IJAS Iranian Journal of Applied Animal Science

Physiological, Biochemical and Metabolic Responses of Japanese Quail (*Coturnix coturnix japonica*) as Affected by Early Heat Stress and Dietary Treatment

Research Article

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Received on: 26 May 2011
Revised on: 21 Oct 2011
Accepted on: 24 Nov 2011
Online Published on: Mar 2013

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Online version is available on: www.ijas.ir

ABSTRACT

The main objective of this study was to elucidate the effect of short- term exposure of Japanese quail eggs to acute high incubation temperature on embryonic development and on the ability of post- hatched chicks to cope with subsequent heat stress conditions during the growing period. A total of 998 Japanese quail eggs were divided into two groups, the first group (470 eggs) was maintained at the recommended incubation temperature (37.5 ˚C), while the second group (528 eggs) was exposed to 39.5 ˚C for two hours at days 3, 7 and 13 of embryogenesis. After hatching, chicks from each incubation temperature were randomly assigned to four dietary treatments, a control, a high-energy (+150 kcal ME/kg diet more than the recommended level); a high-lysine (10% more than in the control diet) and a vitamin C supplemented diet. During the experimental period all of the quail were fed *ad libitum* and they received similar hygienic and managerial conditions. The rearing temperature was 32±2 ˚C during the entire experimental period. Pre-hatching exposure of eggs to 39.5 ˚C did not significantly affect the post-hatching body weight and weight gain during the growing period. While, vitamin C or high-energy diet increased it. Body temperature, respiration rate (RR) and the relative weights of the thymus, spleen and bursa of Fabricius were not significantly affected by the pre-hatching temperature or the post-hatching dietary treatments. However, the high-energy and vitamin C diets caused obvious decreases in RR at 4 weeks of age. Pre-hatching heat treatments caused a slight increase in the plasma triiodothyronine (T_3) and plasma thyroxine (T_4) concentrations, however, was significantly decreased by both the pre- hatching temperature and post- hatching dietary treatments at 6 weeks of age. Based on the above results, it was concluded that pre-hatching exposure of quail eggs to high temperature and post-hatching feeding of a high-energy or vitamin C supplemented diet can be recommended for alleviating the deleterious effects of heat stress during the growing period.

KEY WORDS energy, heat stress, incubation temperature, lysine, quail, vitamin C.

INTRODUCTION

Japanese quail (*Coturnix coturnix japonica*) is becoming more popular as a source of meat and eggs in various parts of the world including Egypt.

Quail have also assumed worldwide importance as laboratory animals (Baumgartner, 1994) who have distinct characteristics such as rapid growth that enable them to be marketed for human consumption at 5-6 weeks of age. The Japanese quail's early sexual maturity results in a short gen eration interval, and their high rate of lay and much lower feed and space requirements also provide some benefit over other domestic fowl.

Commercial quail production has grown steadily in Egypt where strains of Japanese quail that have been selected for rapid growth and heavier body weight are utilized. The nutrient requirements of these strains and the optimum housing temperature along with their physiological responses to acute heat stress environments are still obscure.

Previous research has suggested that acclimatization to heat stress for these strains might be induced through pre-hatch and (or) post-hatch short-time exposure to high environmental temperature (Moraes *et al*. 2003; Leandro *et al*. 2004).

It has previously been shown that the rapid heat stress response can be modulated by early- age thermal conditioning (Yahav *et al*. 1997). Such conditioning may affect the integration of thermal information in the hypothalamus which in turn may lower heat production by reducing the circulating concentration of thyroid hormones (Zulkifli *et al*. 1999).

It is well accepted that the main consequence of heat stress is a reduction in feed intake and consequently a reduction in metabolic heat production (May and Lott, 1992). Reduced feed consumption results in poor growth, reduced feed efficiency, immune- suppression and enhanced fat deposition due to reduced thyroid activity (Puvadolpirod and Thaxton, 2000a; Mashaly *et al*. 2004).

Many practical approaches have been developed in attempts to facilitate the thermotolerance of birds, and to minimize the adverse effects of heat stress on productivity. These approaches include pre and (or) post-hatching acclimation of birds (Abd El-Azim, 1991); use of some electrolytes and vitamins (Cftc *et al*. 2005; Holik, 2009; Joachim *et al*. 2010) and dietary energy or lysine levels' manipulation (Balnave and Brake, 2005; Gous and Morris, 2005). There is, however, very little information on the beneficial effects of such approaches to improve Japanese quail production.

Therefore, the present Japanese quail study was conducted to test the possible effect (s) of increased dietary energy, increased dietary lysine level, and increasedvitamin C, on the effects of heat stress. The productive performance and the physiological and biochemical responses of quail to the various treatments were also studied.

MATERIALS AND METHODS

The experiment was carried out in Egypt at the Poultry Physiology Laboratory, Poultry Production Department, Faculty of Agriculture, Ain Shams University, and the laboratories of the Animal Production Department, National Research Centre, during the summer season.

1. Experimental procedures

998 Japanese quail (*Coturnix coturnix japonica*) eggs were obtained from a private quail farm near Cairo, Egypt. The eggs were incubated in a forced draught laboratory incubator at the recommended incubation temperature (37.5 ˚C) and relative humidity between 55 to 66% (Nitsan, 1992). Turning of eggs was automatically done every four hours until day 14 of incubation. On the first day of incubation, the eggs were divided into two groups. The first group (470 eggs) was maintained at the recommended temperature, while the second one (528 eggs) was exposed to 39.5 ˚C for two hours on days 3, 7 and 13 of incubation.

After hatching, the chicks from both groups were brooded in electrically heat- controlled batteries at 35 ˚C for one week, and they were then maintained at 32±2 ˚C during the whole experimental period. All chicks were fed *ad libitum* on the basal diet (control) during the first week. The basal diet was formulated to cover the recommended requirements of growing Japanese quail birds according to NRC (1994). Chicks were exposed to a 23 hr photoperiod during the growing period.

2. Experimental design

After the first week post-hatching, chicks from each pre-hatch temperature group were sorted by weight and those with extreme weights were discarded. The remaining chicks (480 from both the control and the heat-exposed treatments) were assigned to four dietary sub-groups in such a manner as to achieve equal mean body weights per group. The composition and calculated analysis of the experimental diets are provided in Table 1. Birds of each experimental group were randomly distributed into four treatment groups of 60 chicks each as follows:

1. Control: fed the basal growing diet.
2. High-energy: fed the basal diet supplemented with 150 kcal ME/kg of diet over the recommended level (2900 *vs*. 3050 kcal ME/kg of diet).
3. High-lysine: fed the basal diet supplemented with 10% lysine over the recommended level (1.32% *vs*. 1.45%).
4. Vitamin C: fed the basal diet supplemented with 0.10% of vitamin C (20%). The experimental period lasted for six weeks.

3. Measurements:

Growth performance parameters:

The live body weights (LBW) of all hatched chickswere measured to the nearest gram on day 7 of each week. Body weight gain (BWG) was then calculated by subtracting the average LBW of chicks in a previous perio from the given

Table 1 Composition and calculated analysis of the experimental diets during the growing period

Ingredients (%)	Control	High energy	High lysine	Vitamin C
Yellow corn	54.65	58.30	54.70	54.70
Soybean meal 48%	32.00	31.30	31.70	32.20
Wheat bran	5.10	-	5.20	4.80
Corn gluten meal 62%	4.50	6.00	4.46	4.45
DL-methionine 99%	0.11	0.06	0.11	0.11
L-lysine HCl	0.20	0.15	0.37	0.19
Vegetable oil	0.50	1.20	0.50	0.50
Mono-Ca phosphate	0.83	0.84	0.83	0.82
Premix*	0.30	0.30	0.30	0.30
Limestone	1.49	1.52	1.50	1.50
Salt	0.33	0.34	0.33	0.33
Vitamin C (20%)	-	-	-	0.10
Calculated analysis:				
CP (%)	24	24	24	24
ME (kcal/kg)	2900	3051	2901	2901
Calcium (%)	0.81	0.81	0.81	0.81
Av.Phosphorus (%)	0.32	0.32	0.32	0.32
Methionine (%)	0.50	0.49	0.50	0.50
Methionine + Cystine (%)	0.89	0.89	0.89	0.89
Lysine (%)	1.32	1.32	1.45	1.32
EE (%)	3.16	3.85	3.16	3.15
CF (%)	3.07	2.62	3.07	3.05

Each 3 kg contains: vitamin A: 12000000 IU; vitamin D_3: 2500000 IU; vitamin E: 10 g; vitamin K_3: 2 g; vitamin B_1: 1 g; vitamin B_2: 5 g, vitamin B_6: 1.5 g; vitamin B_{12}: 0.01 g; Niacin (B_3): 30 g; Folic: 1 g; Biotin (B_1): 0.05 g; Pantothenic acid (B_5) 10 g; Copper: 10 g; Iodine: 1 g; Selenium: 0.1 g; Iron: 30 g; Manganese: 60 g; Zinc: 50 g and Cobalt: 0.1 g.
CP: crude protein; ME: metabolizable energy; EE: ether extract and CF: crude fibre.

period being recorded. Feed consumption was recorded weekly for each group and the feed conversion ratio was then calculated as the feed consumed (g) per gram of body weight gain.

Physiological and biochemical parameters:
Respiration rate (RR) was measured weekly by counting the chest movement for one minute with the aid of a stop-watch. Rectal temperature (˚C) was also measured by using a thermocouple thermometer inserted 1-1.5 cm into the Cloaca until a fixed reading was obtained. Blood samples were taken at the end of the 6 wk test period just prior to slaughter. Eight samples per treatment group were collected in heparinized tubes. They were then centrifuged (4000 rpm) for 10 minutes and the plasma was then decanted into Eppendorf tubes and stored at -20 ˚C until the biochemical analyses could be performed. A few drops of fresh blood was also sampled in order to determine blood haemoglobin (Hb) and the packed cell volume (hematocrit, Ht %). Plasma total lipids, cholesterol, ALT and AST levels were spectrophotometerically determined by using available commercial kits as described by the manufacturer companies (Spectrum, Diagnostics, Egypt. Co. for Biotechnology, S.A.E). Plasma thyroxine (T_4) and triiodothyronine (T_3) were determined by the RIA technique using Gamma-Coat ^{125}I RIA Kits, Clinical Assay, Cambridge, Medical Diagnostics, Boston, MA, as reported by Akiba *et al.* (1982).

4. Statistical analysis:
All data were subjected to the analysis of variance by using the General Linear Models Procedure (GLM) of the Statistical Analysis System (SAS, 1994). Differences among treatment means were detected by using Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

1. Productive performance of quail:
Results in which periodic heat exposure of eggs was applied during days 3, 7 and 13 of embryogenesis are listed in Table 2.

Live body weight (LBW) and weight gain:
Final live body weight (LBW) of the Japanese quail chicks in this study was significantly changed with both heat exposure of eggs and dietary treatments. The body weights for the quail from the control (221.65) and lysine (229.59) groups of the non heat treatment supplemented diets and the high energy treatment (228.55) in heat exposed experiment had the lowest LBW. Regardless of heat exposure of eggs, the overall mean LBW of quails fed on vitamin C (vit. C) and high energy diets were significantly (P<0.01) higher than the control quails which have the lowest LBW (226.81) followed by the lysine supplemented ones (231.07).

Table 2 Effect of different treatments on productive performance of quail (6 weeks of age)

Treatment \ Trait	Body weight (g) Initial	Body weight (g) Final	Body weight gain (g)	Feed intake (g)	Feed conversion ratio
Feeding treatment					
A-Non heat treatment					
Control	22.76	221.65^{c}	197.50^{b}	797.00	4.04
Energy	22.83	240.42^{a}	222.00^{a}	847.00	3.82
Lysine	22.92	229.59^{bc}	214.50^{a}	792.50	3.70
Vitamin C	22.91	236.91^{ab}	217.50^{a}	849.50	3.91
B-Heat treatment					
Control	22.92	232.80^{ab}	213.00^{a}	796.50	3.74
Energy	23.02	228.55^{bc}	208.00^{ab}	801.00	3.85
Lysine	22.86	232.65^{ab}	213.00^{a}	816.50	3.83
Vitamin C	22.74	237.12^{ab}	216.50^{a}	841.50	3.89
Feeding treatment overall					
Control	22.84	226.81^{b}	205.25	796.75	3.89
Energy	22.92	234.66^{a}	215.00	824.00	3.83
Lysine	22.89	231.07^{ab}	213.75	804.50	3.76
Vitamin C	22.82	237.02^{a}	217.00	845.50	3.90
Heat treatment overall					
Non heat	22.86	232.03	212.88	821.50	3.87
Heat	22.88	232.83	212.63	813.88	3.83
SEM	0.23	45.74	31.00	75.91	0.03
Source of variation					
Feed	-	0.01	NS	NS	NS
Heat	-	NS	NS	NS	NS
Feed* Heat	-	0.003	0.04	NS	NS

The means within the same columns with at least one common letter, do not have significant difference (P>0.05).
NS: non significant and SEM: standard error of means.

In this concern, it appears that pre-hatching heat exposure of eggs has insignificant effects on post-hatching LBW of quails at 6 weeks of age.Statistical analysis of data reflect the highly significant (P<0.01) effects of dietary treatments and their interaction with heat exposure (P<0.003) on LBW of quails.

Pre-hatch temperature treatments had no effect on the post-hatching body weights of quail during the growing period (6 weeks). Similar results were found by Abd El-Azim (1991) who reported that changes in body weight of broiler chicks as a result of pre- hatch temperature disappeared with age.

The results of this study also show that the body weight gain of quail, when fed the high energy and vitamin C supplemented diets, was higher than that of the other treatments (control and lysine). This trend may be due to the enhancement of feed intake (Table 2) as a result of better palatability and nutrient utilization.

As one would expect, higher feed consumption resulted in higher body weight gains in this study. Growth stimulation in the vitamin C supplemented quail probably reflects the beneficial effect of this vitamin on growth performance. Supplemental vitamin C may improve heat tolerance and thereby reduce body weight reductions that are normally associated with stress conditions.

It is worth noting that the experimental quail were housed at 32 ˚C, which may induce corticosterone synthesis. This hormone has been reported by many investigators to reduce the growth rate of chickens (Attia *et al*. 2003; Sahin *et al*. 2003a; Sahin *et al*. 2003b; Sahin *et al*. 2003c; Hahn *et al*. 2005).

Feed intake and Feed conversion ratio:

As shown in Table 2, the average feed intake of growing quail was not significantly changed with either pre- hatch temperature or feeding treatments. However, even though the differences were not in the highest feed intake were observed in the quail that were fed the high energy and vitamin C supplemented diets.

These increases were what do you mean with the highest values of LBW and weight gain for those groups. A similar trend was also observed for the values of feed conversion ratio which showed non significant differences between all treatment groups. It appears that the post-hatching temperatures that were used in the present experiment (32 ˚C) was insufficient to induce a hyperthermic condition for the quail. This may support that the pre-hatching exposure of eggs could help reduce the deleterious effects of high temperature on feed intake. The present results failed to support previous findings that heat stress reduced feed intake and feed to gain ratio (Deeb and Cahaner, 2002), however, those investigators used different avian species and temperatures.

2. Body temperature and respiration rate (RR):

The pre-hatch temperature treatment did not result in a significant difference in body temperature of quail during the post-hatching period (Table 3).

Table 3 Effect of different treatments on body temperature (˚C) and respiration rate (frequency/min.) of quail at different ages

Treatment	Body temperature		Respiration rate	
	Age (weeks)		Age (weeks)	
	2	4	2	4
Feeding treatment				
A-Non heat treatment				
Control	40.92	41.80	66.67	75.00
Energy	40.67	41.72	79.67	58.33
Lysine	41.58	41.93	68.67	79.00
Vitamin C	40.83	41.65	71.00	71.00
B-Heat treatment				
Control	40.75	41.96	54.67	75.67
Energy	40.83	41.88	79.33	84.67
Lysine	41.08	41.87	77.00	96.67
Vitamin C	40.83	41.87	74.00	70.00
Overall of feeding				
Control	40.84	41.89	60.67[b]	75.34
Energy	40.75	41.80	79.50[a]	71.50
Lysine	41.33	41.90	72.84[a]	87.84
Vitamin C	40.83	41.76	72.50[a]	70.50
Overall of heat				
Non heat	41.00	41.78	71.50	70.83
Heat	40.94	41.90	71.25	81.75
SEM	0.57	0.19	8.29	6.48
Source of variation				
Feed	NS	NS	0.01	NS
Heat	NS	NS	NS	NS
Feed* Heat	NS	NS	NS	NS

The means within the same columns with at least one common letter, do not have significant difference (P>0.05).
NS: non significant and SEM: standard error of means.

The body temperatures measured were somewhat higher at 4 wk than at 2 wk of age. The values obtained were slightly lower than the upper limit of body temperatures (41.5 ˚C) reported by Bobek *et al.* (1980) who concluded that body temperatures of Japanese quail might increase or decrease by 1 or 2 ˚C when birds were exposed to acute cold or warm ambient air temperature above 35 ˚C or below 18 ˚C.

It is worth noting that the quail from the present experiments were exposed to a chronic ambient temperature of 32 ˚C which did not induce an obvious elevation in body temperature. These results are in agreement with those reported by Farrell and Swain (1977) on energy requirements, McNaughton *et al.* (1978) on lysine requirements, and Haazele (1992) concerning the role of vitamin C during heat exposure of chickens.

Results in Table 3 clearly show that respiration rate of Japanese quails during the whole experimental period reflected considerable changes. Statistical analysis of data elucidate that dietary treatments caused highly significant ($P\leq0.01$) effects on RR. This effect was more obvious at 2 week of age, where the value obtained for the control-fed quails was the lowest at 2 weeks (60.67) of age.

Previous research has shown that, in general, the high-energy and vitamin C-supplemented diets cause a pronounced decrease in RR of quails, especially at 4 weeks of age.

Although an increased RR (panting phenomenon) is desirable during higher ambient temperatures to dissipate the excessive heat, via evaporative cooling via the respiratory passages, our results failed to show this phenomena. This may be due to the beneficial effect of vitamin C and (or) the high-energy diets in reducing the metabolic heat production through their effects on thyroidal hormone secretion rates (discussed later) or via the effect of vitamin C on adrenal gland function. It also appears that increasing the dietary energy level could reduce metabolic heat production by decreasing the specific dynamic action (produce more heat) of digestive tract muscles and this effect was greater when the dietary amino acid (lysine) level was increased.

In this respect, Ricklefs (1974) reported that folowing the absorption of dietary energy from the intestine, there is an increase in heat production, which is referred to as the heat increment or the calorigenic effect of the specific dynamic action. This elevation causes an increased respiration or basal metabolic rate by45% for protein, 29% for carbohydrates and 15% for fat molecules, which supports our findings. The present results are in agreement with those of Balnave and Brake (2005) and Gous and Morris (2005) who found beneficial effects of increasing dietary energy to heat- stressed chicks. A similar trend was also reported for vitamin C supplements (Sahin *et al.* 2003a; Sahin *et al.* 2003b; El-Komy, 2004; Seyrek *et al.* 2004).

3. Immune-related organs and some endocrine glands:

The influence of the different treatments on relative weights of the thymus, spleen, bursa of Fabricius, and of some endocrine glands at the end of growing period (6 weeks) are presented in Table 4. Statistical analysis of the data revealed insignificant effects of pre and post-hatching temperature on the relative weights of the studied organs and glands especially thyroid and adrenal gland. However, a slight increase was noticed in the relative weights of thymus, spleen and bursa of quail that were fed the high- energy diets compared by those fed on high- lysine and vit. C-supplemented diets.

Table 4 Relative weights of lymphoid organs and some endocrine glands of growing quail at 6 weeks of age

Treatment \ Trait	LBW (g)	Thymus %	Spleen %	Bursa %	Thyroid%	Adrenal %
Feeding treatment						
A-Non heat treatment						
Control	221.50	0.57	0.04	0.07	0.01	0.02
Energy	247.75	0.68	0.05	0.10	0.01	0.01
Lysine	230.00	0.46	0.03	0.11	0.01	0.01
Vitamin C	226.50	0.55	0.04	0.10	0.01	0.01
B-Heat treatment						
Control	231.25	0.30	0.04	0.08	0.01	0.01
Energy	228.50	0.15	0.05	0.09	0.01	0.01
Lysine	227.25	0.18	0.03	0.06	0.01	0.01
Vitamin C	254.25	0.22	0.04	0.08	0.01	0.01
Overall of feeding						
Control	226.38	0.44	0.04	0.08	0.01	0.01
Energy	238.13	0.42	0.05	0.10	0.01	0.01
Lysine	228.63	0.32	0.03	0.09	0.01	0.01
Vitamin C	240.38	0.39	0.04	0.09	0.01	0.01
Overall of heat						
Non heat	231.44	0.57^{a}	0.04	0.10	0.01	0.01
Heat	235.31	0.21^{b}	0.04	0.08	0.01	0.01
SEM	25.92	0.05	0.003	0.001	0.007	0.005
Source of variation						
Feed	NS	NS	NS	NS	NS	NS
Heat	NS	0.06	NS	NS	NS	NS
Feed* Heat	NS	NS	NS	NS	NS	NS

The means within the same columns with at least one common letter, do not have significant difference (P>0.05).
NS: non significant and SEM: standard error of means.

A similar but negative trend was noticed concerning the main effect of heat exposure which causes an obvious decrease in thymus (%), but no apparent effect was recorded for both spleen and bursa weights. It is likely that under the managerial conditions of our experiment, no significant influences of high ambient temperature on immune responses could be seen which may reflect better immunity, in the experimental flock. As the immunosupression due to heat stress is related to release of corticosterone which cause reductions in the size of the lymphoid organs, since feeding treatments, especially vitamin C may constitute the primary reason that the present treatments can ameliorate the negative effects of stress. The results are in close agreement with (Puvadolpirod and Thaxton, 2000a; Puvadolpirod and Thaxton, 2000b; Puvadolpirod and Thaxton, 2000c) who reported significant decreases in the lymphoid organs (spleen, bursa and thymus) due to heat stress in birds. Also, previous investigations support the present findings about the beneficial effect of lysine (Bhanja *et al.* 2004); high- energy level (Parmentier *et al.* 2002; Rama Rao *et al.* 2004); and vit. C supplementation as reported by Wu *et al.* (2000) and Gheisari *et al.* (2002).

4. Plasma thyroidal hormones:

The influence of pre-hatch temperature and post-hatch dietary treatments on thyroid hormone (T_3 and T_4) concentrations of Japanese quail at 6 weeks of age are presented in Table 5. Short-term acute heat stress (39.5 ˚C for 2 hours) at days 3, 7, and 13 of incubation period resulted in an slight but non-significant increase in T_3 level in both supplemented and heat-stressed groups at 6 weeks of age. It is clear from the present results that, regardless of the dietary treatments, pre-hatching heat exposure did not influence the T_3 concentration of quail at 6 weeks of age. However, plasma T_4 concentrations were significantly decreased in the heat stressed quail at 6 weeks of age (15.46 *vs.* 13.65 ng/mL). Moreover, feeding of a high level of vitamin C resulted in a significant increase in T_4 level at 6 weeks of age. The data also showed a significant increase in the T_3/T_4 ratio in the heat stressed versus the control groups (0.24 *vs.* 0.20) at 6 weeks of age. The dietary treatments had no effect on the T_3/T_4 ratio at 6 weeks of age, where this ratio was significantly higher in vitamin. C treated group in comparison to the other treatments. The lowest ratio was recorded for the control group. From these results it is likely that high pre- hatch temperatures (39.5 ˚C for 2 hours) at days 3, 7 and 13 of incubation) and the high ambient air temperature (32 ˚C) throughout the growing period, resulted in a significant decrease in the T_4 level associated with significantly higher T_3/T_4 ratios at 6 weeks of age. However, the T_3 level in blood was not statistically affected.

Table 5 Plasma triiodothyronine (T_3) and thyroxine (T_4) levels of Japanese quail at 6 wk of age

Trait	Treatment				Overall	Significant		
	1	2	3	4		F	H	F*H
T3 (ng/mL)								
C	2.64±0.17	3.01±0.17	3.20±0.31	3.13±0.26	3.00	NS	NS	NS
HS	3.19±0.13	3.07±0.20	3.14±0.17	3.62±0.22	3.26			
Overall	2.92	3.04	3.17	3.38				
T4 (ng/mL)								
C	14.17±0.88	15.58±1.05	15.21±1.01	16.86±1.03	15.46^a	0.03	0.01	NS
HS	12.94±0.63	12.28±0.56	14.08±0.77	15.29±0.77	13.65^b			
Overall	13.56^b	13.93^b	14.65^{ab}	16.08^a				
T3/ T4								
C	0.19±0.01	0.20±0.00	0.21±0.01	0.19±0.01		NS	0.0001	0.03
HS	0.25±0.01	0.25±0.01	0.22±0.01	0.24±0.01	0.20^b			
Overall	0.22	0.23	0.22	0.22	0.24^a			

The means within the same columns with at least one common letter, do not have significant difference (P>0.05).
NS: non significant
1: control; 2: high energy; 3: high lysine and 4: vitamin C.
C: control eggs; HS: heat exposed eggs; F: feed treatments and H: heat stress.

These results may infer an effect of pre- hatch temperature on thyroid activity of the experimental quail. The data do not support the following sentences, since there was no significant effect of the treatments on T_3 levels. Did you use T_3 when you meant T_4. The reduction in T_3 level (3.00 ng/mL) at 6 weeks of age in the control birds was more pronounced compared by T_4 level (15.46 ng/mL), which is probably due to a lower disappearance rate of T_4 in the blood. Such a decrease in T_4 utilization and (or) conversion to T_3 could explain the higher circulating T_4 level in the control group of quail.

In this respect, May *et al.* (1986) and Iqbal, *et al.* (1990) claimed that heat stress acclimation had no effect on plasma T_3 and T_4 levels. However, studies by Moraes, *et al.* (2003) indicate that incubation temperature did not affect plasma T_4 level but decreased T_3 level. The present results clearly show that plasma T_3 level was lower in the control than dietary- treatment groups with higher values recorded for the vit. C-supplemented group. This may be attributed to more peripheral T_4 to T_3 conversion. Our results are in close agreement with those reported for Japanese quail by Sahin *et al.* (2002) who found that supplemental vitamin C increased both T_3 and T_4 concentration.

5. Some blood parameters:

The influence of early heat treatment of fertile eggs and post-hatching feeding treatments on plasma total lipids, cholesterol, ALT and AST activities, hematocrit values (Ht) and hemoglobin (Hb) for growing quailare shown in Table 6.

5.1. Plasma total lipids and cholesterol:

Plasma total lipids concentration at the end of the growing period was significantly (P≤0.0001) higher in heat-exposed quails than non heat controls.

The overall means were 1497.3 *vs.* 902.9 mg/dL for heat exposed and non heated quails, respectively. Regardless of pre-hatching exposure temperature, plasma total lipids concentration were higher in the high-lysine and high- energy supplied diets compared by the other treatment groups, however, these differences were not significant. The interaction between feeding treatments and heat exposure was, however, significant (P≤0.03) indicating the important role of vitamin C and energy level in reducing the total lipids level of growing quails (Table 6).

In addition, plasma cholesterol level was not significantly affected by either pre-hatch temperature or dietary treatment (Table 6).

In this respect, results show that vitamin C supplemented quails has the highest plasma cholesterol level (373.42 mg/dL) at the end of the growing period, while the lowest level was recorded for the high-lysine supplemented quail (323.26 mg/dL). This reduction in cholesterol level as a result of vitamin C supplementation was also reported in heat-stressed quail (Sahin *et al.* 2002) and broilers (Sujatha *et al.* 2010).

This decrease in the lipid fractions is contradictory to the results of (Puvadolpirod and Thaxton, 2000a; Puvadolpirod and Thaxton, 2000b; Puvadolpirod and Thaxton, 2000c) and Tolba and Hassan (2003) who stated that the responses of broiler chicks to heat stress are associated with higher plasma total lipids, cholesterol and triglycerides. These contradictory results may reflect the differences in bird species and/or the different dietary supplements used.

5.2. Plasma transaminases (ALT, AST) activity:

Concerning plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, analysis of variance of data (Table 6) showed highly significant differences in ALT activity related to feeding and heat treatments

Table 6 Plasma total lipids, cholesterol, ALT, AST, haematocrit (Ht) and haemoglobin (Hb) of growing quail

Treatment \ Trait	Total lipids (mg/dL)	Cholesterol (mg/dL)	ALT (U/L)	AST (U/L)	Ht (%)	Hb mg/100 mL
Feeding treatment						
A-Non heat treatment						
Control	898.7^{bc}	346.12	5.07^{b}	39.03^{c}	58	8.70
Energy	667.3^{c}	329.84	4.99^{b}	62.66^{a}	55	8.25
Lysine	1332.7^{ab}	275.00	4.67^{b}	40.86^{bc}	58	9.65
Vitamin C	714.3^{c}	334.50	3.72^{b}	57.63^{ab}	53	7.65
B-Heat treatment						
Control	1494.2^{a}	359.59	8.49^{a}	35.55^{cd}	53	8.40
Energy	1709.4^{a}	342.73	3.91^{b}	20.00^{d}	53	7.85
Lysine	1354.0^{ab}	371.51	7.90^{a}	57.83^{ab}	48	7.60
Vitamin C	1431.7^{a}	402.62	5.85^{b}	56.89^{ab}	57	8.08
Overall of feeding						
Control	1196.4	353.82	6.78^{a}	37.29^{b}	55	8.55
Energy	1262.8	337.21	4.45^{c}	44.37^{b}	54	8.05
Lysine	1344.9	323.26	6.06^{ab}	49.35^{ab}	53	8.63
Vitamin C	1124.2	373.42	4.78^{bc}	57.31^{a}	54	7.86
Overall of heat						
Non heat	902.9^{b}	317.80	4.61^{b}	50.05	56	8.56
Heat	1497.3^{a}	369.11	6.45^{a}	43.16	52	7.98
SEM	87.21	71.12	0.63	12.24	7.00	2.90
Source of variation						
Feed	NS	NS	0.003	0.01	NS	NS
Heat	0.0001	NS	0.0004	NS	NS	NS
Feed* Heat	0.03	NS	0.007	0.0004	NS	NS

The means within the same columns with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of means.

along with their interaction at the end of the growing period.

The present results show also that AST activity was significantly influenced by dietary treatments during the growing period; however, this response was not significantly changed by the heat exposure treatment. The interaction between both feeding treatments and heat exposure was, however, significant (P≤0.0004). It appears that both ALT and AST enzymes, as indicators of liver function, are changed according to the physiological status of birds and their surrounding environment. Our data revealed that the significant changes which had been observed during the growing period were primarily due to rapid metabolic processes in the hepatocytes, related to growth.

This process may disrupt or result in damage to some hepatocytes, leading to increasing levels of ALT and AST into blood stream. In this regard, Murray *et al.* (2000) reported that both ALT and AST are non- functional plasma enzymes, since their levels are very low in blood plasma compared to that in tissues, which confirm the present findings. In general, the values of ALT and AST in the present study could reflect a protective influence of dietary treatments on liver tissues, which is in close agreement with the results reported by Sahin *et al.* (2002) and Abdel-Fattah (2006).

5.3. Blood haematological parameters:

Results concerning the effect of pre-hatching exposure of eggs to short-term high temperature and post-hatch dietary treatments, on haematocrit values (Ht %) and haemoglobin (Hb) concentration are presented in Table 6.

It is clear from the results that Ht % and Hb level were not significantly changed with all experimental treatments during the growing period. The Ht % values were higher than those reported by many investigators, which may indicate higher blood viscosity associated with a pronounced increase in blood corpuscular volume. This may be due to the increased erythrocytes counts or may reflect chronic macrocytosis related to heat- stress exposure.

These responses are supported by the findings of Iqbal *et al.* (1990), Yahav *et al.* (1997) and Yahav *et al.* (1998) and Zhou *et al.* (1999) who found that blood packed cell volume of heat-stressed birds are significantly affected by environmental temperature, water consumption, dietary contents and the size of red blood cells, which support our present results.

Moreover, the higher Hb content, especially in the high-energy and lysine diets, may reflect an increase in RBC's counts which support the previous findings (Ht %) or may be due to higher oxygen consumption associated with more hemoglobin saturation and dissociation rates.

CONCLUSION

The results of this study suggest that, the exposure of fertile Japanese quail eggs to high incubation temperature leads to increasing thyroid hormone (T3) at 6 wk of age, and this was reflected by the metabolic response of the birds when reared in high ambient temperature. Moreover, the supplementation of 200 ppm of vitamin C or 150 kcal ME/kg of diet more than the recommended levels enhanced the general performance of the quail.

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