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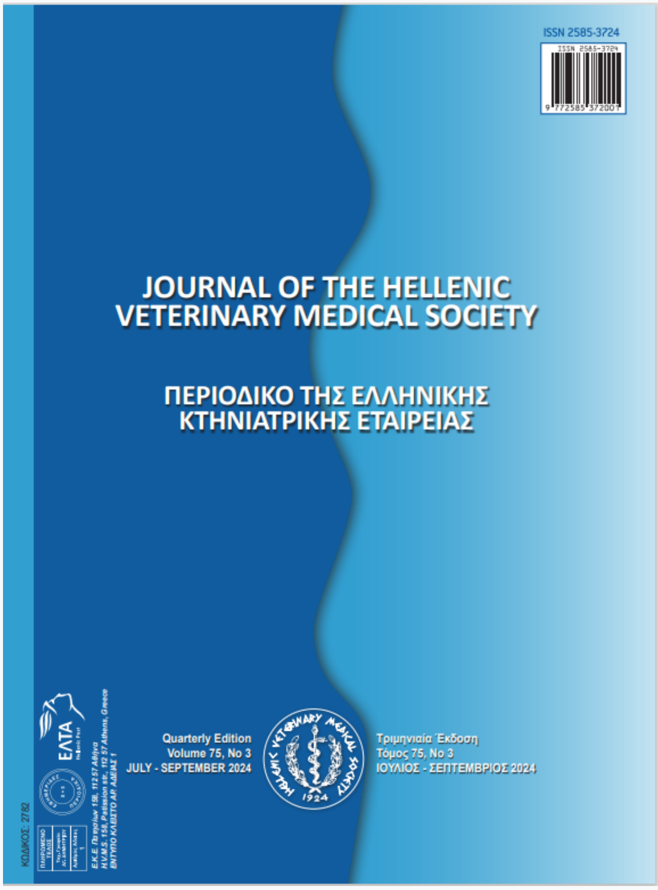

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Research article
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Folic acid, cobalamin, and biotin concentrations in milk samples of sheep, goats, and cows

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ABSTRACT: Milk is an important food obtained from mammals, containing almost all of the nutrients needed for metabolism. The vitamin composition of milk, the first food of mammals, is valuable data for the food industry and public health. This study aimed to determine the levels of folic acid (vitamin B9), cobalamin (vitamin B12), and biotin (vitamin B7) in different milk samples (cow, sheep, goat) using the ELISA method. For this purpose, milk samples from Holstein Cows (n:50), Awassi Sheep (n:50), and Kilis Goats (n:50) available for retail in the Şanlıurfa province of Turkey were collected and analyzed. In this study, the average folic acid content of sheep, goat, and cow milk was found to be 3.45 µg/100 mL, 7.23 µg/100 ml, and 6.64 µg/100 mL, respectively. The average cobalamin content of sheep, goat, and cow milk was 0.70 µg/100 ml, 7.43 µg/100 ml, and 0.58 µg/100 mL, respectively, while the average biotin content of sheep, goat, and cow milk was 2.21 µg/100 mL, 2.21 µg/100 mL, and 2.09 µg/100 mL, respectively. It was noted that the average biotin levels in sheep, goat, and cow milk did not differ significantly. However, significant differences were observed in the quantities of folic acid and cobalamin ($P<0.05$). Different levels of folic acid were found in milk samples from these three species. Cobalamin levels in goat milk samples were significantly higher, while biotin levels were similar in each milk sample of the species.This study underscores the importance of understanding and considering the diverse vitamin compositions of milk from different mammalian species to ensure comprehensive nutritional intake.

Keywords: Milk; Folic acid; Cobalamin; Biotin; ELISA.

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INTRODUCTION

Both consumer knowledge of healthy food production and interest in foods rich in nutrients density are increasing (Vermeir et al., 2006; Park, 2018). Milk, an ancient food containing numerous micronutrients, remains a subject of scientific research regarding its nutritional content. The vitamin profile of milk, encompassing fat-soluble and water-soluble vitamins, is highly variable among mammalian species, strongly influenced by the mother's diet (Haug et al., 2007; Gaucheron, 2011; Vincenzetti et al., 2021). Prior studies have shown that group B vitamins, such as folic acid (vitamin B9) and biotin (vitamin B7), which have high bioavailability, are obtainable from milk and dairy products (Dainty et al., 2007; Matte et al., 2011). Group B vitamins act as important coenzymes, participating in various metabolic activities crucial for energy production, nerve conduction, and DNA and hormone synthesis (Insel et al., 2003). Ruminant milk serves as a significant source of group B vitamins, contributing substantially to the minimum recommended daily intake for individuals of all ages (Magan et al., 2020).

Folic acid, present naturally in a diverse array of foods including milk and dairy products, consists of a pteridine ring, glutamic acid, and p-aminobenzoic molecules, playing a crucial role in nucleotide synthesis and amino acid metabolism (Arkbåge et al., 2003a;Carmel, 2005; Alina et al., 2019). Many countries have implemented national programs to fortify foods with folic acid, addressing its common deficiency worldwide. The National Institutes of Health recommend a daily folate intake of 400-600 µg for individuals over the age of 19 (World Health Organization, 2015; National Institutes of Health, 2023).

Vitamin B12, also known as cobalamin, is a complex water-soluble micronutrient synthesized exclusively by bacteria, containing a cobalt atom surrounded by a tetrapyrrolic ring. Essential for various metabolic processes including energy production, nervous system integrity, and cell proliferation, cobalamin occurs naturally in animal products like milk, dairy, poultry, fish, meat, and eggs (Marchi et al., 2020). Deficiency symptoms include fatigue, weakness, anorexia, constipation, megaloblastic anemia, and weight loss (Jenness, 1980; Dobrozsi et al., 2014; Li et al., 2019).

Biotin, an essential micronutrient, acts as a coenzyme for carboxylase enzymes involved in fat, amino acid, and glucose metabolism. Additionally, it plays a role in histone modifications, gene regulation, and cell signaling. Biotin is naturally present in foods such as milk, meat, eggs, fish, nuts, and certain vegetables. Its content can vary due to factors like plant variety and processing techniques (Dakshinamurti and Chauhan, 1988; Chen et al., 2011).

In 2022, Turkey produced 21,370,116 tons of cow milk, 1,143,762 tons of sheep milk, and 622,785 tons of goat milk, ranking eighth in the world for total milk production with 23,000 tons (Ataseven, 2021; TUIK, 2022). Despite its significant contribution to global milk production, no literature review has simultaneously reported the B-group vitamin content of cow, sheep, and goat milk from Turkey. With increasing consumer awareness, there's a growing interest in understanding food content.

The enzyme-linked immunosorbent assay (ELISA) technique, in use for over four decades, provides results ranging from ng/mL to pg/mL(Ma and Shieh, 2006). It's favored for its simplicity, sensitivity, speed, reliability, and versatility in determining vitamin levels, making it advantageous for perishable raw milk compared to other methods (Reen, 1994). This study aims to determine the levels of folic acid (vitamin B9), cobalamin (vitamin B12), and biotin (vitamin B7) in raw milk samples from different animal species (cow, sheep, goat), assessing their biological value for public health. It seeks to offer updated information on the folic acid, cobalamin, and biotin contents of cow, sheep, and goat milk.

MATERIAL AND METHODS

In this study, raw milk samples (200 mL) available for sale in the province of Şanlıurfa were collected into sterile falcon tubes in the morning and promptly transported to the laboratory with ice packs in a light-protected container. A total of 150 milk samples were collected, comprising 50 samples each of cow milk, sheep milk, and goat milk. The milk samples originated from Holstein cows, Awassi sheep, and Kilis goats. These samples were acquired from animals manually milked daily by their owners or purchased directly from the owners themselves.

Sampling

Since cows, sheep, and goats are widely bred in the province of Şanlıurfa, milk samples were collected from each species. The samples were obtained from family farm herds situated in the southeastern region of Turkey, specifically at latitude 37°10' N and longitude 38°47' E, with an elevation of 510 meters above sea level. These animals were maintained under optimal feeding conditions, receiving *ad libitum* access to native perennial forage grasses outdoors, without supplementary feed. Adequate water was continuously available. All cows (aged three to eight years), sheep (aged two to six years), and goats (aged three to six years) were in good clinical health, subject to repeated health assessments and care throughout lactation. Milk samples were collected from dairy cows, sheep, and goats between the hours of 06:30 and 10:00. Morning milk was obtained from all animals for analysis, as evening milk from sheep and goats was separated due to offspring feeding schedules. All milk samples were manually collected by the farm's milkers during the morning milking shift, transferred into sterile tubes, packed with ice packs, and transported to the laboratory. Upon arrival, the samples were stored in the dark at -19°C until analysis. Stringent measures were implemented during sample collection and handling to minimize the risk of vitamin content degradation, ensuring the reliability of the analysis.

Analysis methods

Vitamin analyses of the collected milk samples were conducted following the procedures outlined in the ELISA test kit at the Food Hygiene and Technology Laboratory of Harran University Veterinary Faculty. Each sample was analyzed in duplicate using ELISA tests, and the results were averaged for accuracy and reliability.

ELISA test procedure for biotin

150 µl of biotin assay medium was pipetted into the wells, followed by 150 µl of standards and diluted samples into the designated wells. The strips were then covered with adhesive foil and placed in an incubator at 37°C (98.6°F) in darkness for 44-48 hours. After the incubation period, the adhesive foil was pressed down once more, and the microorganisms were thoroughly dissolved by shaking the plate on the table's surface. Subsequently, the plate was returned to its regular position, and the adhesive foil was removed. The turbidity was measured using a microtiter plate reader at 610-630 nm.

ELISA test procedure for folic acid and cobalamin

50 µl of each standard or prepared sample was added to the wells, followed by the addition of 50 µl of the conjugate to each well. The plate was then incubated for 15 minutes at room temperature in darkness. Afterward, the liquid was poured out of the wells, and the microwell holder was tapped upside down vigorously three times against absorbent paper to ensure complete removal of the liquid. The wells were then filled with ready-to-use wash buffer (250 µl/well) three times. Subsequently, 100 µl of substrate/chromogen was added to each well and incubated for 10 minutes at room temperature (20-25°C) in darkness. Following the incubation, 100 µl of stop solution was added to each well, and the absorbance was measured at 450 nm within 5 minutes.

Statistical analyses

At the conclusion of the test, the values were reported in µg/100 mL. Detection limits and recovery values were determined during the analysis. To compare the groups, an analysis of variance (one-way ANOVA) followed by Tukey's post-hoc test was performed. Additionally, correlation analysis was conducted to investigate the relationship between vitamin groups in milk samples from each animal. All statistical analyses were performed using SPSS program version 21.0, with a significance level set at 0.05 (SPSS, 2013).

RESULTS

The results of folic acid (vitamin B9), cobalamin (vitamin B12), and biotin (vitamin B7) analysis of sheep, goat, and cow milk samples analyzed within the scope of this study are presented in Table 1.

Mean folic acid contents of sheep, goat, and cow milk were 3.45±1.89, 7.23±0.70, and 6.64±1.49 µg/100 mL, respectively. Mean cobalamin contents of sheep, goat, and cow milk were 0.70±0.40, 7.43±5.8, and 0.58±0.59 µg/100 mL, respectively, while mean biotin contents of sheep, goat, and cow milk were 2.21±1.57, 2.21±0.43, and 2.09±0.91 µg/100 mL, respectively, as shown in Table 1.

Upon examination of the mean folic acid levels, it was observed that sheep milk had the lowest level among the species. Similarly, the mean cobalamin levels indicated that cow's milk had the lowest level among the species. Overall, while cow milk appeared to have the lowest average levels among the species, the average biotin levels of the milk samples analyzed seemed to be quite similar to each other.

Statistical analysis revealed no significant difference in the average amount of biotin among sheep, goat, and cow milk samples. However, significant differences were observed in folic acid and cobalamin amounts (P<0.05), as shown in Table 2.

The maximum amount of cobalamin was found in goat milk, with a level of 7.43±5.8 µg/100 mL, while cobalamin levels were notably lower in sheep and cow milk. Similarly, the maximum amount of folic acid was determined in goat milk, with a level of 7.23±0.70 µg/100 mL. The average folic acid content in sheep milk was lower compared to cow and goat milk.

Upon examining the correlation between the amounts of folic acid, cobalamin, and biotin in sheep, goat, and cow milk, a positive correlation (0.313*) between folic acid and biotin was observed only in cow milk (Figure 1). No such correlation was found in sheep and goat milk.

Table 1. Minimum, maximum, and mean levels of milk samples (µg/100 mL)

Milk	Vitamins	Minimum	Maximum	Mean±SD
Sheep milk (n:50)	**Folic acid**	1.7	8.77	3.45±1.89
	Cobalamin	0.47	1.67	0.70±0.40
	Biotin	1.04	9.06	2.21±1.57
Goat milk (n:50)	**Folic acid**	4.80	9.11	7.23±0.70
	Cobalamin	0.40	19.13	7.43±5.8
	Biotin	1.35	3.08	2.21±0.43
Cow milk (n:50)	**Folic acid**	2.69	8.81	6.64±1.49
	Cobalamin	0.10	3.76	0.58±0.59
	Biotin	1.09	6.18	2.09±0.91

SD: Standard deviation n: number of animals

Table 2. Folic acid, cobalamin, and biotin concentrations and comparisons in cow, sheep, and goat milk samples (µg/100 mL).

	Sheep milk (n:50)	Goat milk (n:50)	Cow milk (n:50)	Pvalue
Folic acid	3.45 ± 1.89^{ax}	7.23 ± 0.70^{bx}	6.64 ± 1.49^{cx}	$<0.001^{***}$
Cobalamin	0.70 ± 0.40^{ax}	7.43 ± 5.8^{bx}	0.58 ± 0.59^{ax}	$<0.001^{***}$
Biotin	2.21 ± 1.57^{ax}	2.21 ± 0.43^{ax}	2.09 ± 0.91^{ay}	>0.05

a-c: The mean values with different letters in the same line are significantly different P<0.05).
x-y: The mean values with different letters in the same column are significantly correlate (P<0.05).
n: number of milk samples

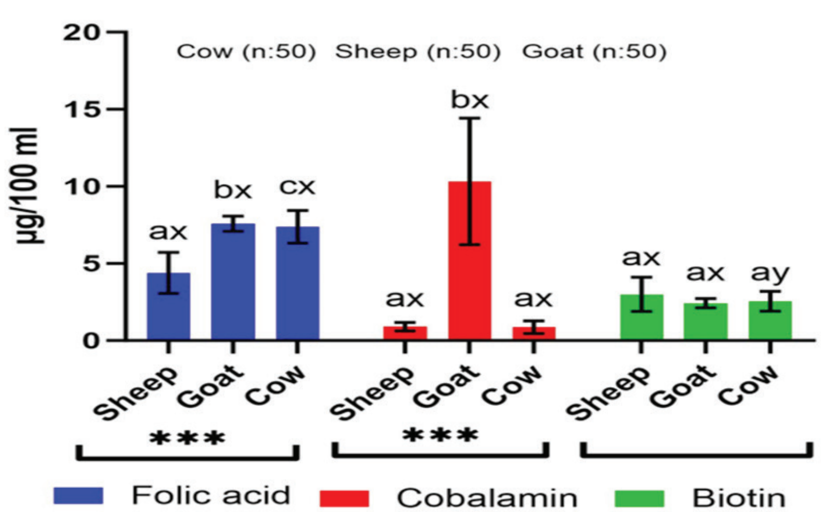

Figure 1. Folic acid, cobalamin, and biotin concentrations and comparisons in cow, sheep, and goat milk samples.

x-y: Values with different superscripts in the same row are statistically different (P<0.05),
a-c: Values with different superscripts in the same column are statistically different (P<0.05).
***P<0.001, n: number of milk samples

DISCUSSION

While studies on the vitamin content of milk may not be entirely up-to-date, their significance in understanding the physiopathology of diseases with increasing incidence rates today, as well as their impact on treatment strategies and prognosis, cannot be understated. Shifts in dietary preferences, such as the adoption of vegan or vegetarian diets, may lead to negative effects resulting from inadequate intake of vitamins, primarily found in foods of animal origin. For instance, although limited information exists on the cardiovascular effects of vegan diets, vitamin B12 deficiency is prevalent among vegetarians and may be partially mitigated by consuming dairy products (Woo et al., 2014).

Studies focusing on the relationship between vitamins and diseases such as cancer, type 2 diabetes, and Alzheimer's, which are prevalent in the 21st century, as well as the potential disruption of vitamin absorption by drugs used for treatment (Sayedali et al., 2023), underscore the growing importance of understanding vitamin content (Tu et al., 2023; Liu et al., 2023).

Milk serves as a vital source of vitamins for human nutrition. Increasing consumer awareness regarding the nutritional composition of foods and their health effects has led to an uptick in studies investigating the nutrient composition of animal products (Insel et al., 2003). However, studies on milk and milk products associated with vitamin values in Turkey remain limited. For instance, a study by Terzi et al. (2008) examined cobalamin levels in milk samples from Holstein, Jersey, Zavot, and Eastern Anatolian Red Breeds. They found cobalamin levels to be 0.98±0.09, 1.15±0.09, 2.93±0.04, and 1.56±0.18 µg/L, respectively, with Zavot cows' milk containing significantly higher cobalamin levels compared to other breeds. In contrast, the cow milk analyzed in our study exhibited higher cobalamin levels. This discrepancy could be attributed to variations in nutrition or season.

Similarly, Paksoy et al. (2018) examined folic acid contents in milk samples from Simental and Montofon cows. They found average folic acid contents to be 2.99±0.88 and 3.27±1.23 µg/100 g, respectively, which were higher than the folic acid levels in cow milk observed in our study. Geographic differences may explain this variance.

Research from other regions provides further insight into vitamin content in milk. For instance, the concentration of cobalamin in milk from commercial dairy herds in Canada ranged from 2.3 to 3.9 µg/L (Duplessis et al.,2016).Additionally, the national food composition databases of Denmark and Switzerland reported cobalamin concentrations in bovine milk ranging from 0.8 to 4.9 µg/L(Gille and Schmid, 2015). These values differ from our findings, indicating potential regional variations.The cobalamin content of skim milk in New Zealand was reported as 2-8 µg/100 g (Indyk et al., 2002). According to the research data reported by Medhammar et al. (2012) , cobalamin and folic acid levels in cow milk were reported to be 0.45 and 5 µg/100 mL, respectively, while buffalo milk was 0.40 and 0.6 µg/100 mL, respectively. In the study conducted by Park et al. (2007) from different animal species, the average cobalamin levels in goat, sheep, and cow milk were 0.065, 0.712, 0.357 µg/100 mL, and folic acid levels were found to be 1.0, 5.0, 5.0 µg/100 mL, respectively. The researchers reported the average biotin levels in goat, sheep, and cow milk as 1.5, 0.93, 2.0 µg/100 mL. Cobalamin levels were measured in parallel in sheep milk and lower in goat and cow milk than in this study. The folic acid level was higher in sheep and cow milk and lower in goat milk. Biotin level was parallel in cow milk and less in sheep and goat milk.

In a study conducted by Haddadin et al. (2008) with camel milk, they concluded that the average folic acid level was 87 µg/100 mL. According to the data reported by Uniacke-Lowe in his doctoral dissertation (2011), the cobalamin levels of sheep, goat, and cow milk were 0.007, 0.7, 0.004 mg/L, respectively, and the folic acid level of cow milk was reported as 0.18 mg/L. When we observe the results of the researchers, it is seen that the average cobalamin level in sheep milk is very similar to the results of this study.

In a comprehensive compilation by Claeys et al. (2014)folic acid levels in goat, cow, and sheep milk were reported to range from 0.24 to 1, 1 to 18, and 0.24 to 5.6 µg/100 mL,respectively, while cobalamin levels ranged from 0.06 to 0.07, 0.27 to 0.7, and 0.30 to 0.71 µg/100 mL, respectively. These levels are lower than those observed in our study.

Similarly, Sharma et al. (2007) found cobalamin levels of 4.91, 21.68, and 3.91 µg/L in cow, buffalo, and goat milk, respectively, which differ from our findings. Moreover, Wijesinha-Bettoni and Burlingame (2013) examined the folic acid content of cow, sheep, goat, and buffalo milk, reporting average folic acid contents of 8.5, 6, 1, and 0.6 µg/100 g, respectively. These values also vary from our study results. These discrepancies underscore the importance of considering regional factors and breeding practices when interpreting vitamin levels in milk.

Given the limited number of articles on milk B-vitamin levels in our literature review, research on the vitamin B levels of dairy products is included in this section. Arkbåge et al. (2003b) found that the amount of vitamin B12 was 0.30 µg/100 g in cottage cheese, and 2.29 µg/100 g and 2.47 µg/100 g in Swedish hard cheese varieties. In a study by Repossi et al. (2017), the average amount of folic acid in milk, whey, ricotta, and exhausted wheywas 5.2±1.3, 2.3±0.8, 5.7±3.3, 2.8±0.9 ng/g, respectively. Park et al. (2003)analyzed vitamin B12 content of infant formulas, follow up formulas, nutritional supplements, medical products, and snakes with average values of 2.8, 2.7, 2.2, 101.7, and 5.6 µg/100 g,respectively. In another study by Campos-Gimenez et al. (2008), the content of vitamin B12 in different milk based infant formulas was investigated, with average values of 1.48-3.07 µg/100 g. Hoppner and Lampi (1992) determined the biotin content in Canadian cheese varieties through microbiological experiments, reporting a content of 5.9 µg/100 g.

CONCLUSIONS

This study sheds light on the vitamin composition of milk from various mammalian species, including sheep, goats, and cows, in the Şanlıurfa province of Turkey. Through meticulous analysis of folic acid, cobalamin, and biotin levels, valuable insights into the nutritional profiles of these commonly consumed dairy products have been gained. The research highlights significant variations in vitamin content among different milk samples, particularly emphasizing higher levels of folic acid and cobalamin in goat milk compared to other species, and elevated biotin content in sheep milk. Notably, cow milk, a staple in global consumption, exhibited lower levels of cobalamin and biotin compared to other species. These findings underscore the importance of considering the source of milk in dietary planning to ensure optimal intake of essential B-vitamins. Such insights are instrumental for both the food industry, aiding in product development and fortification strategies, and for public health, enabling consumers to make informed choices regarding their nutritional needs. Future research endeavors may delve deeper into factors influencing vitamin content variations in milk and their potential implications for human health, thereby enriching our understanding of dairy product nutrition.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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