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Short Communication

Determination of vitamin B9 levels in the milk of Brown Swiss and Simmental cows using the ELISA method

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Abstract

The aim of this study was to determine the levels of folic acid in Brown Swiss and Simmental cows' milk by the competitive ELISA method. Brown Swiss and Simmental cows' milk samples were collected from two dairies between February and August 2017 (20 samples from Brown Swiss and 22 samples from Simmental). The average level of vitamin B9 in Brown Swiss milk was approximately 3.27 ± 1.23 µg/100 g, while for Simmental it was around 2.99 ± 0.88 µg/100 g. In the milk mixture, the mean folic acid level was 3.13 ± 1.07 µg/100 g. There was no statistical difference between breeds in terms of folic acid levels in cow's milk. It was concluded that milk is not a sufficient source to meet the daily folic acid needs of adults in Turkey.

Keywords: Dairy cattle breeds, folic acid, raw milk
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Introduction

Folic acid, a natural form of folate, is a water-soluble vitamin in the B group of vitamins (Anonymous, 1998; Shils *et al*., 2005; Bailey & Gregory, 2006). Folic acid functions as a coenzyme in the synthesis of DNA and RNA, in the transfer of single carbon groups in the metabolism of amino acids (Anonymous,1998; Shils *et al*., 2005; Bailey & Gregory, 2006). A lack of folic acid, which is necessary for healthy growth and reproduction, is a causative factor in chronic, often major, diseases such as cancer and neurological disorders.

The chemical structure of folic acid is composed of a pteridine ring, a molecule of glutamic acid and p-aminobenzoic acid (Bailey & Gregory, 2006). Folic acid taken with food is absorbed via its passage along the intestinal mucosa after being hydrolyzed in monoglutamate form in the intestines. Its monoglutamate form is converted to tetrahydrofolate followed by methyl or formyl groups before entering the bloodstream. The active form of folate in blood plasma is 5 methyl-tetrahydrofolate (Anonymous, 1998; Yetley *et al*., 2011). It is estimated that the total folic acid content in human metabolism is between 10 and 30 mg. Approximately half of this amount is stored in the liver and the other half is found in blood and other tissues (Shils *et al*., 2005; Bailey & Gregory, 2006)

Between 2003 and 2006, according to a survey conducted in the United States of America, folic acid intake varied between 385 and 674 µg/day and, particularly among individuals under the age of 18, was clearly insufficient (Bailey *et al*., 2010a; Bailey *et al*., 2010b). However, there is no information on the long-term uptake of folic acid (Anonymous, 2012). Folic acid deficiency is rare and usually associated with poor nutrition, alcoholism and impaired absorption. The primary clinical symptom of folic acid deficiency is megaloblastic anaemia and other indications such as superficial ulceration of the oral and lingual mucosa, nail and hair depigmentations, osteoporosis, and an increased concentration of homocysteine (Anonymous, 1998; Shils *et al*., 2005; Bailey & Gregory, 2006; Ho *et al*., 2011). In maternal folic acid insufficiency, preterm

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birth and foetal growth retardation were observed (Scholl & Johnson, 2000). Food is supplemented with folic acid to meet the folic acid required during metabolism. Fortification is particularly important in gestation because insufficient folic acid leads to developmental foetal neural tube defects (Lermo *et al*., 2009). Due to its role in the synthesis of DNA and RNA, it is believed that insufficiency is related to carcinogenesis (Hoegger *et al*., 2007).

Folic acid is found naturally in green leafy vegetables, fruits, nuts, beans, poultry, eggs, seafood, cereals, milk and dairy products (Shils *et al*., 2005; Anonymous, 2012). About 50% of the folate found naturally in foods, such as milk, is digestible while the bioavailability of folic acid taken with food is estimated to be 85%. Milk is considered a good source of folic acid because it contains folate binding proteins that increase absorption (Ünal & Besler, 2008).

The amount of folate needed in the first six months of human life is 65 µg/day, 400 µg/day in adults, and 600 µg/day during pregnancy (Anonymous, 1998). To assess the intake of folic acid via food and to examine the relationship between bioavailability and nutrition, the amount of folate in foods should be determined and supplemented as needed (Indyk, 2010). Many countries in the world have agreed that supplementation of folic acid in foods is a common strategy and should be supplemented in a concentration of 30 µg in 100 mL milk (Lermo *et al*., 2009). The aim of this research was therefore to determine the amount of folic acid in the milk of Brown Swiss and Simmental cows and to show the possible differences between these two herds.

Materials and Methods

In this study, milk samples of Brown Swiss and Simmental breed cows were chosen as study material. Since Brown Swiss and Simmental cows are breeding widely in the province of Şanlıurfa, milk samples were collected from both breeds on days 10 to 180 of lactation. The milk samples were obtained from family farm herds located in the same area of southeastern Turkey, at latitude 37°10' north and longitude 38°47' east, at 477 m above sea level. From the beginning of lactation, cows were kept under optimum feeding conditions, and were outdoor-fed ad libitum with native perennial forage grasses, without any supplementary feed. Water was available at all times. All the Brown Swiss and Simmental cows (three to eight years of age) were clinically healthy, and their health condition was supervised and maintained continuously throughout lactation. Twenty raw milk samples were collected from 20 clinically healthy, lactating Brown Swiss cows, and 22 raw milk samples were collected from 22 clinically healthy lactating Simmental cows from Şanlıurfa province in Turkey. The milk samples were collected during the period February to August 2017. Milk samples were obtained from dairy cows between the hours 07:00 and 12:00 and 50 mL milk samples were taken during the first milking of the day. All milk samples were collected during the morning milking shift into sterile tubes, encased in ice packs, and moved to the laboratory where they were stored in the dark at -19 °C until analysis. Sample collection, handling and storage levels were controlled to diminish possible inactivation of the vitamin B9 content of milk samples that could negatively affect the reliability of data.

The ELISA kit (r-BioPharm RIDASCREEN Art No. R3203) used for determining the folic acid content in milk samples is based on the principle of competitive quantitative anti-antigen-antibody reaction. In the ELISA kit there were 48 samples of microplates, six standards (0, 0.75, 1.5, 3, 6 and 12 µg/kg), a sample buffer solution, wash solution, conjugate, substrate/chromoge and a stop solution. All analyses were carried out in duplicate in line with accepted standards. Milk samples were protected from sunlight and thawed at room temperature (~21 °C) prior to analysis and homogenized for testing. All standards and milk samples were tested on the same plate at the same time. Firstly, 50 µL of each standard and 50 µL milk samples were added to the microwells, in duplicate. Following this, 50 µL of the conjugate was added to all wells and incubated for 15 minutes in the dark at room temperature. After incubation, washing was carried out by placing a 250 µL wash solution in each ready-to-use wash solution. Washing was repeated three times. After washing, 100 µL substrate/chromogen were added to all wells and incubated for 10 min in the dark, at room temperature. Following incubation, a 100 µL stop solution was added to all wells and absorption was measured within 5 minutes by a microplate absorbance spectrophotometer at 450 nm wavelength. The ELISA procedure was carried out in accordance with the manufacturer's instructions. Absorption was measured and standards were calculated in the RIDASOFT Win (Art.No. Z9996). The detection limit and specificity of the test were 0.00 µg/kg and 99%, respectively.

The statistical analyses were carried out using SPSS 13.0 (SPSS Inc., Chicago, IL, USA). The Pearson correlation coefficient was used in testing the relationship between numerical variables.

Results and Discussion

The mean folic acid levels in the Brown Swiss (n = 20) and Simmental (n = 22) milk samples were 3.27 ± 1.23 µg/100 g and 2.99 ± 0.88 µg/100 g, respectively (Table 1). In total, the mean folic acid level was 3.13 ± 1.07 µg/100 g. The lowest folic acid level in the milk samples of the Brown Swiss herds was 1.66

μg/100 g, and the highest 6.09 μg/100 g. These variations in vitamin B9 levels could be attributed to samples having been collected during different seasons, for example winter, spring and summer. The lowest folic acid value was 1.09 μg/100 g and the highest 4.68 μg/100 g in the milk samples of the Simmental herds. The folic acid levels in the milk samples of both the Brown Swiss and Simmental herds (P > 0.05) were not statistically different. Based on the ELISA method used in the study, the lowest standard value was 0.00 μg/kg, the highest standard value was 12 μg/kg, and the method of recovery was 99.89%.

Table1 Milk folic acid levels of Brown Swiss and Simmental cows

Sample (n)	Minimum (μg/100 g)	Maximum (μg/100 g)	Mean ± SD (μg/100 g)
Brown Swiss (20)	1.66	6.09	3.27 ± 1.23
Simmental (22)	1.09	4.68	2.99 ± 0.88
Total (42)	1.09	6.09	3.13 ± 1.07

The benefits of folic acid have become the focus of researchers, in particular its protective role against neural tube defects in infants, cardiovascular problems because of corrupted plasma homocysteine and cancer in adults, particularly colon cancer (Giovannucci *et al*., 1995; Forssén *et al*., 2000). The generally low levels of folic acid and the instability of vitamin B9 in foods warrant furher study to determine the amount of dietary folic acid required for optimum health.

In many western and other countries, milk and dairy products provide, on average, 10% to 15% of the folic acid required daily. It has been reported that deficiency in folic acid constitutes a risk factor for occlusive vascular and mental problems (Brattstrom *et al*., 1984; Boushey *et al*., 1995; Jacobsen, 1998; Forssén *et al*., 2000).

Öste *et al*. (1997) stated that the average folic acid content in cow's milk samples was 5 μg/100 g in a range of 3.7 - 7.2 μg/100 g. Wijesinha-Bettoni & Burlingame (2013) reported that the average folic acid content was 8.5 μg/100 g in cow's milk, 6 μg/100 g in sheep's milk, 1 μg/100 g in goat's milk, 0.6 μg/100 g in buffalo milk and 5 μg/100 g in human milk. In this study, the average folic acid content in the cow's milk samples was lower than the previously reported range (Wijesinha-Bettoni & Burlingame, 2013). The mean levels of folic acid of this study were also lower than those reported in previous studies (Andersson & Öste, 1992; Andersson & Öste, 1994; Wigertz & Jägerstad, 1995). Some authors (Souci *et al.,* 1990; Holland *et al*., 1991) reported total folic acid levels in cow's milk by microbiological assay in the range of 5 - 7 μg/100 mL. Forssén *et al*. (2000) reported that both HPLC competitive-binding radio assays and microbiological assays show a similar folic acid content in cow's milk samples. Dong & Oace (1975) reported the mean folic acid levels for Jersey-Guernsey, Holstein, Northern herds and Southern herds as 9.4, 9.5, 9.4 and 9.5 μg/100 mL in cow's milk from California dairy herds, respectively. Average folic acid contents were similar in Brown Swiss and Simmental cow's milk.

The mean folic acid levels found in this study were similar to previously reported results (Hoppner & Lampi, 1986; Müller, 1993; Burlingame *et al*., 1994; Vahteristo *et al*., 1997). Gaucheron (2011) reported that the folic acid content of cow's milk may change seasonally with high levels in summer, compared to winter. Similarly, Wigertz *et al*. (1997) indicated higher folic acid levels for cow's milk throughout summer, and lower in winter.

Conclusion

No differences were observed in the folic acid content of raw milk between the two studied dairy breeds. Obtained values were slightly lower than those reported by other studies but were higher than that of goat's and buffalo milk. It is important that further studies be carried out in different seasons for different breeds. In order to determine good sources of folic acid, it is necessary to ascertain the bioavailability and absorption of milk and dairy folates.

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Authors' contributions

SKA and NP designed the study, conducted statistical analyses of the data, and edited the draft version of the manuscript. HD contributed to laboratory analysis with SKA and NP.

Conflict of interest declaration

Authors declare that there is no conflict of interest for this study.

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