Effects of Dietary Vitamin C Supplementation on Some Serum Biochemical Parameters of Laying Japanese Quails Exposed To Heat Stress (34.8°C)

K. SEYREK[0], C. YENISEY[00], M. SERTER[00], F. KARGIN KIRAL[0], P. A. ULUTAS[0] and H.-E. BARDAKCIOGLU[000]

[0]*Department of Biochemistry, Faculty of Veterinary Medicine, University of Adnan Menderes, Aydin, TURKEY*
[00]*Department of Biochemistry, Faculty of Medicine, University of Adnan Menderes, Aydin, TURKEY*
[000]*Department of Animal Science, Faculty of Veterinary Medicine, University of Adnan Menderes, Aydin, TURKEY*
Corresponding author
Dr. Kamil SEYREK, Departement of Biochemistry, Faculty of Veterinary Medicine, University of Adnan Menderes, Aydin, TURKEY. E-mail:kmseyrek@hotmail.com

SUMMARY

This experiment was conducted to evaluate the effects of vitamin C (L-ascorbic acid) on some biochemical serum parameters such as albumin, globulin, creatinine, cholesterol, VLDL, calcium, triglyceride, glucose concentrations and alkaline phosphatase (ALP) activity. Forty eight laying Japanese quails were divided into four groups and exposed to heat stress (34.8 ± 1.25°C) for 75 days. Animals in control group were fed with a basal diet, whereas experimental animals were fed with a basal diet supplemented with either 150, 250, 500 mg of L-ascorbic acid/kg of diet. Compared to controls, the serum concentrations of cholesterol, VLDL, triglyceride and the ALP activity in birds supplemented with vitamin C decreased significantly ($p<0.01$, $p<0.001$ for cholesterol), whereas albumin concentrations showed significant ($p<0.01$) elevations. Serum concentrations of creatinine, globuline, calcium and glucose were not statistically significantly altered. On the other hand, the variations of serum biochemical marker concentrations or activities were not correlated with the dietary vitamin C doses. As a consequence, dietary vitamin C supplementation reduces the biochemical adverse effects of heat stress on laying Japanese quails. Furthermore, 150 mg vitamin C/kg diet would be enough to prevent the damaging effects of heat stress on laying Japanese quails.

KEY-WORDS : heat stress - vitamin C - Japanese quail - biochemistry - serum.

RÉSUMÉ

Effets d'une supplémentation alimentaire en vitamine C sur les paramètres biochimiques sanguins des cailles Japonaises pondeuses soumises à un stress thermique (34.8°C). Par K. SEYREK, C. YENISEY, M. SERTER, F. KARGIN KIRAL, P.A. ULUTAS et H.-E. BARDAKCIOGLU.

L'objectif de cette étude est d'évaluer des effets de la vitamine C (acide L-ascorbique) sur différents paramètres biochimiques sanguins: concentrations en albumine, globuline, créatinine, cholestérol, VLDL, calcium, triglycérides, glucose et activité des phosphatases alcalines (PAL). Quarante-huit cailles japonaises pondeuses ont été réparties en 4 groupes et exposées à un stress thermique (34.8°±1.5°C) pendant 75 jours. Les animaux du groupe contrôle ont seulement reçu l'aliment de base tandis que les animaux des autres groupes ont reçu cet aliment supplémenté par 150, 250 ou 500 mg d'acide L-ascorbique par kg d'aliment. Les concentrations sériques de cholestérol, VLDL, triglycérides et les activités sériques de PAL ont été significativement diminuées ($p < 0.01$ et $p < 0.001$ dans le cas du cholestérol) chez les oiseaux supplémentés alors que les concentrations sériques en albumine ont augmenté ($p < 0.01$). Les concentrations sériques en créatinine, globuline, calcium et glucose n'ont pas été modifiées par la supplémentation en vitamine C. D'autre part, aucune corrélation entre l'intensité des variations des différents marqueurs analysés et le dosage employé de vitamine C n'a été obtenue.

Par conséquent, une supplémentation alimentaire en vitamine C réduit les effets biochimiques d'un stress thermique chez les cailles japonaises pondeuses. De plus, 150 mg/kg de vitamine C est une dose suffisante pour prévenir les effets néfastes d'un stress thermique dans cette espèce.

MOTS-CLÉS : Stress thermique -Vitamine C - Caille japonaise - Biochimie - Sérum.

Introduction

An important economic goal of the poultry industry is to increase the productivity. However, the productivity of this industry is threatened by climatic, physical, and social stressors [7]. High temperature in poultry-house reduces feed intake, body weight gain and feed efficiency. Furthermore, high ambient temperature causes the release of corticosterone and catecholemines. Corticoids depress immune system function, reduce serum protein concentrations and increase blood glucose concentrations which have damaging effect on poultry performances by decreasing body weight gain and egg production. Therefore, maximum production requires the elimination of the deleterious impacts of environmental stressors [2, 7]. Several methods are available to reduce the adverse effects of high ambient temperature on performance of poultry. Since cooling of animal buildings is expensive, alternative methods are preferred to reduce the negative effects of environmental stressors. By decreasing synthesis and secretion of corticosteroids, dietary vitamin C has been reported to have beneficial effects on poultry housing under heat stress [1, 21, 24, 29].

Vitamin C, also referred to as ascorbic acid or ascorbate, participates in numerous biochemical reactions. Although poultry can synthesise vitamin C, dietary supplementation with vitamin C is thought to be beneficial when metabolic demand likely exceeds endogenous supply [13, 27]. For example, prior treatment with vitamin C reduced the undesirable physical effects (e.g., immunosuppression, intense

adrenocortical activation, weight loss) of diverse stressors, such as high temperatures, transportation and fasting [13, 17, 22, 27, 32].

Plasma ascorbic acid concentrations were reduced in animals stressed by environmental temperature [21]. Moreover, ambient temperature impairs absorption of vitamin C and increases the dietary requirement of this vitamin [8, 14]. Therefore, we intended to study the effects of vitamin C on some biochemical parameters in serums of Japanese quails exposed to heat stress. Furthermore, we also aimed to compare the effects of different dietary doses of Vitamin C on biochemical markers.

Material and Methods

ANIMALS

Forty eight, 11 week old laying Japanese quails obtained from the Poultry breeding Unit of Veterinary Faculty of Adnan Menderes University were used in this study. Animals kept in cages (40 x 40 x 20 cm^3) were divided into four equal groups and fed with basal diet (Table 1) eventually supplemented by ascorbic acid (0, 150, 250, 500 mg L-ascorbic acid/kg of diet). Vitamin C was provided by a commercial company (BASF® Aktiengeselschaft, Germany). Water and diets were offered *ad libitum*. The house of birds lit for 16 h per day, temperature and humidity were measured 3 times a day (at 09h.00, 13h.00, and 20h.00). The mean value of the daily temperature was 34.8 ± 1.25°C. Average relative humidity in the house of animals was 43.8 ± 0.53%. The length of the experiment was seventy-five days.

Ingredients	Dry matter (%)
Crude protein	21
Crude cellulose	6
Crude ash	7
Limestone	10
Lysine	1.26
Methionine	0.45
Cysteine	0.85
Ca	0.90
P	0.60
Na	0.15
NaCl	0.30

Table I : Ingredients of the basal diet consumed by Japanese quails exposed to heat stress.

BIOCHEMICAL ANALYSIS

At the end of experiment, animals were killed by decapitation, and blood samples were collected in tubes. Serum was separated by centrifugation at 1700 g, at room temperature, for 10 minutes. Serum creatinine, albumin, globulin, total cholesterol, VLDL, triglyceride, calcium, glucose concentrations and ALP activities (Alkaline phosphatases) were measured using commercial available kits (Med-Kim, Izmir/Turkey) with an autoanalyser (ILAB 900). The analyses were carried out according to the manufacturer's instructions.

STATISTICAL ANALYSIS

Differences among groups were tested by one-way ANOVA. Duncan test was used to find out the group effects. $P<0.05$ was set as limit of significance.

Results

Serum concentrations of total cholesterol, VLDL, triglyceride and ALP activity in animals received dietary vitamin C supplementation decreased significantly ($P<0.01$, $P<0.001$ for cholesterol), in comparison to control animals (Table II). On the contrary albumin concentrations significantly increased ($P<0.01$), whereas the serum concentrations of creatinine, globulin, and calcium showed no significant alterations. Although the means of glucose concentrations decreased in animals received vitamin C supplementation, differences among groups were not statistically significant. For any biochemical marker, no relation between the dietary vitamin C doses and the alteration of serum marker concentration could be evidenced.

Discussion

High ambient temperature impairs absorption of vitamin C and increases the dietary requirement of this vitamin [8, 14]. Supplementation of vitamin C may be beneficial to poultry exposed to high ambient temperature by improving zootechnical performances such as egg quality and digestibility of nutrients [2, 3, 11, 18, 33]. Results obtained from the present study also indicated that dietary vitamin C has beneficial effects on the laying Japanese quails. Numerous workers [5, 17] have reported that vitamin C supplementation increases serum albumin concentrations. In the present study, we also recorded elevated serum albumin concentrations in birds received vitamin C. At temperatures above thermoneutral zone, corticoid secretion increases as a response to stress. It has been reported [17, 23] that ascorbic acid supplementation reduces the synthesis of corticoid hormones in birds under heat stress. As corticoids induce gluconeogenesis from non-carbohydrate precursors such as lactate, amino acids and glycerol [20], decrease of glucocorticoids secretion could limit lipid and protein catabolism [16]. The increases of serum albumin concentrations observed in experimental groups could be partially explained, by the reduction of synthesis and secretion of corticoids in birds received vitamin C.

Serum biochemical parameters	Control n = 11	Dietary Vitamin C supplementation			P
		150 mg/kg n = 13	250 mg/kg n = 11	500 mg/kg n = 12	
Creatinine (mg/l)	4.30 ± 0.27	4.60 ± 0.23	4.50 ± 0.31	4.00 ± 0.31	NS
Albumin (g/l)	13.0 ± 0.6	14.8 ± 0.6**	16.5 ± 0.5**	16.5 ± 0.7**	< 0.01
Globulin (g/l)	19.1 ± 1.6	18.6 ± 1.1	20.6 ± 1.6	18.3 ± 1.3	NS
Cholesterol (g/l)	2.563 ± 0.341	1.419 ± 0.077***	1.500 ± 0.172***	1.332 ± 0.147***	< 0.001
VLDL (g/l)	3.634 ± 0.361	2.498 ± 0.401**	1.683 ± 0.199**	1.768 ± 0.308**	< 0.01
Triglyceride (g/l)	6.738 ± 0.585	5.418 ± 0.402**	4.901 ± 0.543**	4.103 ± 0.235**	< 0.01
Calcium (g/l)	0.199 ± 0.013	0.212 ± 0.018	0.199 ± 0.018	0.186 ± 0.019	NS
ALP (U/l)	547.90 ± 29.59	336.00 ± 33.19**	388.54 ± 45.46**	354.08 ± 39.99**	< 0.01
Glucose (g/l)	2.528 ± 0.223	2.015 ± 0.162	1.966 ± 0.204	1.920 ± 0.176	NS

***: P < 0.001, **: P < 0.01

Table II : Serum biochemical parameters concentrations in Japanese quails exposed to heat stress. Results are expressed as means ± standard deviations.

Decreased cholesterol concentrations found in the present study were in agreement with previous report [31]. Similarly, the decreases of triglyceride concentrations observed during this experiment confirmed previous studies [12, 15, 16, 30]. Several metabolic pathways would be involved in the reduction of lipid mobilisation and catabolism. Firstly, when birds were supplemented with ascorbate, the corticoid secretion was reduced and the lipoprotein and tissue lipases were consequently not stimulated. As a result, lipids and cholesterol were not mobilised from tissues. Secondly, ascorbate is necessary for the transformation of cholesterol to bile acids by controlling the microsomal 7α-hydroxylation. As this reaction is the rate-limiting step of the cholesterol catabolism in liver, ascorbic acid deficiency induces a marked slowing down of this reaction, leading to cholesterol accumulation in liver and in blood [25]. By contrast, ascorbate supplementation will accelerate the conversion of cholesterol into bile acids, decreasing cholesterol concentrations in liver and in serum. Because cholesterol is transported in blood by lipoprotein complexes (VLDL, LDL and HDL), cholesterol and lipoprotein concentrations were positively correlated [20]. So, in vitamin C-deprived birds, high cholesterol concentrations were accompanied by high VLDL concentrations, whereas in-groups supplemented with ascorbate the VLDL concentrations were significantly reduced. Thirdly, as ascorbate is required for carnitine synthesis [9], the mitochondrial concentrations of this amino-alcohol is increased. By transporting long chain fatty acids from cytoplasma into mitochondrial matrix of muscle cells [10, 19, 26, 28], carnitine improves beta-oxidation of lipids, leading to reduction of serum triglyceride concentrations.

SAHIN *et al.* [30] reported remarkable elevations of ALP activity in Japanese quails exposed to high ambient temperature supplemented with vitamin C. However, in our study, the serum ALP activities in birds received vitamin C were significantly lower than in control birds. But, the relatively low ALP activity in vitamin C-supplemented animals may be connected with the reduced status of corticoids which are well known as strong inductors of this enzyme. Contrary to the finding of SAHIN *et al.* [30], we were not able to detect any significant alteration in serum glucose concentration. A low reduction was noticed in vitamin C-received animals but it remained non-significant.

Ascorbic acid stimulates 1.25 dihydroxy-cholecalciferol synthesis in birds and indirectly increases calcium mobilisation from bone, suggesting that vitamin C could affect serum calcium concentration [4, 6]. Contrary to this, no statistically significant difference in serum calcium concentration were observed between groups in the present study. This discrepancy may be related to the use of limestone which was systematically added in diets of birds and caused high serum calcium concentrations in all birds.

Results from the present study showed that the dietary vitamin C supplementation alleviates the adverse biochemical effects of heat stress on laying Japanese quails. Before to recommend the generalised use of vitamin C supplementation on laying Japanese quails, it will be necessary to analyse the benefit of such a procedure on zootechnical and growth performances of birds. Furthermore, because no relation dose-effect was obtained, 150 mg vitamin C/kg diet would be enough to prevent the damaging effects of heat stress on laying Japanese quails.

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