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Determination of Vitamin A and E in fodder concentrates and cow milk from region of Kumanovo, Macedonia

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Abstract. The main objective of this research was to determinate the vitamins A and E in fodder concentrate and raw cow milk from region of Kumanovo, Macedonia. As material for this research, samples of feed and samples of cow’s raw milk from the cattle fed with the same food were taken. The test material was taken from a farm in the region of Kumanovo. Vitamins A and E have been examined with the methodology of extraction on HPLC – Perkin Elmer, pump: series 200LC, autosampler; ISS – 200, detector LC – 135/LC -235 C DA. The amount of vitamin A in fodder concentrate was 7.5438 mcg/100g, while the amount of vitamin E was 23.115 mcg/kg.

The amount of vitamin A in raw cow milk from region of Kumanovo was 30 mcg/100g, while the amount of vitamin E was 0.86 mcg/100g.

Keywords: vitamin A, vitamin E, fodder, cow milk

1 Introduction

Carotenoids play a vital role in reproduction, they have antioxidant properties and regulate the immune response of both animals and humans (Biard et al. 2005). Vitamin A deficiency is a major public health problem over the world, especially in the poor countries. It occurs mainly in young children and women of childbearing age. Inadequate intake of vitamin A is the main cause of the deficiency. The main animal sources of vitamin A are liver, eggs, milk, and milk products. Plant foods rich in provitamin A represent more than 80% of the total food intake of vitamin A because of their low cost, high availability, and diversity. Fruits, roots, tubers, and leafy vegetables are the main providers of provitamin A carotenoids. Because of their availability and affordability, green leafy vegetables are consumed largely by the poor populations, but their provitamin A activity has been proven to be less than previously assumed. Worldwide, about 250 million children are at risk of vitamin A deficiency (UNICEF, 2004). Carotenoids are called pre-vitamin forms because they can be converted into retinol (yellow and orange fruits and vegetables and dark green leaves). There are
more than 600 forms of natural carotenoids and many of them have provitamin A activity, but food composition data are only available for three of them (α-carotene, β-carotene, and β-cryptoxanthin) (Van Jaarsveld et al. 2005). Vitamin E is a fat-soluble vitamin found in many foodstuffs, such as cereals, eggs, olive oils, and vegetables. Vitamin E occurs in many different forms (α-, β-, γ- and δ-tocopherols and α-, β-, γ, and δ-tocotrienols) and has many health benefits; it is mostly used for treating and preventing heart diseases (Pyka 2001; Zhao, 2014).

2 Material and methods

The main objective of this research was to determinate the vitamins A and E in fodder concentrate and cow milk in farm from region of Kumanovo, Macedonia. As material for this research, samples of feed and samples of cow's raw milk from the cattle fed with the same food were taken. The test material was taken from a farm in the region of Kumanovo. Feed samples comprise two types of concentrates produced by "Agroinvest", feed for molar cows with at least raw protein KMK - 18%, alfalfa silage, and straw. Cows were of the frieze race. Feeding was three times a day, concentrate in the morning, alfalfa and straw at the lunch, and concentrate in the evening. Milking was with the machine, three times a day in the summer period, and twice in the winter period. Methods for the analysis of vitamin A (Retinol) and vitamin E (Tocopherol) in concentrates and milk have been examined, 2 methods of extractions and sample preparation for HPLC analysis – Perkin Elmer, pump: series 200LC, autosampler; ISS – 200, detector LC – 135/LC – 235 C DA.

2.1 Extraction Method 1 (ChemElut)

Weigh 20 g of sample (approx. 0.01 g) into a 500 ml Erlenmeyer flask and add 1 g of ascorbic acid, 150 ml of ethanol (95%) and 40 ml of 50% potassium hydroxide (KOH) aqueous solution. Add the condenser to the flask and place in a water bath (approx. T = 95 ° C). Hydrolysis occurs 30 minutes after the start of the reaction. Shake the sample twice during hydrolysis. After the hydrolysis is complete, the sample is cooled to room temperature. Add 50 ml of distilled water. Transfer the hydrolyzate to a 500 ml volumetric flask and dilute to the ethanol mark (50%). Transfer 10.0 ml to a Chem Elut column (of 20 ml), wait approximately 10 min. The sample was eluted with 100 ml of n-hexane. Then the eluent is collected in a 500 ml flask. Evaporation (evaporation to dryness) is done with some BHT granules. The sample is dissolved in n-heptane and transferred to a volumetric flask (5.0 ml). Dilute to the n-heptane mark.

2.2 Extraction method 2 (separatory funnel)

Weigh 20 g of sample (to the nearest 0.01 g) in a 500 ml Erlenmeyer flask and add 1 g of ascorbic acid, 150 ml of ethanol (95%) and 40 ml of 50% potassium hydroxide solution in water. Add a condenser to the flask and place on a water bath (95 0 C). The hydrolysis lasts 30 minutes from the start of the reaction. During the hydrolysis,
shake the sample flask twice. After complete hydrolysis, the sample is cooled to room temperature and 50 ml of distilled water are added. Transfer the hydrolyzate into a 500 ml volumetric flask and dilute to the mark with 50% ethanol. Transfer 20 ml into a separatory funnel and dilute with 100 ml n-hexane. Shake the funnel for 1 minute, clean, wash (hexane phase, 2 times x 50ml 1M potassium hydroxide in ethanol (40%) and (2x50ml) with distilled water). Next, evaporate, evaporate to dryness in the hexane phase with a few granules of BHT and about 8ml of ethanol (99%) The sample is dissolved in n-heptane and transferred to a volumetric flask (5.0 ml). Dilute to the n-heptane mark. Analyzes are chromatographed on the apparatus used - HPLC - Perkin Elmer, pump: series 200LC, autosampler; ISS-200, Detector LC –135 / LC – 235 C DA.

Vit A and Vit E tests were performed in 5 concentrate samples, as well as raw milk and packaged milk. The table shows the mean of the five samples of each assay.

## 3 Results

The milk quality depends from the health of mammary gland. Milk quality is usually depend from mastitis, milk with a low somatic cell count (SCC) and visibly normal appearance (no clots). But, in accordance with Weiss (2010), the definition of high-quality milk must be expanded. Thus, the quality of milk can also be based on the amount of antioxidants that it contains, protecting the characteristics of milk lifetime by reducing oxidation.

In our research, we determined the amount of vitamin A and E of fodder concentrate, cow raw milk and packed milk in the region of Kumanovo.

As it is shown in Table 1, the amount of vitamin A in fodder concentrate in Kumanovo is 7.5438 mcg/100 g. Mean while the amount of vitamin E in fodder concentrate is 23.115 mcg/kg.

<table>
<thead>
<tr>
<th>Vitamins</th>
<th>Amount (mcg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7.5438</td>
</tr>
<tr>
<td>E</td>
<td>23.115</td>
</tr>
</tbody>
</table>

In Table 2 it is shown the amount of vitamin A and E in raw milk from the region of Kumanovo, as well as in packed milk. The amount of vitamin A in raw cow milk from region of Kumanovo was 30 mcg at 100g, compared with the packed milk which amount (18.48) of this vitamin was drastically lower.

The amount of vitamin E in raw cow milk was 0.86 mcg at 100g, compared with the packed milk which amount (0.12) of this vitamin was drastically lower, as well.
Table 2. Comparison of amounts of vitamins A and E in raw cow milk from region of Kumanovo and package milk

<table>
<thead>
<tr>
<th>Vitamins</th>
<th>Raw cow milk (mcg at 100 g)</th>
<th>Package milk (mcg at 100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>30</td>
<td>18.48</td>
</tr>
<tr>
<td>E</td>
<td>0.86</td>
<td>0.12</td>
</tr>
</tbody>
</table>

4 Discussion

Vitamin A is a fat-soluble vitamin involved in many important biological functions. Vitamin E is considered to act primarily as a lipid-soluble antioxidant, protecting polyunsaturated fatty acids and related substances from peroxidation and hence from rancidity (Bates & Prentice 1994).

Vitamins A (retinol) and E (tocopherol) and the carotenoids are fat-soluble micronutrients that are found in many foods, including some vegetables, fruits, meats, and animal products. Fish-liver oils, liver, egg yolks, butter, and cream are known for their higher content of vitamin A. Nuts and seeds are particularly rich sources of vitamin E (Thomas, 2006).

Vitamin A, found in foods that come from animal sources, is called preformed vitamin A. Some carotenoids found in colorful fruits and vegetables are called provitamin A; they are metabolized in the body to vitamin A. The bioconversion of carotenoids to vitamin A is different from person to person (Krinsky, 2005). Vitamin E activity is derived from at least eight naturally occurring tocopherols, the most potent of which is alphatocopherol. Other less active forms of vitamin E are plentiful in the diet, with gamma-tocopherol being the predominant form. Our results are in accordance with results of other authors such as Mourad (2014), Michlova (2015) and Sanchez-Machado (2006).

5 Conclusion

Based on these researches we can conclude that amount of vitamin A is higher in raw milk cow (30 mcg/kg), while lower in fodder concentrate (7.5438 mcg/kg). The amount of vitamin E is higher in fodder concentrate (23.115 mcg/kg), while it is lower in raw cow milk (0.86 mcg/kg).

By comparing the amount of vitamins A and E in raw milk and packaged milk, we found that their content was higher in raw milk.
References