



**PROCEEDINGS
OF
INTERNATIONAL
BIOLOGICAL,
AGRICULTURAL AND LIFE
SCIENCE CONGRESS**

NOVEMBER 7-8, 2019

LVIV, UKRAIN



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You are welcome to our BIALIC Congress that is organized by Trakya University. The aim of our conference is to present scientific subjects of a broad interest to the scientific community, by providing an opportunity to present their work as oral or poster presentations that can be of great value for global science arena. Our goal is to bring three communities, namely science, research and private investment together in a friendly environment of Lviv, Ukraine in order to share their interests and ideas and to benefit from the interaction with each other.

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Lviv is not only a very nice, lovely and historical city at the edge of Europe, but located just at the heart of Eastern Europe region. We are much pleased to host all of you in Lviv, Ukraine.

We would like to thank all of you for joining this conference and we would like to give also special thanks to our sponsors and collaborators for giving us a big support to organize this event.

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HEALTH BENEFITS OF CONJUGATED LINOLEIC ACID

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ABSTRACT

In recent years, the bioactive compounds present in food, even though in minor amounts, have received increased attention because they may have an important nutritional role.

Conjugated linoleic acid (CLA) is a generic term used to describe a group of geometric and positional isomers of linoleic acid with a conjugated double bond mainly at carbons 9 and 11 or 10 and 12. Milk fat is the richest natural source of CLA. The CLA-isomers are produced via biohydrogenation of the unsaturated fatty acids presented in animal feed into saturated by rumen bacteria and by the enzymatic activity of the $\Delta 9$ -desaturase in the mammary gland.

Numerous physiological properties have been attributed to CLA including action as an antiadipogenic, antidiabetogenic, anticarcinogenic, antiatherosclerotic agent and chronic inflammatory diseases. In addition, CLA has effects on bone formation and the immune system as well as fatty acid and lipid metabolism and gene expression in numerous tissues.

Because potential health benefits have been associated with dietary consumption of CLA, enhancement of CLA concentrations in meat and milk is an important objective in nutrition research.

Key words: milk fatty acids; CLA; human health

INTRODUCTION

There is increasing evidence that nutrition plays an important role in the development of human chronic diseases including cancer, cardiovascular disease, insulin resistance and obesity. Development of foods and diets that promote human health play a central role to public health initiatives for preventing and lowering the economic and social impact of chronic disease (Shingfield and Wallace 2014).

In recent years there is increased interest about foods which contain components that have bioactivity. Milk and dairy products have long traditions in human nutrition (Miller et al., 2007; Rogelj, 2000).

Milk composition is rather than complex. Its components are subject of research for many years. Milk alone is much more than the sum of its nutrients. It is composed of various substances with bioactive properties and therefore milk was given an epithet of functional food. Not only nutritional value but also other physiological properties of milk components are subject of interest (Miller et al., 2000).

The most variable component of milk is milk fat. It is one of the components which determine milk nutritive quality and technological performance of milk.

Mainly milk fat is composed of triglycerides, approximately 98%, while other milk lipids are less present (diacylglycerols about 2%, cholesterol less than 0.5%, phospholipids about 1% and free fatty acids FFA about 0.1%) (Jensen et al., 1991, MecGibbon and Taylor, 2006). According to saturation of fatty acids, bovine milk fat contains 70-75% saturated fatty acids, 20-25% monounsaturated fatty acids, and 2-5% polyunsaturated fatty acids. Profile of fatty acids present in milk fat determines uniqueness of its composition as well as its physiological characteristics (Chilliard et al., 2003). Despite the high content of saturated fatty acids milk fat contains a number of components with health beneficial properties.

Bioactive components of milk fat

Milk fat is composed of components which are needed for normal performing of physiological functions of human. It contains a number of components which are metabolically active such as: sphingolipids, conjugated linoleic acid isomers (CLA), butyric acid, other fatty acids, vitamins A and D. A variety of health benefits have been associated with these compounds.

Bovine milk fat represents a rich source of biologically active molecules, many of which offer potential for commercial exploitation in health-promoting functional food products.

A variety of health benefits have been associated with sphingolipids and their digestion products, ceramides and sphingosines. They are suggested to be important in prevention from carcinogenesis, reduction of serum LDL cholesterol, regulation of the immune system and inhibition of foodborne pathogens.

CLA isomers which are naturally occurring fatty acids found in animal fats, exhibit a number of health benefits. CLA are found in relatively large quantities in the milk and/or meat of ruminant animals and appears to be metabolized differently than linoleic acid. In the diet of many consumers, meat and dairy products would be a significant source of CLA (Barbosa et al. 2003).

Butyric acid has been suggested to have an anti-tumor role and it is especially effective in colon cancer prevention. Vitamin A and D and β -carotene are also offered as natural anti-carcinogens in numerous reviews. As health benefits of these bioactive components of milk fat has been studied mostly in vitro conditions, in vivo researches are not sufficient. So, further research is required to establish the contribution of these dietary components to host metabolism and health (Alkalin et al., 2006).

Biosynthesis of CLA

CLA refers to a mixture of 28 positional and geometric isomers of linoleic acid ($C_{18:2}$, *cis-9*, *cis-12*) with two conjugated double bonds at various carbon positions in the fatty chain. Each double bond can be *cis* or *trans* configuration giving rise to possible CLA isomers (conjugated diene) that can occur in a *cis-trans*, *trans-cis*, *cis-cis* or *trans-trans* geometrical configuration with double bond at positions 8 and 10, 9 and 11, 10 and 12 or 11 and 13.

The most abundant isomer in food products from ruminants is *cis-9*, *trans-11* $C_{18:2}$ (rumenic acid) comprising 80-90% of the total CLA isomers, whereas *cis-12*, *trans-10* $C_{18:2}$ is present in smaller amounts, 3-5%. Both isomers have been proven to have biological activities. The content of rumenic acid and other CLA isomers of most foods are somewhat variable due to differences mainly in environmental conditions and diet of the originating animal species. The major sources of naturally occurring CLA isomers are foods containing ruminant fat. Milk

and dairy products have been shown to contain the highest amounts of CLA isomers (McGuire and McGuire., 1999).

The natural occurring CLA isomers in milk are formed using two different pathways (Fig.1 and Fig.2). First, CLA is formed during ruminal biohydrogenation of PUFA from dietary lipids and the second CLA is synthesized by animal tissues from *trans*-11 C_{18:1} (vaccenic acid; VA), another intermediate in the biohydrogenation of unsaturated fatty acids (Bauman et al., 2003).

In first pathway, reactions proceed via different mechanisms catalyzed by bacterial enzymes. In the rumen, dietary lipids are rapidly hydrolyzed, and the resulting unsaturated free fatty acids can undergo biohydrogenation via the rumen microorganisms. However, when biohydrogenation is incomplete, various CLA as intermediates of this pathway can escape the rumen and can be absorbed through the gastrointestinal tract, thereby providing the peripheral tissues with various isomers of CLA (Harfoot and Hazelwood, 1988).

Another way of forming of rumenic acid in milk is endogenous conversion of *trans*-11 C_{18:1} (vaccenic acid; VA) by enzyme delta-9 desaturase in the mammary gland (Bauman et al., 2003; Bauman and Lock, 2010; Griinari et al., 2000).

The rumen bacteria involved in biohydrogenation have been classified into two groups, A and B, based on their metabolic pathways. To obtain complete biohydrogenation of polyunsaturated fatty acids (PUFA), bacteria from both groups are generally required (Bauman et al., 2003). Two key biohydrogenation intermediates are: *trans*-11 C_{18:1} (vaccenic acid; VA) formed from linoleic and linolenic acids and *cis*-9, *trans*-11 C_{18:2} (rumenic acid) formed from biohydrogenation of linoleic acid. These intermediates are present in appreciable quantities in ruminant fat at a ratio of about 3:1 (Bauman et al., 2003; Bauman and Lock, 2010).

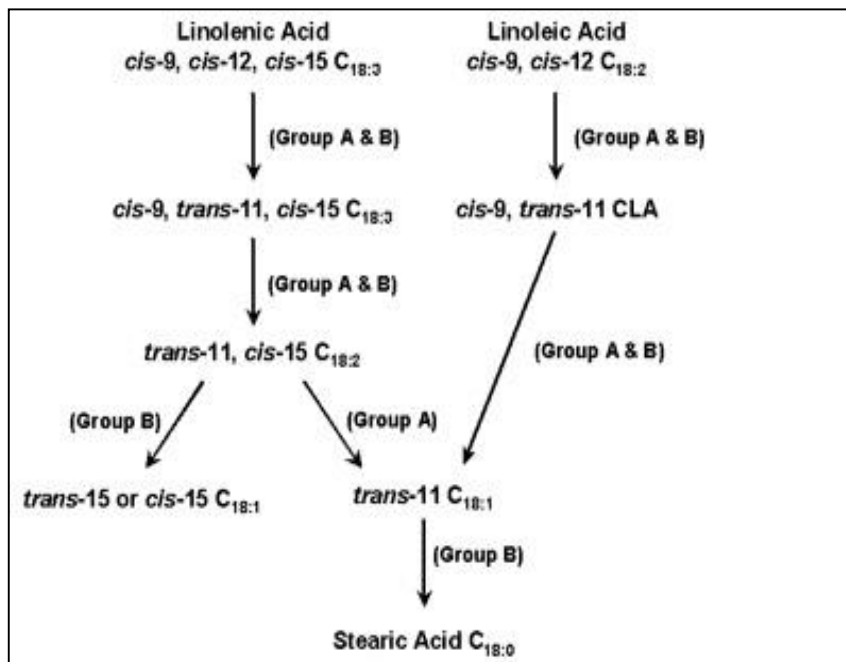


Figure 1: Pathway of biohydrogenation of linoleic and α -linolenic acids to stearic acid by rumen microorganisms (Bauman et al., 2003)

In the rumen *cis*-9, *trans*-11 CLA is only a transitory intermediate. The most of the *cis*-9, *trans*-11 CLA found in ruminant fat originates in the mammary gland and adipose tissue from endogenous synthesis involving desaturation of the *trans*-11 C_{18:1}, rumen-derived VA as a substrate by enzyme delta-9 desaturase (Fig. 2).

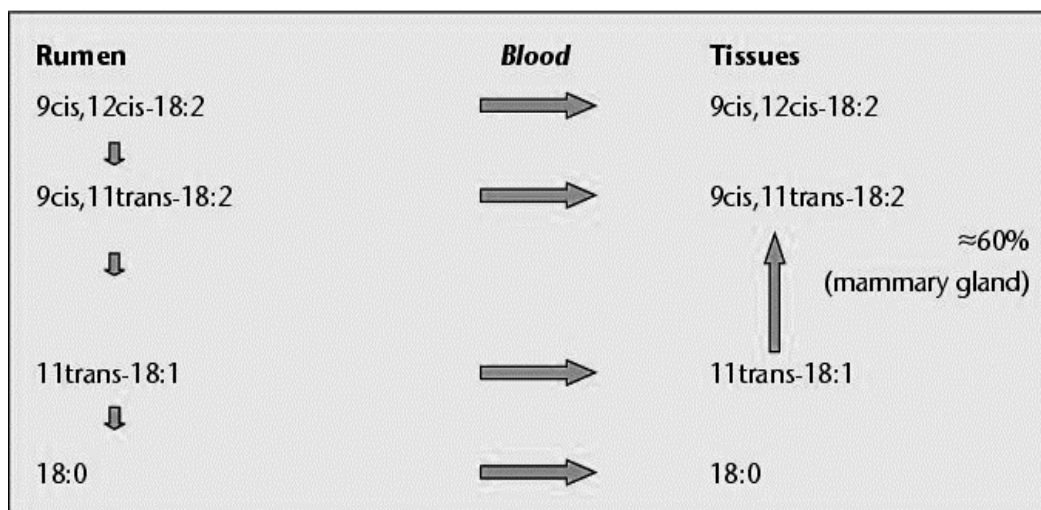


Figure 2: Metabolic pathways involved in the biosynthesis of CLA in ruminants (*Griinari et al., 2000*)

Various factors are known to influence the CLA content in milk, such as the food of the ruminant, the season, the animal breeding type, the number of times the animal has lactated and the current stage of lactation (Sebedio et al., 1999).

Regarding the potential benefits of CLA for human health, a number of research studies are oriented toward finding the possible ways of increasing the CLA concentration in milk fat. The first approach to achieve this objective is to use dietary modification in an attempt to increase the natural production of CLA in the dairy animals. The second approach include the synthetic mixture of CLA isomers in animal feed, protected in some way from the microbial biohydrogenation in the rumen (Silva et al 2014).

Health benefits of CLA

Research conducted in recent years suggests many health benefits leading from the consumption of CLA. Milk fat and meat of ruminants are the richest natural dietary source of CLA. Reported beneficial health-related effects of CLA include anti-carcinogenic effect in many cell culture and animal models. It also has positive effects on cell growth, it has immune modulating properties and improves the function of the immune system. It has anti-atherogenic and anti-diabetic effect and may improve glucose tolerance. Hence CLA is considered as functional food.

Most of the research on CLA is associated with its anti-carcinogenic properties. Inhibitory effects of CLA against carcinogenesis have been demonstrated in a variety of cell type, sites, and animal models including mammary gland, skin, colon, prostate, and forestomach of rats, humans, mice, and hamsters. In contrast to the hundreds of phytochemicals possessing varying degrees of anticancer properties, CLA is unique in that it is a fatty acid, which is found in highest amounts in food products derived from ruminants, and is safe at dietary levels. It is supposed that the effects vary with the specific isomers of CLA and the type and site of the cell/organ as well as the stage of tumorigenesis (Khanal., 2004).

CLA reportedly has anti-carcinogenic effects at various stages of cancer development, including initiation, progression and metastasis. Proposed mechanisms of CLA and its anti-carcinogenic activities include a reduction in cell proliferation, and prostaglandin metabolism. CLA seems to significantly reduce prostaglandin E synthesis which could inhibit tumor formation. It has been demonstrated that CLA has the ability to affect mammary cancer,

stomach cancer, skin cancer and prostate cancer. The mechanisms by which CLA affects carcinogenesis are largely unresolved and may vary for different sites, age, duration of exposure and stage of carcinogenesis. Various studies suggest that CLA may act by antioxidant mechanisms, pro-oxidant cytotoxicity, inhibition of nucleotide synthesis, reduction of proliferative activity and inhibition of both DNA adduct formation and carcinogen activation. Most of the anticarcinogens are of plant origin, but CLA is unique because it is present in food from animal sources and its anti-cancer efficacy is expressed at concentrations close to human consumption level. The unique structural and functional properties of CLA appear to modulate cellular process involved in carcinogenesis. (Ip et al., 1994; Belury, 2002a; Alkalin et al. 2006; Silva et al., 2014).

Experimental animal studies indicate that CLA may have beneficial effects on the inhibition of the atherosclerotic process (Deckere et al., 1999). The anti-atherogenic effects of CLA have been established in animal models, as outcomes of changes in lipoprotein and cholesterol effects (Bauman and Lock, 2010). The effect of CLA on atherosclerosis in humans is studied by an indirect approach by measuring various potential heart disease markers, which are favorably influenced by CLA (Belury, 2002b; Khanal, 2004; Valeille et al., 2004). It has been reported that CLA can reduce plasma lipoproteins and early atherosclerosis in animal models, and can impair glucose tolerance in diabetic rats (Lee et al., 1994; Houseknecht et al., 1998). Moreover, there studies according which CLA has an effect on the reduction of lipid uptake by adipocytes which leads to the reduction in body fat gain (Pariza et al., 2003). In animal models CLA has been shown to decrease body fat while not affecting total body mass. Dietary CLA supplements increase lean tissue deposition and decreases fat deposition in pigs (Ostrowska et al. 1999). The mechanism by which CLA leads to a decrease in fat deposition is unknown. Conjugated linoleic acid seems to cause a loss of appetite in human subjects (Park et al. 1997 a,b). Experimental studies also indicate that CLA treatment can enhance lipolysis. Therefore, the decrease in body fat associated with CLA may be partially a result of reduced fat deposition and increased lipolysis in adipocytes, possibly coupled with increased fatty acid oxidation in both skeletal muscle cells (the principal site of fatty acid oxidation) and adipocytes (the principal site of fat storage) (Pariza et al., 2001; Silva et al., 2014)

Research evidences suggest that CLA influences the immune system by altering the effects of cytokine, interleukin, leukotriene and many immunoglobulins. Yu et al. (2002) has shown that CLA exhibits anti-inflammatory effects by negatively regulating the expression of certain pro-inflammatory genes. Also, CLA has been shown to have immunomodulatory properties by enhancing mitogen induced lymphocyte blastogenesis, lymphocyte cytotoxic activity and macrophage killing ability (Cook et al., 1993). Studies on animal models suggest that CLA is effective in preventing the catabolic effect of immune stimulation, and is a potent immunostimulator in mammals (Cook et al., 1993; Hayek et al. 1999)

According literature data CLA may have therapeutic potential in managing type 2 diabetes. CLA has been shown to improve oral glucose tolerance and delay the development of diabetes in rat models (Silva et al 2014). In a human study, CLA decreased fasting blood glucose concentrations in patients with type 2 diabetes (Belury et al., 2003).

CONCLUSION

Study of bioactive compounds of milk fat can be exploited for their applications in functional foods and for potential pharmaceutical use. Recent discoveries in the functional foods area indicate that specific fatty acids produced in the rumen may have beneficial effects on human health, and there is an increased interest in the possibility of designing natural food products with enhanced levels of these fatty acids.

CLA isomers are predominant polyunsaturated fatty acids (PUFA) found in products originating from ruminant animals whose beneficial effects on the human health are evidenced in numerous researches.

Taking in advance the health benefits of some constituents of milk fat, future research can be forwarded to improve the nutritive value and health benefits of milk. Because the feeding regime of dairy animal is the predominant factor affecting milk fat, future research should be directed to alter the composition of milk fat and enhance the concentration of fatty acids which have beneficial health effects in humans. Natural enrichment of food products through manipulation of animal diet may contribute to the overall goal for obtaining the positive health benefits associated with CLA.

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