World Applied Sciences Journal 29 (12): 1574-1585, 2014
ISSN 1818-4952

DOI: 10.5829/idosi.wasj.2014.29.12.8238

Effect of Different Anti-Stress Feed Additives on Some Blood Metabolites and Lymphoid Organs Histology in Laying Japanese Quail

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Abstract: An experiment was conducted to elucidate the effect of short-term exposure of japanese quail eggs to high incubation temperature on the ability to cope with the heat stress during laying period. A total number of 998 fertile Japanese quail eggs were divided into two groups; the first one was maintained at the recommended incubation temperature (37.5°C), while the second was exposed to 39.5°C for two hours at days 3, 7 and 13 of embryogenesis. At laying period, quails from each incubation temperature were randomly assigned to four dietary treatments, control; high- energy diet (+ 150 Kcal ME/ Kg diet more than the recommended level); high- lysine (10 % more) and vitamin C supplemented diet. Blood metabolites and histological sections for the lymphoid organs and for tibiae of laying quails were examined. Results showed that plasma total protein was insignificantly lower. Also, plasma calcium and phosphorus levels did not significantly influenced by treatments. Histological examination of spleen sections showed considerable variations related to treatments. Both heat exposure and high- lysine diets caused a reduction in lymphocytes number and size. Caecal tonsil sections showed a pronounced effect of early heat exposure on the general developmental patterns of crypts of Lieberkuhn either in their size or number. Harderian gland histology reflected the presence of several lymphocytic cells in the stroma of the gland. Tibia bone sections showed normal appearance of the compact bone layer concomitant by an obvious hypertropic proliferation of bone cells. Based on the present results, it could be concluded that pre- hatching exposure of quail eggs to high temperature and feeding of a high- energy or vitamin C supplemented diets during laying period could be recommended for maintaing the physiological homeostasis of quails during heat stress.

Key words: Incubation · Temperature · Laying quail · Heat stress · Histology · Peripheral lymphoid organs · Energy · Lysine and 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. Commercial quail production has grown steadily in Egypt utilizing strains of Japanese quail selected for rapid growth and higher body weight. The nutrient requirements of these strains and the optimum housing temperature along with their physiological response (s) to acute heat stress environments are still obscure. It is suggested that acclimatization to heat stress may be induced through pre- hatch and (or) post- hatch short- time exposure to high environmental temperature [1, 2]. The rapid heat stress response can be modulated by early- age thermal conditioning [3] which may affect the integration of thermal information in the hypothalamus which in turn reduce heat production by reducing the circulating concentration of thyroid hormones [4].

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It is well accepted that the main consequence of heat stress is the reduction in feed intake as a trial from the bird to reduce metabolic heat production [5]. This will cause immune- suppression and enhanced fat deposition due to hypothyroid activity [6- 8].

To reduce the deleterious effect(s) of heat stress, many practical approaches have been developed to facilitate thermotolerance of birds, leading to minimize the adverse effects on productivity. These approaches include pre and (or) post acclimation of birds [9, 10]; use of some electrolytes and vitamins [11- 13] and dietary energy or lysine levels manipulation [14, 15]. There is, however, a paucity of information on the beneficial effects of such approaches on blood metabolites and the histological features of lymphoid organs.

Therefore, the present study was conducted on Japanese quail to elucidate the possible effect(s) of short-term exposure of quail eggs to acute incubation temperature and subsequent post hatching feeding manipulations (increasing dietary energy, lysine, vit. C) on the physiological and biochemical response of quail to the previous treatments. The histological examination of spleen, cecal tonsils and harderian glands as immune-related organs and tibia bone were also examined.

MATERIALS AND METHODS

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

Experimental Procedures: A total number of 998 fertile Japanese quail (*Coturnix coturnix japonica*) eggs were obtained from a private quail farm near Cairo, Egypt. At first day of incubation, eggs were divided into two groups. The first group was maintained at the recommended incubation temperature (37.5°C), while the second one was exposed to 39.5°C for two hours at days 3^{th}, 7^{th} and 13^{th} of embryogenesis.

Experimental Design: A total of 240 sexually mature Japanese quail birds were obtained from the same two incubation temperature (120 each) used in this experiment. Birds of each group were sub- divided randomly into four dietary experiment groups, 30 birds each (20 females: 10 males). They were fed on a layer diet which was formulated to meet the requirements of Japanese quail laying hens [16].

The first group (control) received the basal laying quail diet, the second group (high- energy) was fed the basal diet supplemented with 150 Kcal ME/ Kg of diet over the recommended level (2900 *vs.* 3050 Kcal ME/ Kg of diet), the third group (high- lysine) was fed the basal diet supplemented with 10% lysine over the recommended level (1.32% *vs.* 1.45%) and the fourth group (vitamin C) was fed the basal diet supplemented with 0.10% of vitamin C (20%).

Feed and water were provided in *ad libitum* basis. All groups received the same hygienic and managerial conditions. Room temperature during the experimental period was maintained at 32 ± 2°C. These treatments were extended till 14 weeks of age).

Measurements:

Physiological and Biochemical Parameters: Blood samples were taken at the end of laying period from birds at slaughtering time. Eight samples per each treatment group (4 per replicate) were collected in heparinized tubes, centrifuged (4000 rpm) for 10 minutes and plasma was then decanted in Eppendorf tubes and stored at -20°C until biochemical analysis. Blood smears were also done, stained with Wright's stain and examined to calculate the number of lymphocytes (L) and heterophils (H) in 100 white blood cells, then the H/ L ratio was calculated.

Plasma total protein and albumine, 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 calcium and phosphorus were determined by commercial kits purchased from Spinreact, S. A. Ctra. Santa Coloma, Spain [17].

Histological Observations: Representative tissue samples from spleen, cecal tonsils, harderian gland and tibia bone were taken during the slaughtering time. Samples were fixed in a 10% Formaline- saline solution before preparing the histological sections by using Paraffin method technique. Bone samples were taken from the center of the shaft, decalcified by Formic acid and then fixed in 70% alcohol solution. All sections were stained with haemotoxylline and eosin (H& E) stains and the thickness of each section was 4- 5 microns. These sections were examined under light microscope and then photographed by using a suitable digital Camera.

Statistical Analysis: Data were subjected to the analysis of variance by using the General Linear Models Procedure (GLM) of the Statistical Analysis System [18]. Differences among treatment means were detected by using Ducan's multiple range test [19].

RESULTS AND DISCUSSION

Some Blood Parameters: Results concerning the effect of pre- hatching exposure of eggs to short- term high temperature and laying dietary treatments, on heterophils to lymphocytes (H/ L) ratio, plasma protein fractions, calcium (Ca) and inorganic phosphorus (P) are presented in Table 1.

Heterophils to Lymphocytes (H/ L) Ratio: It is clear from the results that H/ L ratio were insignificantly changed with all experimental treatments during the laying (14 week of age) period, that both pre-hatching temperature and dietary treatments did not significantly affect the H/ L ratio may reflect a good response of quails related to their early exposure to short- term high temperature, leading to acclimatization, or may be a result of dietary treatments which alleviate the deleterious effects of high temperature during the whole experiment.

It is worse to note that this temperature was 32°± 2°C. In agreement with the present results are the findings of many authers who reported beneficial effects of dietary energy and lysine levels and vit. C in alleviating the negative responses to heat stress condition [20- 24].

Plasma Total Protein, A lbumin (A), Globulin (G) and A/ G Ratio: Plasma total protein (TP) was insignificantly lower in the control and high- lysine supplemented quails than those fed on high- energy and vit. C supplied diets. A similar trend was also observed for the difference between heat and non heat exposed groups of quails. However, plasma albumin concentration showed considerable variation related to feeding treatments but not affected by heat exposure. It appears from the results that both the high- energy level and vit. C supplementation could increase plasma albumin. Statistical analysis of the data revealed significant (P=0.03) effects of feeding treatments on plasma albumin

Table 1: Heterophils to lymphocytes ratio,plasma total protein, albumin, globulin, A/ G ratio,calcium and phosphorus of laying quails.

	Trait						
Treatment	H/ L ratio	Total protein (g/ dl)	Albumin (g/ dl)	Globulin (g/ dl)	A/G ratio	Calcium (mg/ dl)	Phosphorus (mg/ dl)
Feeding treatment							
A-Non heat treatment							
Control	0.79	4.63	1.86	2.77ab	0.70b	19.32	7.41
Energy	0.76	6.57	1.97	4.60a	0.49b	17.16	5.73
Lysine	0.87	3.98	2.21	1.77b	1.08a	19.4	5.85
Vitamin C	0.63	4.85	1.69	3.17ab	0.57b	17.57	5.29
B-Heat treatment							
Control	0.68	4	1.61	2.39ab	0.74ab	17.39	5.31
Energy	0.53	5.51	1.81	3.71ab	0.52b	17.97	7.46
Lysine	0.8	5.35	1.58	3.77ab	0.47b	17.53	4.87
Vitamin C	1.04	4.23	1.59	2.65ab	0.46b	18.52	5.44
Overall of feeding							
Control	0.75	4.32	1.74	2.58	0.72	18.22	6.21
Energy	0.64	6.04	1.89	4.15	0.51	17.56	6.72
Lysine	0.83	4.66	1.89	2.77	0.73	18.47	5.36
Vitamin C	0.8	4.54	1.64	2.91	0.52	18.04	5.36
Overall of heat							
Non heat	0.75	5.01	1.93	3.08	0.69	18.3	6
Heat	0.77	4.77	1.65	3.13	0.55	17.85	5.77
SEM	0.16	0.83	0.36	0.52	0.06	2.05	1.06
Source of variation							
Feed	NS	NS	NS	NS	NS	NS	NS
Heat	NS	NS	NS	NS	NS	NS	NS
Feed* Heat	NS	NS	NS	NS	0.05	NS	NS

level, concomitant with pronounced and significantly (P=0.004) effect of feed by heat interaction. Plasma globulin level was also higher in the same mannar like albumin which may indicate better immune response of quails. Moreover, it is clear from results that A/ G ratio was lower in heat exposed birds indicating possible role of heat stress in disease resistance and viability of quails.It is postulated that plasma protein profile of a given bird is a reflection of the metabolic activities related to protein synthesis and (or) degradation. Since, it is well known that stress conditions could stimulate the adrenal gland cortex for corticosterone secretion, which caused a considerable increase in protein catabolism due to its gluconeogenic activity. This was not the case in the present study, which support the previous findings that feeding treatments applied herein could enhance protein profile of laying quail, which in close agreement with the studies of Gursu *et al.* [25] and Seyrek *et al*. [26].

The effect of feeding treatments by heat interaction on decreasing A/ G ratio was significant (P=0.05) indicating low levels of plasma albumin and higher globulin concentrations. This could be explained by the fact that egg albumen formation during the laying cycle, needs higher rates of protein synthesis and turn- over from blood to egg. Numerous workers [27-29] have reported that high- energy level (i. e. supplementation of oils to diets) and vit. C addition increases blood albumin and total protein by decreasing corticosterone secretion which could limit protein catabolism. The present study recorded higher plasma total protein levels during the laying period. This confirm the previous reports and support the recent f indings of Seyrek *et al*. [26], Sahin *et al*. [30] and Sahin *et al*. [31]on heat- stressed laying quails.

Plasma Calcium (Ca) and Inorganic Phosphorus (P): Regarding the concentration of plasma Ca and P, data showed that the effect of both heat exposure and feeding treatments was not significant. However, some numerically decreases in plasma Ca was recorded in the high- energy fed quails and in heat exposed ones compared with other treatments. This trend was also observed for plasma P levels. It is generally accepted that vit. C stimulates 1.25- dihydroxy- cholecalciferol synthesis in birds which indirectly increases Ca mobilization from bones and hence, P concentration would also be changed. A possible explanation that might be proposed is the role of estrogen in Ca and P metabolism during the laying period, which support the previous studies concerning with the system (via enhancing Ca and P absorption or excretion); vitamin D_3 which stimulates parathyroid glands to secrete PTH and finally the regulatory role of steroids in this process. In agreement with the present results are the findings of Abd El- Azim [10], Seyrek *et al*. [26], Sahin *et al*. [30] and Sahin *et al*. [31] who found the same results.

Histological Observations: The histological structure of the peripheral lymphoid organs of laying quails as influenced by pre- hatching exposure to high incubation temperature (39.5° *Vs*. 37.5°C) for two hours at days 3, 7 and 13 of embryogenesis are illustrated and discussed in the light of the post- hatching dietary treatments.

Spleen Histology: The histological structure of the spleen from the pre- hatching high temperature (HS) group and the control non- heat exposed (NHS) one are illustrated in Figures 1 to 8.

It is generally accepted that the spleen is similar to the lymph nodes, except that blood instead of lymph, flows through the spleenic tissues [32].

It appears from the present sections that the blood capillaries (BC) penetrate into the white pulp (WP) and terminates in a very small dark- stained cords that drains into vinous sinuses (S) in the red pulp (RP) area. Fig. (1) demonstrate that the dominant cell type of the spleen in the HS- control quail sections are the small lymphocytes (LS), which predominantly located in the white pulp (WP) area around numerous blood capillaries (BC), while large lymphocytes (LL) number was very small and tended to be medium sized cells located in the red pulp (RP) area. A similar structure was f ound in the group fed on high- energy diet (HED), however a considerable increase in the size of lymphocytes (LL), especially in the RP area (Fig. 2) was observed. Moreover, it seems that both HS and high- lysine diet (HLD) causes a reduction in lymphocytes number and size (Fig. 3) accompaned by wide area of WP intermingling with the RP area with a large blood sinusoid (S) filled with numerous lymphocyte-like cells. A similar, but with low incidence. The same trend was noticed in spleen section of vit. C supplemented (VCD) diet (Fig. 4) which showed an increase in the WP area with numerous reticular cells and reticular fibers as the main paranchymatic contents of the spleen.

Results showed also spleen histology in non- heat stressed groups (NHS) to be dramatically changed with different treatments.

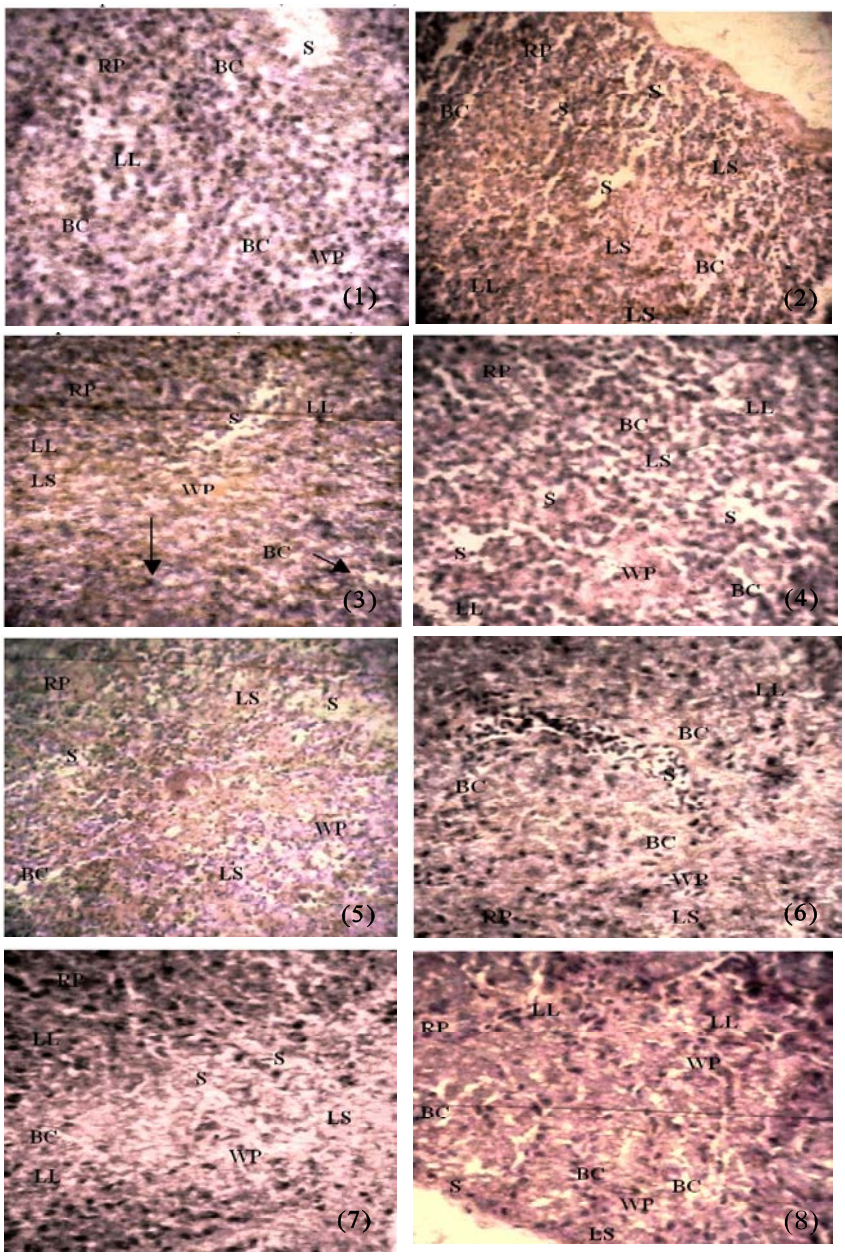

Fig. 1: T. S. of the spleen from HS- control quails (H&E x 200)
Fig. 2: T.S. of the spleen from HS quails fed on HED (H&E x 200).
Fig. 3: T. S. of the spleen from HS quails fed on HLD (H&E x 200).
Fig. 4: T. S. of the spleen from HS quails fed on VCD (H&E x 200).
Fig. 5: T.S. of the spleen from NHS- control quails (H&E x 200).
Fig. 6: T. S. of the spleen from NHS quails fed on HED (H&E x 200).
Fig. 7: T. S. of the spleen from NHS quails fed on HLD (H&E x 200).
Fig. 8: T. S. of the spleen from NHS quails fed on VCD (H&E x 200).

Abbreviation Key for Spleen Sections: BC= blood capillaries, LL= large lymphocytes, LS= small lymphocytes, RP= red pulp, S= sinusoids, WP= white pulp. HS= heat- stressed groups, NHS= non heat- stressed groups, HED= high- energy diet, HLD= high- lysine diet, VCD= vitamin C- supplied diet.

Fig. (5) depicting the spleen section from the control quails with more LL and large WP area, which may reflect the effect of early HS on spleen development. Also, energy level (Fig. 6) causes an increased WP area and reduced lymphocytes sizes but with some dark- stained areas and little sinusoids. This effect was more obvious in HLD quails (Fig. 7) which showed a large WP area being separated from the RP area, both containing numerous small lymphocytes (LS) and few large (LL) cells. However, section from the VCD quails showed a dense stained RP area with a small WP area, both containing numerous large lymphocytes.

These changes may be due to the effect of pre- hatching temperature on spleen growth during the embryonic period, which may stimulate the production of lymphocytes instead of other cell types. In this respect, [33, 34] reported that in the embryonic spleen granulocytes are formed in large numbers in the red pulp, but after hatching (48 h) the spleen becomes predominantly a lymphocyte- producing organ. A similar finding was also reported by [35 and 36] which support our results about the presence of a large number of lymphocytes in the spleen sections.

Caecal Tonsils Histology: The histological structure of the caecal wall is, in general, comparable to that of the intestinal wall with slight variation. Figures 9- 16 presents the main mucosal layer of the proximal part of the caecum where the caecal tonsils are located. The villi (V) being well developed with many crypts (Cr) of variable sizes. These small crypts in the control- HS section (Fig. 9) became larger in the other sections especially those in the HLD and VCD groups (Fig. 11, 12). Many lymphoid cells (dl) diffused in the tunica propria along with some lymphoid nodules (LN) and germinal centers (G) are found, both at the base of and within the villi. Moreover, the epithelial layer (E) is well organized and invaded with different goblet and lymphocytic cells.

The goblet cells are known to secrete mucous substances in the lumen (Lu) of the caecum while the lymphocytic cells being attached with the invaded bacteria [37]. In the non- heat stressed (NHS) sections the caecal tonsil villi was longer with many dilated crypts (Cr) especially in the control (Fig. 13) and HED sections (Fig. 14). This variation in the size of the crypts of lieberkuhn may be related to the nature of feed supplements, also reveal a permenant exposure of the tonsils villi to fecal contents containing vit. C, lysine and (or) high- energy remanents which change the pH of the fluids. The crypts secretions have neutral pH (6.5- 7.5) and their fluids are rapidly absorbed from the lumenal villi making a circulation of fluids from crypts to the villi and this supplies a watery vehicle for the absorption, elaboration and production of lymphocytes and antibodies against soluble antigens. These results support the previous proposed role of caecal tonsils as a peripheral lymphoid tissue that reported by Glick [37], Befus [38], Glick [39] and Glick and I. Olah[40]

Harderian Gland Histology: The Harderian gland (Hg) is a large orbital gland that lubricates the surface of the eyeball [41]. The histological structure of the gland has been well known in the domestic fowl by Bang and Bang [42]. Fig. 17- 24 showed an irregular patterns in the structure of the gland which may be due, in part, to heat exposure of eggs during the embryonic development. In this respect [43] reported histological changes in the Hg of Guinea fowl during days 18, 19 and 21 of incubation period. Many lymphocytes (L) within the stroma of the gland are also observed. At the same time, large contorted lumena (Lu) are present surrounded by lymphocytic cells and a low number of plasma cells, which were, in fact, more pronounced in Hg of HLD and VCD sections either in HS or NHS groups (Fig. 19, 20, 23, 24). There were several compound tubuloacinar glands (AC) with varying structures related to their secretory activity. These acini were well developed and being more active in the Hg sections of HLD (Fig. 19 and 23); HED (Fig. 22) and in VCD (Fig. 20 and 24) irrespective of heat- exposure treatment. Moreover, many secretory vesicles (VC) with different sizes are present in all Hg sections. Also, nerve bundles (NB) are also present especially in HLD and VCD sections. These observations may explain the improved immunoresponse of quails in the present experiment, especially those fed HLD and VCD either in HS or NHS groups. Previous results support our findings where [44] mentioned that the presence of T and B lymphocytes in the Harderian gland of birds could explain the ability of the gland to produce antibodies for improving immunity. A similar explanation was also reported by Onyeanusi *et al.* [43] and Wight *et al*. [45].

Tibia Bone Histology: The histological structure of the tibia from laying quails fed on different dietary treatments after a periodic pre- hatching temperature exposure, are shown in Figures 25- 32.

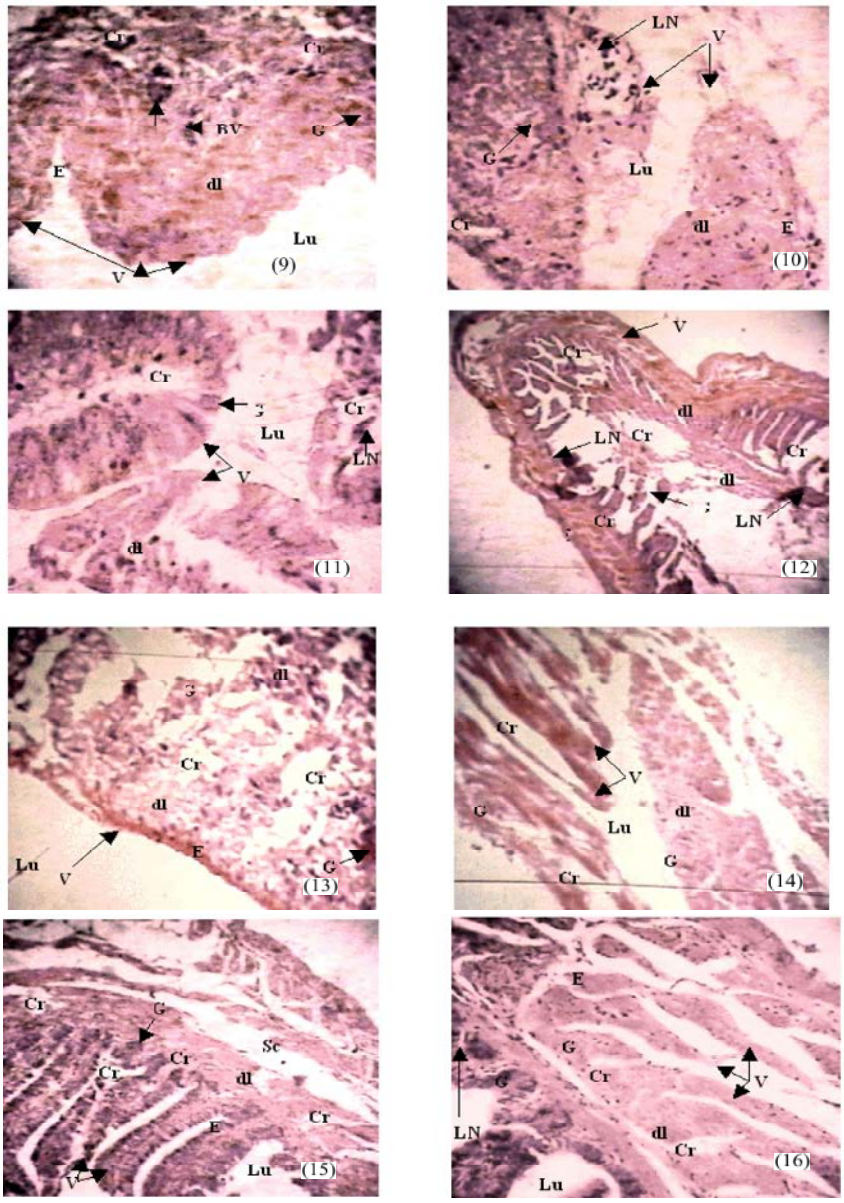

Fig. 9: T. S. of the caecal tonsil from HS- control quails (H&E x 200).
Fig. 10: T. S. of the caecal tonsil from HS quails fed on HED (H&E x 200).
Fig. 11: T. S. of the caecal tonsil from HS quails fed on HLD (H&E x 200).
Fig. 12: T. S. of the caecal tonsil from HS quails fed on VCD (H&E x 200).
Fig. 13: T. S. of the caecal tonsil from NHS- control quails (H&E x 200).
Fig. 14: T. S. of the caecal tonsil from NHS quails fed on HED (H&E x 200).
Fig. 15: T. S. of the caecal tonsil from NHS quails fed on HLD (H&E x 200).
Fig. 16: T. S. of the caecal tonsil from NHS quails fed on VCD (H&E x 200).

Abbreviation Key for Caecal Tonsil Sections: Cr= crypts of lieberkuhn, E= epithi. Invaded lymphocytic cell, Lu=lumen, BV= blood vessels, LN= lymph nodule, G= germinal center, dl= diffuse lymphoid tissue, Sc= submucosa layer, V= villi. HS= heat- stressed groups, NHS= non heat- stressed groups, HED= high- energy diet, HLD= high- lysine diet, VCD= vitamin C- supplied diet.

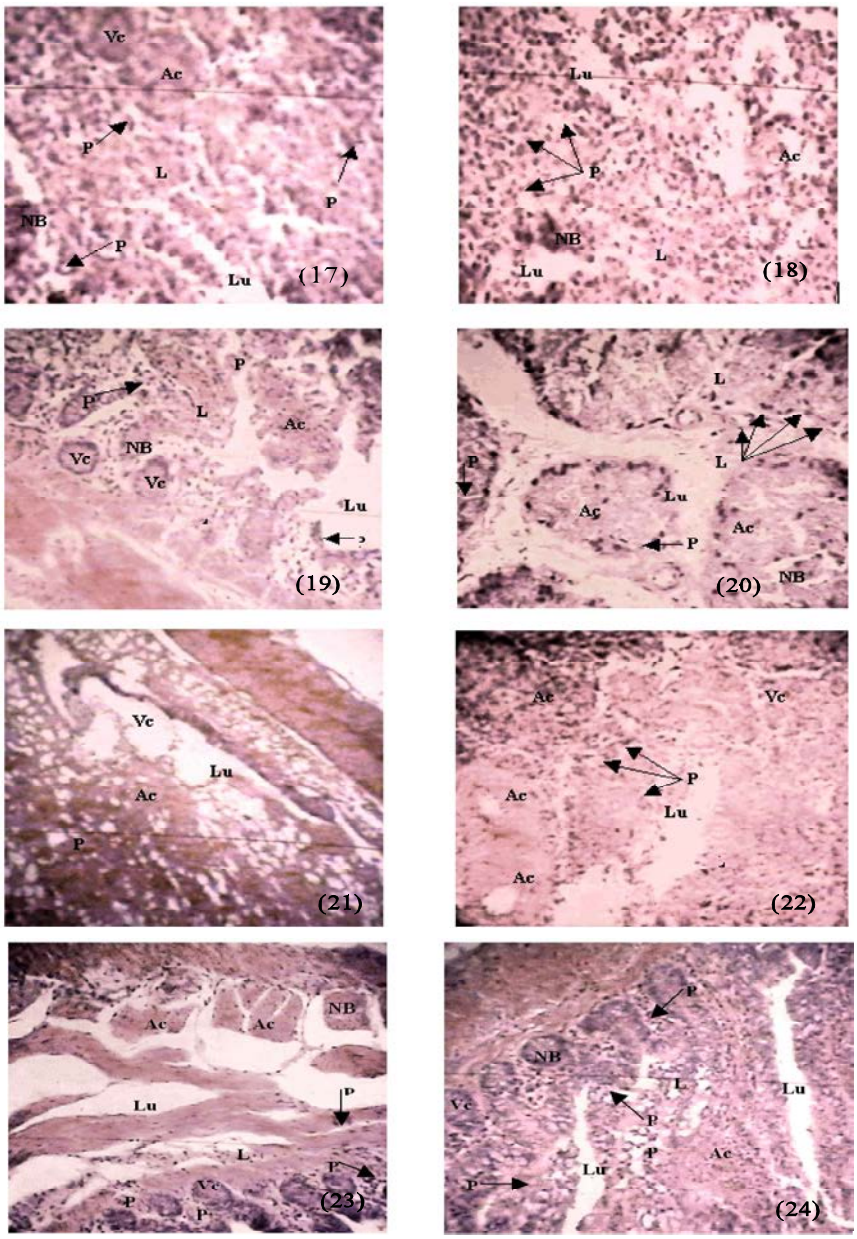

Fig. 17: T. S. of the Harderian glandfrom HS- control quails (H&E x 200).
Fig. 18: T. S. of the Harderian gland from HS quails fed on HED (H&E x 200).
Fig. 19: T. S. of the Harderian gland from HS quails fed on HLD (H&E x 200).
Fig. 20: T. S. of the Harderian gland from HS quails fed on VCD (H&E x 200).
Fig. 21: T. S. of the Harderian gland from NHS- control quails (H&E x 200).
Fig. 22: T. S. of the Harderian gland from NHS quails fed on HED (H&E x 200).
Fig. 23: T. S. of the Harderian gland from NHS quails fed on HLD (H&E x 200).
Fig. 24 T. S. of the Harderian gland from NHS quails fed on VCD (H&E x 200).

Abbreviation Key for Harderian Gland Sections: NB= nerve bundle, L= lymphocytes, Lu= lumen, Vc= secretory vesicles, Ac= acini, P= plasma cell. HS= heat- stressed groups, NHS= non heat- stressed groups, HED= high- energy diet, HLD= high- lysine diet, VCD= vitamin C- supplied diet.

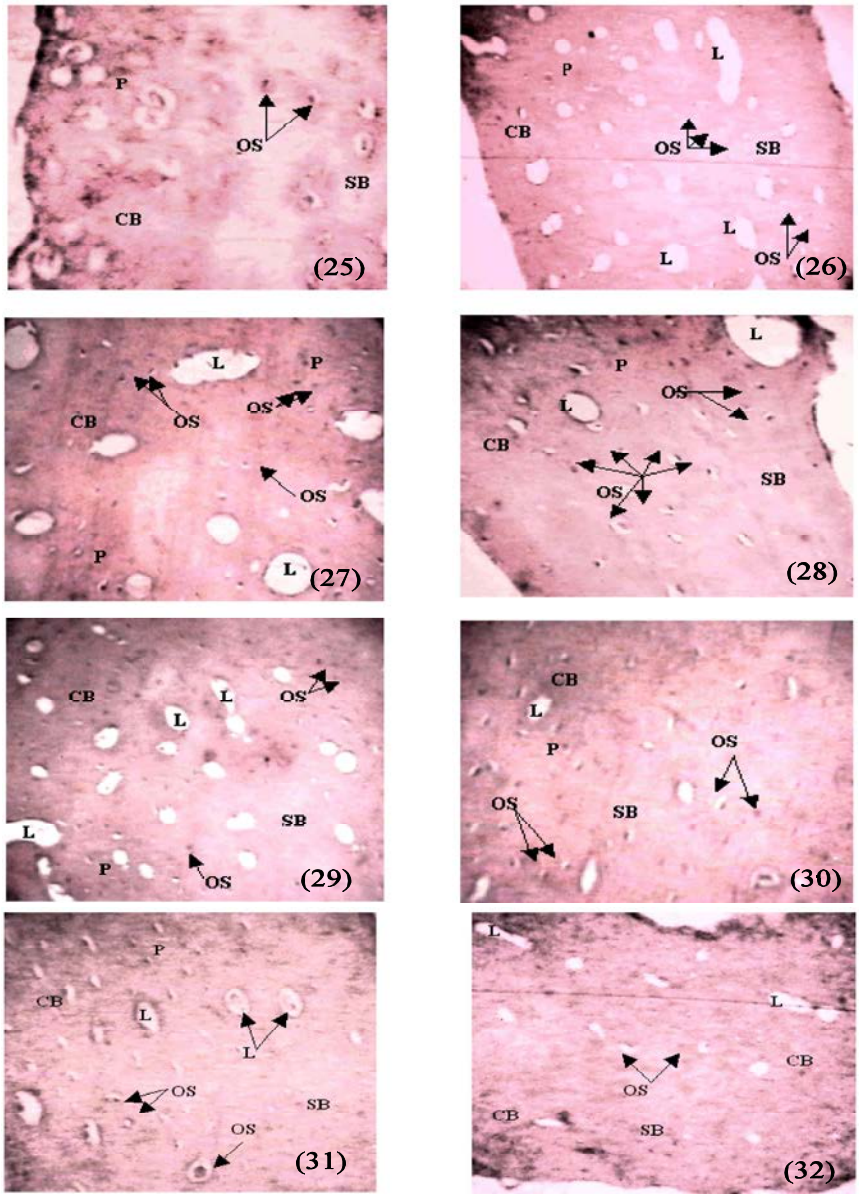

Fig. 25: T. S. of the tibia bone from HS- control quails (H&E x 200).
Fig. 26: T. S. of the tibia bone from HS quails fed on HED (H&E x 200).
Fig. 27: T. S. of the tibia bone from HS quails fed on HLD (H&E x 200).
Fig. 28: T. S. of the tibia bone from HS quails fed on VCD (H&E x 200).
Fig. 29: T. S. of the tibia bone from NHS- control quails (H&E x 200).
Fig. 30: T. S. of the tibia bone from NHS quails fed on HED (H&E x 200).
Fig. 31: T. S. of the tibia bone from NHS quails fed on HLD (H&E x 200).
Fig. 32: T. S. of the tibia bone from NHS quails fed on VCD (H&E x 200).

Abbreviation key for tibia bone sections:
CB= compact bone, SB= cancellous bone, L= Lacunae, OS= osteocyte, P= proliferative zone.
HS= heat- stressed groups, NHS= non heat- stressed groups, HED= high- energy diet, HLD= high- lysine diet, VCD= vitamin C- supplied diet.

It is clear from the tibia bone sections that the general structure of tibia bone was similar, in which the compact (CB) and the spongy (SB) bones tended to be well developed. It appears also that the CB layer of the control- HS quails (Fig. 25) is relatively thicker with well organized proliferative zone (P). This was aslo true in tibia sections of non- heat HLD group (Fig. 31) and in both HED (Fig. 26) and VCD (Fig. 28) sections. In sections of NHS quails, the proliferative zone showed an irregular hypertrophic pattern especially in the HLD (Fig. 27) and HED (Fig. 30) sections. Several osteocytes (OS) were also observed to be fully enclosed within a lacunae of different size. In some sections, there is an obvious vacuoles of resorped Osteoids and some lacunae appear elongated in shape. This appearance, indicative of bone resorption due to egg shell formation, was more obvious in HED (Fig. 26), control (Fig. 29); HLD (Fig. 27 and 31), but it is less observed in the VCD sections.

These histological observations, in general, may suggest the beneficial effect of vit. C and high- lysine supplements in improving bone quality, however, it is likely that bone formation, resorption and remodelling of laying quails depends on the physiological status, nutrition and age of birds. This agreed with the findings of Ali [46] and Bishop *et al.* [47].

CONCLUSION

From the present study, it could be concluded that pre- hatching exposure of quail eggs to high temperature and feeding of a high- energy or vitamin C supplemented diets during laying period could be recommended for maintaing the physiological homeostasis of quails during heat stress.

REFERENCES

1. Moraes, V.M.B., R.D. Malheiros, V. Bruggeman, A. Collin, K. Tona, P. As, O.M. Onagbesan, J. Buyse, E. Decuypere and M. Macari, 2003. Effect of thermal conditioning during embryonic development on aspects of physiological responses of broilers to heat stress. J. Therm. Biol., 28(2): 133- 140.
2. Leandro, N.S.M., E. Gonzales, A. Ferro, M.I.T. Ferro, P.E.N. Givisiez and M. Macari, 2004. Expression of heat shock protein in broiler embryo tissues after acute cold or heat stress. Molecular Reprod. and Develop., 67(2): 172- 177.
3. Yahav, S., A. Shamay, G. Horev, D. Bar Ilan, O. Genina and M. Friedman Einat, 1997. Effect of acquisition of improved thermo tolerance on the induction of heat shock proteins in broiler chickens. Poultry Sci., 76: 1428- 1434.
4. Zulkifli, I., R.T. Doss and M.T. Che Norma, 1999. Acute heat stress effects on physiology and fear related behavior in red jungle fowl and domestic fowl. Canadian. J. Anim. Sci., 79: 165- 170.
5. May, J.D. and B.D. Lott, 1992. Feed and water consumption patterns of broilers at high environmental temperature. Poultry Sci., 76: 627- 633.
6. Geraert, P.A., J.C.F. Padilha and S. Guillaumin, 1996. Metabolic and endocrine changes induced by chronic heat exposure in broiler chickens: growth performance, body composition and energy retention. Brit. Nutr., 75: 195- 204.
7. Puvadolpirod, S. and J.P. Thaxton 2000a. Model of physiological stress in chickens: 1. Response parameters. Poultry Sci., 79: 363- 369.
8. Mashaly, M.M., G.L. Hendricks, M.A. Kalama, A.E. Gehad, A.O. Abbas and P.H. Pattersont, 2004. Effect of heat stress on production parameters and immune responses of commercial laying hens. Poultry Sci., 83: 889- 894.
9. Arjona, A., D.M. Denbow and W.D. Weaver,1990. Neonatally induced thermo tolerance: Physiological responses. Comp. Biochem. Physiol., 95: 393- 399.
10. Abd El-Azim, A., 1991. Changes in some physiological parameters due to different pre and post hatch temperatures in poultry. Ph. D.Thesis, Fac. of Agric. Cairo Univ.
11. Cftc, M., O.N. Ertas and T. Guler, 2005. Effects of vitamin E and vitamin C dietary supplementation on egg production and egg quality of laying hens exposed to a chronic heat stress. Revue de Medecine Veterinaire, 156(2): 107- 111.
12. Holik, V., 2009. Management of laying hens to minimize heat stress. Lohmann Information, 44: 16- 29.
13. Joachim, J.A., O.A. Joseph and A.O. Sunday, 2010. Effects of heat stress on some blood parameters and egg production of Shika Brown layer chickens transported by road. Biol. Res., 43: 183-189.
14. Balnave, D. and J. Brake 2005. Nutrition and management of heat-stressed pullets and laying hens. World's Poult. Sci. J., 61: 399- 406.

15. Gous, R.M. and T.R. Morris, 2005. Nutritional interventions in alleviating the effects of high temperatures in broiler production. World's Poult. Sci., 61: 463-475.
16. NRC "National Research Council", 1994. Nutrient requirement of poultry. National Academy Press, Washington, D. C. USA.
17. Akiba, Y., L.S. Jensen, C.R. Bart and R.R. Kraeling, 1982. Plasma estradiol, thyroid hormones and liver lipids determination in birds. J. Nutr., 112: 299- 308.
18. SAS, 1994. SAS/STAT®User's Guide: Statistics Ver. 6.04, 4th ed. SAS Institute Inc. Cary, NC.
19. Duncan, D.B., 1955. Multiple range and multiple F tests. Biometrics. 11: 1- 42.
20. Arjona, A., D.M. Denbow and W.D. Weaver, 1988. Effect of heat stress early in life on mortality of broiler to high environmental temperature just prior to marketing. Poultry Sci., 67: 226- 231.
21. McKee, J.S. and P.C. Harrison, 1995. Effect of supplemental ascorbic acid on the performance of broiler chickens exposed to multiple concurrent stressors. Poultry Sci., 74: 1772-1785.
22. Puvadolpirod, S. and J.P. Thaxton,2000b. Model of physiological stress in chickens: 2. Dosimetry of adrenocorticotropin. Poultry Sci., 79: 370- 376.
23. Puvadolpirod, S. and J.P. Thaxton, 2000c. Model of physiological stress in chickens: 3. Temporal patterns of responses. Poultry Sci., 79: 377- 382.
24. Ajakaiye, J.J., A. Pérez-Bello, M. Cuesta-Mazorra, J.R Garcia-Diaz and A. Mollineda-Trujillo, 2010. Effects of Vitamin C and E Dietary Supplementation on Erythrocyte Parameters of Layer Chickens Reared in High Ambient Temperature and Humidity. Brazilian J. Poultry Sci., 13: 205- 209.
25. Gursu, M.F., M. Onderci; F. Gulcu and K. Sahin, 2004. Effects of vitamin C and folic acid supplementation on serum paraoxonase activity and metabolites induced by heat stress *in vivo*. Nutr. Res., 24(2): 157- 164.
26. Seyrek, K., C. Yensey, M. Serter; F.K. Kral, P.A. Ulutas and H.E. Bardakcoglu, 2004. Effects of dietary vitamin C supplementation on some serum biochemical parameters of laying Japanese quails exposed to heat stress (34.8°C). Revue de Medecine Veterinaire, 155(6): 339- 342.
27. Kutlu, H.R. and S.M. Forbes, 1993. Changes in growth and blood parameters in heat stressed broilers chicks in response to dietary ascorbic acid. Livestock Prod. Sci., 36(4): 335- 350.
28. Hamdy, A.M.M., M.A.H. Soliman, A.G.M. Abdalla and Z.S.H. Ismail, 2003. Effects of dietary polyunsaturated fatty acids on some immune responses of broiler chicks. Egypt. Poult. Sci. J., 23(3): 601- 616.
29. Abdel-Fattah, S.A., Y.M. El-Hommosany and F. Abdel- Azeem, 2005. Alleviating the adverse effects of summer season on performance and humoral immunity of broiler chicks using different sources of fat. Egypt. Poultry Sci., 25: 279- 293.
30. Sahin, K., O. Kucuk, N. Sahin and M. Sari, 2002a. Effects of vitamin C and vitamin E on lipid per oxidation status, serum hormone, metabolite and mineral concentrations of Japanese quails reared under heat stress (34 degrees C). Intern. J. for Vit. and Nutr. Res., 72(2): 91-100.
31. Sahin, K., N. Sahin and S. Yaralioglu, 2002b. Effects of vitamin C and vitamin E on lipid per oxidation, blood serum metabolites and mineral concentrations of laying hens reared at high ambient temperature. Biol. Trace. Element Res., 85(1): 35-45.
32. Lucas, A.M. and G. Jamroz 1961. Atlas of avian hematology. Agric. Monograph No. 25, U. S. Dept. Agric. Washington.
33. Lucas, A.M., E.M. Dennington, G.E. Cottral and B.R. Burmester, 1954. Production of so- called normal lymphoid foci following inoculation with tumor filtrate. II- liver and spleen. Poultry Sci., 33: 571- 584.
34. Fukuta, K., T. Nishida and M. Yasuda,1969. Comparative and topographical anatomy of the fowl. I. Structure and distribution of the fine blood vascular system in the spleen. Jap. J. Vet. Sci., 31: 303- 311.
35. Glick, B. and K. Sato, 1964. Accessory spleens in the chicken. Poultry Sci., 43: 1610-1612.
36. Hodges, R.D., 1974. The histology of the fowl, pp: 221-230. Academic Press, London, Ny, SanFrancisco.
37. Glick, B., K.A. Holbrook, I. Olah, W.D. Perkins and R. Stinson, 1978a. A scanning electron microscope study of the caecal tonsil: the identification of a bacterial attachment to villi of the caecal tonsil and possible presence of lymphatics in the caecal tonsil. Poultry Sci., 57: 1408-1413.
38. Befus, A .D., N. Johnson, G.A. Leslie and J. Bienenstock, 1980. Gut- associated lymphoid tissue in the chicken. J. Immunol., 125: 2626- 2634.

39. Glick, B., K.A. Holbrook, I. Olah, W.D. Perkins and R. Stinson, 1978b. A scanning electron microscope study of the caecal tonsil: the basic unit of the caecal tonsil. Dev. Comp. Immunol., 5: 95- 112.
40. Glick, B. and I. Olah, 1981. Gut- associated lymphoid tissue of the chicken. In Int. J. Scanning Electron Micros. Chicago, SEM, Inc. pp: 99-106.
41. Burns, R.B., 1977. Possible route of antigen uptake by the Harderian gland of the domestic fowl. Br. Poult. Sci., 18: 407-409.
42. Bang, B.C. and F.B. Bang, 1968. Localized lymphoid tissues and plasma cells in paraocular and paranasal organ systems in chickens. American J. Patholo., 53: 735- 751.
43. Onyeanusi, B. I., J.C. Onyeanusi, A.N. Emma and C.D. Ezeokoll, 1994. The thymus of the Guinea fowl from the eighteen day of incubation until maturity. Anat. Histol. Embryol., 23: 320- 329.
44. Albini, B. and G. Wick, 1974. Delineation of B and T lymphoid cells in the chicken. J. Immunol., 112: 444- 449.
45. Wight, P.A.L., R.B. Burns, R. Rothwell and G.M. Mackenzle, 1971. The Harderian gland of the domestic fowl. J. Anat., 110: 307-315.
46. Ali, S.Y., 1992. Matrix formation and mineralization of bone. In: Bone biology and skeletal disorders in poultry. Carfax Pub. Company, pp: 19- 38.
47. Bishop, S.C., R.H. Fleming, H.A. McCormack, D.K. Flock and C.C. Whitehead, 2000. Inheritance of bone characteristics affecting Osteoporosis in laying hens. Br. Poult. Sci., 41: 33- 40.