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THE IMPORTANCE OF CONJUGATED LINOLEIC ACID FOR MEAT QUALITY

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Abstract - The development of functional foods has led to the investigation of the influence and incorporation of one or more components with the functional activities in the different types of food products, in which the meat and meat products deserve special attention. This research was directed towards providing healthy alternatives to products that have been often identified as the cause of the different types of diseases. Linoleic acid contains 18 carbon atoms and as an unsaturated fatty acid has two *cis*-double bond at positions *cis*-9 and *cis*-12 (*c*9, *c*12)-18:2. It belongs to the essential ω-6 fatty acids. Conjugation of the double bonds occurring conjugated linoleic acid (CLA), which are geometric and positional isomers of *cis* and *trans* (*c/c*, *c/t*, *t/t* or *t/c*) double bonds at positions *c*9 and *c*11; *c*10 and *c*12; *c*8 and *c*10; *c*7 and *c*9; and *c*11 and *c*13. Of these CLA isomers in nature are mostly present *cis*- 9 and *trans*- 11 and *trans*- 10 and *cis*-12 isomers. In foods, such as beef, milk and milk products in a large percentage are present *c*9 and *t*11 conjugated linoleic acid, while the isomer *trans*-10 *cis*-12 is less frequent.

Key words - CLA, animal nutrition, meat quality, health.

I. INTRODUCTION

Anticarcinogenic properties of CLA were first established at the University of Wisconsin (USA) in 1985, analyzing chopped beef [1]. The following researchers have found that CLA affects the inhibition of cancer reducing the risk of atherosclerosis, improves the immune system, accelerates growth, decreases fat and increases muscle tissue in several animal species [2].

Nutritional potential of CLA was recognized in 1978 when dr Michael Pariza with co-workers isolated the substance of roasted beef, which showed mutagenic effects [3]. Later research showed that the "mutagenic" had anticancer effects, and that it was in fact *cis*Δ^9, *trans*Δ^{11}conjugated derivative of linoleic acid [1, 4]. Since then, a number of experiments were conducted to investigate its structural and functional aspects.

Most of the physiological effects related to CLA are assumed to be the result of combined action of two its most important isomers: *cis*-9, *trans*-11 and *trans*-10, *cis*-12 [5]. The literature states that the *cis*-9, *trans*-11 isomer has anti-carcinogenic and other beneficial health effects, while the *trans*-10, *cis*-12 isomer is responsible for the reduction of fat in the carcass [6].

II. BIOSYNTHESIS OF CLA

CLA in nature originates mainly from bacterial isomerisation or/and biohydrogenation of polyunsaturated fatty acids (PUFA) in the rumen and from desaturation of *trans*-fatty acids in the adipose tissue and mammary gland [7]. Bacterial microflora contains enzymes linoleate isomerase and CLA-reductase that unsaturated fatty acids in their fat metabolism converte to CLA or important intermediate precursors of CLA (*trans*-vaccenic acid), on the way to the end product - stearic acid. The functioning of the rumen is closely related to the amount of CLA in milk. Only small amounts of CLA is absorbed directly from the rumen and small intestine suggesting an alternative source for the content of CLA in milk and adipose tissue. Endogenous Δ^9-desaturase converts vaccenic synthesized *trans*-unsaturated acid, with a bond to the position of *c*11 to *c*9, *t*11 CLA. The endogenous synthesis from *trans*-vaccenic acid was also documented in humans but the predominant source of CLA seems to be the dietary CLA intake with meat and meat products as well as milk and dietary products [2].

III. ENRICHMENT OF MEAT WITH CLA

The efficiency of "enrichment" products of animal origin (meat, milk, eggs) varies primarily depending of the species and concentration of CLA, which is present in the feed. For example, the yolk can contain as much as 11% CLA (relative to the total fatty acids), when the CLA is added in an amount of 5% of the food for the hens. When using a 1% CLA in food for fish, the proportion of CLA in fish fillets is as high as 8%. In pigs, higher level of CLA than 6% was found in the subcutaneous adipose tissue, after addition of 2% CLA in feeds used for fattening pigs. The level of CLA in milk and meat of ruminants ranges from 2 to 6%, which is significantly lower compared to non-ruminants. The reason is that an increase in the level of CLA in milk and meat of ruminants is achieved with dietary fatty acids, which are precursors for the synthesis of CLA, while in non-ruminants (pigs, poultry, fish), CLA is directly added to feed [8].

Several studies showed that CLA influences meat quality. CLA improved pork composition by increasing marbling scores and intramuscular fat deposition, while having no detrimental effect on the sensory characteristics of pork [9]. Feeding pigs with CLA tended to increase the firmness of belly and increased lean meat content, and improved other aspects of meat quality in growing-finishing pigs [10]. Du et al. [11] found that dietary CLA improved the oxidative stability of cooked chicken meat patties during aerobic storage.

New technological strategies used to design and develop functional foods based on changes in meat transformation systems, are especially promising. A number of the approaches can be used to remove, reduce, increase, add and/or replace different bioactive components. The modification of the meat formulation process also makes it possible to use traditional ingredients, and other ingredients specifically designed with certain attributes that contain health-promoting properties. Three main goals have been identified for improvement of fat content using meat reformulation strategies: reduction of total and energy, reduction of cholesterol and modification of fatty acid profiles [12]. Fat reduction is usually based on two main criteria: the utilization of leaner meat raw materials and the reduction of fat density (dilution) by adding water and other ingredients (gums, protein-based, fat-based or carbohydrate-based fat replacers) with little or no caloric content. In addition to these factors, the reformulation strategies should take manufacturing and preparation procedures into account [13].

IV. FACTORS INFLUENCING CLA CONTENT OF MEAT

Several influencing factors (seasonal variations, animal genetics, and production practices) have already been mentioned. The most important factor is the diet because it provides the substrates for the CLA formation. Besides animal diet – influencing the naturally occurring CLA content – it is interesting to know whether and how CLA is affected by processing.

Influence of animal diet on CLA content in ruminants

A variety of dietary components influence the CLA level of meat. In general higher CLA concentrations in muscles are associated with a higher intramuscular fat content [14]. The present knowledge clearly demonstrates that certain feed components are necessary to positively influence CLA content in the meat fatty acids.

A switch from concentrate-based diet to pasture has been shown to increase CLA content. French et al. [15] determined in the intramuscular fat of steers (longissimus dorsi muscle) increasing CLA contents consistent with increasing intakes of grass. Levels of 5.4, 6.6, and 10.8 mg CLA/g FAME were detected in grazing steers with increasing grass intake compared to 3.7 mg/g FAME in animals fed concentrate. Grass silage also positively influenced CLA content (4.7 g/g FAME) but not to the same extent.

The increased CLA content in meat from animals grazing on pasture is attributed to the high PUFA content of grass (especially n-3 18:3 with a n-6:n-3 ratio of approximately 1:3–5).

Although not the only determinant, the amount of dietary PUFA determines the generation of trans fatty acids by rumen bacteria as discussed earlier [16].
Pasture feeding does not only cause higher CLA concentrations but also influences fatty acid composition. A decrease in the n-6:n-3 PUFA ratio as well as an increase in the PUFA:saturated fatty acids (SFA) is described in beef adipose and muscle tissue by inclusion of grass in the diet [17]. In lambs a decrease in n-6:n-3 PUFA ratio has been documented as well [18, 19].

Feeding of oilseeds and vegetable oils

Adding oilseeds to the diet has been proven to be an efficient method to increase the CLA content in the muscle lipids. However, not all oilseeds exert the same effect. Casutt et al. [20] supplemented the concentrate feed of Brown Swiss bulls with either sunflower-, rape-, or linseed (increasing the dietary fat content by 3%). Compared to the control group (5.6 mg/g FAME), the CLA concentration of the subcutaneous fat in the sunflower group was significantly increased (7.8 mg/g FAME) whereas no changes were observed in the linseed group (5.5 mg/g FAME) and in the rapeseed group the CLA content even decreased (4.6 mg/g FAME).
In addition to sunflower seed and linseed, safflower seed was also shown to increase the relative CLA content in the muscle tissues of lambs. Kott et al. [21] fed lambs with either a safflower supplemented (containing 6% oil from safflower seeds) or an unsupplemented control diet. The safflower seed supplementation resulted in significantly higher CLA contents in *longissimus lumborum* muscle (4.1 vs. 9.0 mg/g FAME).
Extruded full-fat soybeans were also shown to increase the CLA content in muscle fatty acids of crossbred Angus steers (measured in lipids of rib *longissimus*, eye of round, and chuck tender muscles) [22].
Using chickpeas to replace soybean meal and corn in the diet of lambs also resulted in higher CLA concentrations in the *longissimus dorsi* muscle regardless of whether the dietary chickpeas concentration was 20% or 42% (in mg/g FAME; control: 4.9, 20% chickpeas: 8.5, and 42% chickpeas: 8.9) [23].
Vegetable oils as equivalent to oilseeds show similar effects on CLA content. In beef cattle the addition of 3% and 6% sunflower oil to a barley based finishing diet resulted in increased CLA contents in *longissimus* muscle: 2.0 vs. 2.6 vs. 3.5 mg/g lipid for control, 3%, and 6% sunflower oil, respectively [24]. Rapeseed oil and whole rapeseed do not seem to have positive effects.
Both oilseeds and free oils affect CLA content and fatty acid composition in the tissues in a similar manner. Free plant oils with high PUFA concentrations are normally not included in ruminant diets as high levels of dietary fat disturb the rumen environment and inhibit microbial activity [16, 14]. Additionally, vegetable oils are a rather expensive dietary supplement for ruminants and are more susceptible to oxidation than seeds. Aharoni et al. [25] compared soybean oil with full fat soybeans as supplements over five months in a high forage fattening diet of Friesian bull calves. Extruded full fat soybeans were about 20% more efficient than free oil in increasing the CLA concentration in intramuscular fat. The full fat soybean supplement also resulted in higher PUFA and lower SFA and monounsaturated fatty acids (MUFA) content in the intramuscular fat than supplementation with soybean oil. This may be due to a partial protection of the oils against ruminal biohydrogenation by roughly crushed seeds [26]. Therefore, using oilseeds instead of free oils may be the preferred option.

Feeding of fish oils

Feeding fish oil supplements is another approach to increase CLA. Enser et al. [27] reported an increase in the CLA concentration from 3.2 to 5.7 mg/g FAME in the longissimus lumborum muscles of Charolais steers fed with a fish oil supplemented diet but showed simultaneously that whole linseed was more efficient in increasing the CLA concentration. A comparable feeding design used with three lamb breeds documents no effect of fish oil (mean values of 10.0 vs. 11.0 mg/g FAME) but again a significant increase with linseed [28]. In both studies combining fish oil with whole linseed let to comparable CLA proportions as with linseed alone but another study [29] with lambs found that the linseed fish oil mixture was more efficient than linseed alone. Long chain n-3 fatty acids present in fish oil may interfere with the biohydrogenation of linolenic or/ and linoleic acids or affect Δ^9-desaturase activity [14]. Chow et al. [30] postulate that fish oil increases ruminal accumulation of *trans*-vaccenic acid by inhibiting the final biohydrogenation step to stearic acid. This would supply more substrate for endogenous CLA synthesis.

V. CLA IN NUTRITION OF MONOGASTRIC ANIMALS

Compared with ruminants, dietary fats in monogastric animals are not modified before the digestion and absorption. Therefore, the diet must contain trans fatty acids (eg *trans*-vaccenic acid) as a substrate for the endogenous synthesis of CLA or CLA alone in order to raise the CLA concentration of tissue. CLA used as supplements in studies consist entirely of a mixture of a limited number of CLA isomers (predominantly *c*9, *t*11- and *t*10, *c*12-18: 2).

Glaser et al. [31] have analyzed muscle tissue (muscle *longissimus dorsi*) of large white pigs after feeding 30 to 103 kg of live weight with meal consist of barley-wheat-soybean meal with 6% sunflower oil with high oleic acid or various amounts (1.85%, 3.70%, 5.55%) partially hydrogenated rapeseed oil (high in trans fatty acids). They have reported increased levels of CLA in the neutral lipids of muscle tissue with increased amounts of partially hydrogenated rapeseed oil in the diet (3.8, 6.4, and 8.5 mg CLA/g FAME) 0.9 mg/g FAME in the control group, with sunflower oil.

Similar results (4.4 vs. 0.8 mg/g FAME in *m.longissimus dorsi*) have recently published Lauridsen et al. [32] who have in their essay supplemented diet for 100 Danish sows with 0.5% CLA or 0.5% sunflower oil from 40 up to 100/130 kg of live weight. Joo et al. [33] fed 20 cross breed gilts with meal supplemented with 0%, 1.0%, 2.5% and 5.0% of synthetic CLA preparations based on safflower oil during the 4 weeks (approximately 105 kg of live weight). The concentration of CLA in muscle *longissimus dorsi* increased along with increasing percentage of CLA in the diet (0.1, 3.7, 10.1, and 11.6 mg / g fatty acids, respectively).

When the meals for the growth of broilers (Cobb 500) from 22 days to slaughter 47 days were supplemented with 2.0% or 4.0% of CLA it has resulted in higher concentrations of CLA in chicken tissues (breast, leg meat, skin and abdominal fat) compared to the control group (in abdominal fat: 51.5, 91.4, and 0.3 mg/g fat, respectively) [34]. Similar results have reported Aletor et al. [35] with Ross broilers.

Beside the increased concentrations of CLA in fat and muscle tissue, CLA supplementation affects fatty acid composition of tissue in pigs. Several reports have indicated that CLA supplementation increased the amount of saturated fatty acids (C14: 0, C16: 0, and C18: 0) and decreased MUFA (mainly C18: 1) in tissues of pigs with the activity of Δ^9-desaturase [36, 37, 38].

VI. CLA IN HUMAN NUTRITION

In Germany, according to the Fritsche and Steinhart [39], the average daily intake of CLA in normal diet for men is 0.36 g and 0.44 g in women, while in the US. these data are 0.21 g in males and 0.15 g in women [40].

However, this is only a fifth of the recommended daily intake of 0.1% of the total daily intake of food. This dose of 0.1% CLA is recommended in order to reduce the risk of cancer, and was based on studies conducted on rodents [41].

Fatty acids of n-3 group, particularly those with very long chains, have a positive effect on heart diseases and have anti-cancer properties. CLA helps in prevention and may slow down tumor formation [41], reduces the occurrence of atherosclerosis [42] and helps to maintain normal blood sugar level, which appear to be the prevention of the obesity [43].

VII. CONCLUSION

Conjugated linoleic acids are predominantly present in ruminant products because of the action of microorganisms in the rumen in the process of bio hydrogenation of fatty acids. The concentration of CLA per gram of FAME varies considerably, not only between species but also from animal to animal and within animals, in different tissues. Nutrition has a major impact on the content of CLA. As it has been shown in many studies, there are several ways to increase the level of CLA in meat of ruminants. For non-ruminants only supplementing the CLA or its precursors *trans*-vaccenic acid is an effective way to increase the CLA content. Modification of a meal in order to increase CLA also affects the composition of fatty acids in animal tissue.

Meat and meat products represent for about 25-30% of the total intake of CLA in the body of people in Western populations. This intake can be improved with a stronger orientation to feed containing CLA and enrich the content of CLA in meat through specific nutritional strategies. Until now, the declarations of the promotion of impact of CLA on health were mainly based on experiments on animals and must still be proven in humans. In a study in humans are commonly used synthetic CLA supplements and they do not show the natural composition of the isomers in foods. Further researches should assess if natural sources of CLA (meat and milk from ruminants) have a similar effect on human health as synthetic sources.

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