

# Effect of Supplementing Heat Stressed Japanese Quail Hens with *Withaniasomnifera* root Ethanolic Extract and Crude Powder on Serum Lipids and Proteins

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**Abstract:** This study was conducted to evaluate the effect of treating Japanese quail hens subjected to heat stress with the root of *Withaniasomnifera* as ethanolic extract or crude powder on serum lipid profile and protein fraction. A total of three hundred, 6 weeks old Japanese quail hens was distributed randomly to five treatments: T1= control group (unsupplemented), T2= administered orally with 50mg ethanolic extract/kg body weight, T3= administered orally with 100mg ethanolic extract/ kg body weight, T4= dietary fed with 1g crude powder/kg diet and T5= dietary fed with 2g crude powder/kg diet. Results showed that treating hens with the root as 100mg ethanolic extract or with 2g as crude powder resulted in significant ( $p<0.05$ ) decreasing in low density lipoproteins while high density lipoproteins didn't affected significantly in treating groups at 10 weeks of age in comparison with control while when the birds reach 15 weeks of age, results showed that daily administration with root as ethanolic extract reduced significantly ( $p<0.05$ ) triglycerides, very low density lipoproteins, and high density lipoproteins in comparison with control group. The percentage of serum albumin was increased significantly ( $p<0.05$ ) in all groups treated with the roots while alpha-1 globulin was increased significantly ( $p<0.05$ ) in groups treated with high levels of crude powder or ethanolic extract. Alpha-2 globulin increased significantly ( $p<0.05$ ) in group which daily fed with 50mg ethanolic extract while beta and gamma globulins was significantly ( $p<0.05$ ) reduced in all treated groups as compared with control. **Conclusion:** It can be concluded from these results that supplementing heat stressed Japanese quail hens with the root of *Withaniasomnifera* as ethanolic extract or crude powder could reduce the levels of serum LDL, triglycerides and VLDL whereas the levels of albumin, alpha-1 and alpha-2 globulin will increase.

**Keywords:** Serum lipids and proteins, Japanese quail, heat stress, *Withaniasomnifera* roots

## 1. Introduction

Many practical approaches have been developed to avoid the hazardous effect of drugs that used to maintain health of domestic fowls suffered from heat stress in the tropic area in order to obtain high production from poultry industry. Using medicine could result in stay their residues in animal products, then affect human health<sup>(2)</sup>. One of the approaches that used for this purpose is natural feed additives like herbs<sup>(22)</sup> which have fewer side effects, easy availability, low cost, in addition to their several pharmacological properties.

*Withaniasomnifera* (*W.somnifera*) is one of the herbs belongs to adaptogens which could restore homeostasis against stress in order to increase the capability of individual to resist hazardous effect of environmental factors<sup>(11)</sup>. The active constituents of *W.somnifera* are steroidal alkaloids and steroidal lactones, these compounds are withanolides, in addition to the main active chemical constituent withaferin A which is a phytosteroid<sup>(14)</sup>. Roots are the main portion of the plant which widely uses in polyherbal preparation<sup>(16)</sup> and the preliminary trails suggested its potential therapeutic value for being a source of hypoglycemic, diuretic, hypocholesterolemic and hypolipidemic agents with no detrimental side effects in humans<sup>(3)</sup>. Many studies have been conducted to test the efficacy of *W.somnifera* on serum lipids of domestic fowls. In this field, an improvement was found in broilers serum lipid profile when birds treated with aqueous extract of *W.somnifera* and *A.sativum* at the levels of 10ml/L<sup>(28)</sup>, while<sup>(23)</sup> reported that 2% dose of

*W.somnifera* caused a gradual and significant reduction in LDL-cholesterol.

There are many kinds of avian serum proteins and their percentage, chemical composition and biological function are differing between each other. Avian blood plasma proteins could separate into 12 bands<sup>(27)</sup>. The main portion of blood protein are albumin (50%), the following proteins are globulins which included alpha, beta and gamma in addition to fibrinogen and transferrin. Body physiological condition affects the percentage of blood proteins and under normal conditions it stays constant<sup>(26)</sup> whereas its change when the surrounding environmental condition are change<sup>(14)</sup>. The relation between individual fractions of blood proteins reflects the functional, metabolic and health status of the birds and could be used in evaluation of poultry health condition and production capability<sup>(8)</sup>.

Testing of the efficacy of supplementing Japanese quail hens with *Withaniasomnifera* root as ethanolic extract or crude powder on serum lipid profile and protein fractions has not been investigated enough, thus, the recent study aimed to determine the possibility of the advantage use of active compounds of this herb which belongs to adaptogens and acts as anti-stress.

## 2. Materials and Methods

### Plant material and extraction

Roots of fresh and healthy *Withaniasomnifera* which collected from Baghdad and identified and authenticated at

Iraqi National Herbarium, AbuGhariab, were separated, cleaned, washed and air dried in shades, then crushed and pulverized with the help of an electric grinder. The alcoholic extract was done<sup>(13)</sup> by adding ethyl alcohol (70%) to fresh powder. The suspension was left stirring for 72 hours at room temperature then sieved using sterile gauze to get rid of coarse particulars, filtered through Whiteman filter paper, then, the filtrate was evaporated to dryness in a vacuum oven. A sticky brownish extract was obtained and placed in sterile tube and kept in freeze until use.

**Animal husbandry and experimental treatments**

A total of three hundred Japanese quail hens, 6 weeks old were randomly distributed into 5 treatments and allocated to 12 floor pen (20 hens/pen). The experimental treatments was as follows: T1= control group (unsupplemented), T2=administrated orally with 50mg ethanolic extract/ kg body weight, T3= administrated orally with 100mg ethanolic extract/ kg body weight, T4= dietary fed with 1g crude powder/kg diet and T5= dietary fed with 2g crude powder/kg diet.

**Table 1:** Ingredients and chemical calculations of basal diet

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
Chemical calculated analysis*	
Crude protein%	20.0
ME(Kcal/Kg)	2903
Lycine%	1.11
Methionine%	0.77
Calcium%	2.54
Available phosphorus%	0.35

**According to (21)\***

The doses of ethanolic extract were administrated orally every day at 12 PM along the experimental period using stomach tube which inserts the substance into the crop. For crude powder treatments, the fresh powder were add and mixed for every kg of basal diet and presented to hens daily until the end of experiment. The experimental hens were fed ad libitum with standard production basal diet containing 20% crude protein and 2903 kcal/kg ME (Table 1). Along

the experimental period, the house temperature was kept at 27- 37-27 °C. Blood samples were taken randomly from six birds/treatment at the end of 10 and 15 weeks of age. The fresh blood were centrifuged at 4000rpm for 10 minutes, then serum was separated and refrigerated at -20°C until further analysis. The lipids biochemical tests was done using biochemical analyzer kits (Biosystems, Spain) with the help of spectrophotometer to determine the concentrations of triglycerides , high density lipoproteins (HDL) and low density lipoproteins (LDL). The very low density lipoproteins (VLDL) and the non HDL was calculated according to the following:

$$VLDL = \text{Triglycerides} / 5$$

$$\text{Non HDL} = \text{Total cholesterol} - \text{HDL}$$

The individual protein fractions was determined at 15 weeks of age by electrophoresis in taps of gelled cellulose – acetate (LTD and JOKOHCO Company). The interrelation and absolute concentration of protein fraction were determined by Global – Scan densitometer (type PAV).

**Statistical analysis:**

A completely randomized design CRD within the statistical analysis system<sup>(24)</sup> was used to analyzed the data for the effect of different factors in the studied parameters. Duncan's multiple rang tests was used to determine the significant differences among treatment<sup>(7)</sup>. Pvalue less than 0.05 was considered as statistically significant.

**3. Results and Discussion**

**Serum lipids**

Results of statistical analysis concerning the effect of treatments on serum lipid profile are presented in Table 2. No significant differences were found in the levels of HDL t 10 weeks of age between all experimental groups while triglycerides and VLDL was decreased significantly (p<0.05) in groups supplemented with 50mg ethanolic extract/kg b.wtor 1g crude powder/kg diet in comparison with the higher levels of ethanolic extract or crude powder (T3 and T5) which didn't differ significantly with control group. Concerning LDL, results showed that treating Japanese quail with high levels of ethanolic extract or crude powder resulted in significant (p<0.05) reducing in its level as compared with control and other treating groups (T2 and T4).

**Table 2:** Effect of supplementing *W.somnifera* on serum lipids

Items	Age (Wks.)	Treatments					Signf.
		T1	T2	T3	T4	T5	
Triglycerides mg/dl	10	248.3±6.8b	323.8±4.8b	696.0±3.3a	271.4±2.8b	769.1±6.6a	0.05
	15	764.5±8.4a	270.7±3.2b	301.4±1.9b	748.8±3.4b	812.4±6.9a	0.05
HDL (mg/dl)	10	21.57±3.4	21.53±1.5	21.52±1.5	20.53±3.2	23.66±1.3	NS
	15	15.54±2.0a	12.65±1.8ab	9.188±0.6b	13.3±0.4ab	12.9±1.1 <sup>ab</sup>	0.05
LDL(mg/dl)	10	78.91±2.4b	101.2±8.9ab	10.1±0.0c	128.7±4.2a	13.48±2.8c	0.05
	15	15.0±0.2c	79.22±2.7a	54.15±0.3b	10.0±0.1c	11.1±0.0c	0.05
VLDL(mg/dl)	10	50.0±5.4b	64.76±8.0b	139.2±7.2a	54.3±4.3b	153.8±3.3a	0.05
	15	152.9±6.8a	54.16±6.4b	60.29±3.8b	149.7±2.6a	162.4±5.2a	0.05
Non-HDL	10	128.9±0.8ab	156.9±5.9ab	105.2±4.6b	183.0±8.5a	142.0±7.5ab	0.05
	15	99.41±6.9	127.3±8.9	114.4±8.1	113.26±3.1	97.80±8.6	NS

Mean values with the different superscripts in a row differ significantly (p<0.05)  
 NS=non-significant

According to the above results we can concluded that under high ambient temperature, there are an advantage in birds health when Japanese quail hens supplemented with ethanoic extract or crude powder of *W.somnifera* root.

Concerning non-HDL, results showed that oral administration with 100mg ethanolic extract (T3) led to significant ( $p<0.05$ ) reduction in its level in comparison with 1g crude powder (T4) while all treated groups (T2,T3,T4 and T5) didn't differ significantly with control.

When the hens reach 15 weeks of age, results showed that supplementing *W.somnifera* as ethanolic extract led to significant ( $p<0.05$ ) reduction in triglycerides and VLDL in comparison with control group and with groups received *W.somnifera* as crude powder which in turn reduced ( $p<0.05$ ) the levels of LDL in comparison with groups orally administrated with ethanolic extract. The high levels of ethanolic extract used in recent study (100mg/kg b.wt) resulted in significant ( $p<0.05$ ) reduction in serum HDL in comparison with control group which didn't differ significantly with all other treated groups. Treated and control groups didn't affect significantly the levels of non-HDL at 15 weeks of age. The antioxidant efficacy of *W.somnifera* is related to its flavonoids content in roots<sup>(4)</sup> which offers the herbs antioxidant properties that could maintain cell membrane lipids from oxidation damage<sup>(5)</sup>. In recent study, increasing and decreasing the levels of serum triglycerides and VLDL at 10 and 15 weeks of age respectively could be related to the their increasing in egg production period at 15 weeks of age, other reason of reducing these lipids could be the long time daily oral administration with ethanolic extract.

### Serum protein fractions

Results of the percentage of individual fractions of serum proteins are shown in Table 3 and Fig 1 A, B, C, D and E. There are an significant ( $p<0.05$ ) increasing in the percentage of albumin due to supplementing heat stressed hens with *W.somnifera* roots as ethanolic extract or crude powder especially with those received 1g crude powder/kg diet.

Regarding alpha-1 globulin, results showed significant ( $p<0.05$ ) increasing in groups supplemented with the high

levels of crude powder followed by ethanolic extract (T5 and T3) in comparison with control. The lowest percentage of alpha-1 globulin was found in hens daily administrated with 50 mg ethanolic extract/kg b.wt (Fig.1B) while this treatment increased significantly( $p<0.05$ ) the percentage of alpha-2 globulin in comparison with control and other treated groups (T3,T4 and T5).

Beta and gamma globulin decreased significantly ( $p<0.05$ ) in groups orally received *W.somnifera* roots as ethanolic extract in comparison with unsupplemented group (control).

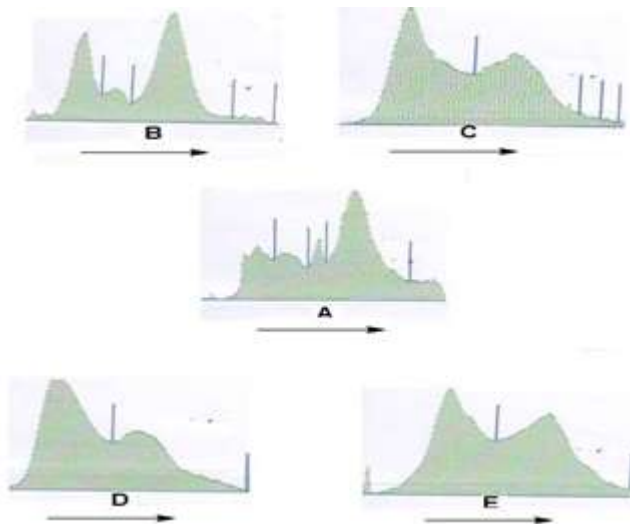
Heat stress affect birds serum total protein, albumin and globulin and led to decrease their levels through reducing protein synthesis<sup>(12,30)</sup> due to the increasing in corticoid secretion. Many studies refers that *W.somnifera* could support the health of adrenal gland and their function under such condition<sup>(28)</sup>, this role was clear in recent study through the increasing in albumin levels in the serum of heat stressed Japanese quail in groups supplemented with this adaptogenic herbs. This result is importance because albumin acts as reservoir of proteins and engage in carriage minerals, fatty acids and vitamins<sup>(9)</sup> in addition to its occupation in transporting thyroid hormones in chickens and quails<sup>(18)</sup>.

Increasing the levels of alpha-1 and alpha-2 globulin in T5 and T2 groups in recent study are most probably happened to satisfy fatty substances needs in egg yolk formation ongoing with increasing in egg production because these proteins functionary moving lipoproteins from blood to eggs<sup>(8)</sup>.

Modulating the immunity of organisms by stimulating other immunity organs like bursa of fabricius and thymus gland is one of the activity of *W. somniferaroot* bioactive compounds like glycowithanilides<sup>(25)</sup>, withaferin A and sitoindosider VIII-X<sup>(5, 17)</sup> and withanolide E has specific effect on T lymphocytes whereas withaferin A affects both B and T lymphocytes and enhance immunostimulatory activity, depending on this, we could explain the reason for reducing the level of gamma globulin in the serum of groups supplemented with *W.somnifera*. To Support this result,(1) found that supplementing Japanese quail hens reread under high ambient temperature with *W.somnifera* root leads to stimulating the activity of their immune system through increasing the follicle diameter of bursa of fabricius and thymus gland, moreover, the tendency of decrease of serum gamma- globulin could associated with the high fertility<sup>(6)</sup>.

**Table 3:** Effect of supplementing *W.somnifera* on serum protein fraction

Protein%	Treatments				
	T1	T2	T3	T4	T5
Albumin	18.82±0.5e	33.18±1.1d	56.79±2.3b	61.81±1.73a	49.26±1.15c
Alpha-1 globulin	15.23±0.13d	11.4±0.23e	41.49±0.2b	38.17±0.0c	50.7±1.1a
Alpha-2 globulin	5.26±0.15b	52.0±1.15a	1.22±0.12c	0.2±0.0c	0.1±0.0c
Beta-globulin	57.9±1.7a	2.35±0.20b	0.43±0.01	0.01±0.0b	0.02±0.0b
Gamma-globulin	2.73±0.11a	1.06±0.03b	0.06±0.00c	0.03±0.00	0.00±0.0c



**Figure 1:** Effect of supplementing *W.somnifera* on serum protein electrophoretic fractions

- A=control group  
 B=group supplemented with 50mg/kgb.wt. (ethanolic extract)  
 C=group supplemented with 100mg/kgb.wt. (ethanolic extract)  
 D=group supplemented with 1g/kg diet (crude powder)  
 E=group supplemented with 2g/kg diet (crude powder)

#### 4. Conclusion

From the results of recent study we can concluded that under high ambient temperature, supplementing Japanese quail hens with the root of *Withaniasomnifera* as ethanolic extract or crude powder could decreased the levels of serum LDL at the early stage of egg production whereas triglycerides and VLDL will decreased at the consequent stags. The percentage of serum albumin, alpha-1 and alpha-2 globulin will increased.

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