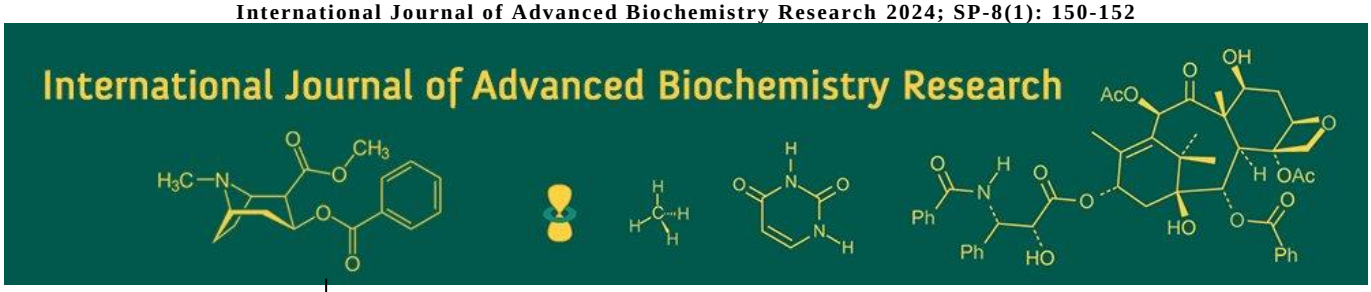

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Altered level of serum calcium during hot humid ambience in broilers

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Abstract
We done our research on the effects of hot humid ambience on serum electrolyte concentration i.e. calcium. Total 240 broilers of different age group were arbitrarily allocated in 2 ambiences i.e. 120 broilers in each moderate and hot humid ambience and samples were taken during both ambiences. We recorded that serum level of electrolyte i.e. calcium was decrease in broiler chickens significantly in hot humid ambience, but highest percent decrease was seen in youngest group of broilers. These results suggested that hest stress have significant impact on serum electrolyte levels which helps in examination of degree of damage of different body organs.

Keywords: Ambiences, hest stress, calcium

Introduction
The poultry sector is continuously suffering from high ambient temperature of environment in past few years. As a result, heat stress causes massive fatalities, particularly in hot areas of the world and the reason of this is its harmful impact on body development rate, mass of different organ, suppression of immunity and death (Smith, 2003; Gupta *et al*., 2014; Habibian *et al*., 2014; Hosseinivashan *et al*., 2016; Wan *et al*., 2017) [20, 11, 12, 13, 25]. A number of earlier research studies on heat stress in poultry are running currently, using specific electrolyte parameters could help by practical assessment of administration practices, dietary status, organ work and health situation (Hosseinivashan *et al*., 2016; Amrutkar *et al*., 2016; Wan *et al*., 2017) [13, 4, 25] with main aimed on the alteration in level of serum biochemical and electrolyte parameters (Azad *et al*. 2010; Hosseinivashan *et al*., 2016; Wan *et al*., 2017) [6, 13, 25]. Therefore, the aim of this research was to assess the impacts of heat stress during hot humid ambience and fluctuation in the serum calcium level during the different age group. This parameter demonstrated to be assist in interpreting metabolic changes in the animal body during heat stress.

Materials and Methods
The research and process of research was done in agreement with the rule of the Ethics Committee and the protocol was permitted by Cvas, Bikaner. Segregation of blood sample was done from slaughter house of Bikaner. The total blood samples of 240 broilers were taken in two ambiences i.e. moderate and hot humid ambience. In moderate ambience 120 blood samples were taken. During hot humid ambience again 120 blood samples were taken. The broilers are categorized in 3 age group i.e.2 weeks, 4-6 weeks and older than 8 weeks of age. The calculation of Data was done by GLM modal of IBM SPSS Version 20.0 software.

Procedure for calcium estimation
The determination of calcium was done by standard modification method of Clark-Collip with Kramer-Tisdall as suggested by Oser (1976) [19] in which correction factor for poultry birds as suggested by Lumeji (1999) [15].

Principle
Calcium estimation is based on the principle that calcium is precipitated from blood in form of oxalate solution and this solution is now titrated with potassium permanganate upto fine pink colour and after that final reading was taken.

Reagents

1. 1 ml of 4% ammonium oxalate solution.
2. Dilute ammonia, 3 ml
3. 2 ml 1 N sulfuric acid
4. N $KMnO_4$.
5. Calcium working standards.

Procedure

Firstly we were taken a graduate centrifuge tube up-to 15 ml marking. Then add 2 ml serum, 2 ml distilled water and 1 ml 4% ammonium oxalate solution in this centrifuge tube and mix well. Keep centrifuge tube in horizontal position for 30 min. After 30 minute, again mix the contents of centrifuge tube. After mixing contents of centrifuge tube, keep centrifuge tube in centrifuge machine and rotate it under 1500 rpm for 5 minutes. Then carefully discard supernatant fluid of centrifuge tube. The precipitated portion is remaining in centrifuge tube. Centrifuge tube kept in inverted position for drying up-to 5 minute. After that, cover the mouth of centrifuge tube with filter paper. Then add 3 ml diluted ammonia in this centrifuge tube with very fine stream. Then centrifuge this suspension and drain again as before. After that add 2 ml sulfuric acid so that precipitate can mix with it and make solution. Then place this centrifuge tube in boiling water bath for about 1 minute. After 1 minute, titrate this solution with 0.01N $KMnO_4$ solution upto coming fine pink colour.

Calculation

One ml of 0.01N $KMnO_4$ is equivalent to 0.2 mg of Ca.
(x-b) X 0.2 X 100/2= mg calcium per 100 ml serum.
Conversion factor of mg/dl to mmol/l for calcium i.e.mg/dl of calcium x0.249 =mmol/l
where x equals the number of ml of permanganate required in the titration, and b is the blank, i.e., the number of ml of permanganate required to titrate 2 ml of sulfuric acid solution to the usual end point.

Result and Discussion

ANOVA and mean ± SEM values of serum calcium are presented in table 2 and 1. Percent changes in serum mean value of calcium of every age group of the broilers are presented in table 3. Overall mean value of serum calcium during moderate ambience was 2.60±0.02 mmol L^{-1}which was obtained from 120 birds irrespective of age group. The overall mean value of serum calcium during hot humid ambience was 1.73±0.02 mmol L^{-1}irrespective of age.
Earlier researchers who detected calcium levels in serum of birds (Igwe *et al.*, 2018; Dimri *et al*., 1994; Makola *et al.*, 2021, Murali and Sherin, 2020) [4, 7, 17, 18].

Effect of hot humid ambience on serum calcium

The overall mean value of calcium was highly significantly ($p \leq 0.01$) decreased during hot humid ambience as compared to overall mean value of moderate ambience.
El Husseiny (1981) [9] detected lowered rates of calcium retention in broilers, whereas, McCormick and Garlich (1982) [16] indicated that heat stress reduced plasma calcium level. Sharma *et al.* (1986) [22] also found lowered levels of calcium in birds under high temperature. Samara *et al.* (1996) [21] related ionized calcium blood and total calcium in plasma to different environmental temperature. Ajakaiye *et al.* (2010) [27] found lower plasma calcium concentration during hot humid ambience in laying hens. Zhu *et al.* (2015) [26] detected serum calcium levels in hy-line brown hens. Gopi *et al.* (2020) [10] recorded higher calcium levels in under hot humid conditions in broiler chicken. Alagawany *et al.* (2021) [2] detected plasma calcium levels in egyptian geese. Hot humid ambience resulted in lowering of calcium in the present study which may be attributed to variation in calcium regulating hormones, so as to maintain calcium homeostasis. Some researchers also correlate decreased calcium levels to respiratory alkalosis. (Edens, 1978 and Arora, 2006) [8, 5].

Effect of age on serum calcium

The highly significant ($p \leq 0.01$) age effects were also revealed by analysis of variance.
Akbarian *et al.* (2015) [1] studied age effect on serum calcium levels of 38 days old broilers. Torki *et al*. (2014) [24] detected mean value of serum calcium in hens at age of 74 weeks. Silva *et al.* (2007) [23] correlated serum values of total calcium with age in broilers. Gharieb and Moursi (2014) determined serum calcium in healthy broiler chicks at 2 weeks of age. Allahverdi *et al*. (2013) [3] detected serum calcium concentration at 1 week of age. Similar pattern of increase in serum calcium levels with growing age was observed during both ambiences in present study.

Interactions of ambience with age

The interaction between age and ambience was highly significant ($p \leq 0.01$) for serum calcium which indicated the impact of environment on the broilers of all age groups.

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

Key effects	Subgroups	Mean ± SEM values	
		Moderate	Humid hot
1. Age	2 weeks (40)	$2.48^{a, x}$±0.03	$1.55^{b, x}$±0.02
	4-5 weeks (40)	$2.60^{a, y}$±0.04	$1.75^{b, y}$±0.02
	>8 weeks (40)	$2.71^{a, z}$±0.03	$1.90^{b, z}$±0.03
2. Overall mean values		2.60^{A}±0.02	1.73^{B}±0.02

$^{A, B}$ marks highly significant ($p \leq 0.01$) differences between overall mean values of both ambience
^{a,b} marks highly significant differences ($p \leq 0.01$) between mean values of different age groups in a row
$^{x, y, z}$ marks highly significant differences ($p \leq 0.01$) between mean values of different age groups in a column

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

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

Table 3: Percent changes in the mean value of calcium (mmol L^{-1}) in serum of broiler of different age groups during hot humid ambience

Effects	Subsets	% Change
Ambiences		Hot humid ambience
Age groups	Overall value	-33.46
	2 weeks	-37.5
	4-6 weeks	-32.69
	>8 weeks	-29.88

Conclusion

In this investigation, serum calcium level decrease during hot humid ambience as compare to moderate ambience

which may be due to the hormones involved in growth of birds which affect calcium metabolism as well.

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