Determination of vitamin A and E in fodder concentrates and cow milk from the region of Gostivar, North Makedonia

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DETERMINATION OF VITAMIN A AND E IN FODDER CONCENTRATES AND COW MILK FROM REGION OF GOSTIVAR, MACEDONIA

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Abstract

Given the importance of oxidative effects on the dairy cows’ health and milk production, a study conducted on the concentration of antioxidants, vitamins A and E. The main goal was to determine vitamins A and E in animal feed concentrate and raw cow's milk from the region of Gostivar, Macedonia. Samples of feed and samples of raw cow's milk from cattle fed taken as material for analysis. Vitamins A and E were tested by high performance liquid chromatography (HPLC) extraction methodology - on Perkin Elmer, pump: 200LC series, self-sample; ISS - 200, detector LC - 135 / LC-235 C DA. Statistical analysis ANOVA test used to determine concentration of vitamin A, E, in feed and milk. The amount of vitamin A in the feed concentrate was 23.92 mcg/100 g, while the amount of vitamin E was 35.7 mcg/kg. The amount of vitamin A in raw cow's milk from the region of was 38.25 mcg/100 g, while the amount of vitamin E is 1.09 mcg/100g. Statistical analysis showed that there is a significant difference in the values of vitamins A and E in the concentration milk, p = < 0.001. It concluded that the concentrations of vitamins A and E in raw milk as well as in the concentrates compared to the standard samples are low, respectively.

Key words: Vitamin A, Vitamin E, Fodder, Cow milk.
1. Introduction

Antioxidant activity results from the presence of antioxidants in animal feed in vitro and from the metabolic processes that take place in the body of living organisms in vivo.

Antioxidants are important in preventing the oxidation and degradation of lipids, vitamins, carotenoids and other components in food that are susceptible to auto-oxidation (Carné, Zaragoza, 2004).

Fodder mixtures or concentrates used to feed dairy cows. Fodder mixtures are composed of grain fodder crops (corn, barley, bran, sunflower and soy husk, vitamin supplement (premix) and minerals, digestible proteins and carbohydrates, minerals, vitamins and have high energy value.

The use of feed mixtures - concentrates balances and completes the daily meal of dairy cows with all the necessary nutrients.

Differences in chemical composition and general nutritional value of forage crops that used as feed for farm animals should be known in order to be able to balance the nutrient and energy needs of each category of farm animals in order to meet the physiological needs (Caisin L, Vasile H, Vasile V. (2012); AHDB Dairy (2018).

Fodder mixtures used for feeding of dairy cows were produce in LLC "Agroinvest", (2017) [4] have their own catalog number and are produce according to the Rulebook for Fodder of the Republic of Macedonia.

As well as controlled from raw material to the final product, by at the Institute of Animal Husbandry - Skopje and the Faculty of Agricultural Sciences and Food from Skopje (2017) [5].

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 causes major public health problems worldwide, especially in 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 sources of vitamin A at animal: 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 consume 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. Materials and Methods

The main objective of this research was to determinate the vitamins A and E in fodder concentrate and cow milk by farm from region of Gostivar, Macedonia.

As material for this research, samples of feed and samples of cow's raw milk from the cattle fed with the same food taken. Feed samples comprise two types of concentrates produced by "Agroinvest", feed for milk cows with at least raw protein KMK - 18%.

Feeding was three times per 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 analysis of vitamin A (Retinol) and vitamin E (Tocopherol) in concentrates and milk

• Extraction method 1 (Chem Elut)

Weigh 20 g of the sample (closest to 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 a 50% aqueous solution of potassium hydroxide (KOH).

The condenser is added to the flask and placed in a water bath (approximately t = 95°C). Hydrolysis occurs 30 minutes after the start of the reaction. After complete hydrolysis, the sample cooled to room temperature. Add 50 ml of distilled water.

Transfer the hydrolyzate to a 500 ml volumetric flask and dilute to the mark with ethanol (50%), than transfer 10.0 ml to a Chem Elut column (20 ml), wait about 10 minutes.

The sample eluted with 100 ml n-hexane.

Then collect the eluate in a 500 ml flask.

Evaporation (evaporation to dryness) done with some BHT granules.

Dissolve the sample in n-heptane and transfer to a volumetric flask (5.0 ml). Dilution is done up to the n-heptane mark.
• Extraction method 2 (separation funnel)

Weigh 20 g of the sample (to the nearest 0.0 1 g) into a 500 ml Erlenmeyer flask and add 1 g of ascorbic acid, 150 ml of ethanol (95%) and 40 ml of a 50% solution of potassium hydroxide in water.

The procedure is similar to Extraction method 1, then after complete hydrolysis, the sample is cool to room temperature and 50 ml of distilled water add and transfer to a 500 ml volumetric flask and dilute to the mark with 50% ethanol.

Transfer 20 ml into a separating funnel and dilute with 100 ml n-hexane. Shake the funnel for 1 minute, clean, wash (hexane phase, 2 times x 50 ml 1 M potassium hydroxide in ethanol (40%) and (2 x 50 ml) distilled water). This followed by evaporation, evaporation to dryness of the hexane phase with a few granules of BHT and about 8 ml of ethanol (99%).

Dissolve the sample in n-heptane and transfer to a volumetric flask (5.0 ml). Dilution is done up to the n-heptane mark.

The analyzes was chromatographed on the applied equipment - HPLC - Perkin Elmer, pump: series 200LC, auto sampler; ISS - 200, detector LC - 135 / LC -235 C DA.

3. Results and Discussion

The milk quality depends from the health of mammary gland, age, feed, age, and milk frequency. Milk quality is usually depend from mastitis, milk with a low somatic cell count (SCC) and visibly normal appearance (no clots). Accordance with Weiss (2010), the definition of high-quality milk must be expansion.

Thus, the quality of milk can also base 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 milk I pack, from the region of Gostivar, Macedonia.

Value of Vitamins A and E according to Cop Rice - Cop Rice (2018) as standard who examined the composition of concentrates in Australia (Concentrate Au) and should contents: dry matter, protein, fiber, fat, urea, calcium, phosphorus, magnesium, manganese, zinc, selenium examined and vitamins A, D and E.

The Table 1 shown the amount of vitamin A in fodder concentrate is 23.92 mcg/100g, while the amount of vitamin E in fodder concentrate is 35.7 mcg/100g.
The amount of vitamin A in raw cow milk from region of Gostivar was 38.25 μg/100g, compared with the packed milk who amount was 18.48 μg/100g of this vitamin was drastically lower.

The amount of vitamin E in raw cow milk was 1.09 μg/100g, compared with the packed pasteurized milk who amount (0.12 μg/100g) of this vitamin was drastically lower, as well.

The Table 2 shown the statistical analysis of amount vitamins A and E in fodder concentrate and milk.

Table 1. Amounts of vitamins A and E in fodder concentrate and milk from region of Gostivar, Macedonia

<table>
<thead>
<tr>
<th>Vitamins</th>
<th>Concentrate (mcg/g = IU/kg)</th>
<th>Concentrate (Au) Standards (IU, mg)</th>
<th>Raw cow milk (μg/100g)</th>
<th>Package milk (μg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>23.92</td>
<td>26.0 IU</td>
<td>38.25</td>
<td>18.48</td>
</tr>
<tr>
<td>E</td>
<td>35.7</td>
<td>90.0 mg</td>
<td>1.09</td>
<td>0.12</td>
</tr>
</tbody>
</table>

The values of amounts vitamins A and E in fodder concentrate and milk from region of Gostivar, Macedonia, are shwon in Figure 1.

Figure 1. Amounts of vitamins A and E in fodder concentrate and milk from region of Gostivar, Macedonia.
The Table 2 shown the statistical analysis of amount vitamins A and E in fodder concentrate and milk made. Statistical analysis ANOVA test used to determine concentration of vitamin A, E, in feed and milk. Statistical analysis showed that there is a significant difference in the values of vitamins A and E in the concentration milk, $p = < 0.001$.

### Table 2. Statistical analysis of vitamins A and E in concentrates and milk

<table>
<thead>
<tr>
<th>Comparison parameters (Vitamins A and E) in concentrates and Row milk</th>
<th>t-test</th>
<th>Arithmetic mean $\bar{x}$</th>
<th>Standard deviation $s$</th>
<th>Significance (S) – $P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A Concentrate 38.25(μg/100g)</td>
<td>$t =$ -96.333</td>
<td>9.240</td>
<td>17.910</td>
<td>0.000</td>
</tr>
<tr>
<td>Vitamin A Packing Milk 18.48(μg/100g)</td>
<td>$t =$ (+inf)</td>
<td>17.910</td>
<td>0.435</td>
<td>0.000</td>
</tr>
<tr>
<td>Vitamin A Concentrate 38.25; Vitamin E 1.09(μg/100g) Milk</td>
<td>$t =$ (+inf)</td>
<td>17.910</td>
<td>0.435</td>
<td>0.000</td>
</tr>
</tbody>
</table>

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 found in many foods, including some vegetables, fruits, meats, and animal products.

Fish-liver oils, liver, egg yolks, butter, and cream was 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, called preformed vitamin A.
Some carotenoids found in colorful fruits and vegetables called provitamin A; they 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 derived from at least eight naturally occurring tocopherols, the most potent of which is alpha tocopherol.
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).

4. Conclusions

Based on this researches we can conclude that amount of vitamin A is higher in raw milk cow (38.25 mcg/g), while lower in fodder concentrate (23.92 mcg/g).
The amount of vitamin E is higher in fodder concentrate (35.7 mcg/g) while it is lower in raw cow milk (1.09 mcl/l).

The obtained concentration of vitamins A and E in food concentrate were compare with values of table expose Cop Rice (2018) [6], Australia as standard where amount of vitamin A is similar, vitamin E is very low.

By comparing the amount of vitamins A (38.25 mcg/g) and vitamins E (1.09 mcg/g) in raw milk, and at packaged milk, we found that amount of vitamin A is (18.48 mcg/g ) and vitamin E is (0.12 mcg/g ) that their content was higher in raw milk.
5. Reference


