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Immunocompetence, Hepatic Heat Shock Protein 70 and Physiological Responses to Feed Restriction and Heat Stress in Two Body Weight Lines of Japanese Quail

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Abstract: The effect of early heat stress either solely or plus feed restriction on the physiological response, antibody titer and hepatic 70-kda heat shock protein expression (HSP 70) of two body weight lines (high and low) Japanese quail chicks were investigated. Chicks of each line were divided into three groups; (1)control, (2) exposure to 39±1°C for six hour (h), form 5 to 21 day of age (DOA) on each of three consecutive d/week (HS) and (3) HS concurrent with 70% feed restriction (HSFR). The results showed that quail chicks of either HS or HSFR exhibited significantly higher respiration rate (RR) compared with the control. While, the rectal temperature (RT) was reduced at 21 DOA, in the HSFR group. The plasma concentrations of total protein, globulin, total lipid, cholesterol, triglycerides and glucose were decreased due to HS or HSFR episode. Likewise, the level of both calcium and phosphorus at 21 DOA. However, at 42 DOA, the HS chicks did not significantly differ from the control. The activities of plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were increased ($p \leq 0.05$) in treated groups (HS and HSFR) at 21 DOA. Either HS or HSFR showed low Newcastle disease antibody titer and a decrease in relative lymphoid organ weights. Unlike the antibody titer, the H/L ratio was increased in the HS chicks. After heat exposure, HSP 70 density of the high body weight line was immediate and pronounced. The combination of heat stress and feed restriction (HSFR) induced even higher response. While in the low body weight line neither the control nor the heat stress treatment showed any response. Chicks of either HS or HSFR had significantly lower live body weight (LBW) and body weight gain (BWG) and poorer fed conversion ratio (FCR) than the control ones. However, during the recovery period (21 to 42 DOA) the HSFR group was the best ($p<0.01$) for BWG and FCR. The high line body weight chicks showed significantly higher plasma level of total protein, albumin, globulin and cholesterol at 21 and 42 DOA than the low line. Similarly, higher antibody titers against NDV and productive performance traits throughout the experimental period.

Key words: Heat stress, feed restriction, physiological, antibody titer, HSP70 and quail

Introduction

The lower tolerance of birds to heat stress in the hot climate is a major limiting factor and a big problem for birds reared in tropic and sub tropic regions. High ambient temperature in Egypt, during the summer generates a status of stress and evokes a combination of behavioral, biochemical, immunological and physiological changes.

Exposure to high temperature significantly reduced live weight gain, feed intake, feed conversion efficiency, relative weights of thymus, bursa and spleen and serum triglycerides (Guo-YuMing *et al*., 1998).

Respiratory rate increases causing respiratory alkalosis (North and Bell, 1990; Mebta and Sbingari, 1999). High temperature is enough to cause increased body temperature also change circulating leucocyte component in broilers and increased in H/L ratio (Altan *et al*., 2000). Heat stress not only adversely affects production performance but also inhibits immune function (Mashaly *et al*., 2004) and cause a reduction in antibody production in young chicks (Zulkifli *et al*., 2000).

There is an abundance of literature on possible strategies to avoid the deleterious effects of heat stress in poultry. One of the most practical and promising techniques in enhancing the bird's ability to withstand high ambient temperature through early neonatal exposure to heat conditioning and feed restriction (Lin *et al*., 2006). The early age thermal conditioning has been shown to ameliorate the deleterious effects of heat stress in poultry (Yahav and Plavnik, 1999). Also, early age feed restriction, leading to acquired heat tolerance, enhanced the ability of broiler chickens to express HSP 70 (Zulkifli *et al*., 2000 and 2002).

Heat shock protein 70 (HSP 70) is a stress-induced protein. It is an important part of the cell's machinery for protein folding. High levels can be produced by cells in response to hyperthermia. The protein acts as a molecular chaperone by binding to other cellular proteins, assisting intracellular transport and folding into the proper secondary structures, thus preventing aggregation of protein during stress (Chirico *et al*., 1988; Hartl, 1996).

Although many studies are available on broiler chickens, few on quail chicks is available concerning effect of early heat stress and feed restriction on immunocompetence, physiological parameters and the relationship between HSP 70 response and stress tolerance. Thus the objective of this study was to determine the effects of early heat stress and heat stress with feed restriction on productive performance, immune response and hepatic HSP 70 expression in the Japanese quail chicks selected for high and low body weight.

Materials and Methods

Birds and experimental design: A total of 240 1-d-old Japanese quail chicks represent two lines body weights (high and low) were used in this study. One hundred twenty chicks from each line were weighed, randomly divided into three experimental groups each of 40 birds with two replicates of 20 chicks each. The first group of each line was served as control. The second group was exposed to high ambient temperature of 39±1°C (HS) and 50% RH for six hour (h), from 0800-1400h, for three consecutive d/wk from 5 d to 21 d of age. While the third group was subjected to the same heat stress as for second group concurrent with 70% feed restriction (HSFR). All chicks were fed *ad libitum* on a grower diet balanced to meet the optimal requirements for quail chicks as recommended by NRC (1994). The chicks were offered fresh water and maintained on a light cycle of 16L: 8D.

Measurements

Productive performance: Weekly individual live body weight (LBW), body weight gain (BWG) g/bird, feed consumption (FC) g/bird and feed conversion ratio (FCR) g feed/g gain were calculated and recorded till the age of six week. Rectal temperature (RT) was measured by an electronic thermometer (0.10) inserted into the colon for a minute. Respiration rate (RR) was measured by counting the movement of body wall for a minute. Both measurements were recorded, at the end of the third d of heat exposure.

Immunization: All chicks were vaccinated at seven DOA and again at 18 DOA with Lasota strain vaccine. At 15 and 28 DOA chicks were bled via heart puncture, blood was centrifuged at 3000 rpm for 10 minutes. Plasma was stored at -20°C until the detection of primary and secondary humoral immune responses. The detection was conducted by Haemagglutination inhibition (HI) test according to Hitchner *et al.* (1980). The total, mercaptoethanol-sensitive (MES-presumably IgM) and mercaptoethanol-resistant (MER-presumably IgG) anti–NDV were determined using a micro-hemagglutination technique (Yamamoto and Glick, 1982). The antibodies were expressed as the $\log_2$ of the reciprocal of the highest dilution giving visible agglutination.

Biochemical analysis: At 21 and 42 DOA, 18 birds (3/ replicates/ group/ line) were randomly taken from each treatment, weighed and slaughtered. Blood samples were collected during the birds exsanguinations in clear tubes containing sodium citrate as anticoagulant. Blood smears were stained using Hema-3 stain for differential leucocytes counts and heterophil to lymphocyte ratios (Gross and Siegel, 1983). The collected blood samples were centrifuged at 3000 rpm for 10 min. The resulted plasma samples were stored at -20°C until their contents were analyzed using commercial kit according to the procedure outlined by manufacturer. The plasma total protein, albumin, total lipids, cholesterol, Aspartate aminotransferase (AST), alanine aminotransferase (ALT) (Diamond Diagnostic, Cairo, Egypt), triglycerides (Biocon Diagnostic, Germany), calcium (Ca) and phosphorus (P) (Spinreact, S.A. Spain). Globulin was calculated by subtraction of plasma albumin from total protein and albumin/globulin (A/G) ratio was calculated. Glucose was determined on fresh plasma just after blood centrifugation.

The bursa of Fabricius, spleen and all lobes of thymus of both sides were removed and weighed to the nearest of milligram. Their weights were expressed as percentage of live body weight.

Total protein extraction and electrophoresis: Liver samples (1g) were homogenized in 15-ml polypropylene centrifuge tubes, using 3 ml lysis buffer (20 mM Tris-HCl, pH 7.5; 0.75 M NaCl; 2 mM β-mercaptoethanol). Samples were homogenized 3 times (30s) using an Ultra-Turrax homogenizer, at 30,000 rpm and ice-bath intervals of 30s. Lysate was centrifuged at 20,000 g for 30 min at 4°C. The resulting supernatant was collected into a fresh microcentrifuge tube. Protein concentrations were calculated using the BCA Protein Assay Kit (Pierce Biotechnology, Rockford, IL) with BSA as the standard. Eight µl of extracted protein were loaded and separated on 10% polyacrylamide gels containing SDS (Laemmli, 1970), using the Mini-Protean II apparatus (Bio-Rad) at a constant voltage (100 V) for 4 h.

Western blotting analysis for HSP70: After fractionation through SDS-polyacrylamide gels, the proteins were electrophoretically transferred to nitrocellulose membranes using the procedure of Towbin *et al.* (1979). The process of transference was performed for 3 h at 4°C at constant voltage (70 V), using a mini trans-blot cell (Bio-Rad). The membranes were washed with deionized water, non-specific interaction sites were blocked using 10 ml of cold TBS buffer (10 mM Tris-HCl pH 8.0; 150 mM NaCl) containing 50 g/l BSA and 0.2 g/l Tween-20, for 1 h at room temperature, in a shaker (100 rpm, approximately). The membranes were then incubated with 10 µl monoclonal anti-HSP70 antibody (H-5157, Sigma) in 10 ml of cold TBS solution (1:1000

Table 1: Effect of heat stress (HS) and heat stress with feed restriction (HSFR) on body temperature (°C) and respiration rate during the first 21 DOA of the Japanese quail with two lines of body weight

Age	Line		Treatment				Sig.		
(d)	L	H	C	HS	HSFR	MSE	Line	Trt.	Line x Trt.
Body temperature									
7	41.18	40.80	40.01^{b}	41.70^{a}	41.74^{a}	0.48	NS	**	NS
14	42.00	41.74	41.40^{b}	42.23^{a}	42.14^{a}	0.32	NS	**	NS
21	41.50	41.27	41.48^{b}	42.20^{a}	41.00^{b}	0.36	NS	**	NS
Respiration rate									
7	94.18	100.0	82.18^{b}	108.54^{a}	104.44^{a}	2.14	NS	**	NS
14	94.66	92.00	83.91^{b}	97.81^{a}	101.60^{a}	2.36	NS	**	NS
21	75.54	76.20	75.25	76.89	75.890	1.59	NS	NS	NS

$^{a-c}$Means within a row with different superscripts are significantly different. NS= non significant; *p = 0.05; **p = 0.01. H = high line; L = low line; C = control group; HS = high temperature of 39°C ±1.0 for six hour (h), from 0800 - 1400h; HSFR = high temperature of 39°C ± 1.0 for six hour (h), from 0800 - 1400h + feed restriction at 70% from 5 DOA till 21 DOA.

dilution) containing 0.2 g/l Tween-20, for 1 h at room temperature in a shaker. Four washings of 5 min each using 10 ml TBST (10 mM Tris- HCl, pH 8.0; 150 mM NaCl; 0.5 g/l Tween-20) and a 10 min washing using 10 ml of cold TBS buffer were performed. The membranes were incubated with 2 µl secondary anti-mouse antibody conjugated to alkaline phosphatase (A-5153, Sigma) diluted in 10 ml of cold TBS-BSA solution (1:5 000 dilution), for 1 h at room temperature with constant shaking. After rinsing with cold TBST and TBS as described above, the membranes were incubated in 12.5 ml of chromogenic substrate until bands were visible. The membrane was washed with deionized water and dried at room temperature, protected from light. the membranes were photographed and the optic density (OD) for each band within lane was recorded by using Gel works 1D advanced software Ver 1.0 UVP-England.

Data were subjected to analysis of variance using general linear model described in SAS User's Guide (SAS Institute, 1994). Differences among means were tested using Duncan's multiple range test (Duncan, 1955). Percentages of slaughter traits were divided by 100 and subjected to arc sin transformation of the square root before analysis; however actual percentage means are presented.

Results and Discussion

Rectal Temperature (RT) response as an estimate of heat tolerance is of primary importance in investigating heat stress. Table 1 indicates that, quail chicks subjected to either heat stress (HS) or heat stress with feed restriction (HSFR) recorded significantly ($p \leq 0.01$) higher values for both RT and RR compared with the control. However, at 21 DOA, the HSFR group achieved the lowest RT.

This result may reveal that the HSFR group could partially tolerate the harmful effect of heat stress. The undesirable effects of thermal stress are in accordance with Altan *et al.* (2000) in broiler chicks and Durgun and Keskin (1998) who found an elevation in the body temperature of Japanese quail exposed to 42°C for 150min. The fact that the body temperature of birds depends on bird size, environmental temperature, age and sex has been reported by Sturkie (1986).

The reported elevation in the respiration rate of treated birds could be attributed to evaporative heat-dissipation mechanism that maintains their normal body temperature (Odom *et al.*, 1986). Moreover, Kalamah (2001) and El-Sheikh *et al.* (2004) postulated that the increased respiration rate (Panting Phenomenon) is desirable in hot weather to dissipate the excessive heat via evaporative cooling from respiratory passage.

Neither line nor its interaction with treatment had significant effects on both RT and RR, up to 21 DOA (Table 1).

Blood constituents: It is clearly observed from Tables 2 and 3, that chicks of the high body weight line showed significantly higher plasma levels of both total protein and globulin at 21 and 42 DOA, comparable to the low line. Therefore, subjecting Japanese quail chicks, during the period from 5 to 21 DOA to 39°C±1 heat stress (HS) or 39±1 C° heat stress plus 70% feed restriction (HSFR) reduced significantly ($p \leq 0.01$) plasma concentration of total protein and its fractions, albumin and globulin, either at 21 or 42 DOA.

These results could be due to reducing the amount of protein consumed and consequently deficiency of essential amino acids as a result of decreased the amount of feed consumed by the treated chicks (Ozbey and Ozcelik, 2004; Abu-Dieyeh, 2006a,b). Bonnet *et al.* (1997) reported that the feed digestibility of proteins, fats and starch was decreased with exposure of broiler chickens to high temperatures.

Additionally, the treated chicks exhibited lesser ($p \leq 0.05$) immune response than the control as obviously noted by their higher A/G ratios. Griminger (1986) reported that low A/G ratio indicates more disease resistance and immune response. Line x treatment interaction was only significant for total plasma protein at 21 DOA.

Data in Table 2 shows that means of plasma total lipid, cholesterol and triglycerides of quail chicks at 21 DOA

Table 2: Effect of heat stress (HS) and heat stress with feed restriction (HSFR) on some blood constituents at 21 DOA of the Japanese quail with two lines of body weight

	Line		Treatment				Sig.		
Item	H	L	C	HS	HSFR	MSE	Line	Trt.	LinexTrt.
Total protein (g/dl)	3.64	3.37	4.04^{a}	2.98^{b}	2.71^{b}	0.009	NS	**	*
Albumin (g/dl)	1.52^{b}	1.72^{a}	1.69^{a}	1.52^{b}	1.39^{b}	0.018	*	**	NS
Globulin (g/dl)	2.13^{a}	1.66^{b}	2.35^{a}	1.46^{b}	1.32^{b}	0.005	*	**	NS
A/G ratio	0.78^{b}	1.03^{a}	0.72^{b}	1.04^{a}	1.05^{a}	0.012	*	*	NS
Total lipids (mg/dl)	1088.20	993.50	1205.60^{a}	988.60^{b}	743.80^{c}	4.950	NS	**	*
Cholesterol (mg/dl)	172.66^{a}	138.75^{b}	175.15^{a}	159.05^{b}	134.44^{c}	2.280	*	**	**
Triglycerides (mg/dl)	381.33	372.57	383.62^{a}	364.70^{b}	365.02^{b}	1.210	NS	*	NS
Glucose (mg/dl)	159.80	159.41	182.76^{a}	154.75^{b}	142.50^{b}	1.610	NS	**	NS
Calcium (mg/dl)	8.64	8.48	10.94^{a}	8.06^{b}	6.97^{b}	0.480	NS	**	NS
Phosphorus (mg/dl)	8.66	8.03	10.75^{a}	7.90^{b}	7.34^{b}	0.004	NS	**	NS
AST (µ/L)	190.22	181.12	167.16b	190.10^{a}	194.55^{a}	1.610	NS	*	NS
ALT (µ/L)	23.42	21.13	19.80b	25.43^{a}	23.80^{a}	0.960	NS	*	NS

$^{a-c}$Means within a row with different superscripts are significantly different. NS = non significant; *p = 0.05; **p = 0.01. H = high line; L = low line; C = control group; HS = high temperature of 39°C ±1.0 for six hour (h), from 0800 - 1400h; HSFR = high temperature of 39°C ± 1.0 for six hour (h), from 0800 - 1400h + feed restriction at 70% from 5 DOA till 21 DOA.

were significantly lower for the treated groups; HS and HSFR than the control group. Moreover, the reduction was more pronounced for the HSFR group. Similar results were obtained at the end of recovering period; 42 DOA (Table 3).

This reduction in plasma lipid profile could be account for lower cumulative feed consumption throughout the experimental period. Consequently lower energy, carbohydrate and fat intake that resulted in fat depletion. Sturkie (1986) reported that the concentration of avian plasma lipids is influenced by the physical and nutritional status of the bird. Thus the present results are in agreement with those of Kalamah (2001); Abdel-Fattah *et al.* (2003) and El-Sheikh *et al.* (2004). They indicated that blood total lipids and cholesterol levels were decreased with feed restriction and/or high ambient temperature.

Concerning the effect of line, the results obtained herein showed that chicks of high body weight line had greater concentration of plasma lipids than the low line (Tables 2 and 3).

The plasma glucose concentrations were not significantly different between the high and low body weight quail chicks. Although, the level was reduced ($p \leq 0.01$) due to treatments effect in both the HS and HSFR groups either at 21 or 42 DOA. In addition, the effect was more prominent in the latter group (Table 2 and 3).

These reductions in plasma glucose concentrations as an induction of HS or HSFR could be attributed to the sever reduction of feed consumed and consequently decrease of carbohydrate consumption and probably the hepatic storage of glycogen which are the available sources of energy. Our interpretation of these results is that the cycle of glucose metabolism is one of the important targets in heat stress.

These results confirmed those found in heat-stressed (34°C) Japanese quails (Sahin *et al.*, 2002) and broiler chicks (Rahimi, 2005). Zulkifli *et al.* (2000) postulated that the decline in blood glucose concentration during heat stress might be due to a decrease in concentration of thyroxine, which is closely associated with energy metabolism during heat exposure.

The plasma concentrations of both Ca and P exhibited similar aforementioned trend for the glucose level at 21 DOA (Table 2). Although, the plasma Ca and P concentrations at 42 DOA were slightly higher in HS group compared to HSFR group. The present results are coincided with the results of Kalamah (2001) and Abdel-Fattah (2006). Conversely, Ching-CY and Ching- Ching-Yen (1992) postulated that serum concentration of Ca was increased in broilers, but not in chickens, maintained under acute heat stress conditions. This result could be due to the increase in respiration rate as reported in the present study (Table 1), that could lead to a reduction in blood CO_2, HCO_3^- and an increase in blood pH, resulting in respiratory alkalosis. The latter resulted in reducing the amount of Ca in the blood (Franco-Jimenez and Beck, 2007) and stimulating glycolysis driving P into cells and reducing its plasma levels (Ait-Boulahsen *et al.*, 1989).

Belay and Teeter (1996) indicated that heat stress exposure elevated urinary Ca and P as a result of increased urine flow rate and osmolar excretion. Furthermore, Rama Rao *et al.* (2002) reported that heat stress reduced calcium intake, as well as the conversion of vitamin D3 to its metabolically active form, 1,25(OH)2D3, which is essential for the absorption and utilization of Ca.

The present results may indicate that heat stress altered the cycle of Ca metabolism. It can be stated that since the metabolism of P is dependent to Ca. the measurement of these parameters (Ca and P) can be used to understand the biological phenomenon related to the mechanism of thermoregulation in birds under heat stress.

Activities of both plasma AST and ALT of quail chicks determined either at 21 or 42 DOA did not significantly

Table 3: Effect of heat stress (HS) and heat stress with feed restriction (HSFR) on some blood constituents at 42 DOA of the Japanese quail with two lines of body weight. Line x Trt

	Line		Treatment				Sig.		
Item	H	L	C	HS	HSFR	MSE	Line	Trt.	LinexTrt.
Total protein (g/dl)	4.03^{a}	3.43^{b}	4.11	3.96	4.01	0.128	*	NS	NS
Albumin (g/dl)	1.70^{a}	1.56^{b}	1.63^{b}	1.93^{a}	1.95^{a}	0.014	*	*	NS
Globulin (g/dl)	2.33^{a}	1.87^{b}	2.48^{a}	2.03^{b}	2.06^{b}	0.142	*	**	NS
A/G ratio	0.73^{b}	0.83^{a}	0.65^{b}	0.95^{a}	0. 94^{a}	0.026	*	**	NS
Total lipids (mg/dl)	1260.05^{a}	1126.05^{b}	1330.34^{a}	1059.49^{b}	1130.14^{b}	13.950	*	**	NS
Cholesterol (mg/dl)	196.25^{a}	169.39^{b}	205.49^{a}	166.10^{b}	169.58^{b}	7.280	*	**	NS
Triglycerides (mg/dl)	370.48^{a}	332.54^{b}	408.48^{a}	296.89^{b}	303.93^{b}	5.210	*	*	NS
Glucose (mg/dl)	205.51	191.95	213.99^{a}	190.00^{b}	187.38^{b}	1.610	NS	**	NS
Calcium (mg/dl)	11.04	10.68	12.29^{a}	11.43^{ab}	10.55^{b}	0.275	NS	*	NS
Phosphorus (mg/dl)	14.01	13.79	15.22^{a}	14.01^{ab}	13.60^{b}	0.979	NS	*	NS
AST (µ/L) 197.82	196.64	200.02	198.34	201.14	2.010	NS	NS	NS	
ALT (µ/L)	19.93	19.03	19.53	20.18	19.94	0.350	NS	NS	NS

$^{a-c}$Means within a row with different superscripts are significantly different. NS = non significant; * p = 0.05; ** p = 0.01. H = high line; L = low line; C = control group; HS = high temperature of 39°C ±1.0 for six hour (h), from 0800 - 1400h; HSFR= high temperature of 39°C ± 1.0 for six hour (h), from 0800 - 1400h + feed restriction at 70% from 5 DOA till 21 DOA.

Table 4: Effect of heat stress (HS) and heat stress with feed restriction (HSFR) on antibodies titers against NDV; H/L ratio and lymphoid organ relative weights at 21 DOA of the Japanese quail chicks with two lines of body weight

	Line		Treatment				Sig.		
Trait	H	L	C	HS	HSFR	MSE	Line	Trt.	LinexTrt.
Primary response									
Total primary Abs	4.250^{a}	4.000^{ab}	4.583^{a}	3.363^{b}	3.384^{b}	0.033	*	*	*
primary IgM	3.000^{a}	2.636^{ab}	3.250^{a}	2.454^{b}	2.307^{b}	0.09	*	*	NS
primary IgG	1.25	1.36	1.333^{a}	1.181^{b}	1.076^{b}	0.031	NS	**	**
Secondary response									
Total Secondary Abs	5.083^{a}	4.182^{b}	5.250^{a}	4.372^{b}	4.384^{b}	0.437	*	**	NS
Secondary IgM	1.667^{a}	1.363^{b}	1.333	1.526	1.363	0.044	*	NS	NS
Secondary IgG	3.416^{a}	2.818^{b}	3.916^{a}	2.858^{b}	3.021^{b}	0.011	*	**	NS
H/L ratio	0.371	0.367	0.369^{b}	0.441^{a}	0.421^{b}	0.01	NS	**	NS
Lymphoid organ relative weights									
Spleen	0.043^{b}	0.060^{a}	0.055^{a}	0.038^{b}	0.050^{ab}	0.003	*	*	NS
Bursa	0.073^{b}	0.102^{a}	0.133^{a}	0.085^{b}	0.056^{c}	0.005	*	**	NS
Thymus	0.208	0.252	0.296^{a}	0.221^{b}	0.164^{c}	0.005	NS	**	NS

$^{a-c}$Means within a row with different superscripts are significantly different. NS = non significant; *p = 0.05; **p = 0.01. H = high line; L = low line; C = control group; HS = high temperature of 39°C ± 1.0 for six hour (h), from 0800 - 1400h; HSFR = high temperature of 39 C ± 1.0 for six hour (h), from 0800 - 1400h + feed restriction at 70% from 5 DOA till 21 DOA.

different among lines as shown in (Tables 2 and 3). While, exposure to either HS or HSFR elevated (p=0.05) the enzymes levels at 21 DOA. The elevation of the enzyme levels reported at 21 DOA was vanished during the recovering period as indicated by the absence of significance among all groups at 42 DOA (Table 3).

These results might signify that Japanese quail chicks are capable to defeat the stressful effect of either HS or HSFR without any adverse effect on the liver functions.

Immune response: Data illustrating the effect of early heat stress either solely or plus feed restriction on antibody production of Japanese quail chicks with different body weights are presented in Table 4. When compared with control group, 8-d-post-primary NDV immunization, the HS and HSFR groups had significantly ($P \leq 0.05$) lower total, mercaptoethanol-sensitive (IgM) and mercaptoethanol-resistant (IgG) antibodies (Abs) against-NDV (primary immune response). Similar trend was attained for both total and IgG Abs 10-d post-second challenge with NDV (secondary immune response).

These results are in harmony with those reported in broiler chickens (Zulkifi *et al.*, 2000) and in laying hens (Mashaly *et al.*, 2004). They showed that heat stress caused a reduction in antibody production. This reduction could be indirectly due to an increase in inflammatory cytokines under stress. Cytokines stimulate the hypothalamic production of corticotrophin releasing factor (Ogle *et al.*, 1997). Gross (1992) stated that corticotrophin releasing factor is known to increase ACTH from the pituitary; ACTH then stimulates corticosterone production from the adrenal gland, corticosterone in turn inhibits Abs production. Additionally, heat stress is known to decrease T-helper 2 cytokines (Wang *et al.*, 2001), which are important for antibody production (Lebman and Coffman, 1988).

The high line body weight birds had slightly higher values of Abs titers against NDV than their counterparts of the low line at primary and secondary responses, respectively. This was unexpected, because several previous studies conducted on different species of poultry other than Japanese quail chicks reported that

Table 5: Effect of heat stress (HS) and heat stress with feed restriction (HSFR) on live body weight(LBW), weight gain (BWG) feed consumption (FC) and feed conversion ratio (FCR) of Japanese quail chicks with two lines of body weight

		Line		Treatment				Sig.		
Item	Age (d)	H	L	C	HS	HSFR	MSE	Line	Trt.	LinexTrt.
BW	Initial body Wt. (5)	13.22^{a}	10.73^{c}	12.64^{a}	12.29^{b}	11.49^{c}	0.08	**	**	**
	21	79.76^{a}	68.40^{b}	84.70^{a}	77.54^{b}	57.54^{c}	0.28	**	**	**
	42	218.06^{a}	191.50^{c}	213.94^{a}	204.51^{b}	196.73^{c}	2.54	**	**	**
BWG	05-21	66.54^{a}	57.67^{b}	72.06^{a}	65.25^{b}	46.05^{c}	0.30	**	**	NS
	21-42	138.30^{a}	123.10^{c}	129.24^{b}	126.48^{b}	139.84^{a}	1.22	*	**	NS
	05-42	204.84^{a}	180.77^{c}	201.30^{a}	192.22^{b}	185.24^{c}	1.51	**	**	NS
FC	05-21	173.37	171.20	196.80^{a}	180.06^{b}	137.02^{c}	0.76	NS	**	NS
	21-42	570.73^{a}	529.66^{b}	536.10^{b}	555.21^{ab}	563.76^{a}	4.94	*	**	NS
	05-42	744.09^{a}	700.86^{b}	732.90^{a}	735.28^{a}	700.78^{b}	5.05	**	*	*
FCR	05-21	2.60^{c}	2.96^{a}	2.73^{b}	2.76^{b}	2.97^{a}	0.03	**	**	*
	21-42	4.12^{b}	4.30^{a}	4.14^{ab}	4.39^{a}	4.03^{b}	0.04	*	**	NS
	05-42	3.63^{b}	3.87^{a}	3.64^{b}	3.83^{a}	3.78^{a}	0.07	**	*	NS

$^{a-c}$ Means within a row with different superscripts are significantly different. NS = non significant; * p = 0.05; ** p = 0.01. H = high line; L= low line; C = control group; HS = high temperature of 39 °C ±1.0 for six hour (h), from 0800 - 1400h; HSFR = high temperature of 39 °C ±1.0 for six hour (h), from 0800 - 1400h + feed restriction at 70% from 5 DOA till 21 DOA.

selection for fast growth rate is often accompanied by a reduction in specific immune responses or increased disease susceptibility (Tsai *et al.*, 1992; Qureshi and Havenstein, 1994 and Bayyari *et al.*, 1997).

The current findings may put forward expectations that, it is possible to conduct selection strategies for growth in Japanese quail against NDV without unfavorable effects on humoral immunity.

Regarding the H/L ratio which has been used as a reliable indicator of the responses of hypothalamic-hypophyseal adrenal axis to the stressors in birds (Gross and Siegel, 1983 and Yalçin *et al.*, 2003). The present findings of H/L ratio (Table 4) indicate that birds of solely heat stress episode (HS) were the most stressful ($p \leq 0.01$) group as compared to the other groups (HSFR and control). Moreover, the H/L ratio of the HSFR birds did not significantly differ from that of the control ones, indicting that the harmful effect of heat stress could be reasonably ameliorated through combination of heat stress with 70% feed restriction (HSFR).

These results are in agreement with those reported by McFarlane and Curtis (1989) and Mashaly *et al.* (2004) in heat stressed broiler chicks and laying hens, respectively. Yalçin *et al.* (2003) found that early heat stress and feed restriction reduced H/L ratios of broilers exposed to prolonged heat stress. The numerical upward shift in the H/L ratios of HSFR group as compared to the control one is apparently related to increased resistance to bacterial diseases and improvements in resistance to neoplasm and immunocompetence (O'Sullivan *et al.*, 1991).

It was notable that, neither line nor its interaction with treatment had significant effect on H/L ratios of Japanese quail at 21 DOA (Table 4).

The spleen, thymus and bursa are the important lymphoid organs involved in the development and differentiation of T or B lymphocytes (Li *et al.*, 2001). From Table 4 it is clearly noted that, the relative weights of lymphoid organs i.e. bursa, thymus and spleen decreased significantly ($p \leq 0.01$) for both treated groups (HS and HSFR) compared with the control. Similar results were obtained by Naseem *et al.* (2005) who reported that, bursa, thymus and spleen of heat stressed birds were atrophied. Moreover, Hangalapura *et al.* (2005) found that layer chickens subjected to severe feed restriction had lower relative spleen and bursa weights.

The present results showed that, birds of low body weight line had significantly higher relative weights of bursa and spleen than those of high line (Table 4).

Molecular analysis of SDS-PAGE using Western blots (Fig. 1) showed that in high body weight line, the response of heat shock was immediate and pronounced as shown by high density of heat shock protein (150 OD) compared with the control (57 OD) (Fig. 1).

Furthermore, the combination of heat shock and feed restriction induced even higher response (215 OD).

On the other hand, in the low body weight line neither the control nor the heat shock treatment showed any response. However, the combined effect of heat shock and feed restriction stresses resulted in marked response of heat shock protein (219 OD), which was slightly higher than that of the combined stress of high body weight line.

These differences in response could be due to the high body weight line being more capable to counteract the effect of heat shock by induction of such high intensity of HSP70 due to inherent factors. While such factors were down-regulated in low body weight line for heat shock.

On the other hand, combined effect of the two stresses (heat shock and feed restriction) was able to trigger high response in this low line. This could be due to some sort of synergism between heat shock and feed restriction in both low and high lines.

Our results agreed with Heydari *et al.* (1993) who reported that caloric restriction increases the induction

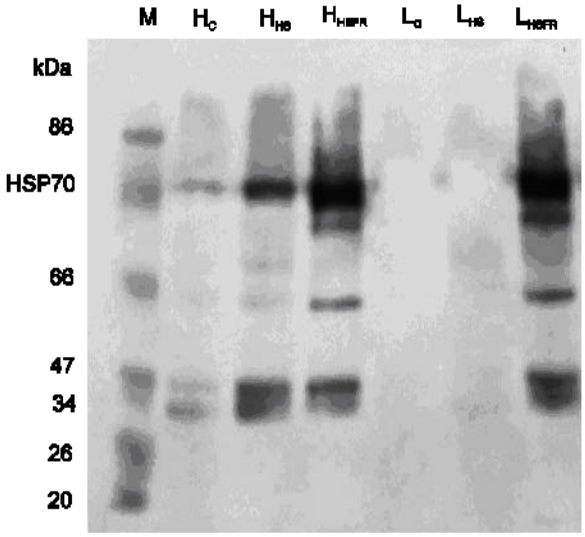

Fig. 1: Effect of heat stress and heat stress with 70% feed restriction (after 6 h at the first day of exposure (5DOA) to 39±1°C for six hour for three consecutive d/wk from 5 to 21 DOA on the optical intensity of hepatic HSP70. M = marker; HC = control of high line; HHS = heat stressed of high line; HHSFR = heat stressed with feed restriction of high line; LC=control of low line; LHS= heat stressed of low line; LHSFR = heat stressed with feed restriction of low line.

of HSP70 transcription and improves thermotolerance (cited by Gabriel *et al.*, 1996). Similar findings have been reported in male broiler chickens subjected to 60% feed restriction on d 4, 5 and 6 and exposure to 36 ± 1°C and 50 to 60% RH for 1 h daily from d 1 to 21 (Liew *et al.*, 2003).

Growth performance: Data of LBW, BWG, FC and FCR of Japanese quail chicks are presented in Table 5. It is clearly observed that the chicks of control group had significantly ($p \leq 0.01$) higher LBW and BWG at either 21 or 42 DOA, than those of the HS and HSFR groups. These findings are in agreement with the results of Koh and Macleod (1999) who found that broilers reared at 32°C with 75, 50 and 25% feed restriction had significantly ($p<0.05$) lower body weight than the control. Yahav and Hurwitz (1996) reported that exposure to 36°C±1 and 70 to 80% RH at the age of 5 d, or 5 and 7 d depressed the body weight of male broiler chickens. On the contrary, Yahav and Plavnik (1999) did not observe significant reduction in LBW of food restricted male broiler chickens exposed to 36°C±1 and 70 to 80% RH at the age of 5 d, or 5 and 7 d, as compared with controls.

The FC and FCR followed similar trends to the aforementioned results of LBW and BWG. Hence, the best FCR was achieved for the control chicks by the end of experiment at 42 DOA compared with the treated ones (HS and HSFR). Similar findings were reported in Japanese quail (Ozbey and Ozceilik, 2004) and in broiler chicks (Abu-Dieyeh, 2006 a,b).

Zuprizal *et al.* (1993) and Siegel (1995) attributed the reduction in body weight gain during heat stress episode to the reduction in both feed consumption and true digestibility of protein and amino acids. Furthermore, plasma triiodothyronine (T3) concentration may decrease during periods of feed restriction and thermal challenge, suggesting a decline in metabolic status, digestive enzymes activities and heat production (Yahav and Plavnik, 1999). This is confirmed by Leeson *et al.* (1992) who stated that high environmental temperature stimulates the peripheral thermal receptors to transmit suppressive nerve impulse to the appetite center in the hypothalamus causing the decrease in feed consumption. Thus fewer substrates become available for enzymatic activities, hormone synthesis and heat production, which minimize thermal load.

It is noteworthy that, during the recovery period (21 to 42 DOA), the HSFR group could nullify, to a certain limit, the harmful effect of heat stress as indicated by their greater ($p<0.01$) BWG and better FCR, as compared to the control and the HS groups (Table 5). However, this improvement was insignificant for the cumulative data from 5 to 42 DOA. This could be due to the insufficient recovery period, as well as the severity of heat stress and feed restriction technique practiced in the present study.

Su *et al.* (1999) reported that the growth rate of broiler chicks was affected by early feed restriction, increasing severity and longer duration being associated with better relative growth rate after restriction was ended. Also, Zulkifli *et al.* (2002) indicated that the severity of the early age stress might have profound impact on the magnitude of improvement in heat tolerance later in life. The present result is in partial agreement with previous findings reported by (Zulkifli *et al.*, 2000; 2002 and Liew *et al.*, 2003).

The evoked compensatory growth, during the recovery period (21to 42 DOA), could be due to the higher plasma growth hormone (GH) concentration of previously feed restricted birds (Gonzales *et al.*, 1998 and Gebhardt and Marks, 1995).

The effect of line on productive performance of Japanese quail chicks, is shown in (Table 5). The high line recorded the highest LBW, BWG and FCR throughout the experimental period as compared to the low body weight line. This improvement in both LBW and BWG is closely associated with the greater feed consumption of the high line chicks.

In conclusion, under the conditions of this experiment the HSFR combination could improved weight gain, immune response and the thermotolerance of heat

stressed Japanese quail chicks. This beneficial effect appears to be closely related to the greater HSP70 expression achieved for the HSFR combination.

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