

Metabolism of fatty acids in tissues and organs of the ruminants - a review*

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Fatty acid metabolism in the rumen is a meaningful, but not the only variation factor of fatty acid profile in milk and meat of the ruminants. Lipids absorbed in the small intestine are transported by lipoproteins to different tissues and organs of an animal, where they are subjected to metabolic modifications. Thus, despite the fact that ruminants absorb mainly long-chain saturated fatty acids, some specific isomers are present in milk and meat, whereas they are absent in the feeds. The impact of many of them on human health is not yet completely understood, but some have proven health-promoting effects in the prevention of lifestyle diseases, therefore are desirable in the food from the point of view of conscious consumer.

KEYWORDS: cattle / fatty acids / metabolism

A growing consumer's awareness about food has been observed in the recent years. People become more educated about food ingredients that may have a beneficial effect on their health and prevention of diseases. Due to the growing knowledge about the impact of individual fatty acids on human health, their origin in food is of considerable interest among researchers. Products derived from ruminants contain for example, a significant level of so-called rumenic acid (conjugated linoleic acid, c9, t11-CLA) of antitumor properties, which is not present in the feed [Strzałkowska *et*

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al. 2009]. Therefore, lipid metabolism in this group of animals is of particular interest. Lipids consumed by the ruminants are subjected to changes in various organs of the animal, which is the cause of the fact that fatty acid composition of the products differs significantly from that of the feed [Jóźwik *et al.* 2010]. Fatty acid metabolism in the rumen is one of the most important factors adding to variation of the fatty acid profile in milk and meat of the ruminants [Brzozowska and Oprządek 2013]. However, lipids absorbed in the small intestine are transported to different tissues and organs of an animal (*inter alia*, liver, adipose tissue, muscles and mammary gland) where the fatty acids are subjected to further metabolic modifications. The aim of this article is to discuss the changes occurring in tissues and organs, which contribute to the diversity of the profiles of fatty acids in products derived from the ruminants.

Blood transport of fatty acids

Lipids absorbed in the small intestine are transported by plasma lipoproteins throughout the body of an animal. Lipoproteins are macromolecular complexes containing various fats and specialized proteins (called apolipoproteins), which are soluble in the blood and lymph. In ruminants, they were divided into five major classes based on their density: chylomicrons (CM), very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), low density lipoproteins (LDL) and high density lipoproteins (HDL) [Bauchart 1993, Jóźwik *et al.* 2012].

Chylomicrons are the largest lipoproteins with the lowest density, that are synthesized in the small intestine of ruminants after ingestion of fat-rich feed. Transport of fatty acids (FA) in the form of triglycerides (TG) to the tissues for fat storage, milk fat production or energy production (oxidation) is the main task of these molecules. The secretion of chylomicrons is stimulated by increased level of fat or polyunsaturated FA in the ration [Bauchart 1993]. VLDL is an alternative form of transport of triglycerides from the intestine. The concentration of VLDL is relatively low in dairy cows, probably due to their rapid rotation especially after milking. They are distinguished by a high content of triglycerides and increased content of phospholipids [Bauchart 1993]. IDL are intermediate molecules between VLDL and LDL, produced during lipolysis. Due to their intense uptake by liver and tissues, their content in blood plasma is very low in ruminants. LDL are the final products of intravascular degradation of VLDL, involved in delivering cholesterol to tissues. As a percentage, it is a small class of lipoproteins (<10%) in bovine lymph and plasma. They consist mainly of cholesterol esters and phospholipids, and to a lesser extent, of free cholesterol and triglycerides. Fatty acids in these lipoproteins depend more on the individual development of an animal, than on the diet in adult animals. HDL are major plasma lipoproteins (>80%) produced and secreted by the liver and small intestine. They are involved in the reverse transport of cholesterol, which returns the excess of cholesterol from tissues to the liver – for bile production and resynthesis of the new VLDL [Bauchart 1993]. Despite the high level of HDL in blood plasma, these

lipoproteins contain a very low amount of triglycerides, therefore play a minor role in their transport [Chilliard *et al.* 2000].

The main preformed FA (i.e., those which arise from a source other than *de novo* synthesis in the liver) in the blood of ruminants are C16:0, C18:0, C18:1 [Mansbridge and Blake 1997].

Hepatic metabolism of fatty acids

Fatty acids can be utilized in four different ways in the liver – they may be secreted to the bile, oxidized completely to carbon dioxide or partially to acetic acid or ketone bodies. After the synthesis of acylglycerol they can also be accumulated in the liver and secreted in the form of lipoproteins [Emery *et al.* 1992].

Short-chain FA (volatile fatty acids and ketone bodies) in the liver derive from the small intestine, from which they are transported by the portal vein. The second source of short-chain FA in the liver is the catabolism of long-chain fatty acids in hepatocytes. The majority of propionic acid is converted to glucose in the liver. The end products of the metabolism of butyric acid in the liver are acetyl coenzyme A (acetyl-CoA), long-chain FA and ketone bodies. However, the production of ketone bodies in the liver is greater when free fatty acids are available as substrates, than by using butyric acid [Hocquette and Bauchart 1999]. Acetic acid is not metabolized in the liver to a greater extent, since this organ uptakes only a small portion of acetic acid, whereas the rest is available for other tissues. The part that goes to the liver is mainly utilized for anabolic reactions, such as *de novo* lipogenesis of long-chain FA [Hocquette and Bauchart 1999].

The pool of long-chain fatty acids (LCFA) in the liver originate mainly from non-esterified fatty acids of blood plasma (NEFA). Delivery of NEFA to the liver depends on their concentration in blood and the blood flow. The concentration of NEFA increases with the mobilization of fat from adipose tissue [Hocquette and Bauchart 1999]. A secondary source of LCFA in the liver is the hydrolysis of circulating triglycerides. Long-chain fatty acids are subjected to esterification or oxidation in the liver - distribution between these two pathways is regulated by two binding proteins - fatty acid binding protein and acyl-CoA binding protein. During the esterification the triglycerides are produced, which can be stored in the cytosol or transported to the microsomes and secreted as VLDL. The fatty acids can also be oxidized in peroxisomes and mitochondria (to acetyl-CoA), to produce energy in the Krebs cycle [Hocquette and Bauchart 1999].

The partitioning between different metabolic pathways in the liver is regulated by the ration (the ratio of fat to carbohydrate, fat content, FA profile of the ration) and hormones [Hocquette and Bauchart 1999]. For example, insulin stimulates *de novo* lipogenesis and esterification of FA, but inhibits their oxidation. After a meal, 76% of palmitic acid is esterified, whereas 24% is oxidized in cow's liver. However, during fasting these values are 33 and 66%. Dysregulation of partitioning between these

metabolic pathways can lead to the accumulation of triglycerides in the liver (leading to its steatosis) or ketosis. Low potential of secretion of triglycerides in this organ also promotes fatty liver in ruminants [Hocquette and Bauchart 1999].

Fatty acid metabolism in adipose tissue and muscles

Triglycerides and neutral fats are the main lipid fractions (>90%) in adipose tissue. In muscle a significant part are phospholipids, which have much higher content of polyunsaturated fatty acids (PUFA) for the proper functioning as the components of cell membranes [Wood *et al.* 2008].

The content of triglycerides in the adipocyte is a result of a balance between *de novo* synthesis of FA, FA uptake, FA esterification, hydrolysis of triglycerides (lipolysis) and re-esterification of FA produced during lipolysis. As *de novo* synthesis of FA in ruminant liver is very small (5%), the main role in this process has the adipose tissue (~90%) [Chilliard 1993].

Despite the fact that, the adipose tissue contains significantly higher proportion of FA than muscles, their composition is very similar (Tab.1). Polyunsaturated fatty acids ingested with the feed are subjected to biohydrogenation processes in the rumen to a large extent, by which for example, only 10% of linoleic acid (C18:2, n-6) may be available for incorporation into tissue [Wood *et al.* 2008]. This acid is present in higher concentrations in muscles than in adipose tissue both in sheep and cattle. α -linolenic acid (C18:3, n-3) is another important acid, which is also subjected to biohydrogenation, and its concentration is higher in muscles than in adipose tissue of the ruminants [Wood *et al.* 2008].

Table 1. Fatty acid composition (g/100g of fatty acids) of subcutaneous fat and *longissimus dorsi* muscle in sheep and cattle [adapted from: Wood *et al.* 2008]

Fatty acid	Sheep		Cattle	
	subcutaneous fat	muscle	subcutaneous fat	muscle
C14:0	4.1	3.3	3.7	2.7
C16:0	21.9	22.2	26.1	25.0
C16:1, cis	2.4	2.2	6.2	4.5
C18:0	22.6	18.1	12.2	13.4
C18:1, cis 9	28.7	32.5	35.3	36.1
C18:2, n-6	1.3	2.7	1.1	2.4
C18:3, n-3	1.0	1.4	0.5	0.7
C20:4, n-6	not detected	0.6	not detected	0.6
C20:5, n-3	not detected	0.5	not detected	0.3
n-6: n-3	1.4	1.3	2.3	2.1

Long-chain polyunsaturated FA (C20-C22) are in substantial amounts in the muscles. They arise from linoleic acid and α -linolenic acid, thanks to the enzymes - delta-5 and delta-6 desaturases and elongases. Important products of these changes are arachidonic acid (C20:4, n-6) and eicosapentaenoic (C20:5, n-3), which have

various metabolic functions, such as the production of prostaglandins [Wood *et al.* 2008, Herrera-Camacho *et al.* 2011].

Different feeds cause different changes in the fatty acid composition of animal tissues. Rations based on concentrates trigger a relatively high level of linoleic acid and its long-chain derivatives in the muscles, whereas the rations based on grass silage increase the level of α -linolenic acid (which represents more than 50% of all FA in the grasses) and its long-chain derivatives including docosahexaenoic acid (C22:6, n-3) [Warren *et al.* 2008]. The ability to incorporate polyunsaturated FA to phospholipids from the ration is limited, and linoleic acid competes for this incorporation much more effectively than α -linolenic acid [Williams and Burdge 2006].

Oleic acid (C18:1, n-9) formed from stearic acid with the participation of stearyl-CoA desaturase is the main component of neutral fats. In ruminants, stearyl-CoA desaturase forms a conjugated linoleic acid C18:2 (CLA), an important component in human nutrition [Wood *et al.* 2008, Bodkowski and Patkowska-Sokoła 2013].

Metabolism of fatty acids in mammary gland

Nutrition is a major environmental factor that impacts milk fat and its composition, therefore, the changes in the composition of the ration of ruminants can be used to effectively modify them [Jóźwik *et al.* 2010, Frelich *et al.* 2012, Kęsek *et al.* 2014].

Milk fat is composed of triglycerides coated with cell membrane, and amounting to 96-98% of the overall milk fat. The remainder is a small amount of phospholipids, cholesterol and cholesterol esters of cell membrane [Strzałkowska *et al.* 2009]. The group of triglycerides includes more than 400 fatty acids, a large part of which are the intermediate products of lipid metabolism in the rumen [Gunstone and Harwood 2007]. However, most of these acids are present in trace amounts, and the main fatty acids of milk fat are saturated FA from C4:0 to C18:0, palmitoleic acid, oleic acid, linoleic acid and trans isomers of C18:1 (Tab. 2). C14:0, C16:0 and C18:0 represent about 75% of the total FA, 21% are the monounsaturated acids, of which oleic acid is the most common. Only 4% of milk fatty acids are PUFA, consisting especially of linoleic and α -linolenic acids [Mansbridge and Blake 1997, Bauman *et al.* 2006].

Fatty acids in milk are derived from two sources - the *de novo* synthesis and the uptake of preformed FA. The substrates for the *de novo* synthesis are acetic acid and β -hydroxybutyric acid formed during digestion of fibre in the rumen. They are used by the epithelial cells for the synthesis of short- and medium-chain FA (C4:0-C14:0) as well as for the part of 16-carbon fatty acids (~50%). The main enzymes involved in this process are acetate CoA carboxylase and fatty acid synthase [Mansbridge and Blake 1997, Sejrsen *et al.* 2007].

Absorption of FA by the mammary gland is the second source, which provides the part of 16-carbon fatty acids and all the long-chain fatty acids, representing FA absorbed during digestion and mobilized from body fat reserves [Shingfield and Griinari 2007]. Fatty acids absorbed from the small intestine into the blood

Table 2. Composition of the major fatty acids in milk fat [adapted from: Jensen 2002]

Fatty acid	Average range (g/100g)
C4:0	2-5
C6:0	1-5
C8:0	1-3
C10:0	2-4
C12:0	2-5
C14:0	8-14
C15:0	1-2
C16:0	22-35
C17:0	0.5-1.5
C18:0	9-14
C18:1 cis-9	20-30
C18:2 cis,cis-9,12	1-3
C18:3 cis,cis,cis-9,12,15	0.5-2.0

are transported by lipoproteins - the majority of preformed FA is delivered to the mammary gland by VLDL or chylomicrons [Chilliard *et al.* 2000]. The uptake by the mammary gland is dependent on the activity of lipoprotein lipase in the walls of capillaries. Long-chain fatty acids from body reserves are transported as NEFA, and their uptake is proportional to the concentration in blood plasma. Under normal conditions, about half of the milk FA (by the molar ratio) is synthesized *de novo* in the mammary gland. Despite the fact that plasma TG and NEFA represent less than 3% of the total plasma lipids, in the molar ratio they are the remaining half of milk FA. This ratio also depends on the physiological state of the animal [Mansbridge and Blake 1997]. Low levels of PUFA in milk may be explained by the fact that they are concentrated in phospholipid fraction and cholesterol esters in HDL particles, whose uptake by the mammary gland is small [Mansbridge and Blake 1997].

The modifications of FA occurring in the mammary gland are catalyzed by desaturases, especially by the delta-9 desaturase which converts trans-11 C18:1 to cis-9, trans-11 C18:2 (the most common CLA isomer found in the fat of ruminants) and C18:0 to cis-9 C18:1 [Huth *et al.* 2006, Sejrsen *et al.* 2007]. Unlike other tissues, ruminant mammary gland is not able to convert C16:0 to C18:0 by elongating the chain [Chilliard *et al.* 2000].

Modification of fatty acid profiles of the products

There has been a considerable interest to alter the fatty acid composition of bovine products (especially milk) with an aim to improve the long-term health of consumers. As was shown in the previous sections, the fatty acid composition of the products reflects the FA profile of the ration only to a certain extent, because consumed FA

undergo various metabolic modifications in the animal's body. The increase in the content of PUFA in tissues is possible only if the amounts of LCFA reaching the small intestine are greater than those observed when feeding conventional rations [Cook *et al.* 1972], therefore proper fat supplementation is essential for obtaining the products with health-promoting properties. The results of the studies indicate that supplementation of ration with vegetable oils can have a positive impact on the FA profile of the ruminants products, but the final result depends on many complex factors, such as parity, stage of lactation, individual variability, season, farming system, milking frequency, basal ration, type and amount of supplement [Kalač and Samková 2010]. Due to the fact that rumen is a place of the most significant metabolic changes of fatty acids in the ruminants, protecting FA against the biohydrogenation in this organ leads to a change in the content of PUFA in blood and other tissues [Douglas *et al.* 2007]. Protecting the fat may be achieved by feeding whole seeds (e.g., rapeseed, linseed), calcium or magnesium salts of oils, heat treated seeds or gelatine encapsulated oils [Patkowska-Sokoła *et al.* 2003]. Similarly, the administration of unsaturated fatty acids directly into the blood (intravenously) bypasses the digestive system and prevents their biohydrogenation in the rumen, therefore also helps to obtain products beneficial in terms of quality and consumer perception [Mashek *et al.* 2005]. Therefore, enhancing PUFA content requires an understanding of the interrelationship between dietary supply of lipid, rumen fermentation, and metabolic changes occurring in liver, muscles, adipose tissue and mammary gland.

Summary

Although the ruminants absorb mainly saturated long-chain fatty acids from the gastrointestinal tract, they have some specific metabolic mechanisms which modify the composition of fatty acids in tissues and organs. As a result, specific isomers of fatty acids such as the isomers of conjugated linoleic acid are found in milk and meat of ruminants though they are not present in the feed. The impact of many of them on human health is not yet fully understood, but some have proven health-promoting effects in the prevention of lifestyle diseases such as atherosclerosis, hypertension, obesity and diabetes, therefore are desirable from the point of view of the consumers. The next task in animal nutrition will be such a composing of the ration, so that the greatest concentration of preferred isomers in the products is achieved, in order to produce food of highest quality from the human health point of view.

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