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THE ALLEVIATION OF THE HARMFUL EFFECTS OF HEAT STRESS ON NUTRIENT DIGESTIBILITY AND SOME BLOOD CONSTITUENTS IN LAYING HENS BY DIETARY POTASSIUM CHLORIDE AND VITAMIN E

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ABSTRACT

A factorial experiment (4×3) was conducted to evaluate the possibility of ameliorating the negative effects of heat stress on nutrient digestibility and some physiological aspects of laying hens by using dietary supplementation with KCl (0.0, 0.8, 1.6 and 2.4%), vitamin E (0.0, 150 and 300 mg/kg) or their combinations. Three hundred and sixty, 20-week-old Hy-Line W-36 pullets were assigned to twelve equal experimental groups. All birds were kept in community battery cages (6 birds per cage), set up in an open-sided laying house, and exposed to a daily photoperiod of 16 hr and managed similarly. Twelve mash experimental diets were formulated to contain metabolizable energy of about 2840 kcal/kg and crude protein of about 18.7%, and offered to pullets during the summer season (20-32 weeks of age). The criteria of response were nutrient digestibility, relative weights of certain lymphoid organs and endocrine glands, antibody titers and some blood parameters of laying hens.

Apart from the effect of dietary vitamin E supplementation, feeding the KCl-supplemented diets during the hot climate of the Egyptian summer significantly improved K retention and ether extract digestibility, compared with those of the control hens; however, digestibility of the other nutrients was not affected. Hens fed the KCl-supplemented diets had significantly higher relative weights of lymphoid organs (spleen, thymus and bursa of Fabricius) and thyroid gland as compared to those of the control hens; however, relative weights of pancreas, ovary, oviduct and liver were not affected. Feeding the KCl-supplemented diets significantly increased the blood concentrations of hemoglobin, erythrocytes (RBCs) and leukocytes (WBCs), and the antibody titers as well as blood plasma concentrations of total protein, albumin, Ca, inorganic P, T_3, T_4, K^+ and Cl^-, and plasma activities of ALT, AST and ALP but significantly decreased blood pH and plasma levels of glucose, total lipids, triglycerides, cholesterol, corticosterone and Na^+ as compared to those of the control hens; however, blood hematocrit percent was not affected.

Regardless of the effect of dietary KCl supplementation, hens fed the vitamin E-supplemented diets exhibited no significant differences in nutrient digestibility of the experimental diets. Laying hens fed the diets containing the high level of vitamin E (300 mg/kg) had significantly higher relative weights of spleen and thyroid gland as compared to those of the control hens and those of hens fed the low supplemental level of the vitamin (150 mg/kg) while relative weights of thymus, bursa of Fabricius, pancreas, ovary, oviduct and liver were not affected. Hens fed the vitamin E-supplemented diets, particularly with the high supplemental level of the vitamin (300 mg/kg), displayed significantly higher means of blood concentrations of hemoglobin, RBCs, WBCs and hematocrit percent as well as antibody titers and blood plasma levels of total protein, albumin, Ca, inorganic P, T_3, T_4 and Cl^-, and plasma activities of ALT and ALP but significantly decreased blood pH as well as plasma levels of glucose, triglycerides, cholesterol, corticosterone, K^+ and Na^+, as compared to those of the control birds; however, activity of AST was not affected.

The effects of the interactions between supplemental dietary KCl and vitamin E were significant on K retention, ether extract digestibility, relative weights of

spleen, thyroid gland, pancreas and oviduct as well as most blood parameters while other measurements were not affected. It could be concluded that dietary supplementation with KCl (1.6%), vitamin E (300 mg/kg), either separately or in combination, can be used as an effective tool for alleviating the adverse effects of heat stress on the physiological status of laying hens.

Keywords: Dietary KCl, Vitamin E, Heat Stress, Nutrient Digestibility, Physiological Aspects, Laying Hens.

INTRODUCTION

High ambient temperature is one of the most important problems for poultry production in many regions of the world. Large economic losses occur because of increased mortality and decreased production in both growing birds and laying hens. At high ambient temperature, growth rate decreases as a result of the reduced appetite and feed intake and respiratory rate increases, causing respiratory alkalosis (Hillman *et al.*, 1985). During heat stress behavioral, physiological, hormonal and molecular adjustments can occur (Etches *et al.*, 1995). The harmful effects of high temperature on the performance of laying hens have been well studied. Feed intake, egg production, egg weight, and egg shell quality are decreased in heat-stressed birds (Marsden and Morris, 1987; Balnave, 1996; Bollengier-Lee *et al.*, 1998; Mashaly *et al.*, 2004). High environmental temperature not only has an adverse effect on laying performance but also can impede disease resistance. Environmental stress may depress the immune function of birds by impeding production of antibodies and effective cell-mediated immunity (Zulkifli *et al.*, 1994). The phagocytic potential of chicken macrophages is decreased during heat exposure (Miller and Qureshi, 1992). In addition, Mashaly *et al.* (2004) reported that mortality rate was increased whereas productive performance, total leukocyte counts and antibody production were significantly inhibited in hens exposed to heat stress, and concluded that heat stress not only adversely affects production performance but also inhibits immune function.

In addition, some studies have indicated that heat stress can adversely affect nutrient digestibility and/or utilization in both broiler chicks and laying hens. The reasons for such an adverse effect of heat stress on nutrient digestibility are not clearly understood but could generally attributed to some reductions in blood flow to the gastrointestinal tract of birds (Wolfenson, 1986), in the digestive enzymes secretion or activity (Osman, 1985; Hai *et al.*, 2000), or in the digestive tract motility (Tur and Rial, 1985). The aim of the present study was to investigate the possibility of ameliorating the negative effects of heat stress, occurring during the summer season, on nutrient digestibility and some physiological aspects of laying hens by means of dietary supplementation with KCl, vitamin E or their combinations.

MATERIALS AND METHODS

The present study was undertaken at the Poultry Research Unit; Agricultural Researches and Experiments Station; Faculty of Agriculture, Mansoura University, Egypt, from July to September, 2004. Daily ambient

temperature (T_a) and relative humidity (RH) were recorded inside the laying house four times per day; at mid-day (12 a.m), afternoon (3 p.m), at mid-night (12 p.m) and at dawn (3 a.m) during the entire experimental period from 20 to 32 weeks of age; and monthly means of maximum and minimum T_a and RH are presented in Table 1.

Table 1: Monthly means of maximum and minimum T_a and RH of laying house during the entire experimental period (20 to 32 weeks of age)

Month	Maximum T_a (°C)	Minimum T_a (°C)	Maximum RH (%)	Minimum RH (%)
July	39.5	28.0	97	47
August	41.8	31.6	98	47
September	40.5	30.4	97	43

Experimental birds and diets:

Three hundred and sixty, 20-week-old Hy-Line W-36 pullets were assigned to twelve equal experimental groups of 5 replications of 6 birds each. All birds were kept in community battery cages (6 birds per cage), set up in an open-sided laying house, and exposed to a daily photoperiod of 16 hr and managed similarly. Four mash experimental diets were formulated to contain supplemental KCl levels of 0.0 (which served as a control), 0.8, 1.6 and 2.4% of the diet, respectively. Each of these diets were fortified with three levels of vitamin E (0.0, 150 and 300 mg/kg diet); and thus, twelve experimental diets were composed. All diets contained approximately similar levels of metabolizable energy (about 2840 kcal/kg) and crude protein (about 18.7%). All the experimental groups of hens were fed their respective diets from 20 up to 32 weeks of age (during the summer season, 2004). All birds had free access to feed and water throughout the experimental period. Composition and chemical analysis of the experimental diets are presented in Table 2.

Criteria of response:

Nutrient digestibility of laying hens:

At 28 weeks of age, 4 laying hens were selected from each treatment, on the basis of average body weight, and placed in a separate metabolic cage and had free access to feed and water. Each group of hens was fed its respective experimental diet for an adaptation period of three days followed by an experimental period of four days in which the daily feed intake and the excreta voided were quantitatively determined. Just after collection, the excreta were sprayed with 1% boric acid to eliminate nitrogen loss due to a possible ammonia release. Any feed spilled over the excreta and feather and other foreign material were carefully removed. The excreta were then dried in a forced-air oven at 70°C for 48 hours. Then, the excreta were allowed to equilibrate in atmosphere before being weighed, finely ground, and stored in plastic bags in pledge of further analysis. Chemical analyses of the experimental diets and excreta were carried out according to the official methods of analysis of the Association of Official Analytical Chemists (AOAC, 1984). The procedure described by Jakobsen *et al.* (1960) was used for

separating the fecal protein fraction in excreta samples. Urinary organic matter was calculated according to Abou-Raya and Galal (1971). The apparent nutrient digestibility of the experimental diets were calculated as follows: Nutrient digestibility (%) = 100 [Nutrient intake (g)–Nutrient excreted (g)] ÷ Nutrient intake (g). The retained percentages of ash (AR), nitrogen (NR) and potassium (RK) were also determined. Digestibility for crude protein (CP) and organic matter (OM), however, were calculated according to the following equation: Nutrient digestibility (%) = 100 [Nutrient intake (g) – Fecal nutrient (g)] ÷ Nutrient intake (g).

Table 2: Composition and chemical analysis of the experimental diets

Feed ingredients (%)	Dietary added KCl level (%)			
	Control	0.8 KCl	1.6 KCl	2.4 KCl
Yellow Corn	61.54	61.42	61.40	61.24
Soybean meal, 44%	6.80	5.80	5.10	4.20
Wheat bran	3.00	2.50	1.80	1.27
Corn gluten meal	15.30	16.10	16.70	17.47
Ground limestone	9.70	9.70	9.70	9.70
Dicalcium phosphate	2.25	2.25	2.25	2.25
Common salt	0.60	0.60	0.60	0.60
Vit.+Min. Premix†	0.30	0.30	0.30	0.30
DL-Methionine	0.10	0.10	0.10	0.10
Lysine-HCl	0.41	0.43	0.45	0.47
KCl	0.00	0.80	1.60	2.40
Total	100	100	100	100
Calculated analysis: (NRC, 1994)				
Metabolizable energy; kcal/kg	2844	2842	2840	2837
Crude protein; %	18.73	18.72	18.70	18.71
Ether extract; %	2.87	2.86	2.85	2.84
Crude fiber; %	2.36	2.24	2.12	2.01
Calcium; %	4.22	4.22	4.21	4.21
Total P; %	0.75	0.74	0.73	0.72
Available P; %	0.47	0.47	0.47	0.47
K; %	0.42	0.80	1.19	1.57
Cl; %	0.40	0.78	1.16	1.53
Na; %	0.26	0.27	0.27	0.28
Lysine; %	0.93	0.93	0.93	0.93
Methionine; %	0.49	0.49	0.50	0.50
Methionine + Cystine; %	0.82	0.83	0.83	0.83
Determined analysis: (AOAC, 1984)				
Dry matter (DM); %	90.44	91.31	91.49	91.39
Crude protein; %	21.87	22.35	22.19	21.62
Ether extract (EE); %	3.62	3.52	3.55	3.49
Crude fiber (CF); %	3.55	3.36	3.03	2.96
Ash; %	7.11	7.88	8.04	8.40
NFE; %	63.85	62.89	63.19	65.53
K; %	0.60	1.20	1.67	2.12

†: Each 3 Kg premix contains: Vit. A, 12,000,000 IU; Vit. D3, 3,000,000 IU; Vit. E, 10,000 mg; Vit. K3, 3,000 mg; Vit. B1, 200 mg; Vit. B2, 5,000 mg; Vit. B6, 3,000 mg; Vit. B12, 15 mg; Biotin, 50 mg; Folic acid 1,000 mg; Nicotinic acid 35,000 mg; Pantothenic acid 10,000 mg; Mn 80 g; Cu 8.8 g; Zn 70 g; Fe 35 g; I 1 g; Co 0.15 g and Se 0.3 g.

Antibody titers of laying hens:

When the birds were 31 weeks of age, sheep red blood cells (SRBC), a thymus-dependent antigen, were used as a test antigen to quantify the specific antibody response as a measure of humoral immunocompetence. Three birds from each treatment were immunized intravenously with 1 mL of 25% SRBC suspension, prepared in 0.9% sterile saline. After 7 days, all immunized birds were bled and the corresponding plasma samples were collected to determine the primary antibody response. Determination of the antibody titers to SRBC was performed using the microtiter technique (Trout *et al.*, 1996).

Certain lymphoid organs and endocrine glands of laying hens:

When the birds were 32 weeks of age, three hens per treatment, with body weights approach the average weight of their respective dietary treatment, were selected and slaughtered. Immediately after complete bleeding and feather plucking, their carcasses were carefully eviscerated and the liver, ovary, oviduct, pancreas and thyroid gland as well as the lymphoid organs (thymus, bursa of Fabricius and spleen) were gently excised and weighed to the nearest 0.001 g. Relative weights (% of live weight at slaughter) of these lymphoid organs, endocrine glands and other organs were also calculated.

Blood parameters of laying hens:

At 32 weeks of age, three hens per treatment were slaughtered within one to two hours post-oviposition in order to take some measurements on blood parameters. Individual blood samples were collected from the jugular veins of laying hens into two heparinized tubes. One group of blood samples was centrifuged at 4000 rpm for 15 minutes to separate blood plasma, and used for determining the concentrations of plasma total protein, total lipids, glucose, cholesterol, triglycerides, albumin, Ca, and inorganic P and activities of plasma alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT); using commercial Kits, according to the methods described by Gornall *et al.* (1949), Frings and Dunn (1970), Trinder (1969), Allain *et al.* (1974), Fossati and Prencipe (1982), Doumas *et al.* (1971), Moorehead and Biggs (1974), Goldenberg and Fernandez (1966), Kind and King (1954), Reitman and Frankel (1957), respectively. At the same time, plasma levels of Na and Cl (Henry, 1974), and K (Tietz, 1987) were also estimated, using commercial Kits. In addition, plasma concentrations of corticosterone, triiodothyronine (T_3) and thyroxine (T_4) were also determined, using commercial Kits, according to the methods described by Sainio *et al* (1988), Sterling (1975) and Liewendahl (1990), respectively. The second group of blood samples was used for the determination of some hematological characteristics (blood pH, hemoglobin, hematocrit, and erythrocyte and leukocyte counts). Blood pH was measured by a pH-meter. Blood hemoglobin was determined according to the method of Van Kampen and Zijlstra (1961). Hematocrit percent and counts of erythrocytes (RBCs) and leukocytes (WBCs) were measured according to the standard procedures (Brown, 1980).

Experimental design and statistical analysis:

A completely randomized design in factorial arrangement of treatments (4×3), with four levels of supplemental dietary KCl (0.0, 0.8, 1.6 and 2.4%) and three levels of dietary vitamin E supplementation (0.0, 150 and 300 mg/kg), was used in the present study. Statistical analyses for various variables were performed, using Statgraphics, Version 5.0 STSC software program (Statistical Graphics Corporation, 1991). The differences were considered significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Nutrient digestibility of laying hens:

Although the test phase of the digestion trial was practiced during the hot climate of the Egyptian summer, no significant differences were detected in either daily feed intake or excreta voided, on DM-basis, among all experimental groups of laying hens; therefore, their means were not tabulated. Apart from the effect of dietary vitamin E supplementation, feeding the KCl-supplemented diets during the hot climate of the Egyptian summer significantly improved the KR and the digestibility of EE as compared to their control counterparts (Table 3). However, there were no significant differences in the digestibility of OM, CP, CF, DM and NFE, or in AR and NR in response to feeding the KCl-supplemented diets. Even though the best mean of KR was achieved by laying hens fed the highest supplemental level of KCl (2.4%) it was not significantly different from those of birds fed the lower levels of KCl added (0.8 and 1.6%). Also, hens fed the highest two levels of KCl (1.6 and 2.4%) attained superior means of EE digestibility, with no significant differences between them; they were significantly higher compared with those of the control group and the experimental group of hens fed the diet supplemented with 0.8% KCl.

There are some reports in the scientific literature that heat stress can adversely affect nutrient digestibility and/or utilization in both broiler chicks and laying hens. In this regard, Wallis and Balnave (1984) found that the influence of environmental temperature on amino acid digestibility was sex-related, with high temperatures decreasing digestibility of amino acids in female but not in male broilers. Larbier *et al.* (1993) found that the true digestibility of protein and amino acids were depressed in broilers when the ambient temperature increased from 21 to 32°C. More recently, Bonnet *et al.* (1997) investigated the effect of chronic heat exposure on feed digestibility of broilers and found that nitrogen retention and apparent metabolizable energy were significantly reduced in birds exposed to constant ambient temperature of 32 as compared to the control group (exposed to constant 22°C). Hai *et al* (2000) reported that the activities of the digestive enzymes: trypsin, chymotrypsin and amylase in broiler chicks were significantly decreased at high ambient temperature (32°C) compared to the normal environment (21°C). High ambient temperature can also alter the absorption and metabolism of minerals in birds. Heat-stressed broiler chicks either reared at 32°C up to 42 days of age (El-Husseiny and Creger, 1981), or subjected to cyclic (24 to 35°C) or chronic (35°C) temperatures from 5 to 7 weeks of age

(Belay and Teeter, 1996) exhibited lower rates of Ca, Fe, Cu, K, Mg, Na, Mn, P and Zn retention compared with the control birds reared at 24°C. High temperatures may also result in less efficient utilization of vitamins (Feenster, 1985; Bollengier *et al.*, 1999). However, Sahin *et al.* (2002c) observed significant depressions in digestibility of DM, OM, CP and EE of Japanese quail hens exposed to high ambient temperature (34°C).

Table 3: Effects of feeding diets supplemented with KCl, vitamin E or their combinations during the hot climate of the Egyptian summer on nutrient digestibility of laying hens at 28 weeks of age

Dietary	Retention (%)			Digestibility (%)					
treatments	AR[2]	KR[3]	NR[4]	EE	NFE	DM	CP	CF	OM
Main factors KCl, % (A)									
0.0	55.70	52.82[b]	64.63	57.05[b]	64.09	62.29	76.45	24.77	59.59
0.8	56.11	62.09[a]	65.91	57.92[b]	63.65	62.27	76.23	23.65	59.87
1.6	56.26	62.09[a]	65.81	61.43[a]	63.47	62.37	76.57	24.91	60.08
2.4	55.62	62.32[a]	64.65	60.90[a]	63.25	62.04	75.49	24.78	59.63
SEM[1]	0.18	0.51	0.50	0.62	0.55	0.47	0.48	0.69	0.56
Sig. level	NS	**	NS	**	NS	NS	NS	NS	NS
Vitamin E, mg/kg (B)									
0.0	55.92	60.23	65.80	59.07	64.10	62.67	76.35	24.38	60.25
150	55.85	59.73	64.70	58.46	63.12	61.78	76.72	24.75	59.35
300	56.00	60.27	65.25	60.45	63.62	62.28	75.48	24.46	59.78
SEM[1]	0.15	0.44	0.43	0.53	0.48	0.41	0.41	0,60	0 48
Sig. level	NS	NS	NS	NS	NS	NS	NS	NS	NS
A×B Interaction									
1×1	56.13	51.43	64.47	57.69	64.78	62.81	77.20	25.53	60.24
1×2	55.39	54.93	64.26	55.93	63.39	61.69	77.06	25.14	59.00
1×3	55.58	52.11	65.16	57.53	64.11	62.38	75.09	23.66	59.54
2×1	55.84	64.52	67.49	59.67	65.28	63.70	77.78	23.18	61.56
2×2	56.25	62.08	64.80	54.66	62.33	61.06	75.67	23.64	58.48
2×3	56.24	62.66	65.45	59.42	63.36	62.05	75.25	24.14	59.57
3×1	56.19	62.09	65.80	59.03	63.31	62.17	76.45	24.45	59.84
3×2	56.12	61.17	65.83	63.36	63.26	62.29	76.24	25.47	59.96
3×3	56.46	63.00	65.80	61.91	63.85	62.65	77.02	24.80	60.44
4×1	55.51	62.89	65.45	59.91	63.05	62.00	73.99	24.36	59.38
4×2	55.64	60.75	63.92	59.87	63.52	62.07	77.92	24.75	59.95
4×3	55.70	63.31	64.58	62.93	63.18	62.06	74.55	25.24	59.56
SEM[1]	0.31	0.88	0.87	1.07	0.96	0.82	0.83	1.20	0.97
Sig. level	NS	*	NS	**	NS	NS	NS	NS	NS

[a-b]: For each of the main factors, means in the same column having different superscripts differ significantly at P ≤0.05.

[1-4]: Refer to standard error of the means, and ash, potassium and nitrogen retention rates, respectively.

Heat stress can also depress K retention in laying hens, and increasing dietary K level can correct the K status of the body (Deetz and Ringrose, 1976). However, Koelkebeck *et al.* (1998) concluded that acute heat stress had no adverse effects on dietary amino acid digestibility in laying hens. The enhanced retention of K by feeding the KCl-supplemented diets, in the present study, is in line with the findings of Deetz and Ringrose (1976), who suggested that dietary K levels of 0.4-0.6% are required for laying hens exposed to heat stress, in order to restore the normal K status in the body. The absence of significant differences (with an exception of the improved

digestibility of EE) in nutrient digestibility and utilization, as a consequence of feeding the KCl-supplemented diets in the present study, is in partial keeping with the findings of Koelkebeck *et al.* (1998) but disagrees with most of the aforementioned reports.

However, it should be pointed out that the lack of significant differences in nutrient digestibility of laying hens in response to feeding the KCl-supplemented diets, under the conditions of the present study, is inconsistent with the achieved improvement in their productive performance exerted by feeding these diets during both the hot climate period (20-32 weeks of age) and the whole experimental period from 20 to 68 weeks of age (Raya *et al.*, 2007). Such a response of the laying hens may be an indication that their superior productive performance was independent from the efficiency of digestion; other factors such as enhanced utilization of certain nutrients at the metabolic level and/or favorable physiological status of these hens could be relevant in that respect. Perhaps the constant high temperature produces a greater negative effect on nutrient digestibility than does the cycling temperature. In addition, severity of heat stress (*i.e.* acute or chronic), species of poultry, and age and strain of birds may be involved as contributing factors for such inconsistent responses of birds to heat stress.

Regardless of the effect of dietary KCl supplementation, feeding the vitamin E-supplemented diets during the hot climate had no significant effects on the digestibility of OM, CP, CF, DM, EE and NFE, or on the retention rates of ash, K and N by laying hens as compared to their control counterparts (Table 3). In disagreement with the present results, Sahin *et al.* (2002c) found that heat exposure decreased digestibility of DM, OM, CP and EE, and they were improved by supplemental dietary vitamin E in laying Japanese quails. Working with growing Japanese quails reared under chronic heat stress, Sahin and Kucuk (2001a) reported that digestibility of DM, OM, CP and EE were higher when their diets were supplemented with a combination of vitamin C (200 mg/kg) and vitamin E (250 mg/kg diet); such a combination gave the greatest performance, carcass characteristics and nutrient digestibility for most parameters measured. In another study they (Sahin and Kucuk, 2001b) also observed that digestibility of DM, OM, CP and EE were significantly higher when growing Japanese quails were fed diets supplemented with 250 mg vitamin E/kg diet as compared to their control counterparts reared under chronic heat stress. Age, gender and strain of birds, species of poultry, severity of heat stress (acute *vs.* chronic) and its rhythm (constant *vs.* cycling high temperatures), course of experiment (short *vs.* long term) and compositional differences of the basal diets within the different experiments are undoubtedly involved in the inconsistent responses of birds to heat stress. Significant interactions between supplemental dietary KCl and vitamin E were observed for KR and EE digestibility by laying hens; all other measurements of nutrient digestibility were not significantly affected.

Lymphoid organs and endocrine glands of laying hens:

Apart from the effect of dietary vitamin E supplementation, significant increases ($P \leq 0.01$) were observed in means of relative weights of spleen, thymus, bursa of Fabricius and thyroid gland of laying hens fed the KCl-supplemented diets during the heat stress period (20-32 weeks of age) as

compared to their control birds (Table 4). However, no significant differences were detected in relative weights of pancreas, ovary, oviduct or liver of laying hens as a result of feeding the KCl-supplemented diets as compared to their control birds. The present results indicated that dietary KCl had beneficial effects on the immune system of laying hens exposed to heat stress. However, the mechanism by which supplemental dietary KCl could alleviate the negative effects of heat stress on endocrine and lymphoid systems is unexplainable yet. Possibly, part of the observed beneficial effect of dietary KCl supplementation is indirectly related to its potentiality in enhancing water intake and/or maintaining normal osmotic pressure and acid-base balance under the heat stress conditions.

The reduced relative weights of lymphoid organs (spleen, thymus, bursa of Fabricius), exhibited herein by the heat-stressed control hens, is in harmony with the findings of Pardue *et al.* (1985) who reported that heat stress decreased the relative weights of bursa of Fabricius and spleen in chicks. Similarly, Williamson *et al.* (1985) observed significant reductions in the weights of bursa, thymus and spleen of chicks which had been exposed to 40°C, from 28 to 63 days of age. More recently, Naseem *et al.* (2005b) found that bursa, thymus and spleen were atrophied in chicks exposed to heat stress. Similarly, the decreased relative weight of thyroid gland in the control hens of the present study is in line with the findings of Moss and Balnave (1978), who measured plasma thyroxine and thyroid weight in chicks kept at 22°C (normal) and 30°C for four weeks, and observed a progressive increase in T_4 in birds reared at 30°C compared to that of birds reared at 22°C; this was associated with a 30% reduction in thyroid weight at the higher temperature. Similar results were also obtained by Donkoh (1989), who observed a decreased thyroid gland weight in chicks housed at higher temperatures (30 or 35°C) compared with that of birds reared at normal temperatures (20 or 25°C).

Apart from the effect of dietary KCl supplementation, feeding the high supplemental level of vitamin E (300 mg/kg) during the hot climate (20-32 weeks of age) significantly increased the relative weights of spleen ($P \leq 0.05$) and thyroid gland ($P \leq 0.01$) of laying hens as compared to those of the control birds (Table 4). However, no significant differences were detected in relative weights of thymus, bursa of Fabricius, pancreas, ovary, oviduct or liver of laying hens in response to dietary supplementation with vitamin E.

Heat stress has been reported to depress thyroid activity and its hormone secretions in poultry (Bowen and Washburn, 1985; Sahin *et al.*, 2002a, b) and increase plasma corticosterone (Etches *et al.*, 1995). Vitamin E is known to be a lipid component of biological membranes (McDowell, 1989). Because of its antioxidant properties, vitamin E has been reported to protect cells involved in immune response, such as lymphocytes, macrophages, and plasma cells, against oxidative damage and to enhance the function and proliferation of these cells (Franchini *et al.*, 1991).

In general, the beneficial effect of dietary supplementation with vitamin E on thyroid size of laying hens, reported herein particularly with the high supplemental level of the vitamin (300 mg/kg diet), is in agreement with the findings of Sahin *et al.* (2002b), who found that supplemental dietary

vitamins C and E either separately on in a combination increased serum T_3 and T_4 concentrations whereas serum ACTH concentration was decreased in heat-stressed laying hens. In broiler chicks exposed to heat stress, Sahin *et al.* (2002a) reported that increased supplemental vitamin E (up to 500 mg/kg diet) resulted in linear increases in serum T_3 and T_4 concentrations and linear decreases in ACTH concentration. Apart from the experimental protocols, the increased relative weight of spleen of heat-stressed laying hens in response to supplemental dietary vitamin E in the present study is consistent with the conclusion of Puthpongsiriporn *et al.* (2001) that vitamin E supplementation enhanced the immune response of haying hens during heat stress. Significant supplemental dietary KCl by vitamin E interactions were observed for relative weights of the spleen, thyroid gland, pancreas and oviduct; however, no significant differences were detected in the relative weights of thymus, bursa of Fabricius, ovary or liver of laying hens.

Table 4: Relative weights[§] of certain lymphoid organs and endocrine glands of 32-wk-old laying hens reared under heat stress as affected by feeding diets supplemented with KCl, vitamin E or their combinations

Dietary treatments	SPL[2]	THY[3]	BOF[4]	THG[5]	PAN[6]	Ovary	Oviduct	Liver
Main factors KCl, % (A)								
0.0	0.077^{c}	0.036^{c}	0.019^{b}	0.0034^{b}	0.148	2.010	3.586	1.88
0.8	0.106^{b}	0.045^{bc}	0.021^{b}	0.0052^{a}	0.148	1.989	3.052	1.99
1.6	0.126^{a}	0.055^{b}	0.023^{ab}	0.0058^{a}	0.148	2.163	3.230	1.77
2.4	0.125^{a}	0.078^{a}	0.028^{a}	0.0059^{a}	0.122	2.396	3.450	1.81
SEM[1]	0.003	0.004	0.001	0.0002	0.008	0.12	0.14	0.09
Sig. level	**	**	**	**	NS	NS	NS	NS
Vitamin E, mg/kg (B)								
0.0	0.105^{b}	0.049	0.022	0.0045^{b}	0.146	1.994	3.431	1.93
150	0.104^{b}	0.053	0.023	0.0049^{b}	0.131	2.195	3.199	1.733
300	0.117^{a}	0.057	0.023	0.0058^{a}	0.147	2.229	3.358	1.92
SEM[1]	0.003	0.003	0.001	0.0002	0.007	0.109	0.12	0.08
Sig. level	*	NS	NS	**	NS	NS	NS	NS
AB Interaction								
1×1	0.08	0.037	0.021	0.0019	0.192	1.819	4.348	2.027
1×2	0.07	0.040	0.020	0.0041	0.142	2.152	3.194	1.995
1×3	0.07	0.030	0.015	0.0042	0.111	2.059	3.215	1.623
2×1	0.09	0.032	0.021	0.0049	0.135	2.001	3.135	1.997
2×2	0.11	0.049	0.021	0.0047	0.158	1.716	2.659	2.008
2×3	0.11	0.053	0.021	0.0059	0.152	2.251	3.361	1.967
3×1	0.11	0.041	0.021	0.0053	0.135	2.054	3.225	1.899
3×2	0.11	0.056	0.022	0.0050	0.110	2.311	3.408	1.474
3×3	0.14	0.067	0.026	0.0070	0.197	2.124	3.057	1.962
4×1	0.12	0.088	0.024	0.0058	0.124	2.101	3.016	1.825
4×2	0.11	0.069	0.029	0.0058	0.114	2.603	3.535	1.453
4×3	0.13	0.078	0.030	0.0061	0.128	2.484	3.797	2.159
SEM[1]	0.006	0.007	0.002	0.0004	0.014	0.21	0.24	0.17
Sig. level	*	NS	NS	**	**	NS	**	NS

[§]: % of live boy weight at slaughter.

[a-c]: For each of the main factors, means in the same column having different superscripts differ significantly at $P \leq 0.05$. [1-6]: Refer to standard error of the means, and relative weights of spleen, thymus, bursa of Fabricius, thyroid gland and pancreas, respectively.

Blood parameters of 32-wk-old laying hens:

Data presented in Table 5 indicated that feeding laying hens on the KCl-supplemented diets during the hot climate of the Egyptian summer (20-32 weeks of age) significantly ($P \leq 0.01$) increased blood hemoglobin (Hb) concentration and the total counts of blood erythrocytes (RBCs) and leukocytes (WBCs) and the antibody titers to SRBC but significantly decreased blood pH as compared to those of the control birds. However, no significant differences were detected in blood hematocrit percent among the different experimental groups of laying hens. As displayed in Table 5, hens fed the KCl-supplemented diets during the experimental period (20-32 weeks of age) had significantly ($P \leq 0.01$) higher blood plasma concentrations of TPR, ALB, Ca and inorganic P but exhibited significantly lower concentrations of GLU, TLI, TRI and CHO as compared to those of their control counterparts.

In addition, data illustrated in Table 6 showed that feeding the KCl-supplemented diets during the experimental period (20-32 weeks of age) significantly ($P \leq 0.01$) increased blood plasma concentrations of T_3, T_4, the electrolytes: K^+ and Cl^-, and plasma enzyme activities of ALT, AST and ALP but significantly decreased concentrations of corticosterone and Na^+ in blood plasma as compared to those of the control birds. These results clearly demonstrated that dietary KCl supplementation could alleviate, at least partly, the depressive effect of heat stress on the cellular components of blood, immune response, certain blood constituents, metabolites, hormones and some enzyme activities in blood plasma of laying hens.

The inhibiting effect of heat stress on blood concentrations of Hb, RBCs and WBCs, and immune responses is well documented in both broiler chicks and laying hens (Donkoh, 1989; Lin *et al.*, 2002; Lin *et al.*, 2003; Mashaly *et al.*, 2004). It should be pointed out that the occurrence of respiratory alkalosis in response to thermal stress has not been consistently observed in all studies in poultry. In laying hens exposed to high temperature, there is an increase in respiration rate which lead to a reduction in blood partial pressure of carbon dioxide (PCO_2) and HCO_3- and an increase in blood pH, resulting in an acid-base disturbance (El-Hadi and Sykes, 1982; Odom *et al.*, 1986). The observed lower blood pH values in laying hens fed the KCl-supplemented diets during the hot climate in this study may reflect a potential role of KCl in correcting the acid-base balance disturbances occurred under such conditions.

In partial agreement with the present results, Raya *et al.* (2003) reported that feeding KCl-supplemented diets to broiler chicks reared under heat stress resulted in significant redactions in body temperature, panting rate and blood pH of chicks. Teeter and Smith (1986) found that supplementing drinking water with 0.15% KCl positively affected live weight gain and feed efficiency but did not affect blood pH. However, Ait-Boulahsen *et al.* (1995) reported that giving 0.6% KCl in drinking water of broiler chicks exposed to heat stress resulted in lower hyperthermic body temperature and pH values than those of the controls. It is interesting to note that the effects of heat stress on blood biochemical parameters in birds are inconsistent. In partial keeping with the present results (Tables 5 and 6)

Table 5: Blood hematological variables and antibody titer, and plasma constituents of 32-week-old laying hens as affected by feeding diets supplemented with KCl, vitamin E or their combinations

Dietary treatments	Ht[2] (%)	Hb[3] (g/dL)	RBCs[4] (10^6/mm^3)	WBCs[5] (10^3/mm^3)	Blood pH	Antibody titer	GLU[6] (g/dL)	TPR[7] (g/dL)	ALB[8] (g/L)	TLI[9] (g/L)	TRI[10] (mg/dL)	CHO[11] (mg/dL)	Ca[12] (mg/dL)	P[13] (mg/dL)
Main factors KCl, % (A)														
0.0	30.33	7.63[b]	2.67[b]	28.09[c]	7.58[a]	5.80[c]	263.5[a]	4.39[c]	2.12[b]	26.91[a]	314.2[a]	322.4[a]	21.47[b]	2.91[b]
0.8	29.44	8.75[c]	2.70[c]	30.37[b]	7.53[b]	6.29[bc]	234.7[b]	5.19[b]	2.44[c]	22.52[b]	243.1[b]	227.9[b]	24.96[c]	3.56[c]
1.6	28.11	10.12[b]	2.71[b]	32.79[a]	7.48[c]	6.43[ab]	230.3[bc]	5.24[b]	2.76[b]	20.73[c]	235.7[b]	218.6[c]	26.92[b]	4.02[b]
2.4	31.77	12.71[a]	2.72[a]	33.24[a]	7.41[b]	6.94[a]	219.6[c]	5.67[a]	3.11[a]	20.78[c]	218.3[c]	204.6[b]	30.14[a]	4.72[a]
SEM[1]	1.22	0.27	0.002	0.23	0.01	0.06	3.88	0.06	0.06	0.47	4.59	0.91	0.25	0.07
Sig. level	NS	**	**	**	**	**	**	**	**	**	**	**	**	**
Vitamin E, mg/kg (B)														
0.0	28.91[b]	8.65[b]	2.69[b]	28.23[c]	7.65[a]	6.11[a]	246.9[a]	4.75[b]	2.61[ab]	23.24[a]	260.1[a]	247.2[a]	24.24[c]	3.66[b]
150	28.67[b]	10.21[a]	2.71[a]	30.86[b]	7.44[b]	6.17[b]	230.4[b]	5.34[a]	2.50[b]	21.81[b]	251.3[ab]	241.1[b]	25.89[b]	3.74[b]
300	32.17[a]	10.54[a]	2.71[a]	34.29[a]	7.42[b]	6.83[a]	233.6[b]	5.28[a]	2.72[a]	23.15[a]	247.0[b]	241.5[b]	27.49[a]	4.01[a]
SEM[1]	1.05	0.23	0.002	0.20	0.01	0.05	3.36	0.06	0.04	0.41	3.98	0.79	0.22	0.06
Sig. level	*	**	**	**	**	**	**	**	**	*	**	**	**	**
AB Interaction														
1×1	22.33	5.54	2.64	24.34	7.88	4.83	290.2	3.52	2.20	29.27	329.8	331.5	18.26	2.53
1×2	32.00	8.38	2.69	27.28	7.44	6.34	253.7	4.88	2.07	26.16	323.9	303.2	20.81	3.04
1×3	36.67	8.98	2.69	32.64	7.43	6.34	246.6	4.76	2.11	25.29	288.8	332.6	25.32	3.16
2×1	28.33	6.92	2.69	26.57	7.71	6.34	238.8	4.81	2.38	22.62	252.0	235.1	23.31	3.65
2×2	30.00	9.46	2.70	29.49	7.44	6.49	229.5	5.24	2.37	20.64	244.9	231.6	24.76	3.42
2×3	30.00	9.87	2.70	35.06	7.43	6.04	235.7	5.52	2.56	24.28	232.3	216.9	26.82	3.59
3×1	31.67	9.45	2.70	29.50	7.58	6.04	240.3	5.34	2.79	20.31	241.9	216.9	25.10	4.07
3×2	21.67	10.44	2.71	34.01	7.45	5.84	220.1	5.37	2.56	20.60	225.7	224.4	27.76	3.79
3×3	31.00	10.46	2.71	34.88	7.41	7.40	230.4	5.03	2.93	21.26	239.5	214.4	27.91	4.19
4×1	33.33	12.71	2.72	32.50	7.42	7.24	218.5	5.35	3.04	20.76	216.7	205.3	30.26	4.37
4×2	31.00	12.58	2.72	32.66	7.43	6.04	218.4	5.85	3.01	19.84	210.8	206.5	30.24	4.69
4×3	31.00	12.87	2.72	34.56	7.40	7.55	221.7	5.84	3.28	21.74	227.5	202.1	29.91	5.09
SEM[1]	2.11	0.47	0.005	0.40	0.02	0.11	6.72	0.11	0.11	0.83	7.96	1.57	0.44	0.13
Sig. level	**	**	**	**	**	**	*	**	NS	**	**	**	**	*

[a-d]: For each of the main factors, means in the same column having different superscripts differ significantly at P ≤0.05.

[1-13]: Refer to standard error of the means, hematocrit, hemoglobin and counts of erythrocytes and leukocytes, glucose, total protein, albumin, total lipids, triglycerides, cholesterol, calcium and inorganic phosphorus respecti

Özbey *et al.* (2004) found that concentrations of blood serum glucose, triglycerides and cholesterol were significantly higher whereas levels of total protein and albumin, and activity of serum alkaline phosphatase were significantly lower in laying Japanese quails exposed to heat stress. However, in laying hens, Koelkebeck and Odom (1995) found that acute heat stress had no effect on blood plasma levels of glucose, total protein, uric acid, creatinine, inorganic phosphorus, calcium, potassium, sodium or activity of serum alkaline phosphatase.

Table 6: Blood plasma enzymes, hormones and electrolytes of 32-week-old laying hens as affected by feeding diets supplemented with KCl, vitamin E or their combinations

Dietary treatments	ALP[2] (U/L)	ALT[3] (U/L)	AST[4] (U/L)	T_3[5] (ng/mL)	T_4[6] (ng/mL)	COR[7] (ng/mL)	Na mEq/L	K mEq/L	Cl mEq/L
Main factors KCl, % (A)									
0.0	192.3^{d}	7.88^{d}	19.72^{d}	2.07^{d}	7.50^{d}	10.61^{a}	169.0^{a}	4.69^{d}	101.7^{d}
0.8	232.2^{c}	10.17^{c}	25.28^{c}	2.80^{c}	10.01^{c}	8.72^{b}	166.0^{b}	5.15^{c}	106.0^{c}
1.6	265.6^{b}	14.89^{b}	29.78^{b}	4.03^{b}	12.87^{b}	8.27^{b}	165.6^{b}	6.42^{b}	110.0^{b}
2.4	355.5^{a}	20.78^{a}	32.72^{a}	5.22^{a}	15.10^{a}	5.16^{c}	163.5^{c}	7.52^{a}	115.4^{a}
SEM[1]	7.4	0.30	0.56	0.07	0.13	0.20	0.69	0.04	0.99
Sig. level	**	**	**	**	**	**	**	**	**
Vitamin E, mg/kg (B)									
0.0	201.2^{c}	13.17^{b}	26.00	2.58^{c}	10.28^{c}	9.37^{a}	166.95^{a}	5.98^{a}	103.6^{b}
150	258.3^{b}	13.08^{b}	26.92	3.87^{b}	11.22^{b}	8.45^{b}	166.82^{a}	5.99^{a}	105.6^{b}
300	324.6^{a}	14.04^{a}	27.71	4.13^{a}	12.61^{a}	6.75^{c}	164.20^{b}	5.86^{b}	115.6^{a}
SEM[1]	6.48	0.26	0.48	0.06	0.12	0.17	0.61	0.04	0.86
Sig. level	**	*	NS	**	**	**	**	*	**
A×B Interaction									
1×1	157.6	6.83	18.50	0.74	5.95	13.33	170.9	4.79	84.3
1×2	157.4	8.50	20.17	2.53	6.60	10.83	169.1	4.91	107.7
1×3	261.8	8.33	20.50	2.95	9.95	7.67	166.9	4.38	113.1
2×1	156.7	10.33	24.17	1.48	8.58	10.50	165.2	5.23	99.9
2×2	244.5	9.50	25.33	3.48	9.45	8.67	169.0	5.05	99.6
2×3	295.4	10.67	26.33	3.45	11.98	7.00	163.7	5.18	118.6
3×1	186.2	14.50	28.33	3.02	11.46	8.50	165.7	6.43	111.8
3×2	262.1	13.67	29.67	4.28	13.65	9.17	165.7	6.52	106.0
3×3	348.4	16.50	31.33	4.80	13.51	7.17	165.3	6.32	112.2
4×1	304.4	21.00	33.00	5.10	15.12	5.17	166.0	7.49	118.4
4×2	369.3	20.67	32.50	5.22	15.18	5.17	163 5	7.51	109.3
4×3	392.8	20.67	32.67	5.35	15.00	5.17	160.9	7.57	118.6
SEM[1]	12.97	0.53	0.97	0.12	0.23	0.34	1.21	0.08	1.73
Sig. level	**	*	NS	*	**	**	NS	**	**

[a-d]: For each of the main factors, means in the same column having different superscripts differ significantly at P ≤0.05.

[1-7]: Refer to standard error of the means, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, triiodothyronine, thyroxine and corticosterone, respectively.

The increased K^+ concentration in blood plasma of laying hens fed the KCl-supplemented diets, in the present study, is in partial harmony with the findings of Raya *et al.* (2003) who reported higher plasma concentrations of K and thyroid hormones (T_3 and T_4) in heat stressed broiler chicks in response to feeding KCl-supplemented diets compared with their control group. Also, in line with the present results Naseem *et al.* (2005a) found that

supplemental KCl at 1.5% in drinking water of heat-stressed broiler chicks significantly increased serum levels of potassium and bicarbonate.

Apart from the effect of dietary KCl supplementation, hens fed the vitamin E-supplemented diets under the conditions of this study significantly ($P \leq 0.01$) increased concentrations of blood Hb, RBCs, WBCs and hematocrit percent ($P \leq 0.05$), and the antibody titers to SRBC, but significantly decreased blood pH as compared to those of the control birds, particularly with the high supplemental level (300 mg/kg) of the vitamin (Table 5). Laying hens fed the vitamin E-supplemented diets displayed significantly ($P \leq 0.01$) higher blood plasma concentrations of TPR, ALB, Ca and P but exhibited significantly lower concentrations of GLU, TRI and CHO as compared to those of their control counterparts, particularly with the high supplemental level (300 mg/kg) of the vitamin (Table 5). In addition, the results presented in Table 6 indicated that feeding the vitamin E-supplemented diets significantly ($P \leq 0.01$) increased blood plasma concentrations of T_3, T_4, and Cl^-, and plasma enzyme activities of ALT and ALP, but significantly decreased concentrations of corticosterone, K^+ and Na^+ in blood plasma, particularly with the high supplemental level (300 mg/kg) of the vitamin E, as compared to those of the control birds. On the other hand, activity of AST in blood plasma of laying hens was not affected by dietary vitamin E supplementation.

As previously mentioned heat stress affects the blood acid-base balance of poultry by decreasing the partial pressure of arterial blood carbon dioxide which causes blood pH to become alkalotic (Koelkebeck and Odom, 1994). Typical responses of heat stress include elevations in plasma concentrations of corticosteroids, protein, glucose, sodium and heterophil/lymphocyte ratio and decreases in potassium and relative weights of adrenal, bursa, spleen and thyroid (Pardue *et al.*, 1985; Kutlu and Forbes, 1993). In laying hens, Arad *et al.* (1983) found that acute heat stress did not adversely affect plasma levels of sodium, potassium and calcium; however, plasma inorganic phosphorus was depressed compared with non-heat stressed birds. The plasma concentrations of very low density lipoprotein (VLDL) and vitellogenin, which are egg yolk precursors, are also reduced during heat stress (Utomo *et al.*, 1994).

In harmony with the present results, Sahin *et al.* (2002b) found that supplemental dietary vitamins C and E either separately or in a combination increased serum concentrations of T_3, T_4 and total protein but decreased serum levels of glucose, cholesterol and ACTH. The increased blood plasma concentrations of T_3, T_4 and the decreased level of corticosterone, reported herein, could be an indication for the beneficial effect of vitamin E in alleviating some of the adverse effects of heat stress on viability and productivity of laying hens. Bollengier-Lee *et al.* (1998) reported that plasma concentration of calcium, vitellogenin and VLDL were significantly higher in laying hens supplemented with 500 mg vitamin E/kg diet. They concluded that dietary supplementation with extra vitamin E can partly alleviate the adverse effects of chronic heat stress, perhaps by maintaining the supply of egg precursors in plasma.

Significant interactions between supplemental dietary KCl and vitamin E were detected on blood parameters, measured herein, except for

levels of albumin and sodium, and activity of blood plasma AST which were not affected. Such significant interactions might indicate that both two factors affected synergistically blood constituents.

Conclusion

It could be concluded that dietary supplementation with KCl (1.6%), vitamin E (300 mg/kg), either separately or in combination, can be used as an effective tool for alleviating the adverse effects of heat stress on some physiological status of laying hens.

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تغذية دجاج البيض علي علائق مزودة بكلوريد البوتاسيوم وفيتامين هـ لتخفيف الأثار السلبية للإجهاد الحراري علي هضم العناصر الغذائية وبعض مكونات الدم

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أجريت تجربة عاملية لتقييم إمكانية تخفيف الأثار السلبية للإجهاد الحراري علي هضم العناصر الغذائية وبعض الملامح الفسيولوجية للدجاج البياض عن طريق التغذية علي علائق مزودة بأربعة مستويات من كلوريد البوتاسيوم (صفر، ٠,٨، ١,٦، ٢,٤%) وثلاثة مستويات من فيتامين هـ (صفر، ١٥٠، ٣٠٠ ملجم/كجم عليقة). تم تقسيم عدد ٣٦٠ دجاجة من سلالة الهاي لاين الأبيض (W36) عمر ٢٠ أسبوعا إلي ١٢ مجموعة تجريبية متساوية وسكنت في أقفاص بياض جماعية (ستة طيور بالقفص) في عنبر بياض مفتوح وكانت الاضاءة اليومية ١٦ساعة وتمت عمليات الرعاية بشكل متماثل لكل الطيور. تم تكوين ١٢ عليقة تجريبية ناعمة (تحتوي علي طاقة قابلة للتمثيل في حدود ٢٨٤٠ كيلوكالوري/كجم وبروتين خام في حدود ١٨,٧%) وتمت التغذية علي تلك العلائق في فصل الصيف (٢٠-٣٢ أسبوعا من عمر الطيور). وتضمنت القياسات المدروسة معاملات هضم العناصر الغذائية والأوزان النسبية لبعض الأعضاء والغدد اللمفاوية والغدد الصماء ومناعة الطيور وبعض مقاييس الدم للدجاج البياض.

وكانت أهم النتائج المتحصل عليها علي النحو التالي: أدت التغذية علي العلائق المزودة بكلوريد البوتاسيوم إلي تحسن معنوي في معدل احتجاز البوتاسيوم ومعامل هضم الدهن الخام بينما لم تؤثر علي معاملات هضم باقي العناصر الغذائية، كما أدت إلي زيادة معنوية في الوزن النسبي لكل من الطحال والغدة التيموسية وغدة فبريشس والغدة الدرقية بينما لم تتأثر الأوزان النسبية لكل من البنكرياس والمبيض وقناة البيض والكبد. أحدثت التغذية علي العلائق المزودة بكلوريد البوتاسيوم زيادة معنوية في كل من عدد كرات الدم الحمراء والبيضاء ومناعة الطيور ومستوي هيموجلوبين الدم وكذلك تركيزات البروتين الكلي والألبيومين والكالسيوم والفوسفور غير العضوي وهرموني الثيروكسين(T_4) والتراي أيودوثيرونين(T_3) وأيونات البوتاسيوم والكلوريد وكذلك نشاط إنزيمات وظائف الكبد (ALT- AST) والفوسفاتيز القلوي (ALP) في بلازما دم الدجاج، وحدث انخفاضا معنويا في محتوي البلازما من كل من الجلوكوز والدهون الكلية والجلسريدات الثلاثية والكولستيرول وهرمون الكورتيكوستيرون وأيونات الصوديوم ودرجة تركيز أيونات الهيدروجين بالدم (pH)، بينما لم تتأثر نسبة خلايا الدم.

لم تتأثر معاملات هضم العناصر الغذائية معنويا بالتغذية علي العلائق المزودة بفيتامين هـ. أدت تغذية الدجاج البياض علي العلائق ذات المستوي العالي من الفيتامين (300 ملجم/كجم) إلي زيادة معنوية في الوزن النسبي لكل من الطحال والغدة الدرقية إذا ما قورنت بمتوسطات مجموعة الكنترول أو المغذاة علي المستوي المنخفض من الفيتامين (١٥٠ ملجم/كجم). لم تؤثر التغذية علي العلائق المزودة بفيتامين هـ معنويا علي الأوزان النسبية لكل الغدة التيموسية وغدة فبريشس والبنكرياس والمبيض وقناة البيض والكبد. أحدثت التغذية علي العلائق المزودة بفيتامين هـ (خاصة مع المستوي العالي) زيادة معنوية في كل من هيموجلوبين الدم وعدد كرات الدم الحمراء والبيضاء ومناعة الطيور ونسبة خلايا الدم وكذلك تركيزات كل من البروتين الكلي والألبيومين والكالسيوم والفوسفور غير العضوي وهرموني T_4 و T_3 وأيونات الكلوريد وكذلك نشاط إنزيمي ALT وALP في بلازما دم الدجاج، وحدث انخفاضا معنويا في درجة pH الدم وكذا في تركيزات الجلوكوز والجلسريدات الثلاثية والكولستيرول وهرمون الكورتيكوستيرون وأيونات الصوديوم والبوتاسيوم، بينما لم يتأثر معنويا نشاط إنزيم AST في بلازما الدم. كان تأثير التفاعل بين كلوريد البوتاسيوم وفيتامين هـ معنويا علي كل من معدل احتجاز البوتاسيوم، معامل هضم الدهن الخام، الأوزان النسبية للطحال والغدة الدرقية والبنكرياس وقناة المبيض ومعظم قياسات الدم بينما لم تتأثر باقي الصفات المدروسة.

يتضح من نتائج هذه الدراسة أن تدعيم علائق الدجاج البياض بكلوريد البوتاسيوم (١,٦%) أو فيتامين هـ (٣٠٠ ملجم/كجم) أو كليهما معا يمكن أن يستخدم كوسيلة فعالة لتخفيف الأثار السلبية للإجهاد الحراري علي الحالة الفسيولوجية للدجاج البياض.