ORIGINAL ARTICLE

Effect of the injection of vitamins AD_3E and the seasons on some blood traits, biochemical components and hormones of Arabi rams

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Abstract The objective of this study was to determine the effect of the injection of different levels of AD_3E vitamins in improving the productivity and reproductive performance of the Arabi rams in different seasons. This study was conducted in the animal field of the College of Agriculture - University of Basrah, Karmat Ali, for the period from December 2017 to March 2018 (three months winter) and June 2018 until September 2018 (three months in summer). The study used 21 Arabi sheep with 2.5 years old and weights from 55 to 60 kg, the animals were randomly distributed to three equal groups (each group of 7 rams). The control group was injected with a saline solution (distilled water), the second group with 2 ml of vitamin AD_3E and the third group with 4 ml of vitamin AD_3E, the injection was performed every two weeks. One month after the injection, the blood samples were collected (4 ml) from the jugular vein. Blood tests (RBC, Hb, PCV, and WBC) and blood plasma separation were performed. The chemical components (total protein, albumin, globulin), some enzymes (AST, ALT), and the hormones (testosterone, ICSH, FSH, and cortisol) were estimated. The summer months significantly ($P < 0.05$) affected the concentration of hormones (testosterone, ICSH, FSH, and cortisol). AST and ALT of Group 3 was significantly higher ($P < 0.05$) compared to the rest of the months. Total protein concentration, albumin, and globulin during December, August, and January were significantly higher ($P < 0.05$) over the rest of the months. G3 was also significantly higher ($P < 0.05$) than G2 and G1 in concentration. The results showed that WBC, PCV, Hb, and RBC of G3 recorded the highest values ($P < 0.05$) compared with G2 and G1 during summer.

Keywords: ALT, AST, summer, testosterone, winter

Introduction

All farm animals need vitamins A, D_3, E for the purpose of increasing and improving the performance and maintenance of health, these requirements are determined by many factors such as the age of the animal, gender, the physiological state, the extent of its availability in the animal feed, as well as the efficiency and preparation of microorganisms in the rumen (Hafez 2012). Vitamin A has an active role in maintaining all epithelial cells in the body and plays an important role in the vision process, sperm and bone growth (Tanumihardjo 2011). Vitamin A is essential for the maintenance of organ function in general and reproductive function in particular. It plays an important role in sexual maturity, maturity, testicular size, and characteristics of semen (Fennema 2008).

Vitamin D_3 has an important role in improving the absorption of calcium, phosphorus in addition to the formation and calcification of the bones of the animal. It also helps prevent the reduction of calcium from the natural levels, thus reshaping the bone plate and the formation of bone. It has an active role in increasing the absorption of iron, zinc, magnesium, and phosphorus from the systemic channel (Institute of Medicine 2013).

Vitamin E is a fat-soluble vitamin that is essential for growth, the perpetuation of reproduction and affects several biological processes and processes, including sperm formation, semen quality, reproduction, libido, as well as it considered as antioxidants (Liu 2006). Several studies have confirmed that vitamin E is positively affecting the immune response, helps to resist oxidation and the production of peroxides, as well as reducing the oxidation process of cell wall phospholipid, the quality of the semen in the mammals is generally affected by the lack of vitamin E in the diet, this effect includes sperm motility, concentration, ejaculate volume, and increased dead and abnormal sperm levels (Koyuncu and Yerlikaya 2007; Ali et al 2009; Yue et al 2010).

The objective of this study was to determine the effect of the injection of different levels of AD_3E vitamins in improving the productivity and reproductive performance of the Arabi rams in different seasons.

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Materials and Methods

Animals and management

This study was conducted in the animal field of the College of Agriculture, University of Basrah, Karmat Ali, Iraq, for the period from December 2017 to March 2018 (three months winter) and June 2018 until September 2018 (three months in summer). Forty-five Arab rams aged 2.5 years and weighed from 55 to 60 kg have been used in this study. The animals were randomly distributed to three equal groups (each group of 15 rams). The control group was injected with a saline solution (distilled water), the second group with 2 ml of vitamin AD_3E and the third group with 4 ml of vitamin AD_3E. The injection was performed every two weeks and one month after the injection, the blood samples were collected (4 ml) from the jugular vein.

Laboratory work

Blood tests were carried out in 5 ml test-tubes containing gel. Blood samples tubes were transferred to the laboratory and blood tests were performed (RBC, Hb, PCV, and WBC), the serum was separated using a centrifuge at 3000 cycles/minute for 15 minutes. The serum was then filled with clean test tubes and kept in a frozen temperature of -16 ° C until all the chemical components (Total protein, albumin, globulin) and some enzymes (AST, ALT) Using optical spectrometer and diagnostic kits produced by Randox UK. The hormones (testosterone, ICSH, FSH, and cortisol) were measured using a mini vidas kit manufactured by the French company BioMerieuxsa, and the kit was manufactured by the company itself. The means of maximum and minimum temperatures in the months of the experiment were shown in Table 1.

Statistical analysis

The obtained data were analyzed statistically using the SPSS (2013). Statistically significant differences were determined by the $P<0.05$ levels of significance. The general statistical model was:

$$Y_{ijk} = \mu + T_i + M_j + TM_{ij} + E_{ijk}$$

where: Y_{ijk}: the k^{th} observation of the i^{th} treatment, within the j^{th} month; μ: overall mean; T_i: the effect of the i^{th} treatment ($i=3$); M_j: the effect of the j^{th} month ($j=6$); TM_{ij}: the effect of the interaction between treatment and months; e_{ijk}: the experimental error, randomly and naturally distributed on all observations.

Table 1 The mean, the maximum and minimum temperatures (°C) during the whole period of the study.

Month		Hot season			Month		Cold season		
		Maximum	Minimum	Mean			Maximum	Minimum	Mean
6	June	39.7	26.2	32.9	1	January	17.7	6.8	12.2
7	July	41.3	27.4	34.3	2	February	20.0	8.4	14.2
8	August	41.8	26.1	33.9	12	December	20.3	8.7	14.5

Results and Discussion

Table 2 showed that the month has a significant effect ($P < 0.05$) on the concentration of testosterone and ICSH hormones. August recorded the highest significant increase ($P < 0.05$) compared to the rest of the study months. In these hormones for all treatments, probably due to the hot climate that affects the reproductive capacity of rams, which depends mainly on the activity of the testes and the increased secretion of sex hormones (Olah et al 2013). These results are consistent with Tajajookeh et al (2007), AL-Damegh (2012), and Hashem (2014) in their study of different breeds of rams. On the other hand, the photoperiod has a direct effect on the hypothalamus by sending the pituitary into the pituitary gland to increase the secretion of the hormone ICSH, which stimulates LIDC cells to secrete the testosterone responsible for the generation of sperm (Bezerra et al 2009). The results of the study were consistent with Hassanin et al (2013) in their study of the Najdi and Heri breeds, demonstrating high levels of testosterone during the hot months and low levels during the cold months.

The results also showed a significant effect ($P < 0.05$) for injections of AD_3E on the concentration of testosterone and ICSH hormones. The concentrations of these hormones were significantly increased ($P < 0.05$) in the G3 treatment, (injection of 4 ml/head) of vitamin AD_3E compared to the G2 (injection 2 ml/head) and G1. The average concentrations of testosterone were 1.74, 1.8, 1.95 ng/mL and ICSH 0.53, 0.62, 0.71 ng/ml respectively. It has an active role in stimulating growth and perpetuation of reproduction and affects several biological activities and processes, including sperm formation, semen quality, reproduction, libido and increased sex hormones (Liu 2006).

The month has a significant effect on the concentration of FSH and cortisol hormones (Table 3; $P < 0.05$). July and August recorded a significant increase ($P < 0.05$) in FSH concentration compared to the rest of the months. This may explain the difference in the reproductive capacity of the rams between the months of the year, which depends mainly on the

activity of the testicles and the secretion of sex hormones and the increase in their secretion (Olah et al 2013). These results are in line with the findings of AL-Damegh (2012) and Hashem (2014) in their study of different rams. In addition, the July and August months were significantly ($P < 0.05$) higher than the rest of the months in the cortisol concentrations for all treatments. The values of cortisol were high during the summer, probably due to the animal's response to heat stress and increased excretion of the adrenal cortex (Marai et al 2009). These results are in line with Habeeb et al (2012) and Al-Samawi et al (2014) in their studies with goat and buffalo, which showed significant increases in cortisol levels during the summer compared to the winter. Results also showed a significant effect ($P < 0.05$) of the injection of AD_3E on the concentration of FSH and cortisol. The FSH concentrations were significantly higher ($P < 0.05$) in G3 compared to the G2 and G1, this is maybe due to the fact that the vitamins AD_3E have an active role in stimulating growth, the perpetuation of reproduction, and affect several activities and biological processes, including the production of sperm, sperm quality, reproduction, sexual desire and increase the secretion of sex hormones (Liu 2006). In addition, results showed that the average concentration of cortisol was significantly reduced ($P < 0.05$) in G3 compared with G2 and G1. This may be due to the role of AD_3E in reducing cortisol secretion as an attempt to reduce animal stress (Gupta et al 2005). The findings came in line with Yasothai (2014), which confirmed that these vitamins play an important role in reducing cortisol secretion.

Table 2 Effect of season and injections with vitamin AD_3E in the concentration of hormone testosterone and ICSH in serum ram (Mean ± SE).

Treatments	Months	Testosterone (ng/ml)	ICSH (ng/ml)
Control G_1	January	1.37 ±0.03c	0.42±0.02c
	February	1.27±0.05d	0.53±0.01c
	June	2.02±0.02a	0.56±0.02b
	July	1.83±0.02b	0.72±0.02a
	August	2.53±0.08a	0.64±0.01a
	December	1.45±0.02c	0.44±0.01c
	Mean	1.74±0.42C	0.53±0.14C
G_2 2 ml/head	January	1.29±0.01c	0.45±0.01c
	February	1.27±0.02c	0.57±0.01b
	June	2.87±0.02a	0.62±0.03b
	July	1.70±0.01b	0.82±0.02a
	August	2.72±0.03a	0.73±0.01a
	December	1.38±0.04c	0.48±0.02c
	Mean	1.83±0.48B	0.62±0.11B
G_3 4 ml/head	January	1.45±0.02c	0.48±0.01d
	February	1.36±0.04c	0.57±0.01c
	June	2.96±0.01a	0.69±0.02b
	July	1.55±0.02b	0.92±0.03a
	August	2.82±0.05a	0.83±0.02a
	December	1.57±0.03b	0.53±0.01c
	Mean	1.95±0.72A	0.71±0.17A

The different capitalize letters indicate significant differences between the treatments; the different small letters indicate significant differences between the months within the single treatment at the level ($P < 0.05$).

The concentration of AST was significantly higher ($P < 0.05$) in December and January compared to the remaining months in all treatments. ALT concentration was significantly higher ($P < 0.05$) in December compared to the remaining months in all treatments. These results are consistent with Giuliano et al (2008) and Morton et al (2010), which found high AST and ALT values during the winter compared with the summer. These authors pointed out that this may be because the animals were exposed to cold stress.

The concentration of these enzymes decreased significantly ($P < 0.05$) in G3 compared to G2 and G1. The average concentration of AST was 41.62, 48.13 and 82.11 IU/L, respectively, while ALT was 30.32, 36.71 and 41.38 IU/L, respectively. Probably, this is due to the important role of AD_3E in reducing animal stress and decreasing the

concentration of these enzymes (Gupta et al 2005; Yasothai 2014).

Table 5 shows that the month had a significant effect ($P < 0.05$) in total protein concentration and albumin. The months of December, August and January were significantly higher ($P < 0.05$). This may be due to variations in temperature during these months, which stimulates the adrenal gland to secrete catecholamine and increase rates of non-carbohydrate substances and thus increase the release of proteins in blood plasma (Habeeb et al 2008a).

Table 3 Effect of season and injections with vitamin AD_3E in FSH and cortisol concentration in the serum of Rams (Mean ± SE).

Treatment	Months	FSH (ng/ml)	Cortisol (ng/ml)
Control G_1	January	1.36±0.02c	12.70±0.10d
	February	1.32±0.01c	13.75±0.05d
	June	1.50±0.01b	16.13±0.05c
	July	1.58±0.02a	17.25±0.05b
	August	1.85±0.01a	20.13±0.02a
	December	1.42±0.02c	12.20±0.05d
Mean		1.47± 0.12C	15.36±2.86A
G2 2 ml/head	January	1.44±0.01c	12.28±0.07d
	February	1.41±0.01c	13.07±0.04d
	June	1.56±0.02b	14.26±0.03c
	July	1.67±0.03a	15.72±0.32b
	August	1.71±0.02a	17.39±0.11a
	December	1.46±0.01c	11.23±0.15d
Mean		1.54±0.14B	13.37±1.10B
G3 4 ml/head	January	1.45±0.02c	11.35±0.05d
	February	1.52±0.01b	12.72±0.07c
	June	1.73±0.02a	14.34±0.11b
	July	1.82±0.02a	15.35±0.13a
	August	1.83±0.03a	16.03±0.07a
	December	1.52±0.02b	10.75±0.09d
Mean		1.67±0.10A	11.87±1.40C

The different capitalize letters indicate significant differences between the treatments; the different small letters indicate significant differences between the months within the single treatment at the level of ($P < 0.05$).

Table 4 Effect of season and injections with vitamin AD_3E in AST and ALT concentration in serum of rams (Mean ± SE).

Treatments	Months	AST (UI/L)	ALT (UI/L)
Control G_1	January	76.17±3.62a	500.11±1.63a
	February	36.80±2.17c	28.13±1.07d
	June	68.22±2.51a	48.22±2.80b
	July	56.22±0.04b	42.72±3.13c
	August	59.34±1.77b	46.11±2.17c
	December	63.77±7.23a	58.11±2.02a
Mean		82.11±5.23A	41.38±7.92A
G2 2 ml/head	January	62.15±2.21a	39.21±1.02b
	February	28.79±1.04c	22.14±1.07d
	June	58.22±1.02a	42.61±1.02b
	July	43.12±1.07b	38.67±2.03c
	August	48.13±1.58b	41.14±2.06b
	December	52.11±1.10a	52.13±2.01a

Mean		48.13±6.28B	36.71±3.04B
G3 4 ml/head	January	58.13±6.02a	33.02±1.07b
	February	27.14±3.04c	11.18±1.02d
	June	41.14±4.05a	35.04±1.11b
	July	38.50±1.07b	28.16±1.28c
	August	42.16±1.92b	32.14±1.34b
	December	45.14±1.78a	42.17±1.82a
Mean		41.62±7.08C	30.32±2.28C

The different capitalize letters indicate significant differences between the treatments; the different small letters indicate significant differences between the months within the single treatment at the level ($P < 0.05$).

Table 5 Effect of Season and Injection with Vitamin AD_3E in Total Protein, Albumin and Globulin in the serum of rams (Mean ± SE).

Treatments	Months	Total Protein (gm/100ml)	Albumin (gm/100ml)	Globulin (gm/100ml)
Control G_1	January	7.35±0.08a	4.37±0.08a	2.99±0.11a
	February	6.80±0.07a	3.85±0.15b	2.95±.14a
	June	5.80±0.05b	3.23±0.13d	2.78±0.42b
	July	4.92±0.05b	2.84±0.16c	2.09±0.18b
	August	4.00±0.10c	2.37±0.26c	1.63±0.07c
	December	7.32±0.09a	4.16±0.04a	3.15±0.06a
Mean		6.03±1.28C	3.47± 0.72C	2.38±0.58C
G2 2 ml/head	January	7.74±0.14a	4.58±0.61a	3.16±0.21a
	February	7.20±0.12a	4.11±0.52b	3.10±0.11a
	June	6.09±0.08b	3.47±0.32c	2.03±0.52b
	July	5.11±0.09c	3.09±0.09c	1.76±0.17c
	August	4.82±0.12c	2.53±0.08d	3.21±0..28a
	December	7.52±0.09a	4.30±0.42a	2.04±0.31b
Mean		6.74±1.32B	3.68±0.74B	2.64±0.39B
G3 4 ml/head	January	8.13±0.34a	4.78±0.12a	3.34±0.06a
	February	7.72±0.28a	4.28±0.17b	3.28±0.11a
	June	6.23±0.27b	3.61±0.28c	2.54±0.09b
	July	5.78±0.25c	3.33±0.21c	2.14±1.35c
	August	5.11±1.27c	2.78±0.25d	1.93±0.07c
	December	7.74±1.09a	4.56±0.24a	3.27±0.51a
Mean		7.11±1.24A	3.90±0.82A	2.75±0.53A

The different capitalize letters indicate significant differences between the treatments; the different small letters indicate significant differences between the months within the single transaction at the level ($P < 0.05$).

On the other hand, the ingestion of nitrogen-rich feedstock contributes to some extent in raising total protein and albumin in blood plasma (Habeeb et al 2008b). In addition, low and high temperatures have an effective effect in stimulating the adrenal glands to increase the secretion of cartons, increased concentration of blood globulin in response to increased animal resistance to stress from temperature variations (Abdelatif et al 2009). Table 5 also showed a significant effect ($P < 0.05$) of AD_3E injection on globulin, albumin, and total protein concentration. The G3 treatment showed the highest concentration ($P < 0.05$) followed by the G2 and the G1 treatments. The average concentration of total protein, albumin, and globulin in G3 was, 2.75, 3.90, 7.11 g/100 ml respectively, in G2 was 2.64, 3.68, and 6.74 g/100 ml, respectively, and G1 was 2.38, 3.47 and 6.03 g/100 ml respectively. The improvement in the total protein average may be due to injecting with a combination of vitamins AD_3E has an active role in maintaining the pressure of the osmosis

of the cells, maintain the process of synthesis of protein and albumin and increase its effectiveness in the synthesis of cellular proteins (Habeeb et al 2008a). It also notes that the values of globulin in G3 treatment, higher than G2 and G1. The reason for the ability of the vitamin AD_3E mixture to stimulate the immune system by increasing the immunoglobulin. The results were consistent with Abdelatif et al (2009) in their study of Nubian goats.

Tables 6 and 7 shows that the month had a significant effect ($P < 0.05$) on the concentration of WBC, PCV, Hb, and RBC blood samples. The reason is that during the summer and at high temperatures, the concentration of oxygen in the atmosphere decreases with changes in osmotic pressure and as a result, an increase in the level of red blood cells because of filling the lack of oxygen concentration (Guyton 1981). Several studies have also found that the summer season increases the ability to transport oxygen within the animal's body by increasing the number of red blood cells and hemoglobin (Elssia 2011).

The increase in blood cell volume (PCV) reflects the adaptation process to resist heat stress and increase blood viscosity (El-Nouty et al 1990). The increase in the number of white blood cells in the summer because of increased heat stress in the animals affect the immune system. The preparation of white blood cells as a means to resist heat stress in the animal (Okab et al 2008). Tables 5 and 6 shows there was a significant effect ($P < 0.05$) for injection with AD3E mixture on the concentration of WBC, PCV, Hb, and RBC. The G3 treatment showed a significant concentration ($P < 0.05$) compared to G2 and G1. The RBC averages, were 8.23, 7.79 and 7.33 ($\times 10^6$ cell/ml), the concentration of Hb was 10.31, 9.28 and 8.48 gm/100ml, the concentration of PCV was 26.89%, 25.26%, and 24.02 %, and WBC mean were 3.07, 2.78 and 2.46 1($\times 10^6$ cell/ml) in G3, G2 and G1 treatment respectively.

Table 6 Effect of season and injections with vitamin AD_3E in the concentration of RBC and Hb in the blood of rams (Mean ± SE).

Treatment	Months	RBC ($\times 10^6$cell/ml)	Hb (gm/100ml)
Control G1	January	6.02±0.02c	7.11±1.10b
	February	6.72±0.03b	7.07±0.08b
	June	8.29±0.07a	11.82±0.05a
	July	8.36±0.04a	12.01±0.03a
	August	8.73±0.07a	12.09±0.06a
	December	6.84±0.02b	6.82±1.02c
Mean		7.33± 1.08C	8.48 ± 2.0C
G2 2 ml/head	January	6.21±1.10b	7.45±0.05b
	February	6.91±1.07b	7.75±0.08b
	June	8.70±0.09a	12.12±0.06a
	July	9.00±0.08a	2.18±0.04a
	August	9.11±1.01a	12.74±0.05a
	December	6.82±0.08c	7.12±0.02c
Mean		7.79±1.20B	9. 28±1.01B
G3 4 ml/head	January	7.15±0.04c	7.91±0.02c
	February	7.72±0.08b	8.12±0.04b
	June	8.92±0.06a	12.35±0.03a
	July	9.11±0.09a	12.74±0.06a
	August	9.25±0.52a	13.15±0.05a
	December	7.21±0.76c	7.61±0.02b
Mean		8.23±0.92A	10.31±2.51A

The different capitalize letters indicate significant differences between the treatments; the different small letters indicate significant differences between the months within the single transaction at the level ($P < 0.05$).

This improvement in blood qualities due to injections with AD_3E vitamins is essential for sustaining vital animal activities and is essential for the maintenance and improvement of blood tissue (Infascell et al 2005).

Table 7 Effect of season and vitamin AD_3E injection in PCV and WBC in the blood of rams (Mean ± SE).

Treatment	Months	PCV (%)	WBC(×10^3cell/ml)
Control G1	January	24.72±0.05b	2.25±0.02b
	February	25.07±0.07b	2.38±0.04b
	June	26.11±0.02a	2.40±0.04b
	July	27.16±0.04a	2.75±0.06a
	August	28.23±0.04a	2.90±0.02a
	December	24.12±0.07c	2.07±0.03c
Mean		24.02±1.47C	2.46±0.29C
G2 2 ml/head	January	25.21±0.05b	2.44±0.04b
	February	25.30±0.07b	2.77±0.06b
	June	26.81±0.07a	2.79±0.06b
	July	27.80±0.05a	3.02±0.08a
	August	28.75±0.05a	3.11±0.05a
	December	24.45±0.04c	2.35±0.04c
Mean		25.26±1.11B	2.78±0.42B
G3 4 ml/head	January	25.70±0.05c	2.52±0.02c
	February	26.18±0.06c	2.92±0.07b
	June	26.74±0.07b	3.08±0.05b
	July	28.08±0.05a	3.34±0.05a
	August	29.11±0.02a	3.85±0.08a
	December	25.61±0.04b	2.62±0.04c
Mean		26.89±1.32A	3.07±0.53A

The different capitalize letters indicate significant differences between the treatments; the different small letters indicate significant differences between the months within the single transaction at the level ($P < 0.05$).

Conclusions

This study concluded that the injection of rams with vitamin AD_3E led to an increase in levels of sex hormones (testosterone, FSH and ICSH), which reflected positively on the sexual behavior, especially in the summer. On the other hand, increase this vitamin led to lower levels of cortisol, AST and ALT enzymes, increase total protein, albumin, globulin, which also improves the sexual performance of rams.

Conflict of Interest

The author declare no conflict of interest.

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