

## EFFECT OF AD<sub>3</sub>E VITAMINS INJECTION ON AGE AND WEIGHT OF WEANING AND REPRODUCTIVE ACTIVITY OF GOATS

### 2- EFFECT OF AD<sub>3</sub>E VITAMINS INJECTION AND DIURNAL VARIATIONS ON PHYSIOLOGICAL RESPONSE AND REPRODUCTIVE ACTIVITY OF GOAT BUCKS EXPOSED TO DIRECT SOLAR RADIATION OF HOT SUMMER SEASON IN EGYPT

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#### ABSTRACT

This study aimed to investigate the effect of AD<sub>3</sub>E vitamins injection and diurnal variations on physiological function, blood components, libido and semen characteristics in goat bucks exposed to direct solar radiation of hot summer season in Egypt. A number of 30 Damascus goat bucks were used in this research. The animals were exposed to direct solar radiation of hot summer season and divided into three equal groups. The first group was kept without treatment as control while the second and third groups were injected every 15 days with vitamin AD<sub>3</sub>E at the rate of 2 and 4 ml, respectively. The experiment lasted 3 months (from 1<sup>st</sup> of July to the end of September, 2014). Temperatures of rectal, skin, hair surface, hair depth, ear and scrotal as physiological measurements in bucks were measured once monthly. Blood sample was collected monthly from the jugular vein of each buck to estimate total protein, albumin, calcium and inorganic phosphorus concentrations and levels of total testosterone, thyroxin (T<sub>4</sub>) and Triiodothyronine (T<sub>3</sub>) hormonal levels. Semen characteristics and semen storage ability after in vitro storage for 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup> and 6<sup>th</sup> hr periods were estimated monthly.

Results showed that physiological parameters in bucks exposed to direct solar radiation were not affected significantly due to vitamins injection but affected significantly by diurnal variations of the day. The highest values were during evening while the lowest values were during morning. Blood hormones and components concentrations increased progressively with increased the level of vitamins injection and also affected significantly by diurnal variations of the day. The highest testosterone hormonal level was during morning while the lowest level was during evening. T<sub>4</sub> and T<sub>3</sub> levels and blood components concentrations in bucks were significantly higher during morning time than during both afternoon and evening times without any significant difference between the later two times.

Injected bucks with AD<sub>3</sub>E vitamins improved significantly number of ejaculates, libido, semen volume, motility percentage and semen storage ability values and depressed dead sperm, abnormality and acrosomal damage percentages. Time of the day affected significantly on number of ejaculates, libido, semen volume and semen storage ability values in bucks goats. Semen storage ability at morning or at afternoon had best values while semen collected at evening had lowest values.

**KEYWORDS:** Vitamins, Goats, Hormones, Semen Characteristics, Blood Components, Diurnal Time

## INTRODUCTION

Vitamins as micronutrients are required in very minute quantities and are considered indispensable for normal cellular metabolism, growth and maintenance including reproduction of animals. Proper levels of vitamins are also very important for successful reproduction and any deficient on certain vitamins will have a detrimental effect on reproductive performance of farm animals (**Yasothai, 2014**). Fat soluble vitamins (A, D<sub>3</sub> and E) are potently antioxidants. Animals cannot produce these vitamins in their bodies; hence an exogenous regular supply is needed to cover the physiological requirements and to sustain high production performance (**Hafez, 2012**).

Vitamin A is required for maintaining healthy tissue in the reproductive tract. In deficient cattle, delayed sexual maturity, abortion, the birth of dead or weak calves, retained placenta, delayed uterine involution, delayed first estrus after calving, delayed ovulation, increased incidence of cystic ovaries and more early embryonic death and abortion (**Gupta et al., 2005**). Vitamin A is required for normal reproductive processes in males; the deficiency of vitamin A produces a decrease of the sexual activity and spermatogenic disorders. Vitamin A participates in the normal development of bones in both male and female of animals by means of the activation of osteoblasts, showing its deficiency in skeletal muscle abnormalities and growth reduction. Vitamin A has an effective role in keeping all the body's epithelial cells and mucous containing and is plays an important role in the process of vision, spermatogenesis and bone growth. Vitamin A also is essential for maintaining healthy immune function and deficiency can lead to an impaired response to infection. (**Fennema, 2008**).

Vitamin D<sub>3</sub> stimulates the absorption of calcium and phosphorus in the intestine performing the function of carrier of these minerals; likewise, it regulates and corrects its metabolism in the blood. Vitamin D<sub>3</sub> acts on the bone tissue, both on the osteodasts increasing the production of osteocalcine and alkaline phosphatase and on the osteoblasts stimulating the cell difference and multinucleation. Vitamin D<sub>3</sub> is essential for metabolism and normal homeostasis of calcium and phosphorus (**Institute of Medicine, 2010**). The action of vitamin D<sub>3</sub> on the intestine, skeleton and kidneys causes an increase of plasmatic levels of calcium and phosphorus. This increase of levels makes the mineralization and normal remodeling of bones and cartilages possible, as well as the maintenance of concentration of calcium in the extracellular fluid, which is required for normal muscular contraction and nervous excitability. Vitamin D<sub>3</sub> has a special importance for the formation and the strength of skeleton and teeth of young animals. Vitamin D is required for normal calcium and phosphorus metabolism. Most commercial concentrates contain supplemental vitamin D in amounts sufficient to meet the animal's requirement (10,000 IU per day). However, deficiency of vitamin D lead to anestrus, milk fever, metritis and retained placenta in dairy cows and impotentia coeundi in bulls (**Mee, 2004**).

Vitamin E acts as a chain split biological antioxidant, neutralizing free radicals and avoiding the peroxidation of lipids, members of cell membranes, minimizing in this way the degeneration and necrosis of tissues, for the irreversible denaturalization of essential cell proteins. These radicals may damage cells, tissues and organs. Its antioxidant actions stabilize the cell membranes, as these consist of polyunsaturated fatty acids. Vitamin E is also important for the formation of red corpuscles and helps the organism to use vitamin K and A accordingly, it protects the biological membranes of nerves, muscles and cardiovascular system. Vitamin E is essential for the normal operation of the reproductive system; consequently, its deficiency affects fertility (**Institute of Medicine, 2013**). On the other hand, it has an important activity

as a biological antioxidant, especially, at level of unsaturated fatty acids. Incidence of retained placenta was 17.5% in cows and it was reduced to 0% in cows receiving both selenium and vitamin E. Vitamin E > 2000 IU/day supplemented during transition phase (3 weeks before to 3 weeks after calving) lower incidence of mastitis and retained placenta (**Robinson, 1990**). **Yasothisai (2014)** found that supplying sufficient amounts of vitamins A and E may improve the immune status of the periparturient cow and reducing the incidence of mastitis and retained fetal membranes which in turn may improve pregnancy rates.

In practical studies, **Hafez (2012)** reported that injecting vitamin AD<sub>3</sub>E mixture in lactating buffalo increase milk production efficiency and enhance the feed efficiency throughout the first 100 day in lactation. **Acatincai et al. (2001)** found that animals treated with AD<sub>3</sub>E, live weight gain of bulls increased from 9.41 to 13.33% higher than those values obtained in control group. **Nasr et al. (2002)** reported that injection ewes with vitamin AD<sub>3</sub>E in Jordan showed an average additional net return of US\$ 5.66/ewe.

This work was aimed to investigate the effect of AD<sub>3</sub>E vitamins injection and diurnal variations on physiological function, blood components and semen characteristics in goats bucks exposed to direct solar radiation of hot summer season in Egypt.

## MATERIALS AND METHODS

The present study was carried out at El Gemmaiza Experimental Station located in mid Nile Delta, Department of sheep and goats research belonging to Animal Production Research Institute, Ministry of Agriculture, Egypt. The experiment lasted 3 months (from 1<sup>st</sup> of July to the end of September, 2014).

### Animals

Total of 30 Damascus goat bucks (22-23 months of age and 42-45 kg body weight) were exposed to direct solar radiation of hot summer season and divided into three similar groups (10 bucks in each). Bucks of the first group was served as control and injected by saline solution, while those in the second and third groups were injected biweekly vitamin AD<sub>3</sub>E (DEVEDRY-MED Injection), Manufactured by ARABCOMED, Egypt). Each ml contained (80000 IU) of vitamin A, (40000 IU) of vitamin D<sub>3</sub> (Cholicacipherol) and (20 mg) of vitamin E (a tocopherol acetate) at levels of 2 and 4 ml/buck, respectively. AD<sub>3</sub>E solution was injected intramuscularly through buck neck area for four weeks prior to the experimental period, which lasted three months. The three experimental groups exposed to direct solar radiation under natural hot summer condition in yard 5 X 21 meters surrounded with brick wall of one meter height. The yard was divided by metal tubes to three partitions; each one was 5x7 meters for each experimental group.

### Feeding System

Animals were offered their requirements from rations (concentrate feed mixture and rice straw) according to **NRC (1985)**. The concentrate feed mixture (CFM) composed of 37.4% wheat bran, 27% yellow corn, 12.5% soybean meal (44% CP), 10.0% decorticated cottonseed cake, 5% rice bran, 4% sugarcane molasses, 3% limestone, 1% sodium chloride and 0.1% vitamin and minerals premix (each kg of vitamin and minerals premix contained 0.1 g selenium and 25 g vit.). A proximate chemical analysis of the concentrate and roughage were carried out according to **A.O.A.C. (2000)**. Chemical composition (on DM basis %) of concentrate feed mixture used in feeding the bucks was 14.46%CP, 12.4%CF, 3.11%EE, 58.49% NFE and 11.36% ASH. The corresponding values of rice straw were 3.5, 35.1, 1.4, 39.6 and 20.4%. Feed mixture

was offered twice daily at 8 a.m. and 4 p.m., fresh drinking water was available all time.

### Environmental Conditions

Ambient air temperature (AT) and relative humidity (RH %) were recorded biweekly at morning (7.00 hr), afternoon (12.00 hr) and evening (17.00 hr) in the same time of carrying out the physiological measurements. Ambient air temperature was recorded using mercury thermometer to the nearest 0.1°C. Relative humidity was recorded using hair-hygrometer to the nearest 1%. Temperature-humidity index (THI) was estimated according to equation of **Livestock Poultry Heat Stress Index (1990)** and modified by **Marai et al. (2000)** as follows:  $THI = db^{\circ}C - \{(0.31 - 0.31 RH) (db^{\circ}C - 14.4)\}$  where  $db^{\circ}C$  = dry bulb temperature in Celsius and  $RH = RH \% / 100$ . Then, the obtained values of THI were classified as follows :  $>22.2$  = absence of heat stress,  $22.2 - <23.3$  = moderate heat stress,  $23.3 - <25.6$  = severe heat stress and  $25.6$  and more = very severe heat stress. The averages of ambient temperatures, relative humidity and THI values estimated at morning, afternoon and evening during July, August and September months are presented in Table (1).

### Thermoregulatory Parameters

Temperatures of rectal, skin, hair, ear and scrotal skin as a physiological measurements of bucks were measured biweekly at morning, afternoon and evening during July, August and September months. Rectal temperature was measured to the nearest 0.1°C by inserting electronic thermometer probe to the depth of 5-6 cm into the rectum. Hair, skin, ear and scrotal surface temperatures were measured by alcohol thermometer. In addition, pulse rate (number of pulses/min) and respiration rate (number of breaths/min) were measured in each season.

### Blood Samples

Samples of blood were collected biweekly at morning, afternoon and evening during July, August and September months from the jugular vein of each buck into glass tubes contained heparin as anticoagulant. Blood samples were centrifuged at 3000 rpm for 15 minutes to obtain plasma and stored at -20°C until plasma analyses. Concentration of total protein and albumin in plasma were estimated using reagent kites manufactured by BIODIAGNOSTIC Company (Egypt). Globulin concentration was calculated by difference between total protein and albumin values. Calcium and inorganic phosphorus was measured by reagent kites manufactured by BIODIAGNOSTIC Company, Egypt. Concentration of testosterone, triiodothyronine (T3) and thyroxin (T4) hormones was estimated by the radioimmunoassay (RIA) technique using the coated tubes kits, kits purchased from Diagnostic Products Corporation, Los Angeles, CA, USA and counting in the Laboratory of Biological Applications Department, Atomic Energy Authority, using computerized Gamma Counter. The tracer in the hormones was labeled with iodine-125 (<sup>125</sup>I).

### Semen Characteristics

Libido and semen characteristics were measured in 5 bucks from each group, three times monthly once day at morning, once day at afternoon and once day at evening during each July, August and September months. Semen ejaculate was collected by artificial vagina. On day of the semen collection, libido (reaction time, sec.), semen pH value, ejaculate volume, percentages of sperm motility, dead spermatozoa, sperm abnormalities and acrosomal damage were estimated. Libido was assessed according to **Chenoweth (1981)**. Hydrogen ion concentration was tested with digital pH meter. Semen ejaculate volume was measured directly in milliliters to the nearest 0.1 ml in a transparent graduated glass tube. Mass motility percentage was assessed according to **Martínez-Rodríguez et al. (2012)**. Live and dead sperm percentages were examined according to **Campbell et al. (1956)**. Acrosomal damage percentage was determined by Gimsa stain

according to **Watson (1995)**.

### **Semen storage Ability Estimation**

After semen collection, pooled semen samples from each buck were immediately transferred to the laboratory. Semen samples were extended with Tris-egg yolk fructose and incubated at 37°C. The changes in the sperm motility percentage for 1, 2, 3, 4, 5 and 6 h was determined.

### **Statistical Analysis**

Data were subjected to statistical analysis using analysis of variance procedure (**SAS, 2004**) and significant differences between means were separated by Duncan's multiple Range test procedure (**Duncan, 1955**). Percentage values were transferred to arc-sign and measured as means.

## **RESULTS AND DISCUSSIONS**

### **1-Effect of AD<sub>3</sub>E Vitamins Injection and Diurnal Variations on Physiological Parameters in Bucks Exposed to Direct Solar Radiation**

Table (2) showed that hair, skin, rectal, scrotal and ear temperatures as well as pulse rate and respiration rate values in bucks were not affected significantly due to vitamins injection. Concerning the effect of diurnal variations on physiological parameters, data in Table (2) showed that hair (surface or deep), skin, rectal, scrotal and ear temperatures, pulse rate and respiration rate in bucks were affected significantly by diurnal variations of the day. The highest values were during evening while the lowest values were during morning. The values during afternoon were significantly higher than the values of morning and significantly lower than the values of evening except pulse rate and respiration rate. No significant differences between pulse rate and respiration rate values in afternoon and evening day.

These physiological adjustments are essential to maintain normal body temperature and to prevent hyperthermia (**Lowe et al., 2001**). RR and RT have been shown to be good indicators of the thermal stress and may be used to assess the adversity of the thermal environment and are the parameters which illustrate the mechanism of physiological adaptation (**Habeeb et al., 1992**). **Brown-Brandl et al. (2003)** found that RT and RR showed significant differences between temperature treatments and both RT and RR had a diurnal pattern which followed the diurnal pattern of the ambient conditions of the day. **Shalaby (1985)** reported that RR of Rahmani and Ossimi ewes reached the maximum during summer at 2.00 pm. and 4.00 pm. which coincided with the hottest time of the day. The higher ST can be attributed to the partially to the fact that exposure to heat stress alter the blood flow and redistribution of blood flow and increase blood flow to the surfaces (**Srikandakumar et al., 2003**). The increase in physiological parameters is associated with marked reduction in feed intake, redistribution in blood flow and changes in endocrine functions that will affect negatively the productive and reproductive performance of the farm animals (**Habeeb et al., 2008a, b**).

### **2-Effect of AD<sub>3</sub>E vitamins injection and diurnal variations on blood components and hormonal levels in bucks exposed to direct solar radiation:**

The effect of AD<sub>3</sub>E vitamins injection on blood components and hormonal levels are shown in Table (3). Testosterone, T<sub>4</sub> and T<sub>3</sub> levels as well as total proteins, albumin, globulin, ca and p<sub>i</sub> concentrations increased progressively with increased the level of vitamins injection. Testosterone, T<sub>4</sub> and T<sub>3</sub> levels increased by 37.81, 11.64 and 50.00% due to injection of 2 ml AD<sub>3</sub>E vitamins and by 65.02, 25.74 and 82.81% due to injection of 4 ml AD<sub>3</sub>E vitamins, respectively. Total protein, globulin, ca and p<sub>i</sub> concentrations increased also by 8.35, 11.36, 17.21 and 12.89% over control when bucks

injected with 2 ml vitamins and by 18.16, 26.18, 33.82 and 33.61% in bucks injected with 4 ml AD<sub>3</sub>E vitamins. Albumin level increased by 10.90% over control in bucks injected with 4 ml vitamins and was not affected significantly by injection 2 ml vitamins.

It has also been reported that serum total protein, albumin, globulin concentrations were increased when dietary vitamins were supplemented. **Sikka et al. (2002)** found that injections of AD<sub>3</sub>E a month prior to expected date of calving enhanced the total immunoglobulins secretion in colostrum of buffaloes. In addition, higher monthly body weight gain was noted in calves of supplemented dams with AD<sub>3</sub>E and concluded that antioxidant and fat soluble vitamins in combination with minerals have a passive immunity and growth in buffalo calves. **El-Nor (2000)** found that dry matter intake and nutrient digestibility were higher significantly in treated group with fat soluble vitamins than in the control group. Milk yield and 4% fat-corrected milk, fat, percentage of milk fat solids-not-fat and total protein yields in lactating buffaloes were significantly higher ( $P < 0.05$ ) in supplementation of vitamins while milk protein, lactose and ash contents tended to increase insignificantly with inclusion of AD<sub>3</sub>E in the diets. **Ahmed et al. (2012)** found that supplementation of low fertile Egyptian cows with AD<sub>3</sub>E improve the reproductive performance and consequently shortens the calving intervals and increases the income from breeding of livestock. **Lavania (2013)** found that vitamin AD<sub>3</sub>E injection can be effectively used for induction of estrus in goats in semi arid environment.

Concerning the effect of diurnal variations on blood components and hormonal levels, data in Table (3) showed that testosterone, T4 and T3 levels as well as total protein, albumin, globulin, Ca and Pi concentrations in bucks were affected significantly by diurnal variations of the day. Concerning, testosterone hormonal level, the highest level was during morning while the lowest level was during evening and the testosterone value during afternoon was significantly lower than the level of the morning and significantly higher than the level of evening. T4 and T3 levels and blood components concentrations in bucks were significantly higher during morning time than during both afternoon and evening times without any significant difference between the later two times.

Plasma protein contents were negatively correlated to environmental temperature (**Habeeb et al., 2008a**). Serum albumin level was found to be significantly lower in summer than during winter season in Karakul (**Baumgartner and Parnthner, 1994**). The decrease in serum albumin concentration was estimated to be about 10 % (**Yousef et al., 1996**). **Sharma and Puri (2013)** found that climatic conditions had significant effect on serum globulin in moderate and total protein in extreme climatic conditions. **Suntorn et al. (2009)** also revealed reduction in albumin level in goats and in sheep during summer. The difference in the globulin may be due to the various physiological adaptation and genetic factors (**Marai et al., 1997**).

It is important to evaluate testosterone levels to determine the development of the reproductive system of goats that are adapted to the hot climate. Testosterone levels are useful in the selection of young sires and to characterize sexual maturity in different breeds, therefore, testosterone is directly involved in the onset of puberty and consequently in the onset of spermatogenesis (**Eloy and Santa Rosa, 1998**). Testosterone is the most important male reproductive hormone; it is related to reproductive behavior, spermatogenesis and secondary sexual characteristics (**Hafez, 2004**). From another point of view, short daylight stimulates the secretion of testosterone, FSH and LH in rams while long daylight inhibits their secretion. Rams sexual activities peak occurs during the autumn breeding season and coincides with a sharp rise in plasma testosterone level and then it declines in late winter, spring and summer (**Jainuden and Hafez, 1987**).

### **3-Effect of AD<sub>3</sub>E vitamins injection and diurnal variations on semen characteristics in bucks exposed to direct solar radiation:**

The effect of AD<sub>3</sub>E vitamins injection on semen characteristics parameters are shown in Table (4). Number of ejaculates, libido, semen volume and motility percentage values in bucks increased progressively with increased the level of vitamins injection. Number of ejaculates, libido, semen volume and motility percentage increased by 36.15, 9.37, 25.00 and 12.31% due to injection of 2 ml AD<sub>3</sub>E vitamins and by 66.92, 22.72, 84.72 and 25.14% due to injection of 4 ml AD<sub>3</sub>E vitamins, respectively. On the other hand, injected bucks with vitamin AD<sub>3</sub>E improved significantly motility percentage and depressed dead sperm, abnormality and acrosomal damage percentages. The improvement percentage was progressively with increased the level of vitamins injection. Percentages of dead sperm, abnormality and acrosomal damage decreased by 28.40, 17.80 and 15.87% in bucks injected with 2 ml vitamins and by 32.29, 41.13 and 40.59% in bucks injected with 4 ml AD<sub>3</sub>E vitamins.

Time of the day affected significantly on number of ejaculates, libido, semen volume values in bucks goats. The highest ejaculate number was at morning while the lowest values were at both afternoon and evening times without significant between them. The highest values of libido and semen volume were at morning while the lowest values were at evening time. The values of libido and semen volume at afternoon time were significantly lower than at morning and significantly higher than at evening. On other hand, semen pH and percentages of motility, dead sperm, abnormality and acrosomal damage were not affected by diurnal variations.

### **4-Effect of AD<sub>3</sub>E Vitamins Injection and Diurnal Variations on Semen Storage Ability in Bucks Exposed to Direct Solar Radiation**

The effect of AD<sub>3</sub>E vitamins injection on semen storage ability showed that the best semen storage ability values were in bucks injected with 4 ml vitamins while the lowest semen storage ability values were in control bucks. Semen storage ability values in bucks increased progressively with increased the level of vitamins injection. Injected bucks with vitamin AD<sub>3</sub>E improved significantly semen storage ability values by 13.66, 33.97, 22.94, 55.83, 64.92 and 133.33% due to injection of 2 ml AD<sub>3</sub>E vitamins and by 24.17, 48.64, 62.14, 95.39, 169.87 and 322.33% due to injection of 4 ml AD<sub>3</sub>E vitamins after 1, 2, 3, 4, 5 and 6 hours, respectively (Table 5).

Concerning the effect of diurnal variations on semen storage ability, Table (5) showed that semen storage ability from 1<sup>st</sup> to 4<sup>th</sup> hour after collection from bucks at morning or at afternoon had best values while semen collected at evening had lowest values either in bucks injected with 0, 2 and 4 ml vitamins AD<sub>3</sub>E. However, after 5 hour from collection, no significant difference in semen storage ability was observed due to diurnal time of the day.

## **CONCLUSIONS**

It can be concluded that physiological thermoregulatory response, hormonal levels and semen characteristics affected by diurnal variations of day time, being at morning better than at both afternoon and evening. In addition, it can be concluded that injection of vitamin AD<sub>3</sub>E mixture could be used to alleviate the effect of heat stress on physiological response and reproductive activity of goat bucks exposed to direct solar radiation of hot summer season in Egypt

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## APPENDICES

**Table 1: Averages of Environmental Conditions of Diurnal Variations of Day During the Experimental Months**

Experimental months	Diurnal time	Environmental Conditions During July, August and September Months			
		Temperature, °C	Relative humidity, %	Temperature-humidity index (THI)*	Status of heat stress (HS)
July month	Morning	28.0±0.5	55±2.0	26.1	Very severe HS
	Afternoon	49.6±1.0	40±2.5	45.2	Very severe HS
	Evening	51.0±1.5	35±1.5	47.3	Very severe HS
August month	Morning	28.0±0.6	55±1.9	26.1	Very severe HS
	Afternoon	47.4±1.3	40±1.6	41.1	Very severe HS
	Evening	50.0±1.4	35±1.5	42.9	Very severe HS
September month	Morning	28.5±0.5	60±2.6	26.8	Very severe HS
	Afternoon	49.4±1.5	40±2.0	42.7	Very severe HS
	Evening	51.0±1.5	35±1.5	46.1	Very severe HS

\* THI =db°C – {(0.31 – 0.31 RH) (db °C – 14.4)} where db °C =dry bulb temperature in Celsius and RH = RH % /100. THI were classified as follows : >22.2 = absence of heat stress, 22.2 -<23.3 = moderate heat stress, 23.3 - <25.6 =severe heat stress and 25.6 and more = very severe heat stress (**Livestock Poultry Heat Stress Index (1990)** and modified by **Marai et al. (2000)**).

**Table 2: Diurnal Variations in Physiological Parameters in Goats Bucks Exposed to Direct Solar Radiation of Hot Summer Season**

Experimental groups	Diurnal time	Thermoregulatory Parameters in Goats Bucks exposed to Direct Solar Radiation of hot Summer Season							
		Hair surface temperature	Hair deep temperature	Dorsal skin temperature	Rectal temperature	Scrotal temperature	Ear temperature	Pulse rate /minute	Respiration rate (rpm)
0 ml vitamin AD <sub>3</sub> E/head	Morning	29.2±0.5 <sup>c</sup>	31.7±0.5 <sup>c</sup>	36.5±0.2 <sup>c</sup>	39.2±0.01 <sup>c</sup>	29.8±0.8 <sup>c</sup>	34.2±0.2 <sup>c</sup>	89.5±0.6 <sup>b</sup>	52.7±2.9 <sup>b</sup>
	Afternoon	45.3±1.6 <sup>b</sup>	44.5±1.4 <sup>b</sup>	43.3±1.2 <sup>b</sup>	39.9±0.02 <sup>b</sup>	35.6±0.4 <sup>b</sup>	39.9±1.6 <sup>b</sup>	101.0±1.7 <sup>a</sup>	112.1±2.0 <sup>a</sup>
	Evening	48.2±0.4 <sup>a</sup>	47.8±0.9 <sup>a</sup>	45.6±0.6 <sup>a</sup>	40.5±0.01 <sup>a</sup>	38.0±0.7 <sup>a</sup>	43.7±0.7 <sup>a</sup>	105.3±1.5 <sup>a</sup>	111.3±0.1 <sup>a</sup>
	Overall	40.90±7.2	41.33±7.1	41.80±2.7	39.87±0.04	34.47±2.4	39.27±2.8	98.60±4.7	92.0±16.7
2 ml vitamin AD <sub>3</sub> E/head	Morning	30.3±0.3 <sup>c</sup>	32.7±0.1 <sup>c</sup>	36.8±0.1 <sup>c</sup>	39.6±0.03 <sup>b</sup>	31.2±0.8 <sup>c</sup>	34.7±0.4 <sup>c</sup>	95.8±1.9 <sup>b</sup>	63.4±4.2 <sup>b</sup>
	Afternoon	44.8±1.3 <sup>b</sup>	44.6±1.3 <sup>b</sup>	41.8±1.3 <sup>b</sup>	39.7±0.01 <sup>b</sup>	34.5±0.5 <sup>b</sup>	38.8±1.8 <sup>b</sup>	98.6±1.2 <sup>ab</sup>	106.7±1.3 <sup>a</sup>
	Evening	48.4±0.4 <sup>a</sup>	47.5±1.5 <sup>a</sup>	44.2±0.7 <sup>a</sup>	40.2±0.01 <sup>a</sup>	36.6±0.5 <sup>a</sup>	42.3±0.7 <sup>a</sup>	100.5±0.6 <sup>a</sup>	103.5±1.4 <sup>a</sup>
	Overall	41.17±6.5	41.6±6.4	40.93±2.2	39.83±0.02	34.10±1.6	38.60±2.2	98.30±1.4	91.2±14.0
4ml vitamin AD <sub>3</sub> E/head	Morning	31.6±0.03 <sup>c</sup>	33.5±0.3 <sup>c</sup>	37.1±0.2 <sup>c</sup>	39.5±0.04 <sup>ab</sup>	31.9±0.4 <sup>c</sup>	35.0±0.2 <sup>c</sup>	97.8±0.5 <sup>b</sup>	68.2±2.4 <sup>b</sup>
	Afternoon	43.9±1.8 <sup>b</sup>	44.7±0.5 <sup>b</sup>	41.3±0.3 <sup>b</sup>	39.4±0.01 <sup>b</sup>	33.9±0.4 <sup>b</sup>	38.0±1.4 <sup>b</sup>	98.1±0.3 <sup>ab</sup>	101.9±0.5 <sup>a</sup>
	Evening	47.9±0.5 <sup>a</sup>	46.8±1.3 <sup>a</sup>	43.3±0.6 <sup>a</sup>	39.7±0.02 <sup>a</sup>	35.9±0.7 <sup>a</sup>	41.4±0.5 <sup>a</sup>	100.1±1.3 <sup>a</sup>	102.9±1.2 <sup>a</sup>
	Overall	41.13±5.6	41.67±5.8	40.57±1.8	39.53±0.01	33.90±1.2	38.13±1.9	98.67±0.7	91.0±11.4

a, b. Means within column in each experimental group with different superscript are significantly differ (P < 0.05).

**Table 3: Diurnal Variations in Hormonal Levels and Blood Components in Goats Bucks Exposed to Direct Solar Radiation of Hot Summer Season**

Experimental group	Diurnal time	Hormonal Levels and Blood Components Concentrations in Bucks Exposed to Direct Solar Radiation of Hot Summer Season							
		Testosterone (ng/ml)	T4 (ng/ml)	T <sub>3</sub> (ng/ml)	T. protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Ca (mg/dl)	P <sub>I</sub> (mg/dl)
0 ml vitamin AD <sub>3</sub> E/head	Morning	3.9±0.1 <sup>a</sup>	39.7±2.80 <sup>a</sup>	0.83±0.04 <sup>a</sup>	7.8±0.1 <sup>a</sup>	4.2±0.1 <sup>a</sup>	3.6±0.1 <sup>a</sup>	8.1±0.3 <sup>a</sup>	3.7±0.2 <sup>a</sup>
	Afternoon	2.7±0.2 <sup>b</sup>	33.9±1.68 <sup>b</sup>	0.52±0.01 <sup>b</sup>	6.4±0.2 <sup>b</sup>	3.5±0.1 <sup>b</sup>	2.9±0.2 <sup>b</sup>	6.3±0.2 <sup>b</sup>	3.5±0.2 <sup>ab</sup>
	Evening	1.9±0.1 <sup>c</sup>	32.8±1.90 <sup>b</sup>	0.56±0.04 <sup>b</sup>	6.3±0.3 <sup>b</sup>	3.3±0.2 <sup>b</sup>	3.0±0.2 <sup>b</sup>	6.0±0.4 <sup>b</sup>	3.3±0.2 <sup>b</sup>
	Overall	2.83±0.58 <sup>C</sup>	35.47±2.1 <sup>C</sup>	0.64±0.10 <sup>C</sup>	6.83±0.5 <sup>C</sup>	3.67±0.3 <sup>B</sup>	3.17±0.2 <sup>C</sup>	6.80±0.7 <sup>C</sup>	3.57±0.2 <sup>C</sup>
2 ml vitamin AD <sub>3</sub> E/head	Morning	5.4±0.5 <sup>a</sup>	44.0±2.38 <sup>a</sup>	1.85±0.02 <sup>a</sup>	8.1±0.1 <sup>a</sup>	4.3±0.1 <sup>a</sup>	3.8±0.1 <sup>a</sup>	9.6±0.3 <sup>a</sup>	4.3±0.1 <sup>a</sup>
	Afternoon	3.6±0.2 <sup>b</sup>	37.8±1.88 <sup>b</sup>	0.52±0.01 <sup>b</sup>	7.0±0.1 <sup>b</sup>	3.7±0.1 <sup>b</sup>	3.3±0.1 <sup>b</sup>	7.2±0.2 <sup>b</sup>	3.9±0.3 <sup>b</sup>
	Evening	2.7±0.1 <sup>c</sup>	37.0±1.80 <sup>b</sup>	0.50±0.02 <sup>b</sup>	7.1±0.3 <sup>b</sup>	3.6±0.1 <sup>b</sup>	3.5±0.2 <sup>b</sup>	7.1±0.5 <sup>b</sup>	3.9±0.2 <sup>b</sup>
	Overall	3.90±0.79 <sup>B</sup>	39.60±2.21 <sup>B</sup>	0.96±0.45 <sup>B</sup>	7.40±0.4 <sup>B</sup>	3.87±0.2 <sup>B</sup>	3.53±0.2 <sup>B</sup>	7.97±0.8 <sup>B</sup>	4.03±0.1 <sup>B</sup>
Change,% and significant		+37.81 <sup>**</sup>	+11.64 <sup>*</sup>	+50.00 <sup>**</sup>	+8.35 <sup>*</sup>	+5.45 <sup>NS</sup>	+11.36 <sup>*</sup>	+17.21 <sup>*</sup>	+12.89 <sup>*</sup>
4ml vitamin AD <sub>3</sub> E/head	Morning	6.1±0.6 <sup>a</sup>	47.5±1.55 <sup>a</sup>	1.93±0.02 <sup>a</sup>	8.7±0.1 <sup>a</sup>	4.4±0.1 <sup>a</sup>	4.3±0.1 <sup>a</sup>	10.2±0.6 <sup>a</sup>	5.4±0.3 <sup>a</sup>
	Afternoon	3.6±0.4 <sup>b</sup>	42.9±1.89 <sup>b</sup>	0.79±0.01 <sup>b</sup>	7.8±0.1 <sup>b</sup>	4.0±0.1 <sup>b</sup>	3.8±0.1 <sup>b</sup>	8.0±0.3 <sup>b</sup>	4.5±0.1 <sup>b</sup>
	Evening	3.3±0.1 <sup>c</sup>	43.4±2.38 <sup>b</sup>	0.80±0.01 <sup>b</sup>	7.7±0.2 <sup>b</sup>	3.8±0.1 <sup>b</sup>	3.9±0.1 <sup>b</sup>	8.6±0.5 <sup>b</sup>	4.4±0.2 <sup>b</sup>
	Overall	4.67±0.81 <sup>A</sup>	44.60±1.5 <sup>A</sup>	1.17±0.38 <sup>A</sup>	8.07±0.3 <sup>A</sup>	4.07±0.2 <sup>A</sup>	4.0±0.2 <sup>A</sup>	9.10±0.6 <sup>A</sup>	4.77±0.3 <sup>A</sup>
Change,% and significant		+65.02 <sup>**</sup>	+25.74 <sup>**</sup>	+82.81 <sup>**</sup>	+18.16 <sup>**</sup>	+10.90 <sup>*</sup>	+26.18 <sup>**</sup>	+33.82 <sup>**</sup>	+33.61 <sup>**</sup>

a , b.. Means within column in each experimental group with different superscript are significantly differ (P < 0.05).

A, B.. Means within overall column in each parameter with different superscript are significantly differ (P < 0.05).

Change %; NS=Not significant, \*P<0.05 and \*\*P<0.001

**Table 4: Diurnal Variations in Seminal Characteristics in Goats Bucks Exposed to Direct Solar Radiation of Hot Summer Season**

Experimental group	Diurnal time	Seminal Characteristics in Goats Bucks Exposed to Direct Solar Radiation of hot Summer Season							
		No. of ejaculates	Libido	Volume, ml	pH	Motility %	Dead sperm %	Abnormality %	Acrosomal %
0 ml vitamin AD <sub>3</sub> E/head	Morning	1.5±0.1 <sup>a</sup>	224±19.4 <sup>a</sup>	0.80±0.10 <sup>a</sup>	7.60±0.1	64.0±4.0	34.8±3.9	24.2±1.2	18.8±0.5
	Afternoon	1.2±0.2 <sup>b</sup>	192±10.7 <sup>b</sup>	0.76±0.02 <sup>b</sup>	7.54±0.1	64.0±4.0	31.6±2.1	22.4±2.8	20.0±1.4
	Evening	1.2±0.2 <sup>b</sup>	138±18.0 <sup>c</sup>	0.60±0.06 <sup>c</sup>	7.54±0.1	59.0±3.6	35.8±3.7	20.0±0.5	18.4±1.1
	Overall	1.3±0.1 <sup>C</sup>	184.7±25 <sup>C</sup>	0.72±0.06 <sup>C</sup>	7.56±0.02 <sup>A</sup>	62.33±1.7 <sup>C</sup>	34.07±1.27 <sup>A</sup>	22.20±1.2 <sup>A</sup>	19.07±0.48 <sup>A</sup>
2 ml vitamin AD <sub>3</sub> E/head	Morning	1.9±0.2 <sup>a</sup>	244±14.7 <sup>a</sup>	1.08±0.01 <sup>a</sup>	7.56±0.1	71.0±3.3	27.2±3.4	17.6±0.5	16.0±0.7
	Afternoon	1.6±0.2 <sup>b</sup>	194±28.1 <sup>b</sup>	0.92±0.07 <sup>b</sup>	7.58±0.1	71.0±1.9	27.8±1.5	18.4±1.2	15.4±0.7
	Evening	1.8±0.2 <sup>ab</sup>	168±17.4 <sup>c</sup>	0.70±0.10 <sup>c</sup>	7.54±0.1	68.0±2.0	30.2±2.0	17.4±0.8	16.2±1.4
	Overall	1.77±0.1 <sup>B</sup>	202.0±22 <sup>B</sup>	0.90±0.1 <sup>B</sup>	7.56±0.01 <sup>A</sup>	70.0±1.0 <sup>B</sup>	28.40±0.92 <sup>B</sup>	17.80±0.31 <sup>B</sup>	15.87±0.24 <sup>B</sup>
Change,% and significant		+36.15 <sup>**</sup>	+9.37 <sup>*</sup>	+25.00 <sup>**</sup>	---	+12.31 <sup>*</sup>	-16.64 <sup>*</sup>	-19.82 <sup>*</sup>	-16.78 <sup>*</sup>
4ml vitamin AD <sub>3</sub> E/head	Morning	2.5±0.2 <sup>a</sup>	264±4.0 <sup>a</sup>	2.00±1.5 <sup>a</sup>	7.54±0.1	80.0±2.0	24.8±2.4	13.8±0.7	11.2±0.6
	Afternoon	2.0±0.3 <sup>b</sup>	236±12.9 <sup>b</sup>	1.10±0.09 <sup>b</sup>	7.50±0.1	76.0±1.6	21.4±1.2	13.0±1.0	10.0±0.5
	Evening	2.0±0.2 <sup>b</sup>	180±8.40 <sup>c</sup>	0.88±0.10 <sup>c</sup>	7.52±0.1	78.0±3.7	23.0±4.8	12.4±0.5	12.8±0.6
	Overall	2.17±0.2 <sup>A</sup>	226.67±25 <sup>A</sup>	1.33±0.3 <sup>A</sup>	7.52±0.01 <sup>A</sup>	78.0±1.2 <sup>A</sup>	23.07±0.98 <sup>C</sup>	13.07±0.41 <sup>C</sup>	11.33±0.81 <sup>C</sup>
Change,% and significant		+66.92 <sup>**</sup>	+22.72 <sup>**</sup>	+84.72 <sup>**</sup>	---	+25.14 <sup>**</sup>	-32.29 <sup>**</sup>	-41.13 <sup>**</sup>	-40.59 <sup>**</sup>

a , b.. Means within column in each experimental group with different superscript are significantly differ (P < 0.05).

A, B.. Means within overall column in each parameter with different superscript are significantly differ (P < 0.05).

Change %; \*P<0.05 and \*\*P<0.001

**Table 5: Diurnal Variations in Semen Storage Ability after different hours from Collection in Bucks Exposed to Direct Solar Radiation of Hot Summer Season**

Experimental group	Diurnal time	Semen storage ability after different hours from collection in goats bucks exposed to direct solar radiation of hot summer season					
		1 <sup>st</sup> hr	2 <sup>nd</sup> hr	3 <sup>rd</sup> hr	4 <sup>th</sup> hr	5 <sup>th</sup> hr	6 <sup>th</sup> hr
0 ml vitamin AD <sub>3</sub> E/head	Morning	55±5.5 <sup>a</sup>	39±3.0 <sup>a</sup>	26±5.1 <sup>a</sup>	17±3.0 <sup>a</sup>	7±1.2	3±1.2
	Afternoon	56±4.0 <sup>a</sup>	40±3.2 <sup>a</sup>	28±3.7 <sup>a</sup>	16±4.0 <sup>a</sup>	7±1.2	4±1.0
	Evening	50±5.0 <sup>b</sup>	30±4.9 <sup>b</sup>	20±4.0 <sup>b</sup>	10±2.5 <sup>b</sup>	6±1.0	2±1.1
	Overall	53.67±1.86 <sup>C</sup>	36.33±3.18 <sup>C</sup>	24.67±2.41 <sup>C</sup>	14.33±2.19 <sup>C</sup>	6.67±0.33 <sup>C</sup>	3.00±0.58 <sup>C</sup>
2 ml vitamin AD <sub>3</sub> E/head	Morning	62±2.0 <sup>a</sup>	54±2.0 <sup>a</sup>	38±3.7 <sup>a</sup>	28±3.7 <sup>a</sup>	12±2.7	7±1.6
	Afternoon	63±3.0 <sup>a</sup>	50±2.5 <sup>a</sup>	38±2.0 <sup>a</sup>	26±4.0 <sup>a</sup>	11±1.2	8±1.2
	Evening	58±4.9 <sup>b</sup>	42±3.7 <sup>b</sup>	24±4.0 <sup>b</sup>	13±3.0 <sup>b</sup>	10±1.0	6±2.2
	Overall	61.00±1.53 <sup>B</sup>	48.67±3.53 <sup>B</sup>	30.33±4.67 <sup>B</sup>	22.33±4.71 <sup>B</sup>	11.00±0.58 <sup>B</sup>	7.00±0.58 <sup>B</sup>
Change,% and significant		13.66 <sup>*</sup>	33.97 <sup>**</sup>	22.94 <sup>*</sup>	55.83 <sup>**</sup>	64.92 <sup>**</sup>	133.33 <sup>**</sup>
4ml vitamin AD <sub>3</sub> E/head	Morning	71±2.4 <sup>a</sup>	58±2.0 <sup>a</sup>	42±3.7 <sup>a</sup>	30±2.0 <sup>a</sup>	20±1.0	13±1.0
	Afternoon	69±2.0 <sup>a</sup>	56±2.5 <sup>a</sup>	44±4.0 <sup>a</sup>	31±2.6 <sup>a</sup>	18±2.1	14±3.4
	Evening	60±3.1 <sup>b</sup>	48±3.1 <sup>b</sup>	34±3.1 <sup>b</sup>	23±4.5 <sup>b</sup>	16±2.5	11±1.6
	Overall	66.67±3.39 <sup>A</sup>	54.00±3.06 <sup>A</sup>	40.00±3.06 <sup>A</sup>	28.00±2.52 <sup>A</sup>	18.00±1.16 <sup>A</sup>	12.67±0.88 <sup>A</sup>
Change,% and significant		24.17 <sup>*</sup>	48.64 <sup>**</sup>	62.14 <sup>**</sup>	95.39 <sup>**</sup>	169.87 <sup>**</sup>	322.33 <sup>**</sup>

a , b.. Means within column in each experimental group with different superscript are significantly differ (P < 0.05).

A, B.. Means within overall column in each parameter with different superscript are significantly differ (P < 0.05).

Change %; \*P<0.05 and \*\*P<0.001