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Research Article

Chromatographic Analysis of Vitamin E (α - Tocopherol) in Blood of Chicken Under Heat Stress and its Correlation with Serum Muscle Injury Markers

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Abstract

Study was carried out to determine the effect of heat stress on broiler birds during hot humid climate with mean ambient temperature of 42 0C and a 58 per cent relative humidity on the serum muscle injury markers and vitamin E. For the study broiler birds from different poultry outlets covering a population of 1.5 lakh birds were selected. Samples were also collected from 25 apparently healthy birds with no indication of heat stress. Approximately 2-4ml of blood was collected in vacutainers without anticoagulant at the time of slaughter. The two muscle injury markers, creatinine kinase (CK) and lactate dehydrogenase (LDH) were found to be significantly raised in the serum of birds under heat stress ($p \leq 0.01$). Concentration of vitamin E in the form of α tocopherol was estimated by Ultra High-Performance Liquid Chromatography (UHPLC). A strong linear correlation was determined between occurrence of heat stress and serum vitamin E concentration at the 0.01 level (2-tailed). This study indicates the vitamin E requirement of birds during hot humid climate is more and supplementation of Vitamin E in diet may prevent losses due to heat stress

Keywords: Chromatography; Heat Stress; Creatinine Kinase; Lactate Dehydrogenase and Vitamin E.

Abbreviations

AST: Aspartate Aminotransferase; CK: Creatinine Kinase; IAEC: Institutional Animal Ethics Committee; LCMS: Liquid Chromatography Mass Spectrometer; LDH: Lactate Dehydrogenase; UHPLC: Ultra High-Performance Liquid Chromatography

Introduction

Poultry production has been reported to be the fastest growing livestock industry, more particularly in tropical and sub-tropical regions of the world. The establishments of poultry farms in new areas means that live birds of all ages have to be transported by road, across different ecological zones throughout the year [1]. Heat stress is the major problem that compromise performance and productivity through reducing feed intake and decreasing nutrient utilization, growth rate, egg production, egg quality and feed efficiency, which lead to economic losses in poultry [2]. It is estimated annually that the damage to agriculture related to heat stress is of the order of 1.69 to 2.36 billion dollars. Of this amount, 165 million is from poultry production [3,4]. As a general term, stress is used to describe the sum of non-specific responses or defense mechanisms of the body when confronted with abnormal or extreme demands [2,3]. The modern broiler chicken is bred for meat production that has been genetically improved to allow for rapid growth and deposition of muscle tissue [5]. These characteristics were obtained due to advances in genetics and applied nutrition. However, the metabolism of these birds is more pronounced and their thermoregulation ability is ineffective under conditions of high temperature and moisture [6] making them highly susceptible to heat stress. Hyperthermia induces blood compositional changes, including metabolic and endocrine alterations with oxidative damages [6].

The importance of vitamin E dietary supplementation to broilers under heat stress in relation to metabolism, growth performance and quality of animal products and its effects on immune system has been the subject of interest [4]. Although several studies have been conducted to determine the effect of vitamin E supplementation on performance of birds and for amelioration of toxicity [7-9],

literature citing the optimum vitamin E concentration in blood of broilers is lacking.

The present work was conducted to observe the effect of heat stress on broiler birds during hot humid climate on the serum muscle injury markers and oxidative stress in correlation of serum vitamin E concentration.

Materials and Methods

Area of Study

The study was conducted in the College of Veterinary Science and Animal Husbandry Jabalpur, Madhya Pradesh, India. Study was conducted during hot humid climate with mean ambient temperature of 42 ^{0}C and a 58 per cent relative humidity. Consent was obtained from Institutional Animal Ethics Committee for the research work (88/IAEC/17-18).

Birds

For the study broiler birds (6 weeks), representing different poultry outlets covering a population of 1.5 lakh birds were selected. Blood samples were collected from birds bought from different farms at the time of slaughter. All the birds selected were having open mouth breathing, difficult respiration and spreading of wings. Examination of the carcass after slaughter revealed pale yellow liver, enlarged heart with right ventricular hypertrophy and pale parboiled muscles lesions indicative of heat stress.

Samples were also collected from apparently healthy 25 broiler birds (6 weeks) with no indication of heat stress.

Approximately 2-4ml of blood was collected in vacutainers without anticoagulant.

Estimation of muscle injury markers in serum

Estimation of known muscle injury markers like creatine kinase (CK), lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) in serum samples was done using Semi-Automatic Biochemical Analyzer with respective kits.

Estimation of vitamin E

Concentration of vitamin E in the form of α tocopherol was estimated by Ultra High-Performance Liquid Chromatography (UHPLC) a unit of High-Performance Liquid Chromatography Mass Spectrometer (LCMS- 8030, Shimadzu, Japan).

Reagents

The chemicals used for estimation of vitamin E. DL-Alpha Tocopherol Acetate Standard for HPLC (Sigma Aldrich, 47786), Hexane for HPLC ≥ 95% (Sigma Aldrich, 2705044), Methanol for HPLC ≥ 99.9% (AS061, Hi-Media).

Chromatography conditions

The UHPLC system was equipped with Photodiode Array UV-Vis Detector. Chromatography conditions were maintained as described earlier [10]. Particle separation was done using Hypersil Gold column with C18 selectivity (Thermo Scientific, USA, column dimensions: 150* 2.1 mm, particle size: 1.9 µm) and the temperature of column was set at 30ºC. The mobile phase is methanol 100% (UHPLC grade) with flow rate adjusted at 0.4 ml/min. Peak separation was accomplished after 8.5 minutes and 10 µl treated samples were required for injection. Figure 1, showing ultra-high-performance liquid chromatography (UHPLC) chromatogram of vitamin E standard.

Procedure

Serum was separated from blood and used for the estimation of vitamin E as per the method described by previous workers [11]. Briefly, 200 µl of ethyl alcohol is added to 200 µl of serum (1:1 v/v), mixed on a vortex and then there is addition of 2 ml of hexane. Samples are shaken for 5 min and centrifuged for 2 min. The hexane layer is transferred to other test tube. Extraction is again repeated; hexane layer is mixed and evaporated to dryness in lyophilizer. The dry residue was dissolved in 200 µl of mobile phase. The obtained samples were filtered through 0.2 µm syringe filter and collected for chromatographic analysis.

Statistical analysis

Data gathered from the study were tabulated and analyzed using statistical descriptive analysis comprising mean, standard error, standard deviation, ANOVA and correlation coefficient. For this purpose, IBM SPSS Statistics Version 24.0 was used. Statistical significance was set at ($p < 0.01$). All data were expressed as means ± standard error of the means.

Results and Discussion

The serum concentration of CK and LDH were significantly increased in birds under heat stress whereas AST showed a nonsignificant increase.

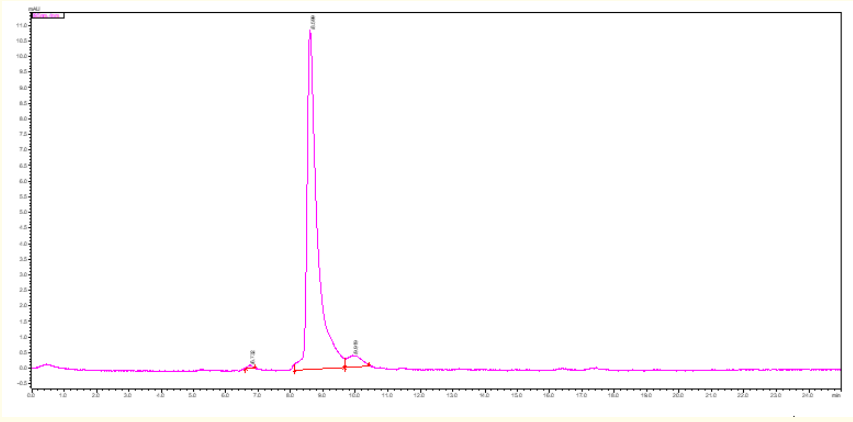

Figure 1: Ultra high-performance liquid chromatography (UHPLC) chromatogram of vitamin E standard.

The mean concentration of CK, LDH and AST in the serum of birds in heat stress was determined to be10.99 ± 1.34, 2131.46 ± 99.99 and 232.03 ± 13.38 (IU/L) respectively. The corresponding mean concentration of CK, LDH and AST in healthy birds was found to be 5.19 ± 0.37, 1671.73 ± 86.09 and 230.30 ± 9.80(IU/L) respectively.

The concentration of vitamin E in serum of birds under heat stress and apparently healthy birds was estimated. The vitamin E concentration in the two groups is given in table 01. The mean vitamin E concentration in serum of birds under heat stress was determined to be 6.85 ± 0.13 µg/ml whereas the Vitamin E concentration in serum of healthy birds was determined as 7.07 ± 0.07 µg/ml. A strong linear correlation was also determined between occurrence of heat stress and serum Vitamin E concentration at the 0.01 level (2-tailed).

The correlation in the concentration of different markers in the serum of healthy chicken and chicken under heat stress was determined and presented in table 2. In birds with heat stress the muscle injury markers CK, LDH and AST were found to be weakly correlated with muscle degeneration whereas a strong correlation was determined between concentration of serum vitamin E and muscle injury.

Animals have been endowed with known zones of thermal comfort that vary by species, physiological status and the envi-

S. No.	Chicken	Mean ± S.E. (µg/ml)	Standard Deviation	Correlation Coefficient (r)
1	Healthy	7.07 ± 0.07	0.28	r= 0.727** (strong linear correlation) p = 0.002* (S)
2	Heat Stress	6.85 ± 0.13	0.50	

Table 1: Concentration of vitamin E in serum of birds.

** Correlation is significant at the 0.01 level (2-tailed).

* Mean concentration of serum vitamin E significantly differs between heat stress and healthy birds.

Group	CK (IU/L)	LDH (IU/L)	AST (IU/L)	Vitamin E (µg/ml)
Heat Stress	10.99	2131.46	232.03	6.85
Healthy	5.19	1212.00	230.30	7.07
	r = 0.299 (weak linear correlation) p = 0.146 (NS)	r = -0.134 (weak negative linear correlation) p = 0.524 (NS)	r = -0.172 (weak negative linear correlation) p = 0.411(NS)	r = 0.727** (strong linear correlation) p= 0.002* (S)

Table 2: Correlation in the concentration of different markers in the serum of healthy chicken and chicken with heat stress.

** Correlation is significant at the 0.01 level (2-tailed).

ronmental conditions of air, temperature and solar radiation [12]. Hyperthermia induces blood compositional changes, including metabolic) and endocrine alterations and oxidative damages [6]. Changes to the serum metabolites also reflect the degree of oxidative damage in poultry tissues caused by heat stress. Increased ROS production may lead to nonspecific modification of lipids and proteins, which may then result in bioenergetic dysfunctions [5]. Vitamin E prevents oxidative damage to cellular structures and tissues through breaking reactions of free radicals and participates in the maintenance of an appropriate structure of cellular membranes [4].

The serum ALP, CK, AST and LDH are used as indicators of muscle and liver damage [6,13]. Few scientists have reported that the muscle isoenzyme (CK-MM) was the predominant form in plasma (99 per cent) and its activity increased in response to an episode of acute heat stress [14]. Similar to our finding several earlier workers also found increased concentration of CK, LDH and AST in birds under heat stress [15-18]. Sustained heat stress exacerbates the oxidative stress in tissues and significantly increases activities of serum CK, LDH and AST [6], which is consistent with the results of this study.

Vitamin E has the potential to reduce the undesirable effects of exposure of broilers to high temperatures [4]. High environmental temperatures decrease the concentrations of vitamins and minerals in the blood serum and increase their excretion [12]. It has been earlier opined that heat stress promotes the release of catecholamines and corticosteroids that induce lipid peroxidation of membranes, including membranes of T and B lymphocytes [19]. Vitamin E stimulates the enzyme glutathione peroxidase activity of circulating neutrophils and macrophages and also promotes increased activity of T lymphocyte. This in turn causes enhanced phagocytic activity with more antibody production and increased immune activity. The inclusion of vitamin E in feed for broiler has resulted in positive effects on growth performance when supplemented at higher levels during heat stress as found by several workers [20,21]. It has been also observed that inclusion of Vitamin E in the diet of broilers enhanced the immune response of birds [23]. Collaborating the above findings a strong linear correlation was also determined in the present study between occurrence of heat stress and serum Vitamin E concentration

Many workers have recommended the supplementation of increasing levels of vitamin E for broilers under heat stress, as a measure to minimize the effects of heat on performance and survivability [25,26]. However, there are contradicting opinions regarding the benefit of vitamin E supplementation at higher levels than recommended in the diet of broilers to combat the effect of high ambient temperature. It has also been observed that in order to reduce the harmful effects of high temperatures in poultry production, vitamin E supplementation is a viable alternative for the sector [4]. Some studies indicate its potential antioxidant effect is able to modulate inflammatory responses and physiological adjustments to mitigate the undesirable effects of exposure of broilers to high temperatures. A meta-analysis was conducted on the effect of vitamin E supplementation on growth performance, meat quality and immune response of male broiler chickens and the study concluded that there is no reason to supplement vitamin E as a growth performance promoter [9].

Conclusion

In the present study significant differences were observed in the concentration of serum vitamin E between the healthy birds and birds under heat stress. This indicates that plausibly the Vitamin E requirement of birds during hot humid climate is more and supplementation of Vitamin E in diet may prevent losses due to heat stress.

More studies on broilers to define the exact role of Vitamin E in preventing the losses due to heat stress involving a larger population size and variable thermal gradient need to be done.

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Conflict of Interest

There is no conflict of interest in the publication of research work.

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