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Short Communication

Seasonal Variation of Vitamin A and Beta-Carotene Levels in Ghezel Sheep

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Abstract: The present study was conducted to investigate the variations in blood serum and hepatic levels of vitamin A and β-carotene in Ghezel sheep throughout the year. From 1000 sheep aged 1-5 years, blood and liver samples were collected before and after slaughter. The levels of vitamin A and β-carotene in both samples were determined spectrophotometrically.

The results showed significant differences between summer and winter values ($P < 0.05$), but no differences were found between the sexes ($P > 0.05$). There were significant correlations between the vitamin A levels in serum and the liver ($P < 0.05$). Due to low levels of vitamin A and β-carotene in Ghezel sheep during the winter, supplementary feeding of vitamin A is recommended during this season.

Key Words: Vitamin A, Beta-carotene, Ghezel sheep, serum, liver, spectrophotometry

Vitamin A ($C_{20}H_{30}O$) is an essential fat-soluble vitamin for food producing animals, including sheep. Green feeds are the major source of carotenoids, including β-carotene, which in turn is metabolized to vitamin A by epithelial cells of the small intestine, which is then mainly stored in the liver. Vitamin A has many physiological functions in different tissues (1) and may provide protection against infectious diseases by enhancing many facets of the immune system. The normal value for serum vitamin A ranges from 25 to 60 µg/dl (2). The present study was carried out to determine the normal values of vitamin A and β-carotene in the blood serum and liver of Ghezel sheep in the Tabriz area of Iran.

The study included 1000 Ghezel sheep that were sampled in an abattoir located in Tabriz, northwest Iran. The animals were 1-5 years old. The sheep were from flocks that grazed on range lands in the spring, summer, and the first half of autumn. They ate sun-cured harvested forage and some barley grain as concentrate when they were in the corral, until the end of March. Samples of whole blood (5 ml) and liver tissue (5 g) were obtained before and after slaughter. Vitamin A and β-carotene levels of the samples were determined spectrophotometrically (3). Briefly, 1 ml of serum was pipetted into a centrifuge tube, followed by the addition of 1 ml of 95% ethanolic KOH and 1.5 ml of hexane. The mixture was stoppered and vortexed vigorously for 2 min and centrifuged at 3000 × g for 10 min. The upper hexane extract (2 ml) was transferred into a dry test tube and the absorbance of the extract was read at 325 nm against a hexane blank using a Biowave spectrophotometer. Then, the absorbance of the extract was read again at 453 nm against a hexane blank. For assaying the liver concentrations of vitamin A and β-carotene, 1 g of liver was crushed in a mortar and mixed with 10 ml of ethanol. From this, a 1-ml aliquot was taken for analysis, using the serum assay. The concentrations of vitamin A and β-carotene in the serum (µg/dl) and liver (µg/g) were calculated using the formulae:

$$C_{\beta\text{-carotene}} = OD_{453}/(2.58 \times 10^{-3})$$

$$C_{\text{Vitamin A}} = (OD_{325} - (C_{\beta\text{-carotene}} \times 1.7 \times 10^{-4}))/(1.82 \times 10^{-3})$$

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where C and OD are the concentration and the optic density, respectively.

Student's t-test, ANOVA, and regression coefficients were used to analyze the data.

The results showed that the mean levels of vitamin A and β-carotene in the serum and liver were significantly higher in the summer season than in winter ($P < 0.05$) (Table 1). The mean vitamin A level in serum increased to its peak in 2-year-old sheep and declined steadily thereafter, but the peak levels of β-carotene in the serum and liver were observed in 4-year-old and 5-year-old sheep, respectively. There were significant differences between the 1-year-old group and the others, except for the 5-year-old group ($P < 0.05$) (Table 2). β-carotene values were higher in females than in males ($P < 0.05$), but sex had no effect on the level of vitamin A in the serum and liver ($P > 0.05$) (Table 3). A close ($r = 0.804$) and significant ($P < 0.01$) correlation was observed between the vitamin A levels of the serum and liver.

Vitamin A deficiency may be a primary disease due to an absolute deficiency of vitamin A or its precursor in the diet, or a secondary disease in which the dietary supply of the vitamin or its precursor is adequate, but their digestion, absorption, or metabolism is interfered with to produce a deficiency at the tissue level (2). The deficiency occurs primarily in growing feedlot ruminants because they require more of the vitamin and feedlot-reared animals may have limited access to succulent plants (4). Clinical signs of vitamin A deficiency in sheep may not become apparent for at least 200 days if the animals have previously grazed good quality pasture (5). Under the same conditions of dietary vitamin A deficiency, male animals are apparently more susceptible to developing clinical signs of deficiency than females. Nyctalopia (night blindness) is a consistent clinical sign of vitamin A deficiency in sheep, along with anorexia and poor body condition. Severely affected animals may be completely blind. In young animals, vitamin A deficiency induces bony

Table 1. Vitamin A and β-carotene content (range and mean) in the blood and liver of Ghezel sheep, according to season.

	Vitamin A		β-carotene		
	Serum (μg/dl)	Liver (μg/g)	Serum (μg/dl)	Liver (μg/g)	
Range	15.9-44.1	29.1-44.1	13.1-23	16.2-26.8	n = 1000
Spring	32.9 ± 3	36.6 ± 3.3	17.0 ± 0.6	20.9 ± 2.2	250
Summer	38.3 ± 4.4[†]	40.0 ± 2.0*	20.3 ± 5.0[a]	24.6 ± 4.3 [b]	250
Autumn	35.2 ± 2.1	37.1 ± 4.8	18.0 ± 1.7	22.3 ± 1.2	250
Winter	30.7 ± 5.2[†]	31.5 ±1.9*	15.8 ± 3.0[a]	19.2 ± 2.8[b]	250

Values with superscript differ ($P < 0.05$).

Table 2. Mean vitamin A and β-carotene content in the serum and liver of Ghezel sheep, according to age.

Age (year)	No. of Sheep (n = 1000)	Mean of Vitamin A		Mean of β-carotene	
		Serum (μg/dl)	Liver (μg/g)	Serum (μg/dl)	Liver (μg/g)
1	272	33.1[§]	37.8	20.5	22.7
2	183	<u>41.5</u>[§]	<u>42.7</u>	19.1	23.3
3	363	40.5[§]	42.6	20.6	27.0
4	94	38.0[§]	37.6	<u>20.8</u>	23.4
5	88	36.2	41.9	19.7	<u>29.0</u>

Peak of concentration is underlined. [§]Differs significantly ($P < 0.05$).

Table 3. Variation of vitamin A and β-carotene in the blood and liver of Ghezel sheep, according to sex.

	Vitamin A		β–carotene	
	Serum (µg/dl)	Liver (µg/gr)	Serum (µg/dl)	Liver (µg/gr)
Male	38.9 ± 3.1	38.9 ± 4.4	17.8 ± 3.3[†]	20.8 ± 3.5[§]
Female	38. ± 4.4	40. ± 3.3	21.1 ± 2.2[†]	25.7 ± 2.9[§]

[†,§] Differs significantly ($P < 0.05$).

remodeling, narrowing of the optic canal, and thickening of the dura mater, which in turn causes ischemic necrosis of the optic nerves; however, remodeling of the optic nerve does not occur in skeletally mature animals and blindness is likely caused by retinal degeneration (6,7). Young sheep and offspring of deficient dams may suffer from vitamin A deficiency (2). For an accurate diagnosis of hypovitaminosis A, it is essential to know the normal values in the blood and different organs of a particular animal species. For the diagnosis of hypovitaminosis A, serum and liver levels are usually measured. The normal values of Ghezel sheep observed in the present study are in agreement with the results of previous investigations of different sheep breeds (8), but there were significant differences between summer and winter values, which could have been due to better access to green feed in summer. In another Iranian sheep breed (Shall), seasonal comparison did not show differences between consecutive seasons in ewes, but there were significant differences in rams (9). In the present study no sex effect was observed for vitamin A levels, most likely because of the identical feeding regimes of the genders.

In the present study the mean winter level of vitamin A in the liver was 31.5 ± 1.9 µg/g; however, a mean level of 220 µg/g in liver samples of lamb fed controlled diets has been reported (10). In comparison, the values we obtained in adult Ghezel sheep were significantly lower than the values reported for adequately fed lambs; therefore, vitamin A supplementation, especially in the winter season, is recommended for meeting the increased vitamin A requirement in late pregnancy.

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