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Metabolic and oxidative stress markers of rabbit bucks at peak of heat stress in Southwest Nigeria

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Target Audience: *Rabbit breeders, Physiologists and Veterinarians*

Abstract

In this cross-sectional study, serum biochemical and oxidative stress indicators in rabbit bucks of different age groups were assessed at peak of heat stress in the tropical condition of South west Nigeria. This investigation was carried out between February and March, when highest temperature-humidity index (THI) is observed in the study location. Thirty-four (34) pubertal rabbit bucks between 4 and 5 months old, thirty-six (36) mature rabbit bucks between 7 and 9 months old and thirty-five (35) adult rabbit bucks above 1 year old were used in this study. Animals were housed individually and allotted randomly into experimental units using the Completely Randomised Design (CRD). After 9 weeks of exposure of the animals to the prevailing heat stress condition in the study area; blood was sampled from all the rabbit bucks through the ear vein into sample bottles for serum biochemical and oxidative status assay using standard procedures. The result revealed that serum glucose, magnesium and sodium in adult rabbit bucks was significantly ($P<0.05$) higher than those of pubertal and mature bucks. Serum lipid peroxidation of adult rabbit bucks was significantly ($P<0.05$) higher than mature bucks, while serum superoxide dismutase, glutathione peroxidase and catalase activities of bucks were not significantly ($p>0.05$) affected by the differences in age. Total antioxidant capacity of adult and mature rabbit bucks was significantly ($P<0.05$) higher than pubertal bucks. The study concluded that at peak of heat stress, adult rabbit bucks are more sensitive to oxidative stress than mature and pubertal bucks. Efforts to combat heat stress in rabbits with antioxidant supplements should cut across three physiological age groups.

Keywords*: Age, Antioxidants, Bucks, Heat stress, Peroxides*

Description of Problem

Rabbits are homoeothermic animals with thermo-neutral zone temperature around 18–21 °C, and can regulate the heat input and output of their bodies using physical, morphological, biochemical and behavioural processes to maintain a constant body temperature [1]. Thus, when rabbits are exposed to elevated ambient temperature, imbalances are induced in their body temperature, which adversely affect their growth and reproductive traits [2] and invoke oxidative stress [3]. Exposing rabbits to heat stress has been reported to cause disturbances in their thermoregulatory system [4]. such disturbances lead to various impairments of their physiological mechanisms. Adult rabbits have been reported to respond to hyperthermic conditions and to adapt themselves to conditions of decreased ventilation and evaporation, compared with young rabbits which responded more sensitively to elevated temperature [5].

Although, it is doubtful that a single theory can explain all the mechanisms of ageing, there is general consensus that, at least in mammals; an increased accumulation of intracellular oxidative damage with time may play an important role in the process of ageing [6]. One explanation of the causes of ageing is the damage of the biological systems caused

by free radical processes [7. It has been postulated that if free radical reactions were the major cause of ageing, a reduction in their levels by antioxidants (e.g. vitamins E and C and glutathione) and antioxidant enzymes {e.g. catalase (CAT), superoxide dismutase (SOD}, and glutathione peroxidases (GPx) should, in principle retard ageing [8]. This study was therefore designed to establish preliminary and comparative results on oxidative stress in clinically healthy rabbit bucks from different age groups at peak of heat stress in Ibadan, Southwest, Nigeria.

Materials and Methods

Experimental site

The research was carried out between January and March (2014) at the rabbitry unit of the Teaching and Research Farm and the Animal Physiology Laboratory, Department of Animal Science, both of the University of Ibadan, Ibadan, Nigeria. Ibadan is situated in the rainforest agro-ecological zone of Nigeria, between lattitude 7˚ 27' 18.74"N and 7˚ 27' 19.17"N and Longitude 3˚ 53' 13.98"E and 3˚ 53' 32.69" E. The highest temperature humidity index is observed in the study area as previously reported [3, 9]. The study was carried out to investigate the physiological response of the rabbit at different age groups to the prevailing heat stress condition in study area. The Temperature Humidity Indices were 29.94 and 28.59 for February? Or January? and March, respectively.

Experimental animals, design and management

Upon commencement of the trial, animals were confirmed to be of good health status, without abnormalities and conformed to the age group categorisation. Within rabbit population in the study area, 105 bucks were categorised into age groups from the parity records of the farm; thirty-four (34) pubertal rabbits between 4 and 5 months old, thirty-six (36) mature rabbits between 7 and 9 months old, thirty-five adult (35) rabbits were above one year old were used in this study. Animals were housed individually, kept in a battery cage system in an open-sided house and allotted randomly into experimental units. The experimental design was a Completely Randomised Design (CRD). The animals were fed 5 % of their body weight with diets containing crude protein 17.05 %, digestible energy 2592.06 Kcal/kg, crude fibre 17.33%, calcium 1.59% and phosphorus 0.51%. Fresh water was made available to the animals always. Other routine and periodic management practices necessary for rabbit production were carried out accordingly.

Serum biochemical and oxidative stress assay

After 9 weeks of exposure of the animals to the prevailing heat stress condition in the study area, blood samples were collected from all bucks through the prominent ear vein into plain sample bottles and were centrifuged at 3000 rpm for 15 minutes and supernatant separated as serum. Serum biochemical indices such as total protein, glucose, total cholesterol, sodium, chloride, phosphorus, magnesium and potassium contents of the samples were determined using spectrophotometric procedure of Randox commercial assay kits. Serum oxidative stress indicators included total antioxidant capacity, lipid peroxidation, superoxide dismutase, catalase and glutathione peroxidase activity and were assayed as outlined [10].

Statistical Analysis

Data obtained in this study were each subjected to one – way analysis of variance (ANOVA) using general linear model procedure to detect significant effects with a confidence level of 95%, the New Duncan's multiple range test was used to separate significant means.

Results and Discussion

Result of serum biochemical indices of

bucks of three physiological ages during peak of heat stress is presented in Table 1. Serum protein, cholesterol and chloride were not statistically influenced by difference in age groups. Serum glucose in adult bucks was significantly (P<0.05) higher than pubertal and mature bucks which shared similar statistical values. Serum magnesium and sodium of adult bucks were significantly (P<0.05) higher than pubertal and mature bucks. However, serum phosphorus of pubertal bucks were significantly (P<0.05) higher than other age groups, but serum potassium of pubertal bucks was significantly (P<0.05) higher than adult group.

The increase in plasma glucose levels in adult bucks as observed in this study might be due to a decrease in glucose utilization in order to preserve energy during their stressed condition [11]. The observed increase in glucose of adult bucks could be due to low glucose utilization and/or increase feed consumption/metabolic body weight in the rabbits. This finding corroborates the results obtained by Ondruska *et al*. [2] who reported differences in serum biochemical parameters and found to be larger in growing animals, which might be due to the instability of regulatory mechanisms for growth and metabolism in growing rabbits [12]. Contrary to the findings of this study, glucose values were decreased in adult females of heat stressed rabbits, and increased in growing males and females of the heat-stressed rabbits [2]. The decrease in glucose levels in the heat-stressed adult rabbits could be due to increases in glucose utilization during muscular movements required for high respiratory activity [13], or due to increases in corticosteroid concentrations [11].

Decline in plasma total protein associated with high ambient temperature could be due to dilution of plasma total protein caused by the increase in water consumed [2, 14], and/ or it could be due to increase in protein utilization and amino acid transamination in the heat-stressed rabbits [12]. However, all age groups considered in this study showed similar serum protein. This signifies that the stage of development in the rabbit bucks does not affect protein and cholesterol activity during heat stress. It could also infer that the bucks across different age groups possessed similar response to heat stress.

Table 1: Serum biochemical indices of rabbits of three physiological age groups during peak of heat stress

	Age groups			
	Pubertal	Mature	Adult	
Parameter	N=34	N=36	N=35	SEM
Glucose (mg/dL)	69.86^{b}	67.73^{b}	96.97^{a}	4.56
Protein (mg/dL)	6.32	6.24	6.09	0.11
Cholesterol (mg/dL)	96.25	95.76	102.84	1.90
Magnesium (mg/dL)	0.72^{b}	0.92^{b}	1.80^{a}	0.13
Phosphorus (mg/dL)	4.61^{a}	2.64^{b}	2.61^{b}	0.28
Sodium (mEq/L)	79.09^{b}	82.25^{b}	99.13^{a}	5.61
Potassium (mEq/L)	7.41^{a}	5.73^{ab}	5.28^{b}	0.39
Chloride (mEq/L)	82.10	84.55	83.15	1.28

^{abc}Means on the same row with different superscripts are significantly (P<0.05) different.
SEM: Standard Error of Mean
Pubertal rabbits were between 4 and 5 months old, Mature rabbits were between 7 and 9 months old, and Adult rabbits were above 1 year old

Serum lipid peroxidation of adult bucks was significantly (P<0.05) higher than mature bucks as shown in Figure 1. Serum total antioxidant capacity of mature and adult bucks were not significantly (P>0.05) different, but were significantly (P<0.05) higher than pubertal bucks as shown in Figure 2. Serum catalase activities of bucks were not significantly (P>0.05) affected by the differences in physiological age as shown in Figure 3. Glutathione peroxidase activity in buck serum was not statistically different across the age groups as shown in Figure 4. Superoxide dismutase activity of the three age groups was statistically (P<0.05) similar (Figure 5). Apparently, adult bucks had higher values across all the antioxidant enzymes assessed, while pubertal bucks had the least antioxidant activity. However, mature and adult bucks had higher total antioxidant activity than pubertal bucks. This shows age related change in total antioxidant activity of rabbit bucks.

The trend of result shows that age group does not differ in antioxidant enzymes production/generation during heat stress, although lipid peroxidation and total antioxidant activity are significantly influenced by age group of bucks. This suggests that ageing does not increase antioxidant enzyme production but influences non - antioxidant enzyme which increases total antioxidant activity in mature and adult rabbit bucks during heat stress and potentially increases peroxides in body system. Reactive oxygen and nitrogen species (RONS) are produced in the body as the result of normal cellular metabolism as well as through exposure to a variety of environmental and physiological challenges [15]. It is now commonly recognized that RONS are involved in a variety of physiological and pathological processes, including gene transcription, regulation of soluble guanylate cyclase activity in cells [16], cellular signal transduction, cell proliferation and differentiation [17], nucleic acid lesions, gene damage, and gene repair activity, leading to subsequent cell death by necrosis or apoptosis [18] and also playing an important role in the progression of a number of diseases [19, 20], including cancer, cardiovascular diseases, cataracts [21], mutagenesis [22], and ageing [15]. Abdel-Kafy *et al*. [23] corroborated the effect of age on serum lipid peroxides in rabbits as observed in this work.

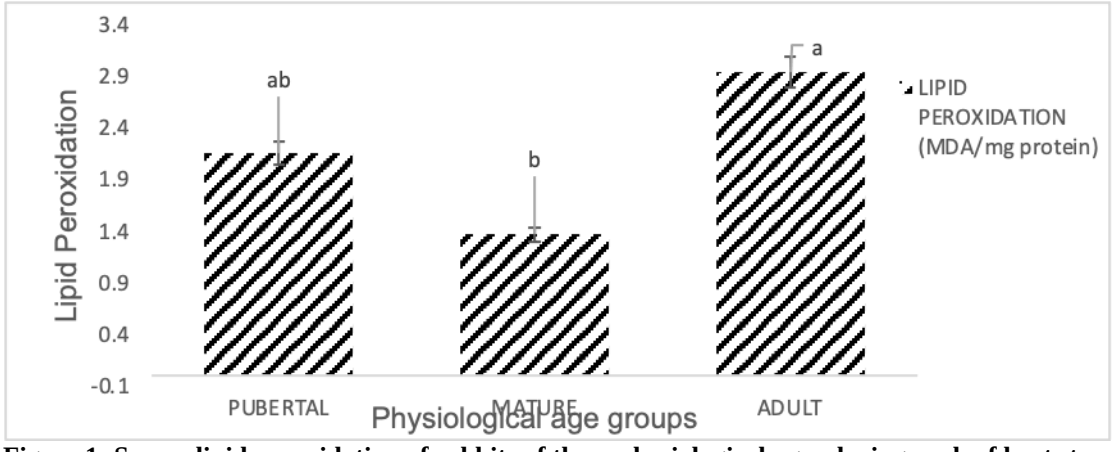

Figure 1: Serum lipid peroxidation of rabbits of three physiological ages during peak of heat stress

[abc]Means with different superscripts are significantly (P<0.05) different.

Where pubertal rabbits were between 4 and 5 months old, mature rabbits were between 7 and 9 months old, Adult rabbits were above 1 year old.

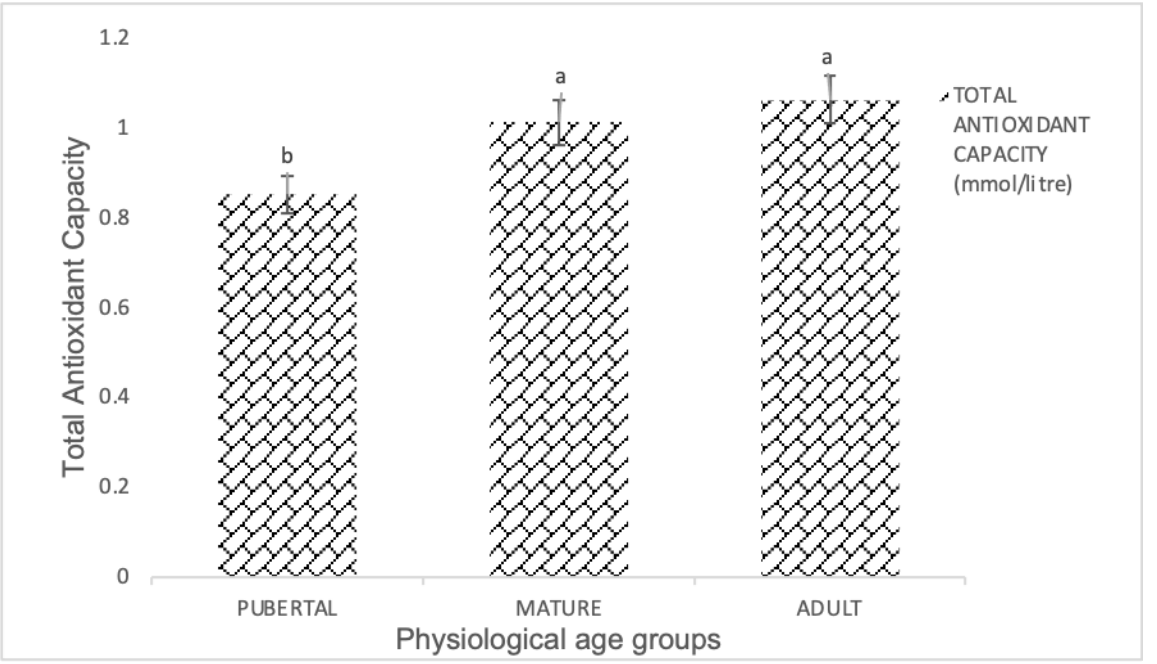

Figure 2: Serum total antioxidant capacity of rabbits of three physiological ages during peak of heat stress

Where pubertal rabbits were between 4 and 5 months old, mature rabbits were between 7 and 9 months old, Adult rabbits were above 1 year old

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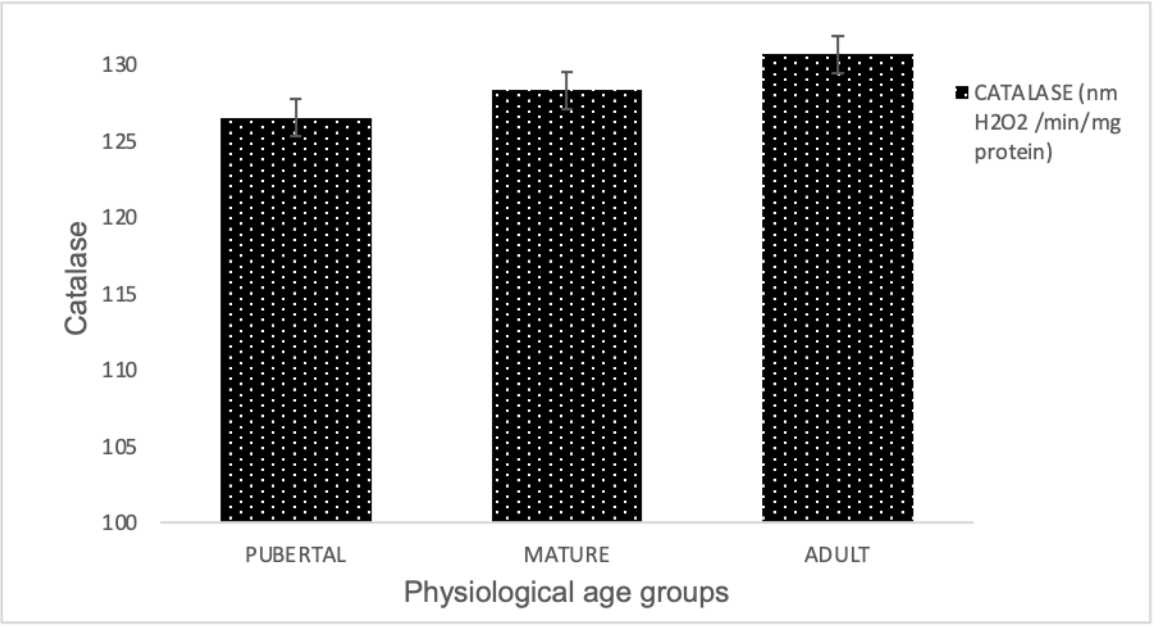

Figure 3: Serum catalase of rabbits of three physiological ages during peak of heat stress

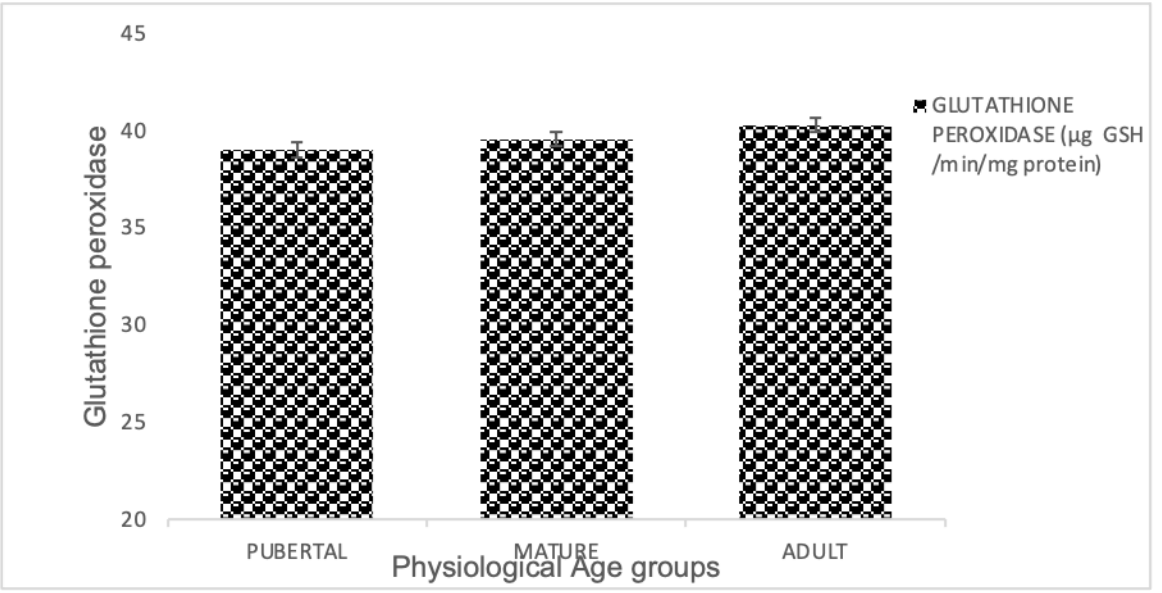

Figure 4: Serum glutathione peroxidase of rabbits of three physiological ages during peak of heat stress

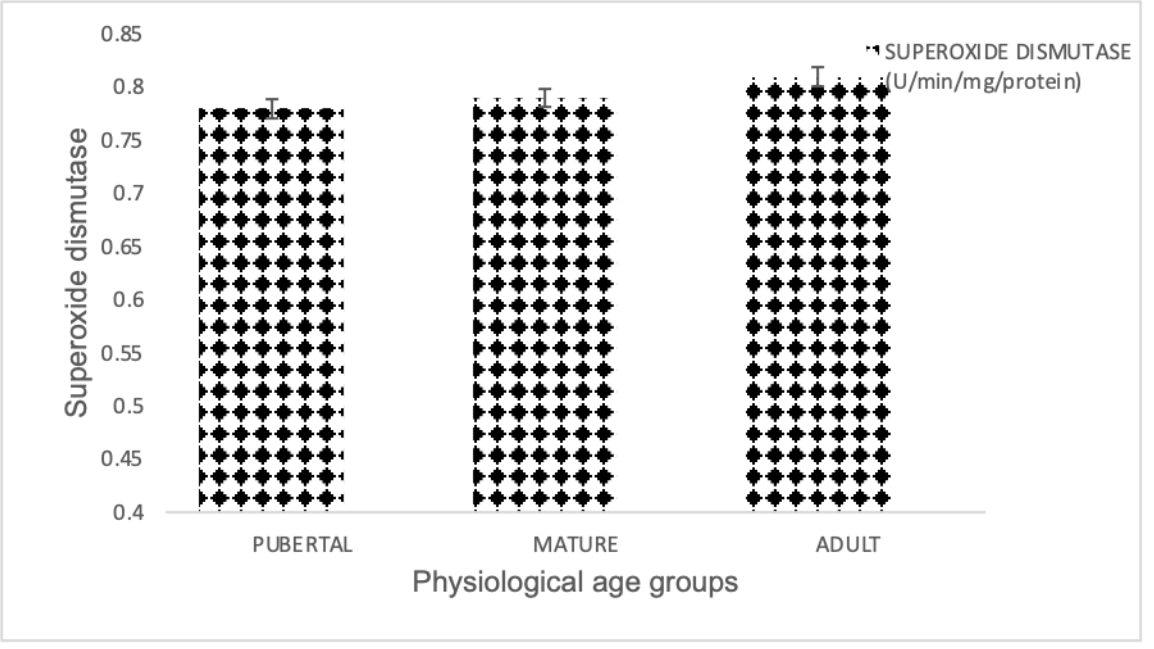

Figure 5: Serum superoxide dismutase of rabbits of three physiological ages during peak of heat stress

Where pubertal rabbits are between 4 and 5 months old, Mature rabbits are between 7 and 9 months old, Adult rabbits are above 1 year old.

Differences were observed in antioxidants activity of rabbit bucks which could indicate a potential effect of ageing on antioxidant system. It has been reported that total antioxidant capacity of local Egyptian rabbits was lower than New Zealand white rabbits at similar age groups, and an increase in total antioxidant capacity was observed with higher ages (45, 60 and 75 days old) in both breeds of rabbit in Egypt [23]. This is in agreement with the findings of this study. One explanation of the causes of ageing is the damage of the biological systems caused by free radical processes [7]. However, [25] reported that there was no evidence of increasing oxidative damage with age among older sheep. It has been postulated that if free radical reactions were the major cause of ageing, a reduction in their levels by antioxidants (e.g. vitamins E and C and glutathione) and antioxidant enzymes (e.g. catalase CAT, superoxide dismutase SOD, and glutathione peroxidases GPx) should, in principle, retard ageing [8]. Also, Salar-Amoli and Baghbanzadeh [15] reported increasing oxidative damage with age among ewes evident from its higher levels of MDA (write MDA in full at first mention). According to Junqueira *et al*. [26], a mild and gradual oxidative stress status in aged humans. However, levels of antioxidants and activities of antioxidant enzymes in different mammalian species appear to be constant. This relationship has been explained by considering the concentrations of antioxidants and antioxidant enzymes relative to the specific metabolic rate rather than absolute concentrations or activities [24]. Similarly, Valls [29] indicated an increase in lipid peroxidation products and glutathione peroxidase, with a decrease in antioxidant enzymes CAT and SOD and an unchanged level of glutathione content in rat liver with age. In addition, Pansarasa *et al*. [27] reported that oxidative stress plays an important role in muscle ageing in humans. Similarly, [28] observed that the liver, red blood cells and plasma are the best tissues to show that the reactive oxygen species (ROS) derived changes with age. In contrast, [27] observed no change in GPx, CAT and gluthatione levels, but reported increased SOD levels in human skeletal muscle. Contrary to the report by [30] that liver damage causes a significant decrease in the levels of these antioxidant enzymes in salinomycin intoxicated sheep. The main target substrates for oxygen radical activity is the polyunsaturated fatty acids that present in membrane phospholipids and result in disorganization of cell framework and function [31].

Conclusion and Applications

1. Adult rabbit bucks had higher serum lipid peroxidation, while mature and adult rabbit bucks had better total antioxidant activity than pubertal buck, despite similar antioxidant enzyme activities.
2. This study reveals that at peak of heat stress, the adult rabbit bucks are more sensitive than mature and pubertal bucks, this infers greater vulnerability of adult bucks to oxidative stress.

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