A review of the effects of different feeding regimes of cows on milk fatty acids profile and antioxidant capacity

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REVIEW

Abstract
This bibliography review analyzes the effects of different feeding regimes of cows the nutritional and sanogenic quality of milk, with a particular emphasis on milk content in functional fatty acids and non-enzymatic lipophilic antioxidants. Thus, using suitable feeding regimes of cows, fatty acid (FA) profile of milk fats can be improved by increasing the concentration in functional FA (C18:1 trans-11, vaccenic acid; C18:3 n-3, acid linolenic and C18:2 cis-9 trans-11, isomer of conjugated linoleic acid), considered as beneficial for consumer’s health. These suitable feeding regimes of cows aim using green fodder (pastures), preserving the fodder by drying or silage, supplementing the food with fats rich FA polyunsaturated (PUFA) and ensuring an optimal ratio between volume and ration concentrates. Milk yielded from grazing cows shows a considerably higher concentration of functional FA and lipophilic antioxidants (tocopherols, retinol and carotenes) and a lower content of saturated FA compared to that yielded from cows fed in shelter with TMR (total mixed ration). Also, hay-based feed rations, mainly those containing small amounts of concentrates or those supplemented with vegetable fats, ensure a milk yield with a fatty acid profile more favorable for human health and a better oxidative stability of compared to those with feed-based rations on silage or those high in concentrates.

Keywords: milk fat; beneficial fatty acids; CLA; pasture; silage; hay; dietary lipids.

INTRODUCTION
Most farmers tend to pay more attention to the quantitative production of milk and less attention to the composition and especially the content in bioactive components with sanogenic effect. However, consumers are increasingly interested in the quality of milk and its effect on their health. Milk is more than a source of nutrients for humans. In addition to the nutritional values of milk, biologically active compounds of milk such as functional fatty acids, lipophilic antioxidants and some protein fractions, have been found to have a favorable impact on metabolism and human health (Simopoulos, 1999).

Milk fat intake is often associated with a high risk of suffering from cardiovascular disease due to its high saturated fat content. However, there is a growing scientific consensus pointing to dairy fat as a natural source of bioactive components (Gomez-Cortes et al, 2018). The milk and whole dairy products promote human health due to the presence of certain bioactive fatty acids. These exert beneficial effects in lowering the risk of cardiovascular disease, in reducing the advancement of the atherosclerotic process in patients with coronary heart disease, in slowing down the growth of cancer, and in
increasing the efficacy of chemotherapeutic treatments, as well as in lowering neuroinflammation and maintaining mental health. In addition, certain bioactive fatty acids are considered potent anti-inflammatory products, able to reduce the risk of insulin resistance and ameliorate obesity-associated disorders (Gomez-Cortes et al., 2018).

Functional fatty acids are represented by: omega-3 fatty acids, conjugated linoleic acid (CLA; isomer C18:2 cis-9, trans-11; also called rumenic acid-RA and isomer C18:2 trans-10, cis-12) and trans-vaccenic acid (C18:1 trans-11; VA). Increasing the concentration of these FAs in the composition of milk fats and dairy products, through sustainable practices of cattle farm management (such as breed, lactation phase), and especially by manipulating nutritional factors (type and structure of feed ratio, the use of pastures or of fresh green fodder, the way to preserve fodder, the ratio of feed/concentrates in food, the supplement of fats in food and its degree of saturation), can improve the health of consumers without a change in diet.

Changing life styles have led the consumer to consume functional foods containing natural antioxidants to prevent oxidative stresses. Antioxidant capacity of milk is largely due to vitamins A, E and carotenoids. The foods containing natural lipophilic antioxidants (tocopherols, carotenoids, retinol) have grown in popularity, as antioxidants can neutralise harmful effects of free radicals both in humans and animals and because oxidation processes in milk can result in a deterioration of its nutritional quality (Gutiérrez-Peña et al., 2021). An adequate daily intake of antioxidant compounds is considered to be of great significance for prevention of health problems like cardiovascular diseases and cancer and/or in the reduction of diseases like diabetes and neurodegenerative diseases (Parkinson’s and Alzheimer’s disease) (Ertan et al., 2017). Concentrations of antioxidant compounds in milk are usually affected by dairy cattle feeding and milk storage conditions (Ertan et al., 2017).

An increase of functional biocomponents content in milk by implementing optimal nutritional strategies in dairy farms could lead to a niche market for milk and dairy products with functional food qualities.

Considering the main role that nutritional factors are playing in changing fatty acid profile of milk fat, this literature review provides recent data from studies on the feeding techniques of dairy cows that may increase the concentration of fatty acids considered beneficial to health human, especially focusing on functional fatty acids (C18:1 trans-11; C18:2 cis-9 trans-11; C18:3n-3 and its metabolism products). On the other hand, the effect of the type of diet on the milk content in lipophilic antioxidants (α-tocopherol, retinol and carotenoids) was analyzed, which would ensure the oxidative stability of milk containing large amounts of FA beneficial to human health.

**Effects of feeding on the milk fat quality**

**Effects of grazing on milk fatty acids profile**

The fatty acid composition of cow’s milk has recently become less favorable to human health due to changes in feeding pattern, based on large amounts of concentrates and silage and less or even elimination of grazing (Elgersma and Tamminga, 2006). Pasture is an important source of PUFA (especially C18:3 n-3, which represents over 60% of total FA - Mierliță et al., 2017) for ruminants, but the fatty acid composition of milk fat is influenced by its nutritional value of pasture, floristic composition, vegetation stage (season) and quantity consumed (Elgersma, 2015). The use of pasture in cow feed has a positive impact on the nutritional quality of milk, increasing the content of some components beneficial to human health, such as omega-3 FA, vaccenic acid (VA, C18:1 trans-11) and conjugated linoleic acid (CLA, C18:2 cis-9 trans-11), in the same time reducing the levels of omega-6 FA and palmitic acid. Significantly higher percentages of functional fatty acids were recorded in the fat in milk obtained from animals kept on pasture (Mierliță, 2017). While feeding TMR cows led to increased concentrations of SFA (saturated FA), the fat in the milk obtained from the pasture has a lower content of SFA and a higher content of unsaturated FA (UFA) with a higher concentration of PUFA; but also, an n-6/n-3 FA ratio and an improved thrombogenicity (TI) and atherogenicity (AI) index (Allothan et al., 2019) and lower concentrations of hypercholesterolemic FA (C12:0, C14:0 and C16:0) (Chilliard et al. 2007; Elgersma, 2015).

Grazing pasture reduces de-novo synthesis in the mammary gland due to intake of longer chain FA, such as ALA. Therefore, it can be assumed that the high percentage of unsaturated FA in milk, especially α-linolenic acid (ALA, C18:3n-3), comes from grass, so it can be used as a biomarker to check the feeding system on pasture (Mitani et al. 2016; Capuano et al., 2014; Gasparo et al., 2010).

Pasture feeding changes the ratio of palmitic to oleic acids and thus can alter the texture or viscosity perception of resultant dairy products (Kilcawley et al., 2018).

The phenological stage of the grass, the botanical composition of the pasture and the management of the pasture show also a particular importance. The young grass has a higher content of lipids and ALA than the mature grass, so the grazing of cows with young grass induced higher levels of this FA (+ 0.3 g / 100 g) and cis-9, trans-11 CLA (+ 0.9 g/100 g) (Ferlay et al., 2011). Milk yielded from cows fed on lush pastures in early spring, showed the highest levels of CLA (Elgersma et al., 2004). Mature pasture, which has higher proportions of C14:0 and C16:0 and less α-linolenic acid, causes a significant decrease in the concentration of CLA in milk (Loor et al., 2002). This effect is determined by the lower content of grass in α-linolenic acid, which is the lipid substrate for the formation of VA
The highest concentrations of ALA and CLA in milk fat were found in Switzerland, in agreement with the feeding of cows on pastures in the mountainous and alpine areas, while the lowest concentrations were found in the Netherlands when cows are fed indoors with mixed rations based on corn silage and starch-rich concentrates (Fernandez and Gonzalez, 2012).

In case of continuous grazing, milk PUFA content decreased continuously during the grazing season, while the share of PUFA in the milk fat structure remained constant when using the rotating grazing system. These differences could be explained by a combined effect of the phenological stage of grass and the selection of grass by cows (Ferlay et al., 2011).

Vanhalato et al. (2007) showed that rations based on mowed and input of red clover improved the profile of milk fatty acids by increasing the ratio of unsaturated fatty acids (monounsaturated and polyunsaturated) compared to rations based on grass silage.

Liu et al. (2016) found that by supplementing the feed of cows held on pasture with concentrates, hay or TMR, in order to increase milk production, affects the FA profile of milk fat, especially by reducing the CLA content. Dhiman et al. (2005) showed that milk yielded from grazing cattle had 5.7 times higher CLA concentration than that yielded from cows fed mixed rations (feed ratio: concentrates of 50:50).

Effects of silage feeding on milk fatty acids profile

The basic feed for dairy cattle in our country is corn silage; it has a lower level of dry matter, but a higher energy and functional fatty acid content than hay (Shingfield et al., 2005; Kalač and Samková, 2010). Milk production is higher when using corn silage than grass silage due to its higher starch content and higher digestibility of organic matter (Nielsen et al., 2006), which makes this system more competitive in terms of production and economic result.

Dewhurst et al. (2006) studied the effect of silage on the fatty acid composition of feed fat. Withering of plants before silage reduced the total FA content by almost 30% (from 24.6 to 17.5 g/kg DM), with a reduction of up to 40% for α-linolenic acid. The authors suggested that the silage process itself has little influence on the fatty acid profile, provided that the compaction and sealing of the silo are good. The input of silage additives (formic acid or formalin) has led to more lower losses of full saturated fatty acids. Hay production reduces the total amount of fatty acids by more than 50%, with a higher loss of α-linolenic acid (Doreau and Poncet, 2000). Similar results were found for semi-hay (70% DM) by Elgersma et al. (2003).

The fat in the milk of cows fed silage-based rations has a higher content of palmitic acid, while the proportion of vaccenic acid (VA) and conjugated linoleic acid (CLA) considered beneficial for human health is lower compared to rations based on green fodder (Table 1). Lipolysis that occurs during forage silage may be the cause of these differences (Chilliard et al., 2007).

Compared to the grass silo, the corn silage caused an increase in the concentration of SFA (62.9% vs. 67.6%) and a decrease in the concentration of PUFA (4.7% vs. 3.6%), thus resulting in a worsening of the quality of milk fats when using corn silage compared to grass silage (Samková et al., 2009). The proportion of grass silage in the diet was positively associated with the levels of fatty acids with chain lengths less than C16, and SFA (Yayota et al., 2012).

The concentration of α-linolenic acid was higher in milk obtained from cows fed clover silage compared to milk produced from grass silage (Dewhurst et al., 2006).
Effects of hay feeding on the milk fatty acids profile

Reduced hay consumption (feed with high crude cellulose content, especially NDF: neutral detergent fibers) can lead to a major reduction in milk fat content (Mentin and Cook, 2006). Bauman and Griinari, (2003) consider that the optimal intake of NDF is 0.9% of the body weight of dairy cows.

Data on the effects of hay on the fatty acid profile of milk fats are limited compared to those on the effects of green or silage. Supplementing the ration of cows with hay, as a source of crude cellulose, in the case of early grazing (young grass) had a low effect on milk composition and fatty acid profile of fat (Wijesundera et al., 2003).

Staszak (2007) observed a higher level of LA (linoleic acid), CLA, ALA and total unsaturated fatty acids in milk fat from cows fed a hay-based ration compared to corn silage. These results were confirmed by Bernardini et al. (2010) in a similar study.

Oelker et al. (2009) reported that alfalfa hay feed rations compared to maize silo feed decreased the total milk CLA content in Holstein cows due to the change in rumen pH that influenced the production of intermediate products, from the rumen, which form the basis of CLA formation.

Rumen microbial enzymes are responsible for the isomerization and hydrolysis of lipids in feed and the conversion of unsaturated fatty acids into various partially and completely saturated fatty acids, including CLA (C18:2 c9, t11), trans-vaccenic acid (C18:1 t11, VA) and stearic acid (C18:0). Although linoleic (C18:2 n-6) and linolenic (C18:3 n-3) acids are the main unsaturated fatty acids in ruminant food, the major fatty acid that forms is rumen bihydrogenation stearic acid (C18:0).

Drying hay in the sun leads to significant losses of unsaturated fatty acids from grass (Boufaied et al., 2003). Shingfield et al. (2005) reported that milk from cows fed hay rations has a higher content of C18:2 n-6, C18:2 c9, t11 and C18:3n-3, compared to cows fed silage-based rations, although PUFA intake was lower.

Effects of forage/concentrate ratio on milk fatty acids profile

Cereal grains (especially corn) are frequently used in dairy cattle rations, as a cost-effective source of energy, for high milk production. A high proportion of cereals in cow feed reduces milk fat content and changes fatty acids composition. Eating large amounts of cereals usually reduces the proportion of milk fatty acids by 6 to 16 carbon atoms and increases the proportion of unsaturated fatty acids by 18 carbon atoms. The decrease in milk fat content in case of feed rations rich in cereal grains is due to the change in the rumen microflora which leads to a decrease in the production of acetate and butyrate to support the synthesis of milk fats and an increase in rumen propionate production which stimulates circulating insulin concentration, which redirects metabolites to reserve tissues (body fat) (Tripathi, 2014). It is recommended that soluble starch and sugars account for 32 to 38% of the DM of the ration to optimize the production of milk fats and proteins (Nyman et al., 2009).

According to Kononoff and Hanford (2006), cereals intake should be limited to a maximum of 10-15 kg/cow/day, so that feces pH exceeds 6.0 values. Increasing the F:C ratio (feed: concentrates) from 34:66 to 45:55 increased short-chain fatty acids without affecting the milk content in total CLA (Kargar et al., 2012). However, Chilliard and Ferlay (2004) reported that concentrate-rich feed rations increase the milk content in conjugated linoleic acid isomers.

Concentrated supplementation of pasture-based feed ratios decreased the nutritionally desired FA content (C18:3n-3; c9, t11-CLA, C18:1 t11; C20:5n-3; C22:5n-3 and total n-3 FA) while the ratio n-6: n-3, atherogenicity

Table 1. The influence of basic feed in cows on the fatty acid profile of milk fats (% of total FA)(Hanus et al., 2018)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Silage of:</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>maize</td>
<td>alfalfa</td>
<td>grass</td>
<td>pasture</td>
</tr>
<tr>
<td>Linoleic acid (C18:2 n-6)</td>
<td>1.92</td>
<td>1.69</td>
<td>1.18</td>
<td>1.04</td>
</tr>
<tr>
<td>Conjugated linoleic acid (C18:2 c9, t11; CLA)</td>
<td>0.45</td>
<td>0.51</td>
<td>0.54</td>
<td>1.30</td>
</tr>
<tr>
<td>Vaccenic acid (C18:1 t11; VA)</td>
<td>0.92</td>
<td>1.24</td>
<td>1.18</td>
<td>3.15</td>
</tr>
<tr>
<td>α-linolenic acid (C18:3 n-3)</td>
<td>0.39</td>
<td>0.95</td>
<td>0.49</td>
<td>0.75</td>
</tr>
<tr>
<td>Hypercholesterolemic fatty acids (HFA)1</td>
<td>51.8</td>
<td>42.1</td>
<td>48.6</td>
<td>38.1</td>
</tr>
<tr>
<td>Polyunsaturated n-6 FA</td>
<td>2.43</td>
<td>2.09</td>
<td>1.33</td>
<td>1.56</td>
</tr>
<tr>
<td>Polyunsaturated n-3 FA</td>
<td>0.43</td>
<td>1.20</td>
<td>0.51</td>
<td>0.99</td>
</tr>
<tr>
<td>n-6 : n-3</td>
<td>6.21</td>
<td>1.95</td>
<td>2.77</td>
<td>1.69</td>
</tr>
<tr>
<td>Atherogenic index (AI)2</td>
<td>3.82</td>
<td>2.83</td>
<td>3.58</td>
<td>2.45</td>
</tr>
<tr>
<td>Desaturation index (DI)3</td>
<td>0.70</td>
<td>0.64</td>
<td>0.62</td>
<td>0.67</td>
</tr>
</tbody>
</table>

1HFA = C12:0 + C14:0 + C16:0;  
2AI = [(C12:0 + 4 x C14:0 + C16:0)/(MUFA + PUFA n-6) + PUFA n-3];  
3DI = [C18:1 c9]/(C18:0 + C18:1 c9).
index (AI) and thrombogenicity index (TI) increased, which means an alteration of the sanogenic quality of milk fats (Mierlița et al., 2021).

**Effects of supplementation of dietary lipids on milk fatty acids profile**

Fat supplements are commonly used in animal feed with high milk production as concentrated energy sources. The inclusion of less than 4% (% of DM) unprotected fats in cow feed during lactation has no negative effects on milk production and composition, instead it improves the fatty acid profile of milk fat by increasing the ratio of polyunsaturated fatty acids (Kass et al., 2012). Increasing the amount of unprotected fat in cow feed reduces the consumption of DM and alters the rumen microecosystem, resulting in a decrease in milk production and sometimes its protein content.

If by-pass fat (protected against rumen fermentation) is used, the total fat content of the ration can safely reach 6-7% of DM. By-pass fats (calcium salts of vegetable oils) can change the composition of milk fatty acids, minimizing the negative effects on rumen fermentation processes that often occur when unprotected vegetable oils are used.

In recent years, new sources of fats such as fish oil, seaweed, chia seeds, lupine, hemp and camelina have been studied as sources of PUFA in cow feed, able to improve the fatty acid profile of fats in milk.

The inclusion of vegetable oils in the form of calcium salts of fatty acids in the diet of dairy cows increased the concentration of vaccenic acid (C18:1\(\text{t-11}\)) and conjugated linoleic acid (C18:2 isomer c-9, t-11) more than when using extruded oilseeds, which in turn increased the levels of these fatty acids in milk more than when raw (untreated) oilseeds were used (Chilliard et al., 2007). The source of fat did not have an impact on the level of the main CLA isomers in milk (Abu Ghazaleh et al., 2003).

Supplementing diet with fish oil was more effective in increasing the milk content in CLA than vegetable oils (Collomb et al., 2006). Supplementation with sunflower oil or fish oil, alone or in combination, increased the content of C18:1 t-11 and CLA c-9, t-11 isomers over that of milk obtained from cows fed pasture. The highest value for C18:1 t-11 was 7.1 g/100 g total FA only with fish oil, compared to 6.98 g/100 g total FA with sunflower oil + fish oil. The combination of oils led to the highest concentration of CLA c-9, t-11, (2.36 g/100 g total FA), while the fish oil led to a higher increase than the sunflower oil. The increase in vaccenic acid in this study is important because VA is converted to CLA in the tissues of consumers, thus increasing the dietary contribution of CLA.

Supplementation of corn silage rations with extruded flaxseeds had no effect on milk fat content but only had moderate effects on milk FA concentrations (increasing the C16:0 percentage and decreasing the C18:0 concentration and C18:1 total trans) (Ferlay et al., 2010).

The degree of change in the acid profile of milk fats is generally proportional to the level of inclusion of oilseeds in diet (Glasser et al., 2008; Hurtaud et al., 2010). The major changes relate to 18:1 trans and total CLA. These FA concentrations increased linearly with increasing flaxseed amounts, while the 18:3n-3 concentration increased slightly, confirming that this FA was strongly biohydrogenated in the rumen. In contrast, the concentration of saturated fatty acids (SFA) in milk decreased linearly with increasing amounts of flaxseed (Ferlay et al., 2011).

The input of rapeseed, sunflower or flax seeds in cow feed reduces the concentrations of short-chain (C4 - C10) and medium (C16:0) fatty acids of carbon atoms and increases the concentrations of C18:0 and C18:1, in milk fat. The input rate of oilseeds in dairy cows’ diet should result in a level of 3-4% fat in the ration (% of DM).

The use of roasted or extruded oilseeds in cow diet has a greater influence on increasing the CLA content of milk fat than raw seeds (Dhiman et al., 2000), due to the reduced release of oil from raw seeds into the rumen, compared to heat-treated seeds. Low oil release can lead to complete biohydrogenation of linoleic acid in the rumen and to a low or no effect on the CLA content of milk (Fernandez and Gonzalez, 2012). Extrusion, micronization, or frying of oilseeds resulted in 2-3-fold increases in the CLA content of milk fat compared to a control ration containing unheated soybeans (Chouinard et al., 2001).

Despite the positive effects of vegetable oil supplementation on milk fatty acid profile (ie high VA and CLA content), it should be noted that in some cases an increase in the concentration of trans-10 C18:1 is observed (Gómez-Cortés et al., 2008), which has negative effects on consumer health.

**Effects of feeding on the oxidative stability of milk fat**

The oxidative stability of milk fats is influenced on one hand by their degree of unsaturation and on the other hand by the content of milk in antioxidants (Havemose et al., 2004). Increasing the concentration in functional FA compromises the oxidative stability of milk fats, as unsaturated FAs are more susceptible to oxidation. Therefore, control of lipid oxidation is necessary to prevent loss of nutritional value and the formation of potentially toxic compounds. In order to maintain a high quality of milk, the concentration of antioxidants should therefore be increased.

Antioxidants are chemicals that can neutralize and eliminate free radicals, which are continuously produced in the human body, being involved in the onset and evolution of complex diseases: diabetes, accelerated aging,
carcinogenesis and cardiovascular disease (Khan et al., 2019). Thus, the demand for foods containing natural antioxidants is growing worldwide, and milk and dairy products can be an important source of natural antioxidants (Budak et al., 2018).

The main antioxidants present in milk can be grouped into lipophilic and hydrophilic antioxidants. Carotenoids, retinol and α-tocopherol are fat-soluble antioxidants, while ascorbic acid is a water-soluble antioxidant. α-tocopherol is largely present in the globular membrane of milk fat and is considered the most effective fat-soluble antioxidant present in milk (Mierliță et al., 2017 and 2018).

The oxidative stability of milk and dairy products depends on fatty acids composition, contamination with metal ions and the concentration of tocopherols and carotenoids (Nielsen et al., 2004). Improving the profile of fatty acids in milk shows the disadvantage of increasing its sensitivity to self-oxidation and the appearance of toxic products (aldehydes, ketones), which can change the psychosensory qualities of milk and dairy products (foreign flavors and tastes). Therefore, there is a special interest in increasing the content of non-enzymatic lipophilic antioxidants (β-carotene, retinol and α-tocopherol), in order to increase the oxidative stability of foods that have a high content of polyunsaturated fatty acids. These are considered effective strategies for improving long-term consumer health, with a positive economic impact, by reducing the implicit social burden of treating cardiovascular disease, in the context in which Shingfield et al. (2008) note that improving the fatty acid composition of fat is more effective in combating cardiovascular disease than reducing fat intake.

Pastures are a good source of antioxidants that are transferred to milk, which explains the higher content of compounds such as β-carotene, terpenes, lutein, vitamins A (retinol), E (tocopherol) and phytoene (a derivative of chlorophyll) in milk obtained on pasture compared to that obtained with TMR type diets (O’Callaghan et al., 2016; Coppa et al., 2011; Faulkner et al., 2018). The yellow color of milk fat and subsequently high-fat dairy products is determined by the β-carotene content. This color of milk and dairy products may be associated by consumers with grazing cows, which may change the attitude (acceptability) of consumers towards these functional food products. Thus, carotenoids can be potential biomarkers for establishing the traceability of grass-milk. Milk from cows of the Guernsey and Jersey breeds is yellow in color, compared to that from other breeds, in which the milk is white. This is because these two breeds convert less carotene (yellow pigments) into vitamin A than other dairy breeds.

Havemose et al. (2004) observed a higher oxidation of lipids in milk obtained from cows fed grass silage compared to that obtained from cows fed corn silage. In contrast, milk from cows fed corn silage was more prone to protein oxidation. In a subsequent study, Havemose et al. (2006), reported a higher degree of lipid oxidation in milk obtained from cows fed grass-clover silage - based diets, compared to that obtained from cows fed hay - based diets. ALA content variation (8% in silage and 4% of total FA in hay, respectively) was considered to be the main cause of oxidation processes. Supplementing the ration of cows with vitamin E introduced into the concentrate mixture reduced the degree of degradation of milk fats by oxidation (Kay et al., 2005).

High concentrations of α-tocopherol and β-carotene in milk can be obtained from a high proportion of grass silage or clover in cow diet, because these feeds are rich in antioxidants (Havemose et al., 2004). The highest content of lipophilic antioxidants (α-tocopherol and β-carotene) and respectively the best oxidative stability of fats, demonstrated by a low concentration of MDA (malonyldialdehyde, which is a by-product of the oxidation of polyunsaturated FA in milk) was made from milk obtained from pasture (Table 2).

### Table 2. Effects of feeding on the fat-soluble antioxidants and oxidative stability of milk

<table>
<thead>
<tr>
<th>Dietary treatment</th>
<th>Fat-soluble antioxidants (µg/100 g fat)</th>
<th>MDA (mg/L of milk)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>α-tocoferol</td>
<td>Retinol</td>
<td>β-caroten</td>
</tr>
<tr>
<td>Pasture</td>
<td>34.2</td>
<td>4.8</td>
<td>40.3</td>
</tr>
<tr>
<td>Grass hay</td>
<td>22.1</td>
<td>4.0</td>
<td>28.2</td>
</tr>
<tr>
<td>Grass silage</td>
<td>18.2</td>
<td>4.0</td>
<td>35.8</td>
</tr>
<tr>
<td>Grass silage,</td>
<td>36.8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Red clover silage</td>
<td>26.8</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Alfalfa silage</td>
<td>29.9</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Milk after storage at 4°C: <sup>1</sup> 24h; <sup>2</sup> 48h.

Timmons et al. (2001) found that full-fat soy introduced into the diet of dairy cows led to an increase in the proportion of C18:2n-6 and C18:3n-3 in milk fat, but decreased its oxidative stability.

Numerous studies have shown that milk obtained from grazing cows contains higher levels of natural antioxidants (tocopherols and retinol) than that obtained from animals fed intensively with TMR, due to the fact that the conservation of green fodder and processing in hay and silage, causes the degradation and inactivation of many bioactive compounds (Elgersma, 2015; Mierliță et al., 2017). The concentration of α-tocopherol in pastures is
4-5 times higher than that found in a TMR (NRC, 2001).

CONCLUSIONS

Feeds, despite the relatively low-fat level contained, are the main source of functional FA in dairy cattle feeding. Milk yielded from grazing cows (including zero-grazing) shows a considerably higher concentration of functional FA and lipophilic antioxidants (tocopherols, retinol and carotenes) and a lower content of saturated FA compared to those fed shelter with TMR (total mixed ration). Also, fodder diets based on hay or those containing small amounts of concentrates, ensure a milk with a fatty acid profile more favorable for human health and a better oxidative stability compared to rations based on silage or those rich in concentrates.

The input of vegetable oils in cow feed in various forms (oil, calcium salts of fatty acids, oilseeds) improves FA profile of milk fat by increasing the ratio of fatty acids considered beneficial to human health (C18:1 t-11; C18:2 c-9, t-11, CLA and n-3 FA). Milk with the highest functional FA concentrations was yielded when cow's diet was supplemented with fish oil or oilseeds (especially flaxseed).

Increasing the concentration of α-tocopherol, retinol and carotenoids as non-enzymatic lipophilic antioxidants, by adopting optimal nutritional strategies is important to ensure the oxidative stability of milk containing higher amounts of FA beneficial for human health. Milk yielded from grazing cows contains higher levels of natural antioxidants (tocopherols and retinol) than that yielded from animals fed intensively with TMR, due to the fact that the preservation of green fodder causes degradation and inactivation of many bioactive compounds, and large amounts of concentrates negatively affect the biohydrogenation processes in the rumen.

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Conflicts of Interest

The authors declare that they do not have any conflict of interest.

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