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Punam
Veterinary Officer, Govt. Veterinary Hospital, Jakhera, Nagaur, Rajasthan, India

Kavita Rohlan
Department of Veterinary Anatomy, Arawali Veterinary College, Bajor, Sikar, Rajasthan, India

Ruchi Maan
Assistant Professor, Department of Veterinary Physiology, CVAS, Bikaner, Rajasthan, India

Kapil Kumar Godara
M.V.Sc. Scholar, Department of Livestock Production and Technology, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

Sanwar Mal
Department of Veterinary Anatomy, Arawali College of Veterinary Science, Sikar, Rajasthan, India

Vijay Kumar Yogi
Veterinary Officer, Department of Animal Husbandry, CVAS, Barmer, Rajasthan, India

Corresponding Author:
Punam
Veterinary Officer, Govt. Veterinary Hospital, Jakhera, Nagaur, Rajasthan, India

Effect of ambience and age on serum cholesterol level of broiler chickens

Punam, Kavita Rohlan, Ruchi Maan, Kapil Kumar Godara, Sanwar Mal and Vijay Kumar Yogi

Abstract
This research designed to assess the influence of age and ambience on the levels of serum cholesterol in broiler chickens of 2 to more than 8 weeks of age. We used 240 blood samples of broilers in a completely randomized experimental design, composed of two ambiences and 3 age groups. The two thermal environments are moderate (control) and hot humid. Concerning the environment, significant differences in the levels of serum cholesterol was observed. There was a significant interaction between the environment and the age for cholesterol values. The age of the birds influenced the values of serum cholesterol.
In conclusion, heat stress, at 39 ºC, from 2 weeks to older than 8 weeks of age increases serum cholesterol indicating that birds may have adapted to heat stress. Age influenced the levels of cholesterol.

Keywords: Broiler, cholesterol, age

Introduction
The present study was conducted on serum samples of recently slaughtered heat stressed broilers of different age group. Many types of stressors i.e. external and internal are responsible for diminishing productive and reproductive profile of birds (Surai and Fisinin, 2016) [17]. The overall performance of body is adversely affected by heat stress as it is responsible for fluctuations of blood parameters level and redox potential in broilers. High rise in environmental temperature causes adverse effect on poultry production, especially in tropical countries (Kadim *et al*., 2008) [8]. When birds comes in heat stress, they use various methods to combat heat stress i.e. biological, hormonal, and physiological etc. (Oguntunji and Alabi, 2010) [13].
The poultry birds metabolic process is well defined and their temperature maintenance capacity is not so pronounced under condition of high temperature and humidity, being highly susceptible to heat stress, with negative effects on the performance ((Uzum *et al*., 2013) [19]. Thus, the purpose of this research was to assess the influence of age and cyclical heat stress, on serum levels of cholesterol.

Materials and Methods
For the conduction of proposed plan, blood samples of 240 apperently healthy broilers were taken. In each environment 120 broilers of different age group were sampled. The data were analysed using IBM SPSS version 20.

Process of cholesterol estimation
Total cholesterol was determined by the method of Sackett as described by Varley (1988) [20].

Principle
Bloor's method is modified in cholesterol estimation procedure in which ethanol-ether mixture is added in blood which leads to precipitation of protein and extraction of cholesterol. After centrifugation, supernatent fluid obtained which was evaporated, cholesterol taken up in chloroform, and recorded colorimetrically by the Liebermann-Burchard reaction.

Reagents
1. Ethanol-ether mixture.
2. Acetic anhydride-sulphuric acid mixture.
3. Chloroform
4. Stock standard solution of cholesterol.
5. Standard salutation for use.

Procedure
Firstly for estimation of optical density of serum cholesterol level in broilers, separate serum from blood by centrifuging blood in centrifuge machine at 3000 rpm for 15 minutes. Then take a glass tube and after that add 0.2 ml serum and 10 ml ethanol ether mixture in it. Shake it vigorously. Keep tube in horizontal position for one hour so that the precipitate can mix properly. After mixing of precipitate, this solution centrifuged for some time. After centrifugation, supernatant fluid was discarded and remaining precipitate was allow to dry for some time. After drying, left residue was dissolved in 5ml chloroform. Then take another test tube and add 5 ml standard cholesterol solution containing 0.4 mg of cholesterol. In both test tube, add 2 ml acetic anhydride and sulfuric acid mixture. Then it was allowed to stand for 15 minutes in dark place. The chloroform was used as blank. After that set optical density of spectrophotometer at zero level and take optical density (O.D.) of unknown and standard solution at 680mμ.

Calculation
Mg cholesterol per 100 ml blood =

$$\frac{\text{Reading of unknown}}{\text{Reading of standard}} \times 0.4 \times \frac{100}{0.2}$$

Conversion factor of mg/dl to mmol/l for cholesterol i.e. mg/dl of cholesterol x0.0258 =mmol/l

Standard curve for cholesterol determination
It was plotted as follows:

Cholesterol (mmol/l)	1.3	2.59	3.89	5.18	7.77	10.36	12.95
O.D.	0.1	0.2	0.30	0.42	0.6	0.81	1.0

Results and Discussion
The mean ± SEM values and analysis of variance of serum cholesterol during different ambiences and age groups are presented in table 1 and 2, respectively. In the present experiment the mean value of serum cholesterol during moderate ambience was found in accordance to (Gumus and Imik, 2016; Leke *et al*., 2018; Ebrahimzadeh *et al*., 2018; Saracila *et al*., 2019; Hassabelrasoul Eljack *et al*., 2020 and Makola *et al*., 2021) [6, 9, 4, 15, 7, 11].

Effect of hot humid ambience on serum cholesterol
The overall mean value of serum cholesterol was highly significant (p≤0.01) during hot humid ambience as compared to overall moderate mean value. A highly significant (p≤0.01) effect of variation in ambience was observed by analysis of variance. The overall mean value during hot humid ambience increase 9.93% as compare to overall mean value of moderate ambience.
Mohamed *et al.* (2020) [12] observed decreased value of serum cholesterol due to heat stress. Attia and Hassan (2017) [2] reported increase in plasma cholesterol levels as compare to thermoneutral environment. Awad *et al.* (2017) [3] found no effect of diet on serum cholesterol levels. Luo *et al.* (2018) [10] found decreased serum cholesterol levels in broilers. Ambience related changes in serum cholesterol levels can be due to physiological modulations which occurs in bird body to cope up with the oxidative stress generated.

Effect of age on serum cholesterol
Age effects showed a non-significant increase in the mean value being highest in the broilers of 2 weeks of age. The non- significant age effects were revealed by analysis of variance.
On percent basis maximum increase in the means value of serum cholesterol was found in broilers of 2 weeks of age (11.50%). Sujatha *et al.* (2010) [16] found decreased in plasma cholesterol levels after 3 weeks of age in broilers. Torki *et al*. (2014) [18] also found age related changes in serum total cholesterol values. Akbarian *et al*. (2015) [1] correlated age with serum cholesterol levels. Oloyo (2003) [14] found higher levels of cholesterol in younger birds and correlated the effect of age on hen eggs. Wood *et al.* (1961) [21] found serum cholesterol levels were higher in 1 week old chicks than mature birds. Zawacka *et al.* (2017) [22] showed the effect of age on plasma concentration of total cholesterol in cockerels and capons. Fakolade (2015) [5] studied effect of age on concentration of cholesterol in chicken meat.
Interactions of ambience with age show highly significant (p≤0.01) effect which showed the effect of ambience on the birds of all age groups.

Table 1: Mean ± SEM values of serum cholesterol (mmol L^{-1}) in non -descript broilers

Key effects	Subgroups	Mean ± SEM values	
		Moderate	Humid hot
Age	2 weeks(40)	$4.26^{a, NS}$± 0.15	$4.75^{b, NS}$± 0.17
	4-5 weeks(40)	$4.42^{a,NS}$± 0.09	$4.86^{b, NS}$± 0.17
	>8 weeks(40)	$4.60^{a,NS}$± 0.09	$4.99^{b, NS}$± 0.12
Overall mean values		4.43^{A} ± 0.06	4.87^{B}± 0.09

$^{A, B}$ marks highly significant (p≤0.01) differences between overall mean values of both ambience
^{a,b} marks highly significant differences (p≤0.01) between mean values of different age groups in a row
^{NS} marks non-significant differences between mean values of different age groups in a column

Table 2: Analysis of variance of serum cholesterol (mmol L^{-1}) in non -descript broilers

Source of variation	p-Value
Ambience	0.000
Age	0.121
Ambience X Age	0.002

Conclusion
The levels of cholesterol increased with increase in age in the present study which may be due to nutrient mobilization with increasing age and hormonal imbalance in birds.

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