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The effect of β-carotene and vitamin E on metabolic profiles in nutritionally flushed sheep

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Abstract

This study aimed to investigate the effects of β-carotene and vitamin E on serum biochemistry, body condition score, and the number of offspring in sheep during the flushing period. To this purpose, forty Romanov sheep 45 (±1 kg) body weight and 3 years of age were allocated into one of four experimental treatments with 10 replicates for 6 weeks: the control (only flushing), and three treatment groups injected intramuscularly with 1, 2, and 4 ml β-carotene + vitamin E in the third week of flushing, respectively. The 4-ml treatment of β-carotene + vitamin E evidently increased the follicle-stimulating hormone level. The 2-ml treatment of β-carotene + vitamin E increased the triglyceride concentration in the serum. Body condition score, offspring number, and biochemical parameters (cholesterol, phosphorus, calcium, magnesium, aspartate aminotransferase, beta hydroxyl butyric acid, total protein, and glucose) were not affected by any levels of β-carotene + vitamin E administration. These findings indicate the positive role of β-carotene + vitamin E administration on pregnancy rate and some blood parameters during flushing.

Keywords: Flushing, β-carotene, vitamin E, serum biochemistry, Romanov sheep
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Introduction

Sheep farming is of great significance in Middle Eastern countries. Nutritional status is an important issue as the reproductive performance of sheep is directly related to the amount of energy, vitamins, and other nutrients received (Ying *et al*., 2011; Safdar *et al*., 2017). The relationship between nutrition and reproduction is quite complex, and the studies conducted in this issue are diverse and contradictory (Somchit-Assavacheep, 2011; Safari *et al.*, 2012). Nutrition is considered to be the most important factor among the environmental factors affecting the reproductive performance of both male and female sheep. The diet of animals can affect both foetal life and their oocyte and embryo quality (Somchit-Assavacheep, 2011). The ovulation rate in sheep can be increased and early embryonic deaths can be reduced by flushing, which is the application of supplementary feed before, during, and after oestrus in sheep (Aidismen & Asturi, 2018; Bari *et al*., 2000). Superovulation may be induced by flushing sheep during the breeding season. Live weight and fertility may be increased by starting the supplementary feeding 3 w before mating. In this way, both the probability of conception and the rate of twinning in the herd is increased as superovulation will occur (Küçük, 2020).

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It has been proven that some vitamins, such as β-carotene and vitamin E, have a substantial effect on metabolism. Previous studies have reported that vitamins, especially β-carotene and vitamins A and E, lead to an increase in reproductive activities (Bari *et al*., 2000; Godfrey *et al.*, 2003). Physiological free radicals may be the result of normal metabolic reactions. There is a balance between lipid peroxidation and the antioxidant system under normal conditions. Increases in lipid peroxidation are an indicator of changes in physiological state, such as pregnancy, temperature, and stress, as the cellular defence system becomes weaker. Vitamins affect reproductive performance by reducing the effect of free radicals formed during pregnancy (Uotila *et al*., 1991; Little & Gladen, 1999; Kamiloğlu *et al*., 2005).
Body condition has an impact on the success of conception in sheep. Animals must have an appropriate body condition score (BCS) during the mating period (Aidismen & Asturi, 2018). Body weight loss and low BCS, which may be caused by malnutrition, delay puberty, extend the interval between birth and re-conception, disrupt the normal sexual cycle, and increase infertility (Robinson, 1996; Nottle *et al*., 1997; Boland *et al*., 2001). Values of BCS between 2 and 3 are acceptable before pregnancy in sheep (Aidismen & Asturi, 2018). Determining how and when nutrition affects ovulation will provide a better understanding of the sheep reproductive system and elucidate the use of exogenous hormones in the regulation of reproductive activities (Scaramuzzi & Martin, 2008). Although the rate of ovulation in sheep is affected by nutrition, genetics, hormones, age, and seasonal factors, the most important of these is the level and type of nutrition (Downing & Scaramuzzi, 1991; Kafi & McGowan, 1997). Metabolic profile tests reported by Payne *et al.* (1970) are used to protect animals from diseases on a herd basis rather than individually (Kida, 2003). The aim of this study was to investigate the effects of the intramuscular injection of β-carotene and vitamin E on the serum metabolic profile, follicle-stimulating hormone (FSH), calcium (Ca), phosphorus (P), magnesium (Mg), beta hydroxyl butyric acid (BHBA), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP), glucose (GLU), triglyceride (TG), cholesterol (CHOL) and body condition score (BCS) in Romanov sheep undergoing nutritional flushing.

Materials and Methods

A total of 40 Romanov sheep (45 ± 1 kg) body weight and 3 years of age were allocated to one of four experimental treatments (group 1 to group 4) with 10 replicates for 6 weeks: control (only flushing) and the remaining three groups were injected intramuscularly with 1-, 2-, and 4-ml β-carotene + vitamin E (15 mg β-carotene, 20 mg alpha tocopherol acetate) per body weight in the third week of the flush, respectively. The basal diet was formulated to meet or exceed National Research Council (National Research Council, 1994) guidelines. During the experiment, all sheep were fed a flushing diet composed of 450 g of concentrate feed, 1500 g of lucerne grass, and 500 g of lentil straw daily, *ad libitum.* The ingredients and nutrient composition of the concentrate feed used in the study are given in Table 1. At the end of the study, BCS of all sheep was evaluated using the method described by Russel *et al*. (1969) (BCS = 1 for emaciated ewes to BCS = 5 for obese ewes at 0.5 intervals). The blood samples were collected from all sheep into tubes without anticoagulants and then centrifuged at 3500 rpm for 10 min at 4 °C to obtain serum and stored at - 20 °C. BHBA analysis was measured using the Abbott Free Style Optimum Neo H instrument. Biochemistry analyses of the study were performed using the Beckman Coulter Chemistry Analyzer AU5800. FSH analyses were performed using the Siemens ADVIA Centaur XPT Analyzer. After blood collection, rams were added to the herd. The pregnancy status of the sheep used in the study was determined using ultrasound examination 30 days after mating. Statistical analysis of the data obtained at the end of the experiment was performed using SPSS 18.0 package program (SPSS, 2018). The analysis of variance of the averages was performed using a General Linear Model (GLM) ANOVA. Tukey's multiple comparison test was used to compare differences between means.

Ethical Approval

Ethical approval for this study was obtained from the Siirt University Local Ethics Committee for Animal Experiments (Approval Date and Number: 23.09.2021-2021/02/26).

Table 1. Ingredients and nutrient composition of the basal diet

Ingredients	**%**
Wheat bran (13,6% CP)	349.5
Barley (10,5% CP)	175.0
Cracked wheat (11,5% CP)	134.8
Maize (7,3% CP)	125.0
Rice bran (14,5% CP)	90.0
Molasses (11% CP)	30.0
Distillers dried grain solubles (26% CP)	25.3
Marble dust	17.3
Soy meal (44,5% CP)	10.6
Salt	6.1
Ammonium chloride	5.0
Sunflower meal (27% CP)	4.4
Lamb premix	1.0
Cylactin	1.0
Ecomass 52	25.0
Nutrient composition	
Dry matter	88
Metabolizable energy, kcal/kg*	14.0
Crude protein	2725.0
Crude ash	4.5
Ether extract	6.3
Crude fibre	7.6
Calcium	1
Phosphorus	0.6

Lamb premix: Vitamin A 20.000.000 IU, vitamin D3 3000000 IU, vitamin E 30000 mg, vitamin B1 4000 mg, vitamin B2 8000 mg, vitamin B6 5000 mg, vitamin B12 20 mg, calcium-D-pantothenate 12000 mg, choline chloride 100000 mg, niacinamides 20000 mg, manganese sulphate 50000 mg, ferrous sulphate 50000 mg, copper sulphate 10000 mg, zinc sulphate 50000 mg, cobalt sulphate 200 mg, calcium iodide, 800 mg, sodium selenite 300 mg
* Metabolizable energy, by calculation

Results and Discussion

The effects of vitamin E and β-carotene administration together with flushing on serum parameters are given in Table 2. The highest FSH value was obtained as 0.255 in the Group 4 where flushing was applied and β-carotene + vitamin E were administered intramuscularly at a dose of 4 ml/BW (P <0,05). The highest ALT concentration was found in Group 4. The 2-ml β-carotene + vitamin E administration increased the TG concentration in serum. However, other biochemical parameters measured (CHOL, P, Ca, Mg, AST, BHBA, TP, and GLU) were not affected by β-carotene + vitamin E administration (P >0.05).

Table 2. The effects of β-carotene + vitamin E administration on serum parameters in Romanov sheep

Parameters	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**SEM**	***P* value**
FSH (mIU/dl)	0.074^{b}	0.077^{b}	0.080^{b}	0.255^{a}	0.021	0.001
TRIG (mg/dl)	24.600^{b}	23.100^{ab}	31.700^{a}	29.300^{ab}	1.176	0.026
CHOL (mg/dl)	55.500	49.600	53.800	51.300	1.543	0.557
AST (IU/L)	73.810	76.700	76.550	75.830	1.540	0.913
ALT (IU/L)	14.910^{ab}	13.950^{ab}	12.860^{b}	15.930^{a}	0.349	0.009
BHBA (mmol/L)	0.45	0.40	0.42	0.43	0.009	0.524
TP (g/L)	73.150	71.200	71.820	71.880	0.789	0.859
GLU (mg/dl)	56.400	53.660	60.920	55.830	1.205	0.188
P (mg/dl)	6.179	6.930	7.057	7.282	0.210	0.282
Ca (mg/dl)	9.720	9.760	9.650	9.420	0.122	0.774
Mg (mg/dl)	3.102	2.922	2.917	2.853	0.064	0.562

SEM: standard error of mean, FSH: follicle stimulating hormone; TRIG: triglyceride; CHOL: cholesterol; P: phosphorus; Ca: calcium; Mg: magnesium; AST: aspartate aminotransferase; ALT: alanine aminotransferase; BHBA: β-hydroxy butyric acid; TP: total protein; GLU: glucose

The measures for BCS and numbers of offspring are given in Figure 1. Numbers of offspring were higher in sheep administered with 4-ml β-carotene + vitamin E (Group 4). However, no differences were noted in BCS or the number of offspring (P >0.05).

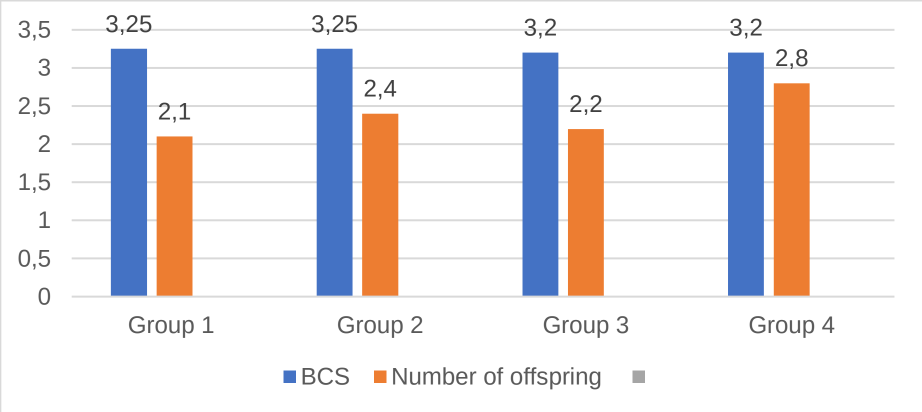

Figure 1. The effects of β-carotene + vitamin E administration on number of offspring and body condition score in Romanov sheep

Many serum biochemical parameters, such as the hormone concentrations in the serum, provide important indicators of the nutritional status, health, fertility, and welfare of sheep (Opara *et al.*, 2010; Antunović *et al*., 2011). Consumption of nutrients acts as a metabolic signalling mechanism for spermatogenesis and folliculogenesis (Scaramuzzi *et al.*, 2006). Various nutritional supplementation methods are used to increase the fertility of sheep. The flushing method, which is one of these methods, has been used in sheep breeding for many years (Coop, 1966; Kida, 2003). In the current study, both flushing and flushing + β-carotene and vitamin E supplementation applied at different doses were used. Follicular fluctuation in ruminant animals begins with an increase in follicle stimulating hormone (FSH). Since the developmental stage of the follicle depends on the release of FSH, this developmental stage is also called the FSH-dependent stage. The highest FSH values detected in the current study were detected in Group 4, and this value was found to be statistically different from the other groups. It is thought that this treatment will have positive effects on conception and multiple births as it will induce better ovulation with the stimulating effect of FSH. The number of offspring obtained confirms this. Although other studies have reported similar results, contradictory statements are used because the effect of nutrition on FSH gives rapid, transient, and normal blood concentration responses (Scaramuzzi *et al*., 2006). Other researchers report that nutrition stimulates FSH release and stimulates folliculogenesis (Somchit-Assavacheep, 2011). Although there was no statistical difference in the number of offspring between the groups, the highest number of offspring in numerical terms was obtained in Group 4 (Figure 1). Scaramuzzi & Martin (2008) reported in their study that the injection of vitamin E together with β-carotene increased multiple births. Their 60 mg β-carotene + 80 mg α-tocopherol acetate treatment together with flushing may be beneficial in creating multiple conceptions.

ALT is one of the enzymes frequently used to measure liver function. The highest ALT value was observed in Group 4. This may be due to multiple conception. Triglyceride was highest in Group 3 and was statistically different from the other groups. A difference was found between the groups in terms of ALT and triglycerides (P <0.05). On the contrary, Avci *et al.* (2000) reported that they did not find a difference in ALT and triglyceride in their selection of merinos administered vitamins A and E and selenium.
As a result of supplementation in the current study, heat was observed in all animals, and pregnancy was positive in all animals by ultrasound performed on the 30th day after mating. The high pregnancy rates indicate that both flushing and the β-carotene–vitamin E combination can be used successfully before the mating season. Other studies reporting the positive effects of flushing and β-carotene–vitamin E applications on pregnancy rates also support this conclusion. It has been reported in studies that ovulation, oestrus interval, and corpus luteum activities continue in their normal course in animals with sufficient β-carotene in the diet, and disruptions occur in the specified fertility parameters in animals fed a diet deficient in β-carotene (Hemken & Bremel, 1982; Scaramuzzi *et al.*, 2006; Scaramuzzi & Martin, 2008). Studies have reported that vitamin E deficiency produce mummified foetuses and resorption problems (Kott *et al*., 1998). Kott *et al*. (1998) reported that vitamin E supplementation did

not make a difference in live weight, body condition, or fertility of sheep, but they found that the mortality rate in lambs born was higher in those who did not receive vitamin E supplementation (Kott *et al.*, 1998). The supplements in the current study did not make a difference to body condition scores, and it was found that they were similar to other studies in this sense. In the current study, when P, Ca, Mg, AST, TP, CHOL, GLU, BHBA, number of offspring, and BCS were considered, no statistical difference was found between the groups (P >0.05). Although there was no statistical difference in terms of pregnancy rates and the number of offspring in this study, Group 4, which received β-carotene and vitamin E supplementation, and Group 1, which did not, had numerically more offspring. The results obtained are similar to previous studies (Coop, 1966; Scaramuzzi & Martin, 2008; Aidismen & Asturi, 2018). Köse *et al.* (2013) found that oestrus in sheep was induced by intravaginal progesterone sponge + PMSG (400 IU) + PGF2α application during the anoestrus period; injection of β-carotene or Vit E + Se combinations in the pre-oestrus period did not produce a positive effect on fertility in sheep.

Conclusion

Results obtained from this study indicate that there is a positive role of β-carotene + vitamin E administration on pregnancy rate and some blood parameters in Romanov sheep during nutritional flushing. In addition, different doses of β-carotene + vitamin E administration may be tested in different periods of flushing or at different BCS.

Authors' contributions

MI and VK designed research; MI, TT, DÇ, and CÖ conducted experiment; MD analysed the data; MI, TT, and ÖYÇ wrote manuscript. All authors read and approved the manuscript.

Conflict of interest declaration

All authors declare that they have no conflicts of interest.

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