

## Araştırma Makalesi

### DETECTION OF VITAMIN B<sub>12</sub> IN DIFFERENT COW MILK USING ELISA TECHNIQUE

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### Farklı Sığır Irklarına Ait Süt Örneklerinde B<sub>12</sub> Vitamin Düzeyinin Elisa Tekniği İle Tespiti

**Özet:** B<sub>12</sub> vitamini, B kompleks vitaminlerin bir üyesidir. Bu vitamin kobalt içerdiğinden dolayı kobalamin olarak adlandırılmaktadır. B<sub>12</sub> vitamini balık, et, tavuk, yumurta ve süt ürünlerinde bulunur ve bakteriler tarafından sentezlenir. B<sub>12</sub> vitamini yetersizliğine bağlı olarak mide ve bağırsak bozukluklar, kol ve bacaklarda uyuşukluk ve megaloblastik anemi şekillenir. Bu çalışmada farklı sığır ırklarına ait süt örneklerinde B<sub>12</sub> vitamin düzeyleri araştırıldı. Bu amaçla Samsun ve Kars bölgesinden Holştayn, Jersey, Zavot and Doğu Anadolu Kırmızısı sığır ırklarına ait süt örnekleri toplandı. Süt örneklerinde B<sub>12</sub> vitamini miktarı ELISA yöntemi ile tespit edildi. B<sub>12</sub> vitamini Holştayn, Jersey, Zavot and Doğu Anadolu Kırmızısı sığır ırklarına ait süt örneklerinde sırasıyla 0.98µg/L, 1.15µg/L, 2.93µg/L ve 1.56µg/L olarak bulundu. Yapılan istatistik çalışması sonucunda vitamin B<sub>12</sub> miktarı Zavot ırkına ait süt örneklerinde diğer ırklardan daha yüksek bulundu ( $p<0.05$ , ANOVA).

**Anahtar Kelimeler:** B<sub>12</sub> vitamini, inek sütü, ELISA.

**Abstract :** Vitamin B<sub>12</sub> is a member of the vitamin B complex. It contains cobalt, and is also known as cobalamin. It is exclusively synthesised by bacteria and is found primarily in fish, meat, poultry, eggs, milk and milk products. Vitamin B<sub>12</sub> deficiency have an underlying stomach or intestinal disorder that limits the absorption of vitamin B<sub>12</sub> and might be caused to megaloblastic anemia. In this study cow milk samples were collected from in two different region in Turkey. In milk samples the amount of vitamin B<sub>12</sub> was detected by using ELISA. The vitamin B<sub>12</sub> concentrations of milk samples obtained from the cows of Holstein, Jersey,

Zavot and East Anatolian Red species were determined as 0.98µg/L, 1.15µg/L, 2.93µg/L and 1.56µg/L, respectively. The concentrations of vitamin B<sub>12</sub> in Zavot cows milk were higher than the other species.

**Key Words:** Vitamin B<sub>12</sub>, cow milk, ELISA.

## Introduction

Vitamin B<sub>12</sub> (cyanocobalamin) has an important function in human health. Vitamin B<sub>12</sub> is synthesised by bacteria and it is naturally found in foods that come from animals, including fish, meat, poultry, eggs, milk, and milk products. In many animals gastrointestinal fermentation supports the growth of these vitamin B<sub>12</sub> synthesising microorganisms, and subsequently the vitamin is absorbed and incorporated into the animal tissues **(18)**.

Although it changes due to age, lactation and pregnancy status, daily amount of vitamin B<sub>12</sub> needed to be taken by foodstuffs is 2.4µg/day for an adult human. Hematological, neurological and gastrointestinal symptoms are formed due to lack of this vitamin in humans **(10)**.

Vitamin B<sub>12</sub> deficiency in infants often produces haematological and neurological deficits, including macrocytic anaemia **(15)**, fatigue, weakness, constipation, loss of appetite, and weight loss **(4, 7, 13)** decreased red blood cell count, pallor of the skin, numbness and tingling in the hands and legs **(10, 12)**, excessive fatigue, depression and memory loss **(6)**.

Milk is the most nutritionally complete food, containing nearly all the constituents of nutritional importance to humans **(10)**. There are total 9 million bovines and 18 million ovines in Turkey. 5 million of these bovines are cattle. 28% of cattle is also used in production of meat and 87% in production of milk. Produced milk amount is 10.5 million ton/year. Annual milk consumption in Turkey per person is 15kg 36.40% of bovine existence in Turkey is native race, 43.77% is hybrid of cultivated race, and 19.83% is cultivated race **(20, 21, 22)**. There are Grizzly Race, Native Black, East Anatolia Red, South Anatolia Red and Zavot cattle among native cattle races in Turkey. Common characteristics of native cattle races in Turkey are their small size, low milk productivity and growth speed and high endurances. Picking up of movement cattle between regions in country has caused the mixture of native races within each other and amount of hybrid has increased among them. So, Grizzly race has nearly disappeared, South Anatolia Red and Zavot cattle have decreased very much.

East Anatolian Red cattle race having high decrease rate is a race having narrow body structure with color changing between light red and chestnut. It has spread over East and Northeast Anatolia region. Kars province is the most intense spreading region

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of East Anatolia Red. It is an animal that adapted hard climate of East Anatolia. It has fattening characteristics. Although it changes due to feeding, its annual milk productivity is about 600 and 1200 litres (2, 3).

Zavot Cattle race as other race faced to be disappeared has grizzly-gray color and is a race that adapted in Kars region in Turkey. It has been obtained by hybridization of Simental and Swiss Swarty. Its milk has 4% fat and annual milk productivity is 1500 litres. Its fattening characteristics are better than East Anatolia Red. Zavot cattle are among the most important cattle race in Turkey and have higher productivity rate than other native cattle (3).

There is no information about vitamin B<sub>12</sub> in milk of Zavot and East Anatolia Red cattle races. So, to determine values of vitamin B<sub>12</sub> in Zavot and East Anatolia Red cattle race as native cattle races of Turkey and to compare those values with Holstein and Jersey cattle races as cultivated race are purposed in this study.

## Material and Methods

**Sampling:** Four groups of individual milk samples obtained from Samsun and Kars regions of Turkey were studied. The study was carried out in winter season of 2007. 40 milks were obtained 10 from each group. Holstein and Jersey cows were obtained from Samsun, Zavot and East Anatolian, while Red cows were obtained from Kars in Turkey. Milk samples were randomly collected after the complete morning milking. Samples were collected from 8 different cow farms. The animals used in study were selected from 2nd and 3rd months of lactation period, dry rough feed and straw breeding in same stable conditions. The milk samples were brought to laboratory with sterile glass bottles under the cold chain prevented from light.

**Apparatus:** For the Vitamin B<sub>12</sub> analyses, a Digital and Analog Systems DAS RS 232 ELISA plate reader was used. Measure the optical density at 450nm.

**Preparation of samples:** For degreasing centrifuge the sample at 3500 g for 10 min at 10°C (50 °F). Remove upper cream layer by aspirating through a pasteur pipette under vacuum stream. Employ 50 µl of the defatted milk per well in the assay.

**ELISA test procedure:** Vitamin B<sub>12</sub> levels in milk samples were determined according to test procedure of Ridascreen Fast Vitamin B<sub>12</sub> ELISA (R-Biopharm A.G. Kit, Art No: R2102 Darmstad, Germany) and results were evaluated in ELISA reader (DAS). Insert a sufficient number of wells into the microwell holder for all standards and samples to be run. Record standard and sample positions. Measure the absorbance at 450 nm against an air blank. Read within 10 minutes.

**Data analysis:** Statistical analysis of data was carried out using SPSS statistical package programs. All experimental results were means  $\pm$  SE of three parallel measurements. The results were evaluated by using one-way ANOVA and any significant differences further evaluated using the Tukey multiple-comparison test. The level of significance was set at  $p < 0.05$ .

## Results and Discussion

The vitamin B<sub>12</sub> concentrations of milk samples obtained from the cows of Holstein, Jersey, Zavot and East Anatolian Red species are presented in Table 1 with ranges, means and SE. The results indicate that the concentration varied from 0.77 to 1.20 with a mean of 0.98 µg/L for Holstein cows milk, from 0.94 to 1.36 with mean of 1.15 µg/L for Jersey cows milk, from 1.96 to 3.89 with mean of 2.93 µg/L for Zavot cows milk, from 1.15 to 1.97 with mean of 1.56 µg/L for East Anatolian Red cows milk. Statistical analysis of results were evaluated by ANOVA. The concentrations of vitamin B<sub>12</sub> in Zavot cows milk were significantly higher than the other species ( $p < 0.05$ ).

In this study, Vitamin B<sub>12</sub> level in races of Holstein and Jersey having higher annual milk productivity (approximately 3000 litres/year) has been determined lower than Zavot and East Anatolia Red cattle races having lower annual milk productivity (approximately 1500litres/year).

Vitamin B<sub>12</sub> is synthesized by rumen microbes from dietary cobalt and the clinical signs of cobalt deficiency in sheep and cattle are due to reduced vitamin B<sub>12</sub> concentrations in tissue (14). Vitamin B<sub>12</sub> level shows difference due to race, environment, feeding, and cobalt addition to food and lactation period. Cobalt is a necessary element for synthesis of vitamin B<sub>12</sub>. Microorganisms in rumen synthesize vitamin B<sub>12</sub> by using cobalt. Clover, melas, corn, corn silage, cotton seed pulp, fish flour, meat flour, fowl side products, rice bran, sorgum, soybean pulp are food material having richer cobalt amount.

Cattle races used in study have been grown in same stable conditions and feed by rough food and straw and effect of cobalt on formation of vitamin B<sub>12</sub> has been examined.

The mean value of vitamin B<sub>12</sub> found in this study was higher than that reported by Sawaya et al.(16) for goat milk. According to Sawaya et al. (16) the concentration of vitamin B<sub>12</sub> in Aardi and Masri goat milk samples were 0.7 and 0.6 µg/L, respectively. The average content of vitamin B<sub>12</sub> was lower than that reported by Bestuzheva <sup>5</sup> for camel milk on the other hand, the concentrations of vitamin B<sub>12</sub> in Zavot cows milk were similar to the content reported by Alais (1) for cow milk.

In studies made in human milk, the amount of vitamin B<sub>12</sub> was determined in level of 0.4-0.8 µg/L (**9, 8, 19, 23, 11**). In this study, it is seen that amount of vitamin B<sub>12</sub> in cattle milk is higher than human milk when compared.

Shrimpton and Duckworth (**17**) motioned that the vitamin B<sub>12</sub> concentrations of milk samples obtained from the sheep of Lowland and Hill species were found as 1.3 to 5.0 µg/L, respectively In study performed, it is observed that level of vitamin B<sub>12</sub> shows difference in different races of same animal type.

### Conclusions

Zavot and East Anatolia Red among native cattle race of Turkey is faced to be disappeared. Native races have adapted to special conditions of environment in which they have been grown for centuries. Although their low productivity, they have special characteristics, and are resistant animals that can reproduce in insufficient environment condition. So, necessary measures must be taken to protect animal gene sources in Turkey.

**Table 1.** Mean concentrations (µg/L) and standard error of Vitamin B<sub>12</sub> in different cow milk

**Tablo 1.** Farklı süt örneklerinde ortalama Vitamin B<sub>12</sub> düzeyi (µg/L) ve standart hata

Milk sample	n	Vitamin B <sub>12</sub> (Mean±SE)
Holstein	10	0.98±0.09 <sup>a</sup>
Jersey	10	1.15±0.09 <sup>a</sup>
Zavot	10	2.93±0.42 <sup>b</sup>
East Anatolian Red	10	1.56±0.18 <sup>a</sup>

<sup>a, b</sup> Show differences among cow milk ( $p < 0.05$ ), tukey test.

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