during experimental periods except at 9 wks of age. Hens supplemented with root as extract (T_1 and T_2) or as powder (T_4) had significantly ($p \leq 0.05$) lower EM than control group and T_3 at 7 wk of age, while there were no significant differences between hens in T_3 and hens in T_0 .

Hens supplemented with WSRP (T_3 and T_4) had significance ($p \leq 0.05$) increased EM compared to WSRE and control groups at 11 wk of age. At 13 wk of age, T_3 had the higher EM value than others and differed significantly ($p \leq 0.01$) from T_1 , T_2 , T_3 and T_0 .

Total average of EM showed that supplemented hens with 1g/kg diet WSRP (T₃) gave higher values (p≤0.05) of EM compared to other treatments(T₁, T₂, T₄ and T₀).

Table 4. Effect of supplementing *Withania somnifera* roots as ethanolic extract or crude powder on feed conversion ratio and egg mass of Japanese quails reared under high environmental temperature.

Age (wks)	Treatment					Significant level
	T ₀	T ₁	T ₂	T ₃	T ₄	
7	3573±0.260	5.551±0.333	4.844±0.371	3.218±0.104	4.247±0.092	**
9	3069±0.032	3.153±0.333	3.025±0.378	3.154±0.350	2.965±0.169	NS
11	3071±0.112	3.204±0.131	3.017±0.200	2.668±0.104	2.472±0.101	**
13	2535±0.135	2.397±0.079	1.551±0.089	1.375±0.119	1.468±0.075	**
Total average	3062±0.058	3.576±0.041	3.109±0.246	2.603±0.196	2.788±0.049	*
7	2.14±1.46	26.17±2.51	28.69±2.65	46.04±1.18	32.99±0.54	**
9	50.84±2.28	50.86±6.40	53.30±7.43	49.45±5.42	53.59±1.84	NS
11	1.03±3.63	51.22±1.98	46.94±2.93	64.68±0.32	61.19±0.65	**
13	55.97±3.67	57.49±2.49	52.40±1.80	69.30±0.63	62.16±2.90	**
Total average	49.95±0.21	46.43±5.86	45.33±1.55	57.367±0.48	52.482±0.50	*

T₀ = control, T₁ = 50 mg/Kg b.wt WSRE, T₂ = 100 mg/Kg b.wt WSRE, T₃ = 1g/Kg diet WSRP, T₄ = 2g/Kg diet WSRP, *Significant differences between treatments at (p≤0.05) in the same raw, **Significant differences between treatments at (p≤0.01) in the same raw), NS= No Significant differences between treatments, Values are expressed as mean ± SE

From Table 5 it can be observed that at 7 and 9 wks of age, no significant differences between treatments were found in egg albumin height (AH). At 11 wk of age, T₃ group had higher AH (p≤0.01) than T₁. At 13 wk of age, T₃, T₄ and T₀ had significantly (p≤0.05) increased AH as compared with T₁ group. Total average showed no significant difference in all treated groups compared with control. Concerning yolk height (YH), no significant differences were found between treatments throughout the experimental periods (Table 5).

Statistical analysis of yolk diameter (YD) presented in Table (6) shows that there was conflict in differences among treatments through experimental periods. At 7 wk of age T₁ reduced significantly as compared with other treatments while at 9 wk T₃ decreased significantly with T₂ meanwhile at 11 wk T₃ reduced significantly compared with T₀, T₂ and T₄ also at 13 wk T₁ increased significantly as compared with T₀, T₃, T₄.

Total average of YD revealed that T₄ had significantly (p≤0.05) higher values than T₁ and T₃. On the other hand, all supplemented quails (T₁, T₂, T₃ and T₄) did not differ significantly from control.

Yolk index (YI) parameters that presented in Table 6 showed no significant differences between treatments at 9, and 13 wks of age and the total average.

Table 5. Effect of supplementing *Withania somnifera* roots as ethanolic extract or crude powder on albumin height and egg yolk height of Japanese quails reared under high environmental temperature.

Age (wks)	Treatment					Significant level
	T ₀	T ₁	T ₂	T ₃	T ₄	
7	5.16±0.27	5.21±0.03	5.44±0.23	4.79±0.10	5.18±0.47	NS
9	4.60±0.29	4.67±0.30	5.21±0.07	5.24±0.42	4.81±0.42	NS
11	4.59±0.12	4.18±0.02	4.51±0.17	4.70±0.21	4.58±0.06	**
13	5.59±0.64	3.97±0.21	4.74±0.03	5.31±0.30	5.50±0.31	*
Total average	4.98±0.01	4.50±0.04	4.97±0.04	5.01±0.01	5.01±0.08	NS
7	11.47±0.44	11.67±0.10	11.37±0.14	10.96±0.07	11.00±0.20	NS
9	12.05±0.05	12.41±0.47	12.34±0.33	12.00±0.23	11.87±0.19	NS
11	12.62±0.28	12.45±0.01	12.29±0.27	12.73±0.12	12.24±0.20	NS
13	13.19±0.94	13.11±0.16	12.92±0.09	12.94±0.03	13.18±0.12	NS
Total average	12.33±0.09	12.41±0.00	12.25±0.03	12.15±0.04	12.07±0.05	NS

T₀ = control, T₁ = 50 mg/Kg b.wt WSRE, T₂ = 100 mg/Kg b.wt WSRE, T₃ = 1g/Kg diet WSRP, T₄ = 2g/Kg diet WSRP, *Significant differences between treatments at (p≤0.05) in the same raw, **Significant differences between treatments at (p≤0.01) in the same raw), NS= No Significant differences between treatments, Values are expressed as mean ± SE

Table 6. Effect of supplementing *Withania somnifera* roots as ethanolic extract or crude powder on egg yolk diameter and index of Japanese quails reared under high environmental temperature

Age (wks)	Treatment					Significant level
	T ₀	T ₁	T ₂	T ₃	T ₄	
7	24.26±0.08	21.43±0.21	23.44±0.92	24.52±0.30	25.26±0.935	**
9	24.38±0.26	23.92±0.02	24.72±0.02	23.46±0.53	24.13±0.41	*
11	25.20±0.00	25.11±0.35	25.10±0.28	23.57±0.23	25.21±0.47	**
13	24.08±0.02	25.33±0.50	24.77±0.06	24.42±0.04	24.45±0.24	*
Total average	24.48±0.09	23.94±0.36	24.50±0.12	23.99±0.05	24.76±0.30	*
7	0.47±0.01	0.54±0.00	0.48±0.02	0.44±0.00	0.43±0.00	*
9	0.49±0.00	0.51±0.01	0.49±0.01	0.51±0.02	0.49±0.00	NS
11	0.50±0.01	0.51±0.00	0.48±0.01	0.54±0.00	0.48±0.00	*
13	0.54±0.02	0.51±0.01	0.52±0.00	0.52±0.00	0.53±0.01	NS
Total average	0.49±0.00	0.51±0.01	0.49±0.00	0.50±0.00	0.48±0.00	NS

T₀ = control, T₁ = 50 mg/Kg b.wt WSRE, T₂ = 100 mg/Kg b.wt WSRE, T₃ = 1g/Kg diet WSRP, T₄ = 2g/Kg diet WSRP, *Significant differences between treatments at (p≤0.05) in the same raw, **Significant differences between treatments at (p≤0.01) in the same raw), NS= No Significant differences between treatments, Values are expressed as mean ± SE.

Quail hens supplemented WSRE at the rate of 50 mg/kg b.wt (T₁) had increased YI significantly (p≤0.05) compared with T₀, T₂, and T₃ and T₄ at 7 wk. At 11wk of age, T₃ had significantly (p≤0.05) increased YI compared with T₀, T₂ and T₄, which did not differ significantly between each others.

Regarding Haugh unit values, no significant differences were obtained between treatments throughout the study wks, and in total average (Table 7).

Egg tests during experimental periods showed that there were no appearances of blood spot and meat segments.

Statistical analysis presented in Table 8 shows no significant differences between treatments concerning with egg flavor test (EF).

Table 7. Effect of supplementing *Withania somnifera* roots as ethanolic extract or crude powder on haugh unit of Japanese quails reared under high environmental temperature

Age (wks)	Treatment					Significant level
	T ₀	T ₁	T ₂	T ₃	T ₄	
7	71.13±2.77	72.37±0.65	70.51±0.91	67.95±0.465	68.22±0.46	NS
9	74.71±0.35	76.94±2.97	76.52±2.08	74.40±1.43	73.61±1.43	NS
11	78.24±1.78	77.21±0.09	76.19±1.68	78.94±0.80	75.26±0.80	NS
13	81.77±0.25	81.28±0.03	80.12±0.59	80.22±0.21	81.71±0.21	NS
Total average	76.46±0.70	59.33±0.02	75.83±0.12	75.37±0.29	74.70±0.46	NS

T₀ = control, T₁ = 50 mg/Kg b.wt WSRE, T₂ = 100 mg/Kg b.wt WSRE, T₃ = 1g/Kg diet WSRP, T₄ = 2g/Kg diet WSRP, NS= No Significant differences between treatments, Values are expressed as mean ± SE.

Table 8. Sensory evaluations for egg flavor of heat stressed Japanese quails supplemented with *Withania somnifera* roots as ethanolic extract or powder

Treatments	Evaluation degree*
T ₀	3
T ₁	3
T ₂	3
T ₃	3
T ₄	3
Significant level	NS

T₀ = control, T₁ = 50 mg/Kg b.wt WSRE, T₂ = 100 mg/Kg b.wt WSRE, T₃ = 1g/Kg diet WSRP, T₄ = 2g/Kg diet WSRP, NS = No significant differences between treatments.

*Ibrahim and Hill (1980), Flavor: 1=No flavor, 2=little flavor, 3=intermediate flavor, 4=strong Flavor, 5=very strong flavour.

The results of EP(H.D%) indicated that the usage of WSR did not significantly affect egg production of Japanese quail reared under heat stress conditions in comparison with unsupplemented group. Yet, it was found an improvement in EP when the roots were added as powder at the rate of 1g/kg diet. This effect could be happened as a result of the significant increased in the amount of feed consumption in this group under heat stress conditions; this means that females body made use of bioactive

components available in the roots powder which in –turn may improve the exportation of egg yolk precursors from the liver (Asli et al.,2007), perhaps through mediated protection of the liver during heat stress (Harikrishnan et al.,2008), and therefore increased egg production, whereas daily oral administration with ethanolic extract for several wks under high environmental temperatures may reduce the advantage usage of WS.

The significant reduction in average egg weight of T₂ group could be attributed to the reduction in feed intake and FCE in this treatment.

The reduction of FCE, which was obtained in groups supplemented with ethanolic extract, could result from their reduction in feed intake and egg weight in comparison with groups received root as crude powder.

Regarding EM, the possible explanation for significant increase in T₃ group as compared with both extracts and unsupplemented groups may relate to; T₃ superiority in EP percentage and the inequality in EW, as, EM consequential EP x EW.

The absence of significant differences among supplemented groups and control in HU may correlate with the disimprovement in EW and AH due to treatments. This result indicated that treated of heat stressed quails with WSR whether as powder or ethanolic extract did not had any effect on HU values.

The absence of blood spots and meat segments in eggs of quails reared under heat stress condition could be attributed to the possible role of WSR in normalizing blood pressure inside ovarian follicle vessels.

Results of EF revealed that supplementing WSR as ethanolic extract or powder did not affect this parameters in Japanese quail eggs and this could be a good indicator for its safety use in layer hens' nutrition with a good possibility for marketing.

CONCLUSIONS

Regarding egg production performance of the hens, root powder 1g/kg diet resulted in a significant improvement in egg mass and feed conversion ratio; whereas egg production, egg weight, yolk diameter, yolk index, Haugh unit and egg flavor were not affected by experimental treatments.

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