# EFFECT OF VITAMIN $AD_3E$ INJECTION ON AGE AND WEIGHT OF WEANING AND REPRODUCTIVE ACTIVITY OF GOATS.

## 1- PHYSIOLOGICAL RESPONSE AND REPRODUCTIVE PERFORMANCE OF GOAT BUCKS DURING DIFFERENT SEASONS IN EGYPT.

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## ABSTRACT

The present study aimed to evaluate the effect of vitamins $AD_3E$ administration during different seasons of the year in Egypt on physiological thermoregulatory response, blood components and semen characteristics of Damascus bucks. Thirty Damascus goat bucks aged 14-15 months and weighing 32-35 kg were divided into three similar groups (10 bucks in each). The first group (Control, G1) was kept without treatment (injected by saline solution) , while the second (G2) and third (G3) groups were injected intramuscularly biweekly with vitamin $AD_3E$ at levels of 2 and 4 ml/buck, respectively. Thermoregulatory parameters, some Blood component and hormones, Semen characteristics, semen storage ability and Scrotal measurements were measured biweekly. The treatment period lasted during different seasons for one year.

Results showed that hair, skin, rectal, scrotal skin, and ear temperatures were the highest ($P<0.05$) in summer and the lowest ($P<0.05$) in winter, but these traits were not affected by vitamins injection.

Concentrations of total protein (TP), albumin (A), Ca and P decreased ($P<0.05$) in summer compared with other seasons, while globulin (G) concentration increased ($P<0.05$) in summer and autumn compared with winter and spring. Concentrations of TP, G, Ca and P increased ($P<0.05$) by vitamins injection. Concentration of testosterone was the highest ($P<0.05$) in autumn and the lowest ($P<0.05$) in summer. The highest ($P<0.05$) $T_3$ and $T_4$ levels was in winter .

Cortisol concentration was highest ($P<0.05$) in summer and the lowest ($P<0.05$) in winter and autumn. Concentration of testosterone, $T_3$ and $T_4$ increased ($P<0.05$), while cortisol concentration decreased ($P<0.05$) by vitamins injection.

No significant changes in scrotal circumference were observed due to change of season or due to vitamins injection. The highest testicular length was in bucks injected with 4 ml vitamins while the lowest testes length was in control bucks. Testes size increased ($P<0.05$) by increasing level of vitamins injection.

The lowest ($P<0.05$) number of ejaculate and semen volume were in summer, while the highest values were in autumn. Number of ejaculate, libido and semen volume increased ($P<0.05$) by vitamins injection and the opposite trend was observed in pH value. The highest motility percentage and the lowest dead sperm, abnormality

and acrosomal damage percentages were during autumn while the lowest motility percentage and the highest dead sperm, abnormality and acrosomal damage during summer. Semen characteristics including percentages of mass motility, dead sperm, abnormality and acrosomal damage improved ($P<0.05$) by vitamins injection. The best semen storage ability was in autumn.

From these results it can be concluded that vitamins $AD_3E$ injection improved semen quality of heat stressed goats bucks, especially at a level of 4 ml/buck.

**Keywords:** $AD_3E$, Goat bucks, body temperature, blood, semen, scrotum.

## INTRODUCTION

Goat bucks, like other animals,require vitamins for optimal performance and health and each vitamin performs a unique function and cannot be replaced by any other vitamin. Because of wide variations in content of vitamins A, $D_3$, and E in normal feedstuffs for animals, particularly harvested forages, and because of the multitude of factors which affect their utilization and bioavailability. Animals cannot produce fat soluble vitamins in their bodies; hence an exogenous regular supply is needed to cover the physiological requirements and to sustain high production performance and anyway, vitamins A, $D_3$, and E must be furnished in farm animals diets, while the water-soluble vitamins and vitamin K are synthesized in apparently adequate amounts by rumen microbes (Hafez, 2012).

Vitamin A has an effective role in keeping all the body's epithelial cells and is plays an important role in the process of vision, spermatogenesis and bone growth (Tanumihardjo, 2011). Vitamin A also is essential for maintaining healthy immune function and deficiency can lead to an impaired response to infection (Fennema, 2008). Vitamin A helps maintain internal and external linings and is necessary for a healthy reproductive tract (Deshmukh and Honmode, 1988). Clinical signs of male infertility related to vitamin A deficiency include delayed onset of puberty, reduced testis size and inferior semen characteristics (Kupfer et al., 1986). Advanced deficiency of vitamin causes degeneration of the somniferous tubules and testicular atrophy (Unni et al., 1983). Rode et al. (1990) reported that hypovitaminosis A significantly decreased paired testis weight, daily sperm production and epididymal sperm reserves, but did not affect daily weight gain.

Vitamin $D_3$ known as cholecalciferol has an important role to increases Ca and P absorption in bone calcification and mineralization. It is responsible for enhancing intestinal absorption of Ca, Fe, Mg, P and zinc. Vitamin $D_3$ promotes Ca absorption in the gut and maintains adequate serum Ca and phosphate concentrations to enable normal mineralization of bone and to prevent hypocalcemic tetany. It is also needed for bone growth and bone remodeling by osteoblasts and osteoclasts (Institute of Medicine, 2010). It has other roles in the body, including modulation of cell growth, neuron-muscular and immune function, and reduction of inflammation (Holick, 2006). Vitamin $D_3$ is interested in reproduction because its role in absorption and retention of calcium and phosphorus. Principle effects of vitamin $D_3$ deficiency have been related to development of the skeletal system of fetus.

Vitamin $D_3$ may influence the time of first postpartum estrus and calving interval (Bearden and Fuquay, 1997).

Vitamin E (tocopherol) is a fat soluble antioxidant prevents oxidation of unsaturated fatty acids and vitamin A in the intestine and acts as an important role in growth, fertility and increase body resistance (National Institutes of Health, 2013). It removes the free radicals which are unstable compounds that damage the cell structure by combining with free oxygen and destroys free radicals (Mayo Clinic, 2013). In addition, immunity levels improve when vitamin E is consumed (Institute of Medicine, 2013). A lack of vitamin E causes failure in the reproductive system. Laflamme and Hidiroglou (1991) found that overall pregnancy rate was increased significantly with vitamin E supplementation.

Climatic heat is one of the major constraints for growth, milk production and reproduction which are impaired as a result of the drastic changes in biological functions caused by heat stress (Habeeb et al., 1992). During the hot period the concentrations of these vitamins reduce dramatically in the peripheral blood (Weiss et al., 1994). Thus, animals are venerable to different metabolic disorders, contagious diseases and a reduction in milk production and quality during this period. Increasing the proportion of both A and E vitamins starting few weeks pre-partum and post-partum was found to increase milk production in cattle (Panda et al., 2006).

Aim of the current study was to evaluate the effect of vitamins $AD_3E$ administration during different seasons of the year in Egypt on physiological thermoregulatory response, blood components and semen characteristics of Damascus bucks.

## 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 started from December 2013 and up to the end of November 2014.

**Animals:**

Total of 30 Damascus goat bucks (14-15 months of age and 32-35 kg body weight) were divided into three similar groups (10 bucks in each). Bucks in the first group (G1) was kept without treatment as control (injected by saline solution), while those in the second (G2) and third (G3) groups were injected biweekly with vitamins $AD_3E$ (DEVEDRY-MED Injection), manufactured by ARABCOMED, Egypt. Each ml contained (8000 I.U) of vitamin A, (4000 I.U) of vitamin $D_3$ (Cholicalcipherol) and 20 mg of vitamin E (a tocopherol acetate) at levels of 2 and 4 ml/buck, respectively. $AD_3E$ solution injected intramuscularly through buck neck area for two months prior to the experimental period, which lasted about one year (four seasons). Bucks were housed in concrete floored, partially asbestos roofed in semi-open sheds during the experimental period. The surface area of each shed

pen 4 x 6 meters and was surrounded by brick walls of two meters height except south wall was 4.5 meters.

**Feeding system:**

Animals were offered their requirements from concentrate feed mixture (CFM) and rice straw according to NRC (1985). The 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. Chemical analysis of CFM and rice straw were carried out according to A.O.A.C. (2000). Chemical composition (on DM basis %) of CFM 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 the times of carrying out the physiological measurements. Ambient air temperature was recorded using mercury thermometer to the nearest $0.1^0$C. Maximum and minimum temperatures were recorded using thermometer. 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$^o$C – {(0.31 – 0.31 RH) (db$^o$C – 14.4)} where db$^o$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 and relative humidity values and THI estimated monthly and seasonally are presented in Table (1).

**Table (1): Average Monthly of ambient temperatures, relative humidity (%) and THI values during different months of the year.**

| Season of year | Months of year | Environmental conditions | | | |
|---|---|---|---|---|---|
| | | AT ºC | RH, % | THI | HS |
| Winter | December | 18.0 | 55 | 17.5 | Absence |
| | January | 18.0 | 45 | 17.4 | Absence |
| | February | 20.0 | 45 | 19.1 | Absence |
| | Overall | 18.7 | 48 | 18.0 | Absence |
| Spring | Marsh | 25.0 | 45 | 23.2 | Moderate |
| | April | 27.0 | 45 | 24.8 | Severe |
| | May | 30.0 | 40 | 27.1 | Very severe |
| | Overall | 27.3 | 43 | 25.0 | Severe |
| Summer | June | 35.5 | 55 | 32.5 | Very severe |
| | July | 37.5 | 55 | 34.3 | Very severe |
| | August | 40.0 | 50 | 36.0 | Very severe |
| | Overall | 37.7 | 53 | 34.3 | Very severe |
| Autumn | September | 32.5 | 55 | 30.0 | Very severe |
| | October | 28.5 | 45 | 27.0 | Very severe |
| | November | 20.5 | 45 | 19.8 | Absence |
| | Overall | 27.2 | 48 | 25.2 | Severe |

**AT= Ambient air Temperature, RH= Relative humidity ,THI= Temperature humidity index, HS=Status of heat stress**

**Thermoregulatory parameters:**

At 13.00-14.00 h temperatures of hair (HT), skin (ST), rectal (RT), scrotal skin (ScT) and ear (ET) as a physiological measurements of bucks were measured biweekly by alcohol thermometer. Rectal temperature was measured to the nearest 0.1ºC by inserting electronic thermometer probe to the depth of 5-6 cm into the rectum. 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 in the same time of thermoregulatory parameters 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$^{o}$C until analyses. Concentrations of total protein (TP) and albumin (A) in plasma were estimated using Biuret reaction and bromocresol green reagent kites, respectively, manufactured by BIODIAGNOSTIC Company (Egypt). Globulin (G) concentration was calculated by difference between (TP) and (A) values. Calcium and inorganic phosphorus was measured by direct method with ammonium molybdate reagent kites manufactured by BIODIAGNOSTIC Company, Egypt. Concentrations of testosterone, triiodothyronine ($T_3$), thyroxin ($T_4$)and cortisol hormones ware 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 computerizes Gamma Counter. The tracer in the hormones was labeled with iodine-125 (125I).

**Semen characteristics:**

Semen ejaculates were collected biweekly by artificial vagina. On day of collection, number of ejaculates and volume, libido (reaction time, sec.) were measured in the 1$^{st}$ Semen ejaculate, pH value, percentages of sperm motility, dead, abnormalities and acrosomal damage were also measured. Libido was assessed according to Chenoweth (1981). Hydrogen ion concentration was tested with digital pH meter. 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, semen samples from each buck were immediately transferred to the laboratory which extended with Tris-egg yolk fructose and incubated at 37ºC. The changes in the sperm motility percentage for 1, 2, 3, 4, 5 and 6 h was determined.

**Scrotal measurements:**

Scrotal length (distance between abdominal margin and scrotal tip), scrotal circumference and testicular length (from top of the tail to the head of the epididymis for each testis) were measured with a flexible metal tape biweekly according to Oberst et al. (2011) in the same time of physiological parameters measured.

**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 DISCUSSION

**Effect of seasons of the year and vitamin $AD_3E$ Injection on:**
**Thermoregulatory parameters:**

Data in Table (2) revealed that Hair (HT), skin (ST), rectal (RT), scrotal (ScT) and ear (ET) temperatures, pulse rate (PR) and respiration rate (RR) of bucks were affected significantly ($P<0.05$) by season, being the highest in summer and the lowest in winter. However, the corresponding temperature values were higher in spring and autumn than in winter, and lower than that in summer. No significant differences were observed in most of these thermo-regulatory parameters between spring and autumn seasons. On the other hand, the above mentioned parameters were not affected significantly by vitamins injection.

**Table (2): Effect of $AD_3E$ vitamins injection on thermoregulatory response of goat bucks during different seasons.**

| Group | Season | Temperatures ($^oC$) | | | | | PR (pulse/ min) | RR (Br./min) |
|---|---|---|---|---|---|---|---|---|
| | | HT | ST | RT | ScT | ET | | |
| G1 (Control) | Winter | 21.0±1.83c | 32.8±0.56c | 38.4±0.08c | 17.9±1.41c | 28.3±1.68c | 77.8±1.01c | 36.6±2.95d |
| | Spring | 30.4±3.58b | 34.4±0.44bc | 38.8±0.18bc | 26.2±2.11b | 32.6±1.00b | 86.8±0.86b | 56.4±10.00c |
| | Summer | 40.1±2.25a | 37.8±1.21a | 39.6±0.37a | 31.5±1.08a | 36.2±1.18a | 96.7±2.17a | 104.4±4.43a |
| | Autumn | 33.7±2.46b | 35.2±0.86b | 38.9±0.34b | 26.7±2.08b | 33.1±1.28b | 88.5±5.48b | 67.9±17.88b |
| Overall mean | | 31.30±3.98 | 35.05±1.04 | 38.93±0.25 | 25.58±2.82 | 32.55±1.63 | 87.45±3.88 | 66.33±14.24 |
| G2 (2 ml $AD_3E$/h) | Winter | 22.0±1.82c | 33.9±0.35c | 38.6±0.09c | 19.8±1.01c | 31.3±1.19c | 78.8±0.62c | 40.4±3.54d |
| | Spring | 30.7±3.00b | 34.8±0.09bc | 38.9±0.01b | 26.3±1.94b | 32.9±0.88b | 87.8±1.11b | 61.2±5.67c |
| | Summer | 38.9±2.04a | 36.8±1.44a | 39.9±0.34a | 30.3±0.83a | 35.0±1.26a | 92.6±1.31a | 98.8±3.74a |
| | Autumn | 33.7±2.30b | 35.2±0.64b | 38.7±0.08bc | 26.6±1.27b | 33.3±0.99b | 85.0±2.94b | 66.3±16.93b |
| Overall mean | | 31.33±3.54 | 35.18±0.61 | 39.03±0.30 | 25.75±2.18 | 33.13±0.76 | 86.05±2.88 | 66.68±12.08 |
| G3 (4ml $AD_3E$/h) | Winter | 22.6±1.78c | 34.4±0.51c | 38.8±0.07b | 20.8±0.91c | 32.2±0.93c | 81.1±0.81c | 44.2±4.98d |
| | Spring | 30.7±2.74b | 34.9±0.09c | 38.9±0.12b | 25.9±1.54b | 33.1±0.37b | 86.9±0.52b | 57.9±3.76c |
| | Summer | 38.3±1.72a | 36.6±1.39a | 39.8±0.22a | 29.5±0.59a | 34.9±0.90a | 93.1±3.73a | 96.1±3.55a |
| | Autumn | 33.8±1.87b | 35.3±0.22b | 38.7±0.13b | 26.4±1.14b | 33.6±0.47b | 84.5±1.64b | 69.7±13.43b |
| Overall mean | | 31.35±3.31 | 35.30±0.47 | 39.05±0.25 | 25.65±1.80 | 33.45±0.56 | 86.40±2.53 | 66.98±11.02 |

**a , b.. Means within the same column with different superscript are significantly differ (P<0.05).**
**HT= Hair temp, ST= Skin temp, RT= Rectal temp, ScT= Scrotal temp, ET= Ear temp PR= Pulse rate, RR=Respiration rate**

These results are in agreement with Ghosh et al. (1993), who reported that the average RR of goats was 29.09 breaths /min and was, being significantly and positively related to ambient temperature. During heat stress, cardiac output and respiration are accelerated and reach the stage of panting to increase evaporative cooling, increased amounts of $CO_2$ are exhaled (Tsigos and Chrouso, 2002). In this respect, Shinde et al. (2002)

reported a marked seasonal change in RT, RR and energy expenditure in goats under tropical conditions. Also, Banerjee et al. (2015) found significant increase in RT and RR in summer as compared to in winter in goats. Phulia et al. (2010) reported increase in PR and RR as affected by increased environmental temperature. The same authors reported increase in RT and RR from 38.97$^0$C and 43.66 breath /min to 39.35 $^0$C and 77.33 breath/min, respectively, when goats were kept for 6 hours in hot ambient temperature in summer. AL-Haidary et al. (2012) found that exposure to heat stress results in a significant increase in RT and this was associated with a significant increase in RR and ST. Moreover, Popoola et al. (2014) found that THI had significant effects on RR of goats; the RR differs significantly when THI was greater than 27.50 and the highest RR (70.40 breaths/min) was obtained when THI ranged between 25.50-27.50, while the least RR (49.27 breaths/min) was obtained when THI was less than 23.50. Increased RR is an attempt to increase heat loss by evaporative cooling and changes of metabolism and muscle activity of goats (Habeeb et al., 1992).

Exposure to high ambient temperature augments the animal efforts to dissipate body heat by increasing RR (Marai et al., 2007). Increasing heart rate and PR is attributed to increase in muscular activity controlling the rate of respiration, concurrent with elevated RR and the reduction in resistance of peripheral vascular beds and arteriovenous anastomoses (Devendra, 1987). In addition, increase in PR increases blood flow from the core to the surface as a result of this more heat is lost by sensible and insensible means (Marai et al., 2007).

In goats, El-Sherbiny et al. (1983) found that in Arabi and Zaraiby goats that increasing of air temperature (AT) from 10 to 40$^o$C increased ST significantly when air temperature was higher than 30$^o$C and ST reached 40-40.5$^o$C, when AT was 40$^o$C, while it was 37.8$^o$C at 25$^o$C AT. Shalaby and Johnson (1993) studied that the physiological responses of Anglo-Nubian goats kept under cyclic hot environmental conditions (25-35$^o$C AT and 25-65% RH). They found that ST was significantly increased with increasing AT and the overall mean of ST in goats was 32.9$^o$C. Marai et al. (2009) found that exposure to heat stress conditions was accompanied with significant increase in ST, whereas it is regulated by blood flow to the skin, evaporation, conduction and radiation from the skin.

**Blood biochemical changes:**

Table (3) showed that season of the year affected significantly blood biochemical components. Concentrations of TP, A, Ca and P in goats were significantly ($P<0.05$) lower in summer than in other seasons. The highest values of TP, A, Ca and P were observed in winter and autumn without any significant difference. Concentration of G showed an opposite trend, while Ca/P ratio was higher in summer than in other seasons due to a decline in P more than in Ca in summer. As affected by vitamins injection, concentrations of TP, G, Ca and P increased progressively by increasing level vitamins, being significantly ($P<0.05$) the highest in G3 group, while the lowest values were in control bucks G1 group. However, concentration of A and Ca/P ratio were insignificantly higher in G1 than G2 and G3.

The plasma proteins provide an efficient way of transferring the heat from inside the body to the outer surface in the skin for heat dissipation by non evaporative process during heat stress, since it holds an adequate percentage of water in the intra-vascular fluids and maintains the viscosity of the blood (Habeeb et al., 1992). This finding is compatible with the role of AL in maintaining plasma osmotic pressure and transportation of protein in the blood. The significant decline in plasma proteins with rising temperature seems to be due to dilution of plasma proteins as a result of the increase in body water content, and decrease of protein synthesis as a result of the depression of anabolic hormonal secretion and the increase in the catabolic hormones such as glucocorticoids and catecholamines (Habeeb et al., 2008a). In addition, the decrease in plasma protein may also be due to the decrease in feed nitrogen and mineral intake, which occurs under heat stress conditions (Habeeb et al., 2008b). The recorded increases in TP and G in bucks treated with vitamin mixture are supported by other reports. El-Nor (2000) found that serum TP and G were slightly increased in lactating buffaloes supplemented with $AD_3E$ vitamins. Similar results were obtained by Gado et al. (2014). They indicated that plasma TP, G and A/G ratio in lactating Ossimi ewes were decreased ($P<0.01$) under summer conditions in comparison with winter. Vitamins supplementation increased significantly ($P<0.01$) TP, G and A/G ratio. Abdelatif et al. (2009) obtained similar results in female Nubian goats.

**Table (3): Effect of $AD_3E$ vitamins injection on components concentrations in blood plasma of goat bucks during different seasons.**

| Group | Season | Blood components | | | | | |
|---|---|---|---|---|---|---|---|
| | | Total protein (g/dl) | Albumin (g/dl) | Globulin (g/dl) | Ca (mg/dl) | P (mg/dl) | Ca/P ratio |
| G1 (Control) | Winter | 7.7±0.07a | 4.5±0.03a | 3.2±0.12bc | 11.0±0.64a | 5.6±0.27a | 1.96 |
| | Spring | 7.4±0.06b | 4.3±0.10b | 3.1±0.06c | 8.5±0.38b | 4.5±0.46b | 1.89 |
| | Summer | 7.2±0.03b | 3.6±0.23c | 3.6±0.23a | 8.2±0.19b | 3.5±0.30c | 2.34 |
| | Autumn | 7.7±0.10a | 4.3±0.06b | 3.4±0.06b | 10.5±0.25a | 5.2±0.13a | 2.02 |
| Overall mean | | 7.50±0.12C | 4.18±0.20 | 3.33±0.11C | 9.55±0.70C | 4.70±0.46C | 2.05±0.10 |
| G2 (2 ml $AD_3E$/h) | Winter | 8.0±0.09a | 4.4±0.06a | 3.6±0.03bc | 12.1±0.50a | 6.3±0.12a | 1.92 |
| | Spring | 7.6±0.03b | 4.2±0.07b | 3.4±0.06c | 9.8±0.34b | 5.1±0.44b | 1.92 |
| | Summer | 7.4±0.03b | 3.7±0.24c | 3.7±0.19ab | 8.4±0.25c | 4.1±0.19c | 2.05 |
| | Autumn | 8.0±0.07a | 4.3±0.03ab | 3.8±0.03a | 11.8±0.23a | 5.9±0.22a | 2.00 |
| Overall mean | | 7.75±0.15B | 4.15±0.16 | 3.63±0.09B | 10.53±0.87B | 5.35±0.49B | 1.97±0.03 |
| G3 (4 ml $AD_3E$/h) | Winter | 8.1±0.09a | 4.4±0.09a | 3.8±0.01ab | 12.7±0.64a | 6.1±0.26a | 2.08 |
| | Spring | 7.8±0.10b | 4.2±0.03b | 3.6±0.12b | 10.2±0.23b | 5.4±0.17b | 1.89 |
| | Summer | 7.6±0.06b | 3.7±0.25c | 3.9±0.19a | 9.1±0.33c | 4.5±0.29c | 2.02 |
| | Autumn | 8.3±0.09a | 4.4±0.03a | 3.9±0.06a | 12.0±0.33a | 6.1±0.12a | 1.97 |
| Overall mean | | 7.95±0.16A | 4.18±0.17 | 3.80±0.07A | 11.00±0.82A | 5.53±0.38A | 1.99±0.04 |

**a , b.. Means within the same column with different superscript are significantly differ (P<0.05).**

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

**Blood hormonal levels:**

Table (4) showed that season affected significantly (P<0.05) hormonal concentration of testosterone, $T_3$ and $T_4$ in plasma of bucks, being lower in summer than in other seasons. Testosterone was recorded highly significant (P<0.05) in autumn, while the highest $T_3$ was observed in winter and autumn but the highest $T_4$ was observed in winter. On other hand, cortisol was significantly (P<0.05) the highest in summer and the lowest in winter. Concentration of testosterone, $T_3$ and $T_4$ increased significantly by increasing level of vitamins injection. The highest concentrations of testosterone, $T_3$ and $T_4$ were observed in G3, while the lowest values were in G1 group. An opposite trend was observed in cortisol, since level of cortisol decreased progressively with increased the level of vitamins injection.

In accordance with the present results, Curtis (1983) reported that testes testosterone content decreased from 1.1 to 0.4 ng/ml and spermatic vein plasma concentration decreased from 8.2 to 1.9 µg /dl, when rams were exposed to an average environmental temperature of 30°C for 14 days. In this respect, Delgadillo and Chemineau (1992) documented that plasma testosterone concentrations are related to season. The lowest serum testosterone level was recorded during hot environmental conditions in Ossimi rams (El-Darawany, 1999). contrarily, Zarazaga et al. (2009) found that the plasma testosterone concentrations were higher in summer and autumn than in spring and winter. The reduction in thyroid activity ($T_3$ and $T_4$) under heat stress is a process of adaptation to the environmental conditions (Habeeb et al., 2014). This depression in summer has an important role in the animal adaptation to environmental changes (Habeeb et al., 1992).

Several studies showed that the cortisol level increased significantly under high ambient temperature. In this way, Marai et al. (2009) confirmed that plasma cortisol level in Ossimi x Suffolk ram was significantly higher in summer than in winter and autumn under Egyptian conditions. In buffaloes, Habeeb et al. (2012) showed that exposure to elevated ambient temperature were associated with increase of cortisol level. Also, Al-Samawi et al. (2014) found that serum cortisol concentrations were significantly higher in summer than in winter. The increase in cortisol was estimated as 38 % after 1 h and 62% after 2 h reaching a peak of 120% at 4 h when goats were exposed to hot conditions. Generally, secretion of cortisol stimulates physiological adjustments that enable an animal to tolerate the stress caused by a hot environment (Habeeb et al., 1992). During heat stress, one of the main functions of cortisol is to promote protein catabolism, converting the protein into amino acids to support gluconeogenesis (Ribeiroa et al., 2014). In consistence with the present results, Gupta et al. (2005) reported vitamin E supplementation decreased concentrations of plasma cortisol in crossbred dairy cattle. Finally, Yasothai (2014) reported an importance role of vitamins on reproduction and hormonal levels in dairy cattle.

**Table (4): Effect of $AD_3E$ vitamins injection on hormonal levels in blood plasma of goat bucks during different seasons.**

| Group | Season | Hormonal level (ng/ml) | | | |
|---|---|---|---|---|---|
| | | Testosterone | Triiodothyronine ($T_3$) | Thyroxin ($T_4$) | Cortisol |
| G1 (Control) | Winter | 5.9±0.88b | 1.16±0.02a | 43.8±1.68a | 12.0 ±0.57c |
| | Spring | 4.3±0.12c | 1.00±0.03b | 41.7±2.80b | 12.8 ±0.87bc |
| | Summer | 3.6±0.31d | 0.84±0.05c | 33.9±1.68c | 20.0 ±0.69a |
| | Autumn | 8.3±0.15a | 1.11±0.01a | 40.7±1.90b | 13.3 ±0.67b |
| Overall mean | | 5.53±1.04C | 1.03±0.07C | 40.03±2.14C | 14.53±1.84A |
| G2 (2 ml $AD_3E$/h) | Winter | 6.6±0.86b | 1.26±0.03a | 46.8±2.82a | 10.9±0.62c |
| | Spring | 5.0±0.12c | 1.12±0.05ab | 44.0±2.38ab | 11.9±0.42bc |
| | Summer | 4.7±0.22c | 0.97±0.05b | 39.8±1.88c | 18.0 ±0.69a |
| | Autumn | 9.2±0.27a | 1.26±0.06a | 42.7±1.80b | 12.9±0.22b |
| Overall mean | | 6.38±1.03B | 1.15±0.07B | 43.33±1.45B | 13.43±1.58B |
| G3 (4 ml $AD_3E$/h) | Winter | 6.8±0.70b | 1.32±0.06a | 48.8±2.88a | 10.9 ±0.74b |
| | Spring | 5.3±0.26c | 1.16±0.06b | 47.5±1.55a | 10.2 ±0.42c |
| | Summer | 5.1±0.03c | 1.03±0.07c | 42.9±1.89b | 16.9 ±0.40a |
| | Autumn | 9.4±0.46a | 1.30±0.04a | 43.4±2.38b | 10.9 ±0.40b |
| Overall mean | | 6.65±0.99A | 1.20±0.07A | 45.65±1.47A | 12.23±1.57C |

**a , b.. Means within the same column with different superscript are significantly differ (P<0.05).**

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

## Reproductive measurements:

### Scrotal and testicular measurements:

Data in Table (5) showed that scrotal length, testicular length and size of bucks were significantly (P<0.05) affected by season, while scrotal circumference was not affected significantly by season. In all groups, scrotal length was significantly (P<0.05) higher in summer and autumn than in winter and spring. Testicular length was significantly (P<0.05) the highest in autumn and the lowest in summer. However, testicular size was significantly (P<0.05) the highest in winter and the lowest in summer. Concerning the effect vitamins injection on testicular measurements, only testicular length and size significantly (P<0.05) by vitamins injection at a level of 4 ml (G3), while the lowest values were recorded in G1. However, scrotal length and circumference were not affected significantly by vitamin injection.

In accordance with the present results, Marai et al. (2009) found that exposure to heat stress conditions was accompanied with significant increase in scrotal length and significant decrease in scrotal circumference and testes length. However, Mikelsen et al. (1981) reported the lowest scrotal circumference values in summer and the highest values in autumn. Elsheikh and Elhammali (2015) found that the scrotal circumference was significantly higher in winter than in summer and autumn. Scrotal circumference and testicular consistency tone size and weight are excellent indicators of sperm production capacity and spermatogenic function. It is important to emphasize the close relationship between testosterone concentration and scrotal circumference. Scrotal circumference is directly positive correlated to testosterone concentration (Bezerra et al., 2009). Scrotal circumference of rams showed significant effect on number of mounting without ejaculation

(libido), testicular measurements and testosterone concentration after ejaculation. Higher testosterone concentration in the group having scrotal circumference >25 cm may be the main factor affecting testes measurement, and better libido compared to rams having scrotal circumference ≤25 cm (Mahmoud, 2013). Scrotal circumference is a simple repeatable method of measurement of testicular size which is highly correlated with testicular weight, semen quality and with fertility (Kastelic et al., 2001; Karakuş et al., 2010).

**Table (5): Effect of $AD_3E$ vitamins injection on scrotal and testes measurements of goat bucks during different seasons.**

| Group | Season | Scrotal and testes measurements, cm | | | |
|---|---|---|---|---|---|
| | | Scrotal length | Scrotal circumference | Testes length | Testes size |
| G1 (Control) | Winter | 11.6±0.5b | 21.8±1.1 | 7.9±0.1b | 74.0±3.5a |
| | Spring | 12.0±0.1b | 20.3±0.2 | 7.6±0.2bc | 69.3±1.3b |
| | Summer | 15.1±1.3a | 20.9±0.8 | 7.4±0.1c | 64.7±0.7c |
| | Autumn | 14.1±1.2a | 22.1±0.5 | 8.4±0.3a | 66.8±2.6bc |
| Overall mean | | 13.2±0.8 | 21.3±0.4 | 7.8±0.2B | 68.7±2.0C |
| G2 (2 ml $AD_3E$/h) | Winter | 10.5±0.3b | 22.3±0.5 | 8.3±0.1b | 92.7±2.8a |
| | Spring | 11.6±0.8b | 21.9±0.9 | 8.2±0.2b | 82.7±2.4b |
| | Summer | 15.9±1.5a | 21.1±0.3 | 8.1±0.1b | 81.3±1.3b |
| | Autumn | 14.0±1.6a | 21.6±1.1 | 8.9±0.1a | 81.1±3.7b |
| Overall mean | | 13.0±1.2 | 21.7±1.0 | 8.4±0.2AB | 84.5±2.8B |
| G3 (4 ml $AD_3E$/h) | Winter | 9.3±0.10b | 23.8±0.5 | 8.6±0.3b | 100.7±3.9a |
| | Spring | 11.4±1.0b | 22.9±1.0 | 8.4±0.1bc | 89.3±4.8b |
| | Summer | 16.9±1.3a | 20.9±0.7 | 8.2±0.1c | 89.3±2.4b |
| | Autumn | 15.0±1.6a | 21.1±1.8 | 9.2±0.1a | 89.8±4.6b |
| Overall mean | | 13.2±1.7 | 22.2±1.4 | 8.6±0.2A | 92.3±2.8A |

**a , b.. Means within the same column with different superscript are significantly differ (P<0.05).**

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

**Semen characteristics:**

Table (6) showed that season affected significantly (P<0.05) number of ejaculates (NE), libido (LI), volume (V), pH value, percentages of motility (SM), livability (DS), abnormality (SA) and acrosomal damage (AD) of semen in bucks. The lowest NE, LI, SV and SM and the highest DS, SA and AD percentages were recorded in summer, while the highest NE, LI, SV, SM and the lowest DS, SA and AD percentages were observed in autumn. However, the lowest pH value was always in autumn. Generally, semen characteristics were better in winter than in summer and at the same time lower than in autumn. Values of NE, LI and SV increased significantly (P<0.05) by increasing the level of vitamins injection. $AD_3E$ injection significantly (P<0.05) improved SM and depressed DS, SA and AD percentages, being the best in G3. However, no significant differences were observed in percentages of SM, DS, SA and AD in G2 and G3. From these results, it can be concluded that the best semen of bucks was produced in autumn, but was better for G3 bucks. Therefore it can be recommended vitamins $AD_3E$ injection to improve semen quality of bucks during summer season.

Buck semen volume as determined by various workers showed clear seasonal variation. In this line, El-Gamal (1975) reported SV to be low in summer and winter and high in autumn. Gunzel et al. (1982) confirmed that ejaculate volume tended to diminish with the increase of daylight, reaching a minimal in June (summer). Abi-Saab and Hamadah (1984) recorded ejaculate volume as 0.65 ml during winter and early summer and 1.30 ml in autumn. Also, the lowest ejaculate volume was reviewed to be in summer in Ossimi by El-Darawany (1999). Elevated body temperature during periods of high ambient temperature leads to testicular degeneration and reduction in percentage of normal and fertile spermatozoa in the ejaculate (Marai et al., 2002). A high percentage of live and progressively motile sperm cells is essential for accepted semen quality and high conception rates.

Sperm motility differs also according to season of the year. Most studies indicate that live sperm percentages decrease with the increase in ambient temperature (Habeeb et al., 2013). Recently, Elsheikh and Elhammali (2015) found that percentage of SA increased significantly and the percentage of live sperms decreased in summer. The seasonal differences in semen quality seemed to be attributed to both meteorological and nutritional factors (Aboul-Ela and Chemineau, 1988) as well as the sperm output and semen characteristics are adversely affected following exposure to long daylights (Habeeb et al., 2008c).

In association with improving semen quality of bucks injected with vitamins in the present study, Sing et al. (1989) recorded that vitamin E improved significantly the percent of live spermatozoa in Indian buffalo bulls. Velasquez-pereira et al. (1998) indicated that feeding vitamin E to bulls increased the percentage of bull spermatozoa and improved the number of mounts in the first test and the time to first service in the second test. In Merino rams, Gokcen et al. (1990) found that semen characteristics and acrosomal morphology were significantly better in the supplemented animals with 2500 mg/kg vitamin E or 50 mg/kg selenium than in the controls. Abdel-Rahman and AI-Karablieh (2000) found that injection of vitamins $AD_3E$ caused an increase of 8.8% in lambing rate increased the fertility of ewes by 8.0% compared with the control. In addition, Luo et al. (2011) and Habeeb et al. (2013) found that vitamin E supplemented in diet can improve density of spermatogenic cell, Sertoli cells, and diameter of seminiferous tubule and thickness of germinal epithelium and concluded that vitamin E has a positive role in improving the development of testis in sheep.

**Semen storage ability:**

Table (7) showed storage ability of buck semen in term of sperm motility percentage in semen stored at 37$^{o}$C for 6 h from collection. Results revealed that semen collected in autumn showed significantly ($P<0.05$) the highest sperm motility as compared to other seasons in G1,G2 and G3 groups at all storage times, while the lowest values were recorded in summer. However, sperm motility percentage was significantly ($P<0.05$) higher in winter than in summer. In addition, vitamins injection had beneficial effects on semen storage ability, being significantly ($P<0.05$) the highest for bucks in G3, moderate for G2, and the lowest in G1 at all storage times and this may be due to injected with vitamin mixtures.

**6**

**7**

## CONCLUSION

It can be concluded that injection of vitamin $AD_3E$ mixture is effective, safe and practical technique in improving libido and semen quality of bucks under heat stress conditions. In addition, using $AD_3E$ injection caused reduction of heat load on bucks and alleviates the physiological response of goats bucks, especially, during hot summer season in Egypt.

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# تـأثير الحقـن بفيتامينـات أد٣هـ علـى عمـر ووزن الفطـام والنشـاط الجنسـي للمـاعز. ١-الاسـتجابة الفسـيولوجية والاداء التناسـلي لـذكور المـاعز خـلال فصـول السـنة المختلفة في مصر.

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تم اجراء البحث على ثلاثين ذكر ماعز دمشقي بعمر ١٤-١٥ شهر وبمتوسط وزن بين ٣٢-٣٥ كجم والتي قسمت الى ثلاث مجموعات متماثلة عشرة في كل منها وذلك لدراسة أهمية الحقن بفيتامينات أد٣هـ بمستويات مختلفة على الأداء الفسيولوجي والكفاءة التناسلية لذكور الماعز الدمشقي تحت الظروف المناخية المختلفة لفصول السنة في مصر. حيث تم حقن المجموعة الأولى (المقارنة) بمحلول فسيولوجي كل أسبوعين أما المجموعة الثانية والثالثة حقنتا بخليط فيتامينات أد٣هـ بمعدل ٢ و٤ مل/رأس كل أسبوعين على الترتيب وتم الحقن قبل البداية بشهرين. تم قياس مقاييس التنظيم الحراري وتقدير بعض مكونات الدم والهرمونات وتم جمع عينات السائل المنوي وتم تقدير القدرة التخزينية له وتم قياس بعض مقاييس كيس الصفن والخصيتين كل أسبوعين طوال مدة التجربة والتي استمرت (أربعة مواسم) عام.

**وكانت أهم النتائج كالتالي:**

ارتفعت درجة حرارة كل الشعر وسطح الجلد و الشرج و كيس الصفن والأذن معنويا في فصل الصيف بينما كانت الأقل معنويا في الشتاء و لم تتأثر هذه المقاييس معنويا بالحقن بالفيتامين.

انخفض تركيز كل من البروتينات الكلية والألبيومين والكالسيوم والفسفورالعضوى في الدم معنويا صيفا مقارنة بالفصول الأخرى بينما ارتفع تركيز الجلوبيولين معنويا في فصلى الصيف والخريف مقارنة بالشتاء والربيع. ارتفع تركيز كل من البروتينات الكلية والألبيومين والجلوبيولين والكالسيوم والفسفورالعضوى معنويا بالحقن بالفيتامين. ارتفع تركيز هرمون التستوستيرون معنويا في فصل الخريف وانخفضت معنويا في فصل الصيف. ارتفع تركيز هرمونات الغدة الدرقية معنويا في فصل الشتاء. ارتفع تركيز هرمون الكورتيزول معنويا أثناء فصل الصيف وانخفض معنويا في الشتاء والخريف. ارتفع تركيز كل من هرمون التستوستيرون وهرمونات الغدة الدرقية معنويا بينما انخفض تركيز هرمون الكورتيزول معنويا بالحقن بالفيتامين.

لا يوجد تغير معنوي لمحيط كيس الصفن بتغير فصول السنة او الحقن بالفيتامين. زاد طول الخصية لذكور الماعز معنويا بالحقن بالفيتامين وكان الاقل في مجموعة المقارنة وكان الأعلى في الحيوانات المحقونة ب ٤ ملى فيتامين . زاد حجم الخصيتين معنويا مع زيادة معدل الحقن بالفيتامينات.

انخفض عدد القذفات وحجم قذفة السائل المنوي معنويا صيفا بينما كانت الأعلى في الخريف. عدد القذفات والرغبة الجنسية وحجم القذفة ارتفعت معنويا بالحقن بالفيتامين وقد لوحظ عكس هذا الاتجاه في رقم حموضة السائل المنوي .أعلى نسبة لحركة الحيوانات المنوية وأقل نسبه للحيوانات المنوية الميته وتهتك الاكروسوم كانت في فصل الخريف بينما أقل نسبة لحركة الحيوانات المنوية وأعلى نسبه للحيوانات الميته وتهتك الاكروسوم كانت في فصل الصيف .خصائص السائل المنوي وخاصة حركه الحيوانات المنوية والحيوانات المنوية الميته وتهتك الاكروسوم تحسنت معنويا بالحقن بالفيتامين وكانت أفضل قدرة تخزينية للسائل المنوي في الخريف.

من هذه الدراسة نستنتج أنه يمكن تحسين جودة وخواص السائل المنوي وتخفيف الاجهاد الحرارى لذكور الماعز بالحقن بفيتامين أد٣هـ كل أسبوعين خاصة مستوى ٤ملى/رأس.

**Table (6): Effect of $AD_3E$ vitamins injection on semen characteristics of goat bucks during different seasons.**

| Group | Season | Semen characteristics* | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | NE | V, ml | LI, sec. | pH value | SM, % | DS, % | SA, % | AD, % |
| G1 (Control) | Winter | 1.7±0.35b | 0.8±0.03b | 64.3±12.9c | 7.5±0.01ab | 68.7±0.67b | 30.3±0.68b | 18.1±0.41b | 15.9±0.98b |
| | Spring | 2.1±0.47a | 0.8±0.03b | 97.3±8.6b | 7.7±0.04a | 61.3±0.90c | 38.1±0.70a | 18.1±0.88b | 17.4±0.99ab |
| | Summer | 1.6±0.23b | 0.7±0.04c | 40.0±7.01d | 7.7±0.04a | 62.7±0.67c | 37.1±0.93a | 21.9±0.80a | 20.3±0.24a |
| | Autumn | 2.3±0.37a | 1.3±0.54a | 118.0±10.6a | 7.4±0.02b | 75.3±0.67a | 23.8±0.81c | 14.0±0.31c | 13.8±0.36c |
| Overall mean | | 1.9±0.17C | 0.90±0.14C | 79.90±10.3C | 7.58±0.08A | 67.0±3.2B | 32.3±3.3A | 18.0±1.6A | 16.9±1.4A |
| G2 (2 ml $AD_3E$/h) | Winter | 2.3±0.35bc | 1.0±0.02a | 117.3±12.6b | 7.4±0.02ab | 74.0±0.58b | 24.5±0.82b | 12.7±0.47b | 11.9±0.37b |
| | Spring | 2.7±0.55b | 0.9±0.05b | 70.0±11.2c | 7.4±0.01ab | 70.7±0.40bc | 28.2±0.36ab | 14.4±0.69ab | 13.7±0.70ab |
| | Summer | 1.9±0.47c | 0.9±0.04b | 66.0±3.47c | 7.5±0.01a | 66.7±0.67c | 32.5±0.93a | 16.4±0.35a | 15.1±0.65a |
| | Autumn | 3.5±0.44a | 1.0±0.05a | 132.0±12.1a | 7.3±0.06b | 83.7±0.86a | 15.2±0.45c | 10.9±0.76c | 9.9±0.34c |
| Overall mean | | 2.60±0.34B | 0.95±0.03B | 96.33±11.7B | 7.40±0.04B | 73.8±3.6A | 25.1±3.7B | 13.6±1.2B | 12.7±1.1B |
| G3 (4 ml $AD_3E$/h) | Winter | 2.8±0.35bc | 1.2±0.16a | 114.0±12.0b | 7.3±0.06ab | 75.3±0.86b | 23.1±0.21b | 11.5±0.29b | 12.0±0.73b |
| | Spring | 3.0±0.50b | 1.1±0.13ab | 160.0±11.5a | 7.4±0.11a | 73.0±0.65bc | 25.6±0.69ab | 12.9±0.37ab | 12.3±0.29b |
| | Summer | 2.4±0.42c | 1.0±0.12b | 80.0±5.3c | 7.4±0.04a | 70.0±0.66c | 29.5±0.54a | 14.8±0.42a | 14.6±0.78a |
| | Autumn | 3.5±0.68a | 1.2±0.06a | 170.0±10.1a | 7.2±0.03b | 84.7±0.67a | 13.7±0.68c | 9.9±0.41c | 10.3±0.29c |
| Overall mean | | 2.93±0.23A | 1.13±0.05A | 131.0±10.9A | 7.33±0.05C | 75.8±3.2A | 23.0±3.4B | 12.3±1.0B | 12.3±0.9B |

**a , b.. Means within the same column with different superscript are significantly differ (P<0.05).**
**A, B.. Means within overall column with different superscript are significantly differ (P<0.05)**

**NE= Number of ejaculates , LI =libido, V= volume , SM=percentages of motility , DS=livability , SA=abnormality and AD=acrosomal damage.**

**Table (7): Effect of $AD_3E$ vitamins injection on sperm motility percentage in buck semen stored for 6 h during different seasons.**

| group | Season | Sperm motility (%) at different storage times | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 h | 2 h | 3 h | 4 h | 5 h | 6 h |
| G1 (Control) | Winter | 62.0±1.2b | 54.7±1.8b | 47.3±3.3a | 38.7±1.8a | 26.7±3.5a | 18.7±4.4ab |
| | Spring | 54.7±3.5c | 44.7±2.9c | 34.0±4.6b | 27.3±4.7b | 17.0±5.5b | 15.0±4.1b |
| | Summer | 50.3±2.6c | 40.7±2.4c | 31.3±3.5b | 20.7±2.9c | 11.0±2.9c | 6.3±2.2c |
| | Autumn | 70.7±0.67a | 60.0±1.2a | 47.3±2.9a | 38.7±1.8a | 28.7±1.8a | 23.3±1.8a |
| Overall mean | | 59.4±4.5C | 50.0±4.4C | 40.0±4.3C | 31.4±4.5C | 20.9±4.2C | 15.8±3.6C |
| G2 (2 ml $AD_3E$/h) | Winter | 68.7±0.3b | 61.3±1.8b | 51.3±1.8b | 42.7±4.7b | 36.0±3.1ab | 26.3±5.6ab |
| | Spring | 63.3±3.7bc | 57.3±4.1b | 48.0±4.6b | 40.0±3.5b | 32.0±7.2b | 23.0±6.0b |
| | Summer | 58.0±2.0c | 49.3±3.7c | 39.3±5.2c | 31.3±6.4c | 19.7±6.7c | 11.0±3.6c |
| | Autumn | 75.8±3.0a | 68.0±4.0a | 57.7±5.1a | 49.3±5.5a | 38.7±6.8a | 31.3±2.9a |
| Overall mean | | 66.5±3.8B | 59.0±3.9B | 49.1±3.8B | 40.8±3.7B | 31.6±4.2B | 22.9±4.3B |
| G3 (4 ml $AD_3E$/h) | Winter | 72.3±0.9b | 64.0±2.0b | 57.0±2.1b | 48.0±3.1b | 40.0±4.0b | 31.0±6.3a |
| | Spring | 66.7±2.4c | 61.3±2.9b | 52.0±4.2b | 44.0±4.6b | 33.0±7.2c | 25.3±7.1b |
| | Summer | 62.0±3.5c | 53.3±3.7c | 43.3±5.7c | 35.3±6.8c | 24.7±7.7d | 15.0±4.2c |
| | Autumn | 79.3±1.8a | 71.3±3.5a | 64.0±4.2a | 57.3±4.4a | 46.0±6.4a | 34.7±2.7a |
| Overall mean | | 70.1±3.7A | 62.5±3.7A | 54.1±4.4A | 46.2±4.6A | 35.9±4.6A | 26.5±4.3A |

**a , b.. Means within the same column with different superscript are significantly differ (P<0.05).**
**A, B.. Means within overall column with different superscript are significantly differ (P<0.05).**