



Effect of herbal immunomodulators on immune status, haematological and serum biochemical parameters of Japanese quails (*Coturnix japonica*) during summer stress

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High temperatures, especially when coupled with high humidity, impose severe stress on birds and leads to reduced performance. High environmental temperatures stimulate the hypothalamo-hypophyseal-adrenocortical axis which increases corticosteroid secretion in response to stress (Maini *et al.* 2007). During heat stress, most of the production energy is diverted to thermoregulatory adaptations which results in oxidative stress induced immunosuppression, predisposing birds to various infectious diseases and high mortality rates (Jadhav *et al.* 2014). Blood biochemical profile of birds could serve as an index in predicting the effect of any ration given to the birds. Biochemical changes have clinical importance in assessment of health, nutritional status and diagnosis of disease.

In the last few decades, a number of ayurvedic herbal immunomodulators and antistressor products have been extensively used in poultry to alleviate the negative effects of high environmental temperature. The present experiment was conducted to study the effect of herbal immunomodulators on the haematological, serum biochemical and immune status of Japanese quails.

The experiment was conducted during the extreme hot summer to early monsoon period (April 2017 to May 2017). April, May and June are the hottest months with average daily maximum temperature of 44.8°C and the relative humidity in the afternoon varies between 25 and 40% outside the shed.

Day-old Japanese quail chicks (120) were distributed randomly into 4 treatments of 6 replicates with 5 chicks in each replicate. At day one, chicks were wing banded and housed under battery brooder with optimum brooding conditions. Standard management practices were followed

during the entire experimental period. The birds were fed with maize and soybean meal based diets containing 2900 kcal ME and 25% crude protein during overall experimental period (Table 1). The experimental design consisted of T1, control; T2, Stressroak liquid (contains *Withania somnifera*, *Ocimum sanctum*, *Mangifera indica* and Shilajit) through water @ 1 ml/30 birds; T3, *Withania somnifera* and *Phyllanthus emblica* liquid through water @ 1 ml/30 birds; T4, *Glycyrrhiza glabra*, *Tribulus terrestris* and *Asparagus racemosus* through water @ 1 ml/30 birds. Blood samples were collected at 5th week of age for estimation of haematological and biochemical parameters. Blood was drawn from the wing vein and was subjected to estimation of haematological parameters (TEC, TLC, PCV, HB, MCV and MCH) by using auto hematology analyzer (BC-2800, Mindray). Blood glucose levels were estimated by using capillary blood glucose method. Serum samples were separated from the blood and were used for the estimation of serum parameters (protein, albumin, globulin, triglycerides and cholesterol) by using standard diagnostic kits (Erba Pvt. Ltd). On 21st day of experiment, 6 birds from each group were selected for cell mediated immune response study using delayed type of hypersensitivity reaction to 2,4 dinitro-chloro benzene as per method adopted by Tiwary and Goel (1985). Total serum immunoglobulin was estimated by using zinc sulphate turbidity test (Mondesire 2004). The statistical analysis was done by using SPSS version 20.0.

Mean temperature and RH were 93.5°F and 30.1% inside the shed. The temperature-humidity index (temperature 93.5°F and humidity 30.1%) (90±1.10) were above the threshold established for poultry indicating that the birds were subjected to heat stress.

The haematological data revealed that there was significant difference (P<0.05) in total erythrocyte count (TEC), packed cell volume (PCV), haemoglobin (HB), mean corpuscular volume (MCV) and mean corpuscular Hemoglobin (MCH) in all test groups compared to control (Table 2). Mean corpuscular volume and mean corpuscular haemoglobin were significantly (P<0.05) higher in T4 and

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Table 1. Chemical composition of experimental diet (%)

Maize	51
Broken rice	6
Soyabean meal	38.5
Maize gluten meal	3
Shell grit	0.6
DCP	0.5
Salt	0.3
Lysine	0.05
Methionine	0.11
Chemical composition (% DM basis)	
ME (kcal/kg)	2900
Crude protein	25
Crude fibre	5.46
Ether extract	3.84
Total ash	7.01

followed by T3, T2 and T1. Similarly, Lateef *et al.* (2016) observed that supplementation of *Moringa oleifera* leaf meal significantly ($P<0.05$) increase mean corpuscular volume and mean corpuscular haemoglobin compared to control. Dwivedi *et al.* (2015) reported that supplementation of Ashwagandha (*Withania somnifera*) and Mangrail (*Nigella sativa*) increases Hb, PCV, TLC and MCH concentration values in broilers. Similar results were also reported by Ravi *et al.* (2015). On the contrary, Tariq *et al.* (2014) reported that supplementation of aloe vera and clove did not show any influence on TEC, TLC, PCV, Hb, MCV and MCH in Japanese quails.

Cell mediated immune response showed a significant ($P<0.05$) effect of all herbal immune modulators supplementation as mean skin thickness values in T2, T3 and T4 groups were significantly higher than those in T1 group, however, the values were similar in T2, T3 and T4. Supplementation of all immune modulators significantly ($P<0.05$) increased the total serum immunoglobulin levels as compared to control. These findings indicated the immunomodulatory role of herbal products in Japanese quails during summer stress. The results were in accordance with Tariq *et al.* (2014) who reported that aloe vera and clove supplementation improved cell mediated immunity and serum immunoglobulins in Japanese quails. Mehala and Moorthy (2008) reported significant rise in titre value against Newcastle disease with herbal products in broiler diets. Similar results were also reported by Najafi and Torki (2010).

The values of total serum protein, albumin, globulin, A/

G ratio, triglycerides, cholesterol, and blood glucose were similar in all the treatments indicating that supplementation of herbal immune modulators did not have any significant effect on these parameters (Table 3). Similarly Tariq *et al.* (2014) reported that supplementation of aloe vera and clove had no significant effect on biochemical parameters in Japanese quails. Ravi *et al.* (2015) also reported nonsignificant difference in albumin, globulin and A/G ratio values in broilers supplemented with neem leaf powder. Similar results were also reported by Mehala and Moorthy (2008). On the contrary, Khwairakpam Ratika *et al.* (2016), Lanjewar *et al.* (2008) and Dwivedi *et al.* (2015) reported that supplementation of herbs to broilers causes significant reduction in serum LDL cholesterol, total cholesterol and triglycerides.

Supplementation of all herbal immunomodulators ameliorated the heat stress by improving the immune status of Japanese quails without any significant effect on serum biochemical profile. However, herbal immunomodulators supplementation significantly improved the TEC, PCV, Hb, MCV and MCH levels. Thus it can be concluded that supplementation of herbal immunomodulator preparations to Japanese quails during summer season can overcome the heat stress.

SUMMARY

As the body temperature of birds rise, growth and production parameters tend to decline. In order to address the problem of heat stress, an experiment was conducted to investigate the effect of different herbal immunomodulators on the haemobiochemical and immune status of Japanese quails. Japanese quails (*Coturnix japonica*) (120; day-old) were randomly divided into 4 treatment groups, viz. T1 (control diet), T2 (Stressroak liquid), T3 (*Withania somnifera* and *Phyllanthus emblica* liquid), T4 (*Glycyrrhiza glabra*, *Tribulus terrestris* and *Asparagus racemosus* liquid) with 6 replicates of 5 birds each. The haematological data revealed that total erythrocyte count, packed cell volume, haemoglobin, mean corpuscular volume and mean corpuscular haemoglobin were significantly high in all test groups (T₂, T₃ and T₄) compared to control. Total leukocyte count, mean corpuscular haemoglobin concentration, total serum protein, albumin, globulin, A/G ratio, triglycerides, cholesterol and blood glucose were not affected by herbal immunomodulators at 5th week of age. Immune response as revealed by delayed type of hypersensitivity

Table 2. Effect of supplementation of herbal immunomodulators on haematological profile of Japanese quails

Treatment	Parameter						
	TEC (10 ⁶ /μl)	TLC (10 ³ /μl)	PCV (%)	Haemoglobin (g/dl)	MCV (fl)	MCH (pg)	MCHC (%)
T1	2.67 ^c ±0.07	27.42±0.89	40.60 ^c ±0.82	10.78 ^c ±0.17	136.53 ^b ±1.82	35.85 ^b ±0.40	26.26±0.38
T2	3.14 ^{ab} ±0.10	27.17±0.66	44.55 ^b ±1.58	11.85 ^b ±0.47	132.63 ^b ±1.14	34.93 ^b ±0.46	26.36±0.25
T3	2.97 ^b ±0.49	27.02±0.40	42.15 ^{bc} ±1.40	11.01 ^{bc} ±0.37	131.15 ^b ±1.78	34.13 ^b ±0.44	27.71±1.72
T4	3.27 ^a ±0.05	27.75±0.87	49.93 ^a ±0.80	13.8 ^a ±0.21	161.88 ^a ±5.35	43.38 ^a ±1.42	26.86±0.02

^{a,b,c}Mean values with different superscripts within a column differ significantly ($P<0.05$).

Table 3. Effect of supplementation of herbal immunomodulators on immunological and serum biochemical profile of Japanese quails

Treatment	Parameter								
	Skin thickness (mm)	Serum immuno globulin (mg/dl)	Total serum protein (mm)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio	Triglycerides (mg/dl)	Cholestrol (mg/dl)	Glucose (mg/dl)
T1	1.92 ^b ±0.05	2.37 ^b ±0.02	4.39±0.03	1.28±0.04	3.10±0.04	0.41±0.03	143.35±1.14	143.24±1.55	125.92±1.34
T2	2.50 ^a ±0.05	2.67 ^a ±0.02	4.44±0.02	1.27±0.06	3.17±0.02	0.40±0.04	142.23±0.74	141.08±1.25	125.30±1.32
T3	2.47 ^a ±0.04	2.68 ^a ±0.02	4.39±0.02	1.24±0.05	3.17±0.01	0.38±0.05	142.39±1.04	144.47±1.56	125.36±1.53
T4	2.47 ^a ±0.02	2.65 ^a ±0.02	4.39±0.02	1.24±0.04	3.14±0.03	0.39±0.07	142.26±0.83	143.12±1.25	124.70±1.42

^{a,b}Mean values with different superscripts within a column differ significantly (P<0.05).

(measurement of skin thickness) and serum immunoglobulin were significantly high in all the test groups compared to control. It can be concluded that herbal immune modulators improve haematological and immune status of Japanese quails without affecting serum biochemical parameters.

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