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DETERMINATION OF VITAMINS A, E AND C IN MILK AS IMPROVERS OF ITS ANTIOXIDANT ACTIVITY

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ABSTRACT

As a part of food and feed, nutrients including proteins, fats, carbohydrates, minerals, vitamins and water serve to meet animal physiological needs, maintain basal metabolism and reproduction, and the productive needs of milk production. Antioxidants have important role in inhibition of oxidative processes not only in living organisms, but also in food and feed preventing their oxidation and degradation. In this study determined were vitamins A, E and C in cow milk from three production farms as nutrients that increase the effect of antioxidant activity in caw milk. Vitamin A (Retinol) and vitamin E (Tocopherol) has analyzed by HPLC technique, using Perkin Elmer apparatus with pump: series 200LC, auto sampler, ISS - 200, detector LC - 135 / LC -235 C DA. Vitamin C (Ascorbic acid) was analyzed spectrophotometrically (Spectroquant Pharo 300 - Merck) at 520 nm. Vitamins A and E have shown higher values in samples of raw milk compared to those determined in pasteurized milk. The determined values of vitamin A, E and C in investigated milk samples were in agreement with the literature data, and they have shown statistical significance of difference in concentrations of some particular vitamins.

Key words: cow milk, vitamin A, vitamin E, vitamin C.

INTRODUCTION

The basic nutrients such as proteins, fats, carbohydrates, minerals, vitamins and water assure physiological and nutritional requirements of humans and livestock. Those that are a part of feed serve to meet animal physiological needs, maintain the basal metabolism and reproduction, as well as the productive needs of milk production (Ferris et al., 2003; Theodoridou & Koidis, 2005; EFSA, 2006; AOAC Official Method, 2000-2010; FAO, 2011; Caisin et al., 2012; Marin et al., 2016; Cornell, 2018; AHDB, 2018).

Vitamins are essential micronutrients what an organism needs in small quantities for proper functioning of its metabolism. They cannot to synthesize in the organism, either at all or in sufficient quantities, so therefore they must be taken in through the diet (Vaughan et al., 2009).

Antioxidants are substances that inhibit the oxidative processes not only in living organisms, but also in food/feed. They are important in preventing the oxidation and degradation of food components such as lipids, vitamins, carotenoids, and other that are susceptible to auto-oxidation. The content of antioxidants in milk are important ingredients that contribute to the neutralizing of free radicals and their amount is quite low (Clausen et al., 2009; Carlsen et al., 2010; Hayajneh & Mahmoud, 2014; Grazyna et al., 2017).

In this paper presented are the results obtained from investigation on the content of vitamins A, E and C in cow milk obtained from dairy farms at three production regions. Vitamins A, E and C in this study has examined as nutrients that increase the effect of antioxidant action in milk.

Milk and its nutritional value

Milk long time ago have known as "medicine and food for a long time". Cow milk is a product of the caw mammary gland obtained by milking healthy and properly fed animals. The first milk obtained immediately after cow calving have called colostrum, which has a yellowish color and salty-bitter taste being turn into milk after 3-4 days.

Milk is a highly valuable food that contains a sufficient amount of easy digested and used of the all nutrients such as proteins, fats, sugars, minerals and vitamins, and that are necessary for a proper development of the calf. Due to its composition, the cow milk is use in human daily diet for the all age groups from young children, sick and recovering people, athletes to the adults, so being the most consumed milk in the daily diet (Penny, 1993, Hurley, 2009).

The man's daily protein needs can met by consuming 200 g of meat and 0.5 l of milk, and the rest of the protein need is provided through foods of plant origin. Besides that, milk is a rich source of the essential amino acids, fats, some essential vitamins, and that is also very rich source of calcium that easily digest and use. Due to this, great attention today is to paid to the milk production in the world, especially in highly developed countries. The largest world milk producers are the highly developed countries such as the United States, Canada, Germany, France, Belgium, the Netherlands, Switzerland, Austria, Denmark and others.

The composition and quality of milk

The average chemical composition of cow milk is based on about of 87% water and 13% dry matter. The dry matter consists of proteins that participated around of 3.2%, milk fat with of 3.6%, milk sugar (lactose) with of 4.6% and minerals with of 1% (Hurley, 2009; Cornell, 2018).

Milk proteins are the most important nutrients that assess the quality of milk among which dominated is casein being followed by lactoalbumins and lactoglobulins. Milk proteins are quite constant comparing to the milk fat (Jones & Heinrichs, 2017). The second most important milk ingredient is milk fat that is the main source of energy. It contains fat-soluble vitamins, especially vitamin A, and on its content depends the color of the fat. Milk fat is quite variable and it is present in smaller quantities at the beginning of the lactation when milk production is the highest, while it increases and is the most present at the end of the lactation. The content of milk fat is greatly influence by the composition of the daily dairy cow ration. If there is a rough bulky feed with a higher cellulose content, the content of milk fat will be higher.

The main energetic nutrient present in milk is also the milk sugar (lactose) with prevalence of 4.6%. Lactose also like proteins shows the least variability. Milk minerals are also important nutrients for both the milk quality and its nutritional value. They easily used by the human body, because they easily digest. The most common minerals present in milk are calcium (up to 1200 mg/L), phosphorus (up to 950 mg/L), potassium (up to 1200 mg/L) and magnesium (up to 110 mg/L). Milk is quite poor in trace elements such as iron and cobalt.

Milk vitamins and their properties

Vitamins are generally classified as water-soluble, that easily dissolve in water and are readily excreted from the body, and as fat-soluble, that are absorbed through the intestinal tract with lipids (fats).

Vitamins A, D, E, and K are fat-soluble vitamins present in human body, while the vitamin C and the vitamins of the B group are water-soluble. Vitamins C and E function as antioxidants (Bender, 2003). Milk also contains a sufficient amount of biostimulants that are necessary for a proper development of the newborn animals. Fat-soluble vitamins A, D, E and K mainly found in the milk fat (Hurley, 2009). Milk has limited amounts of vitamin K, and B vitamins is found in the aqueous phase of the milk. Vitamin A have a role in the growth and development of cells, mucous membranes, skin, bones and the health of teeth, eyes, reproduction and immunity. Vitamin A is a biostimulant and nutrientand and it is known in the forms as all-*trans*-retinol, all-*trans*-retinyl-esters, as well as all-*trans*-beta-carotene and other provitamin A carotenoids (Palozza et al., 2000; World Health Organization (WHO), 2005; Noziere et al., 2006; Ross, 2006a; Ross, 2010b).

There are eight groups of related molecules (vitamers) of vitamin E- four tocopherols and four tocotrienols (Harvard Health, 2009). Vitamin E is an antioxidant that protects the cells from damage and supports the function of the immune system. Tocotrienol and tocopherol protect lipids and have significant antioxidant properties. Vitamin E protects lipids, and it is present in milk at a very low level (Hurley, 2009). The combination of selenium (Se) with vitamins A and E has an important role for the normal fetal development during pregnancy. The combination of vitamins A and E is also required for the development of antibodies that are present in large amounts in colostrum (ARD, 2009). Vitamin E acts as an antioxidant and help lipid membranes to be intact. Vitamin E reacts with selenium (Se), what is an excellent combination of antioxidants. If one of the two nutrients is deficient, the metabolic activity is impaired and therefore its addition is necessary. Products that have sufficient content of vitamin E and selenium (Se) that characterized by greater sustainability due to the antioxidant activity (Surai, 2003; Naziroglu et al., 2004).

Vitamin C (ascorbic acid) contributes in collagen forming, and as antioxidant restores the vitamin E to its active form, helps also the hormon synthesis, supports the immune function, and aids the iron absorption (Vaughan, 2009).

MATERIALS AND METHODS

Cow milk that were obtained from livestock farms (A, B and C) at three different production regions was used in investigation on the content of vitamins A, E and C. Milk was analyses immediately after the milking.

The samples of animal feed included: two types of concentrates (fodder mixture for dairy cows with the lowest crude protein concentration of 18%), alfalfa hay and straw. The cow breeds from the three farms were of the Holstein-Friesian breed. The cows from the farm A (Kumanovo) and from the farm C (Gostivar) has fed with two types of concentrate (K_1 and K_2) in the morning, with alfalfa and straw at lunchtime and with concentrate in the evening. The cows from the farm B (Tetovo) has fed with concentrate K_2 and alfalfa. The all cow breeds was milking of done by a milking machine.

Vitamin A (Retinol) and vitamin E (Tocopherol) were analyzed by HPLC technique, using Perkin Elmer apparatous with pump: series 200LC, auto sampler; ISS - 200, detector LC - 135 / LC -235 C DA. Vitamin C (Ascorbic acid) was analyzed spectrophotometrically (Spectroquant Pharo 300 - Merck) at 520 nm.

RESULTS AND DISCUSSION

Determination of vitamins A and E

The determinate concentrations of vitamins A and E in the samples of raw caw milk that were analyses in this investigation is present on the Graphs 1 and 2, respectively.

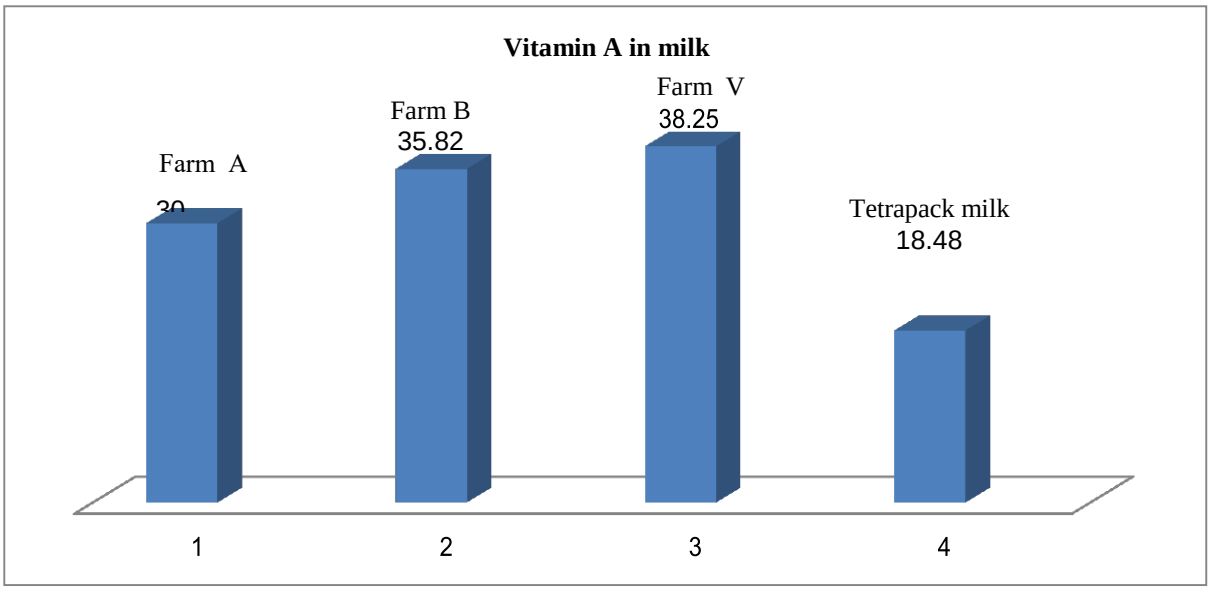

Figure 1. Concentrations of vitamin A in raw cow milk from three farms and pasteurized milk

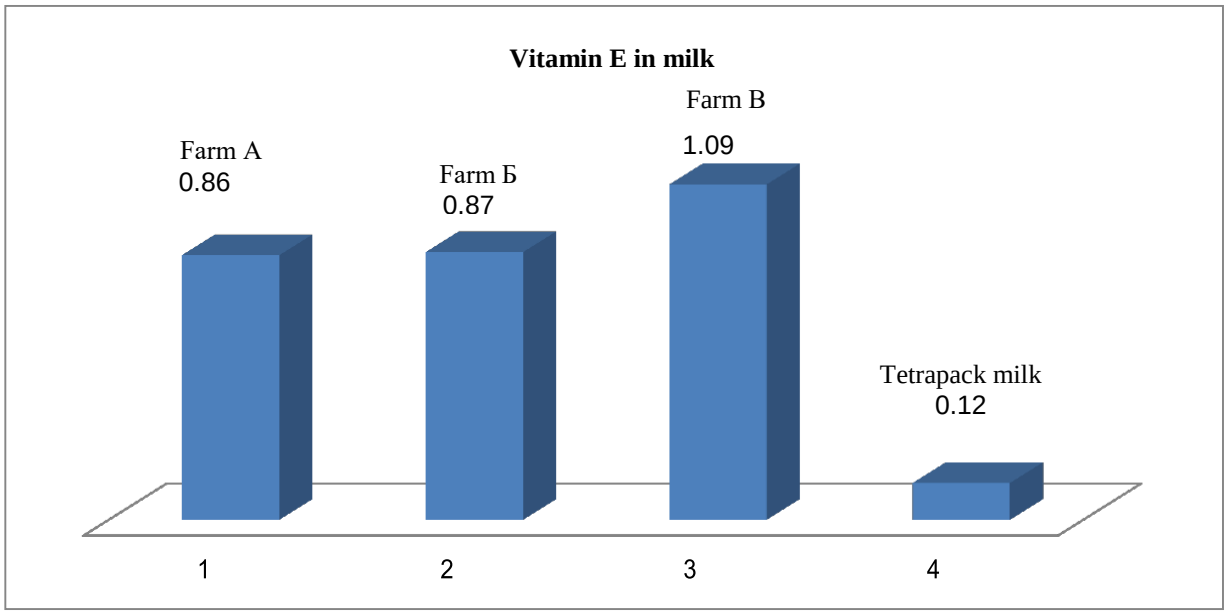

Figure 2. Concentrations of vitamin E in raw cowmilk from three farms and pasteurized milk

The milk from the farm C showed the highest values of concentration of vitamin A (38,25 μg/100g), the lowest values were found in milk from the farm B (35,8 μg/100g) and the lowest values were from the farm A (30 μg/ 100g). Vitamin A has shown higher values than that found in the commercial milk-tetrapack (18,48 μg/100g). Vitamin E had higher values in the milk from the farm C (1,09 μg/100g), following by the milk from the farm B (0,87 μg/ 100g) and the farm A (0,86 μg во 100 g). It was also shown that the values of vitamin E were higher than that in the milk of tetrapack (0,12 μg/100g).

A comparison among the concentrations of vitamins A and E determined in the samples of caw milk made and using statistic ANOVA test. The results are presented in Table 1 where a significant difference was noticed between the value of vitamin A in milk from the farm B (35,82 μg/100g) and the value of vitamin E in milk from the farm B (0,87 μg/100g). There was

also a significant difference between the values of vitamin A in milk from the farm C (38,25 μg/100g) and the value of vitamin E from the same farm (1,09 μg/100g).

Table 1. Statistical analysis by ANOVA test of cow milk vitamins A and E

Parameters of comparison (Vitamins A and E) in milk	**t = test**	**Arithmetic mean– $\bar{x}$**	**Standard Deviation – s**	**Significance – P**
Vit. A Farm A: Vit. A Farm B, 30,0:35,82	t = -1.006	15.000 17.910	2.828 2.956	Ns, P= 0.420
Vit. A Farm A: Vit. A Farm V, 30,0:38,25	t = -1.890	15.000 19.125	2.828 1.237	Ns, P= 0.199
Vit. A Farm B: Vit. A Farm V, 35,82:38,25	t = -0.536	17.910 19.125	2.956 1.237	Ns, P= 0.645
Vit. A Farm A: Vit. A Terapack milk, 30,0 :18,48,	t = -5.760	9.240 15.000	0.000 1.414	S, P= 0.029
Vit. A Farm B: Vit. A Tetrapak milk, 35,82:18,48	t = -96.333	9.240 17.910	0.000 0.127	S, P= < 0.001
Vit. A Farm V: Vit. A Tetrapack milk, 38,25:18,48	t = -79.080	9.240 19.125	0.000 0.177	S, P= < 0.001
Vit. A Farm A: Vit. E Tetrapack milk, 30,00:0,86	t = (+inf)	15.000 0.430	0.000 0.000	S, P= < 0.001
Vit. A Farm B: Vit. E Tetrapack milk, 35,82:0,87	t = (+inf)	17.910 0.435	0.000 0.000	S, P= < 0.001
Vit. A Farm V: Vit. E Tetrapack milk, 38,25:1,09	t = (+inf)	19.125 0.950	0.000 0.000	S, P= < 0.001

Ns – Insignificant value (a negligible value), S – Significant value

Determination of vitamin C

Determination of the concentrations of vitamin C in the cow milk samples investigated has performed by using a standard curve of ascorbic acid. The results is present on Graph 3.

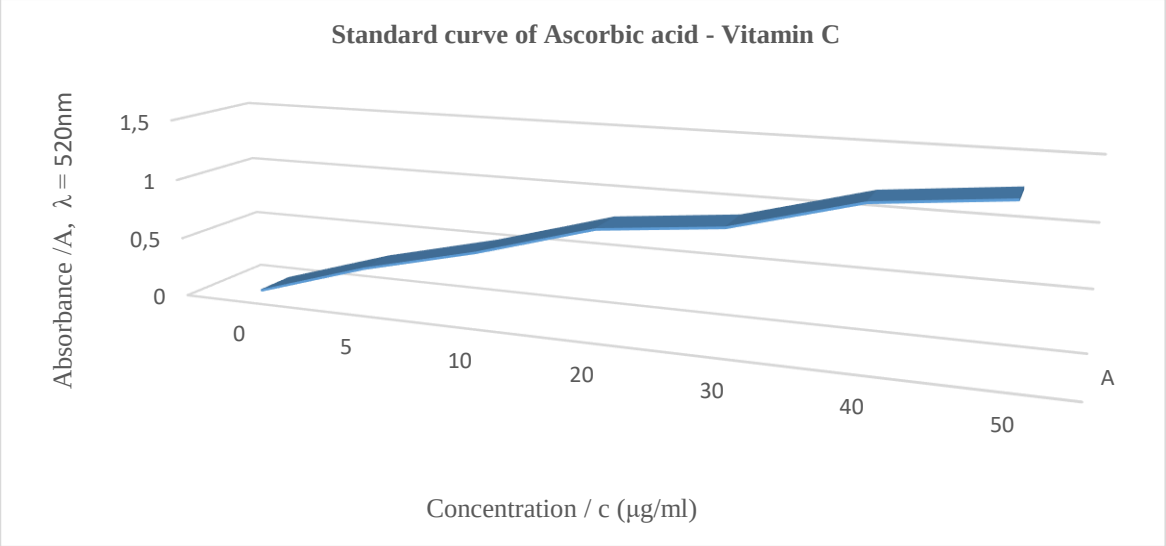

Figure 3. Standard curve of ascorbic acid

The values of the standard curve of ascorbic acid were in the measuring range between 0,00 and 40,00 µg/ml. The measured values of absorbance A for the milk samples analyses are present in Table 2. The absorbance values have transferred to a calibration curve for ascorbic acid from which the values of concentrations (µg/ml) have read. On the Graph 4 is present the determination concentrations of vitamin C in milk samples from the three farms.

Table 2. Measured concentrations and absorbance for standard curve of ascorbic acid

Concentration (c) µg/ml	**Absorbance (A) λ =520nm**
0	0.006
5	0.30
10	0.53
30	0.91
40	1.18
50	1.28

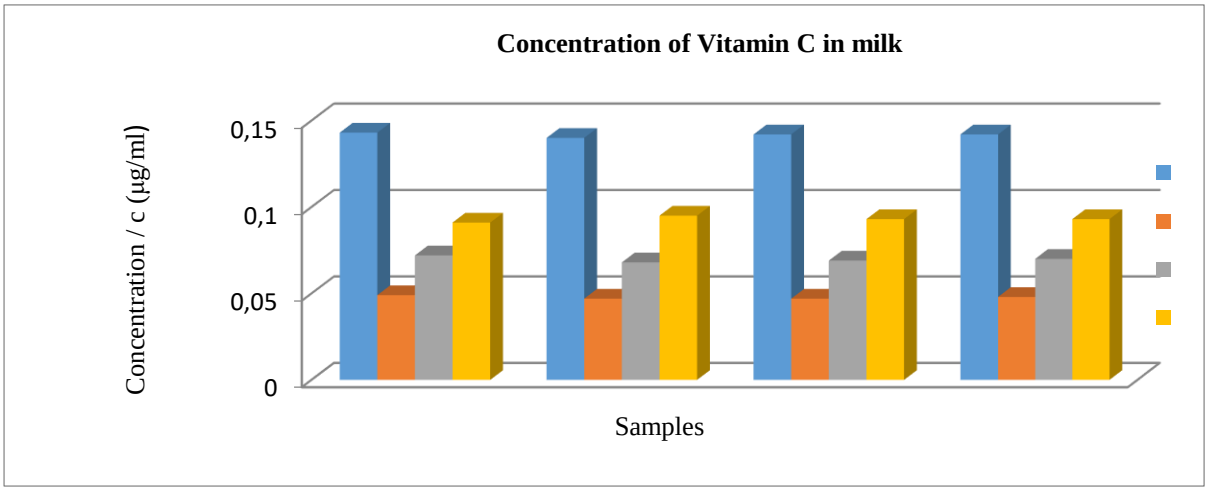

Figure 4. Concentrations of vitamin C in row caw milk from three farms

The highest value for vitamin C was determined in milk from the farm A (2,8 μg/ml), while the lowest was in the milk from the farm B (1,4 μg/ml). The values of the content of vitamins A and E were higher in raw milk compared to those found in the pasteurized milk, while the content of vitamin C was lower. Statistical analysis of absorbance and concentrations of vitamin C in cow milk samples is present in Table 3.

Table 3: Statistical analysis of absorbance and concentrations of vitamin C in milk samples

Number of measurements	**Number of samples** Absorbance (A), λ = 520nm			
	1	2	3	4
1	0.143	0.049	0.072	0.091
2	0.142	0.047	0.068	0.095
3	0.142	0.047	0.069	0.093
n = 3				
$\bar{x}$ =	0.142	0.048	0.070	0.093
s =	0.001	0.001	0.002	0.002
RSD	0.0040	0.0242	0.0298	0.0215
(c)/ (μg/ml)	2.8	1.35	1.4	2.3

Several types of milk were study on presence of vitamins such as cow and sheep milk, sterilized whole milk, skim milk, milk with added vitamins and chocolate milk. It has shown that the content of vitamin C in a vitaminized milk was the highest of 1.20 mg/dL (Tomovska et al., 2018). Chocolate milk also contains a significant amount of vitamin C, up to a maximum of 0.22 mg/dL, which is probably due to the cocoa used as a basic supplement in the production of chocolate milk. Skim milk has the lowest content of vitamin C, up to a maximum of 0.25 mg/dL, and a minimum of 0.1 mg/dL (Al-Ani et al., 2007). The adding of vitamin C in milk also improves milk antioxidant capacity, so the milk with supplements has the status of a functional food, providing a number of health benefits to consumers. The whole milk has a maximum content of vitamin C up to 0.35 mg/dL, which is higher than that in the chocolate milk. Skim milk, which contains 1% milk fat commonly used in the production of chocolate milk, has a small content of vitamin C. This is probably due to the degreasing processes, where along with the fat, the larger amount of vitamin C probably removed. This closely related to vitamin E, which, in turn, is the most concentrated in the membranes of the fat drops.

CONCLUSIONS

The investigation on the presence of vitamins A, E and C in cow milk originated from three farms has shown that results was a significant difference in the concentrations of particulate analyses of vitamins in the milk samples analyses. It was also determined that the

concentrations of vitamins were higher in the milk simples which were taken immediately after the milking, than in those that were determined in the homogenized and pasteurized commercial milk- tetrapack. It can be also concluded that the composition of the animal feed: concentrates, alfalfa and straw in the feed samples from three different farms, as well as the cow breed, might have an influence on the concentrations of the vitamins in the cow milk investigated, and consequently on the milk antioxidant activity. The world milk production aims to produce more milk with better quality, including more essential nutrients having high antioxidant activity that are important in daily human food and animal feed for achievement human and animal wellbeing. The feed intended for dairy cows feeding should have an excellent quality and safety, and to contain the necessary all nutrients in quantity and ratio, in order they be easily digested and used by the animal organism.

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