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Annals of Biological Research, 2012, 3 (2):1161-1176  
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### Importance essential fatty acids (n-6 and n-3) in animal nutrition: I: Ruminant

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#### ABSTRACT

*Fatty acids, n-3 as well as n-6, are essential for normal physiological functioning and for the health of humans and all domestic species. In humans, not all fatty acids can be produced endogenously due to the absence of certain desaturases. Thus, specific fatty acids termed essential (linoleic, alpha-linolenic) need to be taken from the diet. Other fatty acids whose synthesis depends on essential fatty acid intake include eicosapentaenoic acid and docosahexaenoic acid, found in oily fish. Dietary sources of saturated fatty acids are animal products and tropical plant oils, whereas sources of unsaturated fatty acids are vegetable oils and marine products. In recent years there has been a greater demand for foods with increased levels of functional fatty acids, such as long-chain omega-3 fatty acids and conjugated linoleic acids, because of their biological roles in cells. Hence, there is a need to develop alternative food sources to increase consumption of n-3 polyunsaturated fatty acid. The main dietary sources of eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) are marine algae, fish, and fish oils. However, this review consists of two parts. The present part aims at describing fatty acid chemistry, nutrition, metabolism functions and increase essential fatty acid content of milk and meat in ruminants. The second part of further article, present knowledge and approaches to increase essential fatty acid content of egg and meat in poultry will reviewed.*

**Key words:** Essential fatty acids, (n-6 and n-3) polyunsaturated fatty acids, chemistry, metabolism, ruminant, poultry.

*Abbreviations: EFA, essential fatty acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; CLA, Conjugated linoleic acid; FA, fatty acid; ALA, α-linolenic acid; LA, linoleic acid; LNA, linolenic acid; MFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acids; VLDL, very low density lipoproteins; TMR, total mixed rations; DM, dry matter; VLC, Very long chain.*

## INTRODUCTION

The EFA, although key to normal growth and reproduction, have not historically been a topic for animal nutrition. It is only because of the increasing interest in EFA for human nutrition and health and the potential to increase their content in human diets through animal feeding have EFA become of interest in animal nutrition [1]. In recent years there has been a greater demand for foods with increased levels of functional fatty acids, such as long-chain omega-3 fatty acids and conjugated linoleic acids, because of their biological roles in cells [2]. Higher intakes of long chain omega-3 fatty acids in the diet have been reported to improve the functions of immune, nervous, and cardiovascular systems in humans, and the reproductive performance and carcass quality in [2]. Hence, there is a need to develop alternative food sources to increase consumption of n-3 polyunsaturated fatty acid. The main dietary sources of eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) are marine algae, fish, and fish oils [3]. Concentrations of *cis*-9, *trans*-11, the major CLA isomer in milk, can be increased by feeding whole oilseeds or by supplementing the diet with plant oils or marine lipids [4]. Essential fatty acids are fatty acids that humans and other animals must ingest because the body requires them for good health but cannot synthesize them. The term "essential fatty acid" refers to fatty acids required for biological processes, and not those that only act as fuel [5]. Holman (1964) introduced the concept of grouping EFA by families according to the position of the terminal carbon-carbon bond in the molecule. He called these "ω families." Omega 6 (or v6) indicates that the double bond nearest the terminal methyl group lies after a 6 carbon saturated terminal structure. Omega 3 indicates a 3 carbon saturated terminal structure. This terminology continues in popular literature, whereas the proper chemical designation for these families is "n-". Thus, Linoleic acid (*cis*-9, *cis*-12 18:2) is of the n-6 family, and linolenic acid (*cis*-9, *cis*-12, *cis*-15 18:3) is of the n-3 family. As noted above, increasing interest in the role of foods as nutraceuticals has led to research that has explored opportunities to increase the content of biologically active agents in animal foods [6]. However, the present part aims at describing fatty acid chemistry, nutrition, metabolism functions and increase essential fatty acid content of milk and meat in ruminants.

### Chemistry of fatty acid

Essential FA includes LA for n-6 family and ALA for n-3 family. Essential FA are polyunsaturated fatty acid which have their first double bond located on the third (n-3 family) or the sixth (n-6 family) carbon atom counting from the methyl terminus of the hydrocarbon chain [7] (Fig. 1).

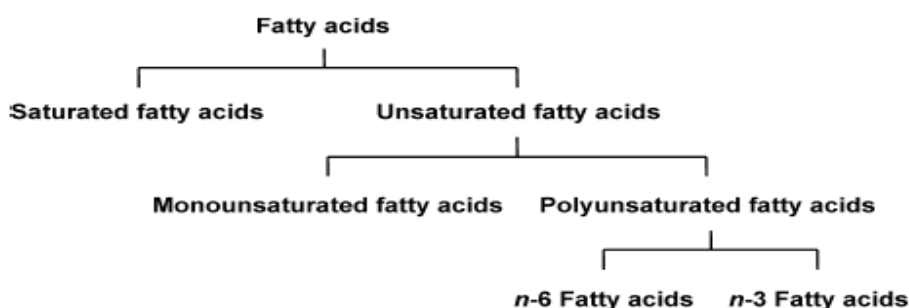


Fig. 1: Summary of fatty acids fate [8]

The position of a double bond is indicated by the position of the first carbon of the bond; thus the 9,10 monoenoic C18 fatty acid is  $\Delta^9$ -octadecenoic acid. Most unsaturated fatty acids in biological systems have the *cis* configuration, so this molecule would be characterized further as *cis*-9 18:1. Most naturally occurring polyunsaturated fatty acid have the double bonds in the *cis*, *cis* 1,4 pentadiene structure (multiple double bonds are separated by a methylene group); this structure is assumed unless designated otherwise [1]. Important fatty acids involved in metabolic pathways are summarized in (Table 1) [7].

**Table 1: Important fatty acids involved in metabolic pathways [7,9]**

Notation	Systematic name by IUPAC	Trivial name	Abbreviation	Molecular mass	Melting point (°C)
16:1n-9	<i>cis</i> -7-hexadecenoic			254.411	
16:3 n-3	<i>all-cis</i> -7,10,13-hexadecatrienoic acid	n/a			
18:1n-9	<i>cis</i> -9-octadecenoic	oleic	OA	282.47	16.3
18:1n-9	<i>trans</i> -9-octadecenoic	elaidic		282.47	44–46
18:2n-6	<i>cis, cis</i> -9,12-octadecadienoic	linoleic	LA	280.46	–5 (–9)
18:3n-6	<i>all cis</i> -6,9,12-octadecatrienoic	$\gamma$ -linolenic	GLA	278.44	
18:3n-3	<i>all cis</i> -9,12,15-octadecatrienoic	$\alpha$ -linolenic	ALA	278.44	–11 (–17)
18:4n-3	<i>allcis</i> -6,9,12,15-octadecatetraenoic	stearidonic		276.417	
20:1n-9	<i>cis</i> -11-eicosenoic	gondoic		310.518	
20:2n-6	<i>cis, cis</i> -11,14-eicosadienoic			308.502	
20:3n-9	<i>all cis</i> -5,8,11-eicosatrienoic	Mead		306.487	
20:3n-6	<i>all cis</i> -8,11,14-eicosatrienoic	dihomo- $\gamma$ -linolenic	DHGLA	306.487	
20:3n-3	<i>all-cis</i> -11,14,17-eicosatrienoic acid	Eicosatrienoic acid			
20:4 n-3	<i>all-cis</i> -8,11,14,17-eicosatetraenoic acid	Eicosatetraenoic acid			
20:4n-6	<i>all cis</i> -5,8,11,14-eicosatetraenoic	arachidonic	AA	304.471	–49.5
20:5n-3	<i>allcis</i> -5,8,11,14,17-icosapentaenoic	timnodonic	EPA	302.455	–54
22:1n-9	<i>cis</i> -13-docosenoic	erucic		338.58	33.8
22:2n-6	<i>cis, cis</i> -13,16-docosadienoic acid			336.556	
22:3n-6	<i>all cis</i> 10,13,16-docosatrienoic acid			334.540	
22:4n-6	<i>allcis</i> -7,10,13,16-docosatetraenoic	adrenic		332.524	
22:5n-3	<i>allcis</i> -7,10,13,16,19-docosapentaenoic		DPA-3	330.509	
22:5n-6	<i>allcis</i> -4,7,10,13,16-ocosapentaenoic		DPA-6	330.509	
22:6n-3	<i>allcis</i> -4,7,10,13,16,19-ocosahexaenoic	clupadonic	DHA	328.493	–44.7, –44.5
22:6 n-3	<i>all-cis</i> -4,7,10,13,16,19-ocosahexaenoic acid	Docosahexaenoic acid			
24:1n-9	<i>cis</i> -15-tetracosenoic	nervonic	NA	366.625	42–43
24:5 n-3	<i>all-cis</i> -9,12,15,18,21-docosahexaenoic acid	Tetracosapentaenoic acid			
24:6 n-3	<i>all-cis</i> -6,9,12,15,18,21-etracosenoic acid	Tetracosahexaenoic acid (Nisinic acid)			

There are three essential omega-3 fatty acids important nutritionally to humans include  $\alpha$ -linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA).  $\alpha$ -linolenic acid (18:3, *n*–3; ALA), eicosapentaenoic acid (20:5, *n*–3; EPA), and docosahexaenoic acid (22:6, *n*–3; DHA).

### n-3 Polyunsaturated fatty acids

Fatty acid  $\alpha$ -linolenic (ALA), parent the family of *n*-3 polyunsaturated fatty acid. Its main metabolic products are eicosapentaenoic acid (EPA) (timnodonic acid) and docosahexaenoic acid (DHA) (clupadonic acid), and to a lesser extent docosapentaenoic acid (DPA, 22:5n-3). These metabolites are termed long-chain *n*-3 polyunsaturated fatty acid. Dietary sources of ALA are seeds and leaves of some plants – soybeans (*Glycinemax*), linseed (*Linum usitatissimum*), blackcurrant seeds (*Ribes nigrum*) and borage leaves (*Borago officinalis*), as well as their oils. Its metabolites, EPA and DHA, can be taken from the diet through oily fish which are excellent sources containing approximately 2 g of EPA plus DHA per portion of fish (150g). Among other fish, oily fish include sardines, mackerel, trout, salmon, fresh (not canned) tuna, and herring.

Other sources of long chain n-3 polyunsaturated fatty acid include fish oils, the liver of non-oily fish (such as cod and haddock), and the flesh of some white non-oily fish but in much lower amounts. Conversion of ALA into 20-22 carbon number metabolites is much more effective in marine animals than in human species [7].

### Feedstuff Sources of n-3 Fatty Acids

Sources of n-3 fatty acids as feedstuffs for livestock and other domestic animals are limited [10]. Although both soybean oil and rapeseed (canola) oil contain significant amounts of LNA (Table 2), high contents of LA in soybean oil and oleic acid in canola limit their general usefulness, although these feed ingredients may be used to advantage in certain applications. The supply of EPA and DHA is limited to fish oil, usually menhaden in commercial applications and, increasingly, marine algae oil [11].

### n-6 polyunsaturated fatty acids

In the n-6 polyunsaturated fatty acid family the parent fatty acid is LA. Its metabolic products are  $\gamma$ -linolenic (GLA; 18:3n-6), dihomogamma-linolenic (DHGLA; 20:3n-6) and arachidonic acids, and in minor amounts also adrenic (22:4n-6) and docosapentaenoic (22:5n-6) acids. High concentrations of n-6 polyunsaturated fatty acid (>60%) are found in soybean oil (Glycine soja), sunflower seed oil (*Helianthus annuus*), safflower oil (*Carthamus tinctorius*), evening primrose oil (*Oenothera biennis*), grape seed oil (*Vitis vinifera*), poppy seed oil (*Populus nigra*), borage seed oil (*Borago officinalis*), blackcurrant seed oil (*Ribes nigrum*), and lower concentrations (40-50%) in wheat germ oil (*Triticum vulgare*), corn oil (*Zea mays*), walnut oil (*Juglans regia*), cottonseed oil (*Gossypium*) and sesame oil (*Sesamum indicum*), as well as the seeds of some of these plants [7]. Percentage of individual FA for oils rich in polyunsaturated fatty acid shows in (Table 2) and (Table 3) shows the percentage of individual FA for oils rich in monounsaturated fatty acid (MFA).

**Table 2: Average percentage of individual fatty acids in oils rich in monounsaturated fatty acids [1,12,13]**

Fatty acid	Olive	Rape seed*	Rape seed**	Canola	Canola, gene modified	Almond	Rye grass, fresh	Peanut	Hazelnut
12:0	—	—	—	—	—	—	—	—	—
14:0	—	—	—	—	—	—	—	—	—
16:0	11.0	2.7	4.0	4.0	7	9.5	5.2	—	—
18:0	2.0	1.0	7.0	2.0	2	2.2	2	—	—
18:1n-9	71.0	11	52.0	62.0	69	44.8	77.8	—	—
18:2n-6	10.0	13.8	30.0	18.6	8	17	15	32	10.1
18:3n-6	—	—	—	—	17	—	—	—	—
18:3n-3	1.0	6.2	7.0	9.0	32	—	68	—	—
18:4n-3	—	—	—	—	23	—	—	—	—
20:0	—	—	—	—	—	—	—	—	—
SFA	13.0	3.7	11.0	6.0	9.0	11.7	7.2	—	—
MFA	71.0	52.0*	52.0	62.0	69	44.8	77.8	—	—
POLYUNSATURATED FATTY ACID n-6	10.0	13.8	30.0	18.6	32	17	68	32	10.1
POLYUNSATURATED FATTY ACID n-3	1.0	6.2	7.0	9.0	—	—	—	—	—

SFA, saturated fatty acids; MFA, monounsaturated fatty acids; polyunsaturated fatty acids  
 Note: Percentages may not add up to 100% due to some FA traces not listed; where % FA varied average values were used.

\*Non-cultivated rape seed (mustard seed) oil (*Brassica napus*); contains about 41% erucic acid (22:1n-9)

\*\*Cultivated rape seed oil (*Brassica campestris*)

Table 3: Average percentage of individual fatty acids in oils rich in polyunsaturated fatty acids [1,12,14]

Fatty acid	Sunflower seed	Safflower seed	Pumpkin seed	Sesame seed	Corn	Soya bean	Soybean, gene modified <sup>1</sup>	Flaxseed (linseed)	Wheat germ	Hemp seed	Evening primrose	Borage seed
12:0	–	–	–	–	–	–	–	–	–	–	–	–
14:0	–	–	–	–	–	–	–	–	–	–	–	–
16:0	7.0	7.0	9.0	9.0	11.0	11.0	–	5.0	16.0	6.0	6.0	10.3
18:0	5.0	2.0	–	4.0	2.0	4.0	–	4.0	0.5	2.0	2.0	3.6
18:1n-9	19.0	13.0	33.5	42.0	28.0	22.5	–	21.0	14.6	12.0	7.3	16.0
18:2n-6	68.0	78.0	50.5	45.0	58.0	50.0	31	16.0	55.0	58.0	74.0	37.0
18:3n-6	–	–	–	–	–	–	5	–	–	2.0	9.0	23.3
18:3n-3	1.0	–	–	–	–	–	11	53.0	–	–	–	–
18:4n-3	–	–	–	–	–	–	17	–	–	–	–	–
20:0	–	–	–	–	–	–	–	–	–	–	–	–
SFA	12.0	9.0	9.0	13.0	13.0	15.0	–	9.0	16.5	8.0	8.0	13.9
MFA	19.0	13.0	33.5	42.0	28.0	22.5	–	21.0	14.6	12.0	7.3	16.0
POLYUNSATURATED FATTY ACID n-6	68.0	78.0	50.5	45.0	58.0	50.0	–	16.0	55.0	60.0	83.0	60.3
POLYUNSATURATED FATTY ACID n-3	1.0	–	7.0	–	1.0	7.0	11	53.0	6.9	20.0	–	–

SFA, saturated fatty acids; MFA, monounsaturated fatty acids; POLYUNSATURATED FATTY ACID, polyunsaturated fatty acids

Note: Percentages may not add up too 100% due to some FA traces not listed; where % FA varied average values were used.

### Metabolism of essential fatty acids:

#### Absorption and Transport

Majority of absorbed fatty acids are incorporated into triacylglycerols and transported from the intestine to peripheral tissues in chylomicrons or very low density lipoproteins (VLDL), or both. There is evidence in rats and in cows that n-6 fatty acids are transported preferentially in the phospholipids of triacylglycerol-rich lipoproteins, whereas it has been shown in rats that n-3 fatty acids are transported mainly in the triacylglycerols. Long-chain and total n-3 polyunsaturated fatty acid were mainly in cholesteryl esters and phospholipids in bovine plasma taken from jugular puncture. However, distribution of fatty acids in the general circulation may not be representative of distribution in lipid classes of intestinal lymph. Consistent with the latter idea, although n-3 fatty acid proportions were greater in cholesteryl esters and phospholipids than in triacylglycerols of both high- and low-density lipoproteins of lactating cows, n-3 fatty acid proportions were comparable or greater in triacylglycerols of the VLDL [15], which would be representative of intestinal lipoprotein composition. Incorporation into polar versus apolar lipids of intestinal lymph may influence the site of tissue uptake and metabolism. Triacylglycerols of chylomicra and VLDL are taken up by the mammary gland by action of lipoprotein lipase (LPL) [1].

Palmquist and Mattos (1978) reported that 76% of absorbed radiolabeled LA is taken up directly by the mammary gland [16].

#### Biosynthesis of essential fatty acid

Essential fatty acid are polyunsaturated fatty acid which have their first double bond located on the third (n-3 family) or the sixth (n-6 family) carbon atom counting from the methyl terminus of the hydrocarbon chain. Essential FA cannot be synthesized in humans due to lack of  $\Delta^{12}$ - and  $\Delta^{15}$ -desaturases which are present only in plants and marine algae, and thus, the human organism

is completely dependent on their dietary intake. Further elongation and desaturation of these fatty acids to produce long chain polyunsaturated fatty acid, including EPA, DHA and arachidonic acid, is performed but not that efficiently in humans. Thus, these fatty acids may be characterized as conditionally essential depending on essential fatty acid availability. The metabolic pathways of EFA are schematically shown in (Fig. 3) [7].

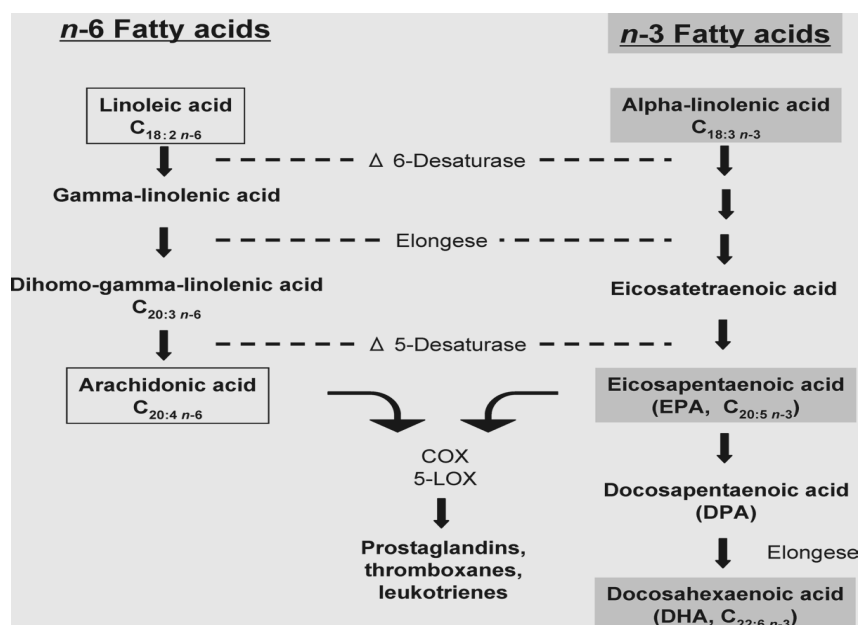


Fig. 3: Elongation and desaturation of essential fatty acids of the n-3 and n-6 families [8]

### Increasing n-3 fatty acids in animal tissues and products

With greater interest in increasing the n-3 fatty acid content of human diets, interest has also developed to increase these fatty acids in animal products.

#### Ruminants

Challenges in increasing the n-3 content of ruminant tissues and products differ considerably from those of nonruminants. In the last 25 yr, developments in manufactured feed fats ("rumen-inert" fats) and advances in feeding management have minimized the effects of fatty acids on the ruminal microbiota. However, not all feed fats are amenable to these processes [1]. Untreated vegetable oils high in unsaturated fatty acids have only limited ability to alter milk fatty acid composition. The reason for this was established decades before the 1980s, and is attributed to the microbial population located mainly in the rumen that transforms dietary unsaturated fatty acids. Therefore, delivery of unsaturated fatty acids to mammary tissue is limited even when their dietary concentration is high [17]. Of major concern in modifying the fatty acid composition of animal products is ruminal biohydrogenation; under almost all feeding conditions, >80% of dietary unsaturated fatty acids are partially biohydrogenated to myriad trans unsaturated products or completely BH to saturated products, mainly stearic acid (18:0) [1]. The ruminal microorganisms transform unsaturated fatty acids in a process called biohydrogenation, in which hydrogen addition via microbial enzymes removes double bonds in a fatty acyl chain converting it from unsaturated to saturated (Figure 4).



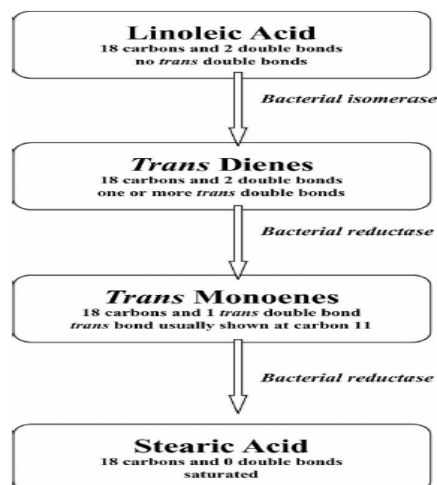


Fig. 4: Major steps in the biohydrogenation of linoleic acid by ruminal microorganisms [17].

Microbial biohydrogenation of linoleic acid and  $\alpha$ -linolenic acid by an anaerobic rumen bacterium *Butyrivibrio fibrisolvens* is highly dependent on rumen pH [18]. Depending on conditions in the rumen, various proportions of stearic acid and trans intermediates are produced from linoleic acid. The trans diene intermediates usually include various conjugated isomers or conjugated linoleic acid. There has been considerable interest over the last 25 yr in finding ways to shield dietary unsaturated fatty acids from biohydrogenation to enhance their absorption and delivery to the mammary gland. One of the first successful rumen-protected fats was prepared by embedding unsaturated oils within a protein shell made resistant to microbial attack by cross-linking with formaldehyde. The formaldehyde-protected fats had dramatic effects on enhancing unsaturated fatty acid in milk. biohydrogenation due to the nature of their hard outer seed coats, although the protection is probably best described as limited and inconsistent. Oilseeds are commonly processed (ground, extruded, pelleted) to enhance their handling, intake, or digestibility, which can significantly reduce their resistance to biohydrogenation [17]. Commercial fat supplements that have been processed to increase rumen-inert properties ("rumeninert" means that the fat has no or little effect on the ruminal microbiota, but may be BH or otherwise modified) include calcium salts of palm fatty acid distillate, hydrogenated fatty acids, and hydrogenated triacylglycerols [1]. Another significant finding bringing a great deal of attention to biohydrogenation intermediates in milk fat was the discovery that conjugated linoleic acid had beneficial effects on human health, most notably cancer-fighting properties [17]. one must assume that for most ruminant fat supplements (as well as the basal diet), approximately 15% of dietary unsaturated fatty acids escape ruminal BH [1]. It was the cis-9, trans-11 conjugated linoleic acid isomer in particular that received the most attention for its anticarcinogenic properties, which was known to arise from the biohydrogenation of linoleic acid [17].

### Conjugated linoleic acid (CLA)

As mentioned above, most polyunsaturated fatty acids are characterized by pentadiene configuration (i.e. methylene interruption) of the double bonds, with the exception of conjugated FA. Most abundant FA with a conjugated system of double bonds are isomers of LA (conjugated linoleic acid; CLA). These FA appear in red meat and dairy products; cows grazing pasture have a several times higher content of conjugated linoleic acid in meat and milk fat than cows fed

typical dairy diets. There are 28 possible isomers of conjugated linoleic acid, which differ in the position (e.g. 7 and 9, 8 and 10, 9 and 11, 10 and 12, 11 and 13 – counting from the carboxyl group) and configuration (cis or trans) of double bonds. The type most commonly found in meat and dairy products is rumenic acid (18:2 $\Delta$ 9c, 11t). Also, the isomer 18:2 $\Delta$ 10t, 12c has important metabolic effects. Compared to previous generations, the current human population consumes less conjugated linoleic acid in their diet preferring white to red meat and very low fat dairy products. Thus, conjugated linoleic acid, containing equal amounts of 18:2 $\Delta$ 9c, 11t and 18:2 $\Delta$ 10t, 12c isomers, is frequently used as special dietary supplement [7]. Dietary supplements of conjugated linoleic acid reduce milk fat synthesis in lactating cows [19] and decrease body fat content of several species of growing animals [20].

### **Ruminal protection of fat**

There have been several attempts to develop fat supplements that have true ruminal bypass value. The goal is to increase the LA content of ruminant products this is achieved to excess levels of 18:2 in milk fat and adipose tissues of beef and lamb often exceeded 20% of the total fatty acids, and oxidation of the products is a large problem. The quality of the products has been improved, with the focus on using protected canola seed to decrease saturated fatty acids in ruminants, with increases mainly in 18:1. Despite the successful protection of fats from ruminal BH by formaldehyde-treated protein, this practice has not been widely adopted. The most frequent criticisms are the limitations of using formaldehyde in feedstuffs (formaldehyde is generally accepted today), the cost of the process, and the inconsistent quality of the product [1]. Jenkins, T. C (2000) investigate the effect of oleamide on lactation performance and milk fatty acid composition. Diets were total mixed ration containing 47% corn silage and 53% concentrate (dry matter basis) and were supplemented with no added fat (control), or with 3.5% added fat from either higholeic canola oil, a commercial source of oleamide, or oleamide synthesized from oleic acid and urea. The canola oil supplement had no effect on milk yield or composition. Compared to canola oil, the oleamide supplements reduced milk yield, dry matter intake, and milk fat and protein contents. Milk oleic acid concentration increased from 17.4% of total fatty acids for the control diet to 22.1% for the canola oil diet. Both oleamides further increased milk oleic acid to 30.0 and 27.1% of total fatty acids for the commercial and synthesized oleamides, respectively. Milk palmitic acid was reduced and stearic acid was increased by all fat supplements but more so by the oleamides than by the canola oil [21]. Consistent with previous reports [22] that fatty acyl amides resist ruminal biohydrogenation, feeding oleamide to Jersey cows in this study increased milk oleic acid concentration but had negative effects on feed intake and milk yield. Inasmuch as the BH process requires the substrate to have a free carboxyl group, fatty acid amides are only slowly BH, probably by some ruminal degradation of the amide bond. No amide is transferred to milk [22]; however, amides reduce feed intake [21], severely limiting their application in dairy diets. Acyl amides are not in commercial use as a protected fat supplement. Abel-Caines, et. al (1999) investigate the influence of non enzymatically browned soybeans on ruminal fermentation and lactational performance of dairy cows and reported that milk C18:2 and C18:3 were increased as the inclusion of non enzymatically browned soybeans in the total mixed rations (TMR) increased. All percentages of the non enzymatically browned soybeans fed to cows resulted in fat corrected milk yields that were similar to those of cows fed the TMR that contained Ca salts of fatty acids. Treatment of soybeans was accomplished by heating cracked soybeans with wood-processing sulfite liquor (lignosulfonate) containing 40% xylose as a reducing sugar for the non enzymatic browning reaction. The mixture was heated to



100°C, followed by evaporative cooling. Protection of supplemental LA from BH was reported to be 50%; although the polyunsaturated fatty acid content of milk fat clearly was increased, the methods of fatty acid analysis and the milk fatty acid data reported caused the reliability of the data to be questionable. The product was highly acceptable by lactating cows, with no decrease in feed intake reported with inclusion of as much as 22.5% non enzymatically browned soybeans in the diet. Many publications refer to the calcium salt (soap) of fatty acids as “protected.” The proper term is ruminally inert [23]. Sukhija and Palmquist (1990), investigate the dissociation of calcium soaps of long-chain fatty acids in rumen fluid and reported that the dissociation was maximum at pH 5.0, minimum at pH 6.5, and dependent on unsaturation of fatty acids in the soaps. Soluble calcium in the acetate-buffered rumen fluid was higher than predicted from pKa of calcium soaps, due to formation of soluble calcium acetate; however, the relative patterns were similar to their pKa values. Unsaturated soaps are less satisfactory for maintaining normal rumen function, because dissociation is relatively higher. Calcium soaps of palm fatty acid distillate were satisfactorily stable to pH 5.5. Calcium salts of canola, soybean, and fish oils have been manufactured and marketed; because more highly unsaturated fatty acids have higher pKa, they are more completely BH [24]. The differences in pKa also means that the equilibrium between dissociated fatty acids and calcium salts tends to result in greater unsaturation in the dissociated fatty acids, with a greater proportion of saturated fatty acids in the insoluble salt [25]. Shingfield *et al.* (2006) suggested that supplementing diets with fish and soybean oil enhances milk fat cis-9, trans-11 conjugated linoleic acid content, but the high level of enrichment declines because of changes in ruminal biohydrogenation that result in trans-10 replacing trans-11 as the major 18:1 biohydrogenation intermediate formed in the rumen. In other words, BH of long-chain polyunsaturated fatty acid increases as length of time on feeding increases [4].

### **ruminal biohydrogenation and Forages role**

Forages are important to promote a healthy ruminal microbial environment and stabilize the ruminal fermentation; slow degradation of complex carbohydrates provides an even supply of fermentation products, and rumination of forages ensures a continuous supply of buffering saliva. Excessive amounts of rapidly degradable carbohydrates supplied by high grain feeding causes low ruminal pH, inhibition of cellulolytic microorganisms, decreased fiber digestion, and changes in ruminal BH. Because the BH bacteria are attached to feed particles and forages compete with microbial cells as adsorption sites for fatty acids, forages in the rumen minimize the inhibitory effect of fat on microbes. Although forages generally are low in total lipid, a high proportion (>50%) of the fatty acids in forages are LNA. Therefore, because forage constitutes a large part of the diet of grazing ruminants and lactating cows, fresh forages often are the major dietary source of LNA [1]. The fatty acid content of grass (eg. Perennial ryegrass) is very low on a dry matter basis (2–2.5%) and mainly present as esterified fatty acids. Depending on the species of grass, the FA composition varies with 55–70% as linolenic acid (18:3n-3) and 10–20% as linoleic acid (18:2n-6). On the basis of ingredients used in this study, it can be stated that cattle fed grass would have received a high proportion of the polyunsaturated fatty acid as 18:3n-3 while the short term grain-fed and long term grain-fed groups would have a high proportion of 18:2n-6 in their diets [2]. Maintaining the more favorable lipid profile in grass-fed beef requires a high percentage of lush fresh forage or grass in the ration. The higher the concentration of fresh green forages, the higher the aLA precursor that will be available for conjugated linoleic acid and n-3 synthesis. Dried or cured forages, such as hay, will have a slightly lower amount of precursor for conjugated linoleic acid and n-3 synthesis [26].

## **1- Meat Animals**

Wachira et al, (2002) reported that, red meat provides significant levels of essential polyunsaturated fatty acids, protein, vitamins and minerals in human diets. However, the type of feeding regimens used in beef cattle production can influence the level of essential fats in red meat, due to variations in the fatty acid composition of diet. Red meat from cattle and sheep fed grain is often perceived as not healthy due to presence of relatively high levels of fat in meat cuts [27]. For many people, however, meat is the only source of n-3 FA in the diet, and red meat enriched with long-chain n-3 FA could make a significant contribution to n-3 FA consumption for people consuming little or no fish [28]. There is an increasing interest in conjugated linoleic acid (CLA) from ruminant milk and meat because of the potential benefits for human health. Advances in agricultural technology with increased use of grains and formulated feedlot rations in animal feeding systems has resulted in changing levels of functional lipids, saturated and trans fatty acid content in farm animals. This, in turn, can alter the carcass components and product quality or nutritive value of the meat [2].

### **1-1- Beef**

Red meat provides significant levels of essential polyunsaturated fatty acids, protein, vitamins and minerals in human diets. However, the type of feeding regimens used in beef cattle production can influence the level of essential fats in red meat, due to variations in the fatty acid composition of diet. Red meat from cattle and sheep fed grain is often perceived as not healthy due to presence of relatively high levels of fat in meat cuts, 18 particularly saturated fatty acids, a perception that may impact on consumer choice. If the saturated FA can be reduced and replaced with FA of known health benefits, then it could be expected that consumers would look more favorably on animal products [2]. Steen et.al (2003), investigate the Effects of pasture and high-concentrate diets on the performance of beef cattle, carcass composition at equal growth rates, and the fatty acid composition of beef and reported that muscle from pasture-finished cattle had higher concentrations of omega-3 polyunsaturated fatty acids (141 versus  $49 \pm 8.2$  mg 100 g<sup>-1</sup> muscle) and long-chain omega-3 polyunsaturated fatty acid (58 versus  $27 \pm 3.8$  mg 100 g<sup>-1</sup> muscle) than muscle from concentrate-fed cattle. These results highlight the potential of high quality ryegrass pasture for finishing cattle, and meat from pasture finished cattle as a source of omega-3 polyunsaturated fatty acid [29]. Ponnampalam, et.al (2006) investigate the Effect of feeding systems on omega-3 fatty acids, conjugated linoleic acid (CLA) and trans fatty acids in Australian beef cuts: potential impact on human health. In this study, the main finding was a diet-induced change in functional FA such as n-3 FA, conjugated linoleic acid, trans fatty acids and ratio of n-6/n-3 FA. The results showed that the essential lipid components of beef could be altered by the feeding system. As noted in other studies some of the dietary ALA escapes hydrogenation in the rumen and is subsequently metabolized to eicosapentanoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA), which are found in ruminant tissues (cell membranes). Rumen bacteria (*Butyrivibrio fibrisolvens*) have the ability to produce conjugated linoleic acid as an intermediate substrate through biohydrogenation of linoleic acid (18:2n-6). It is possible that the main route for conjugated linoleic acid (cis-9, trans-11-C18:2) production in ruminants originates through the delta-9 desaturation of vaccenic acid which originates from rumen metabolism of 18:2n-6.12. [2]. Overall, grass-based systems increased concentrations of LNA and EPA by approximately 2.5-fold when expressed as grams per 100 g total fatty acids, and by approximately 4.0-fold when expressed as milligrams per 100 g muscle, respectively, compared with confinement feeding. Comparable factors for DHA are 1.5 and 2.5,

respectively. Thus, EPA appeared to increase as a function of the LNA supply, with less response for DHA. Further, concentrations of LA and other n-6 fatty acids were reduced with grass feeding systems [1]. Noci et.al (2005), reported that muscle fat and s.c. adipose tissue (SAT) fatty acid profile was improved from a human health perspective by pasture feeding, and that this improvement depended on the duration of grazing [30].

### 1-2- Lamb

Wachira et.al (2002), reported that red meat from cattle and sheep fed grain is often perceived as not healthy due to presence of relatively high levels of fat in meat cuts [27]. Ponnampalam et.al (2001), investigate the Effects of diets containing n-3 fatty acids on muscle long-chain n-3 fatty acid content in lambs fed low and medium-quality roughage diets and reported that, fish meal (80 g DM) can increase the muscle long-chain n-3 FA content and decrease the ratio of n-6/n-3 in lamb meat. Feeding soy meal (75 g DM) modestly increased both the long-chain n-3 and n-6 FA content of meat, resulting in no difference in the n-6/n-3 ratio of meat. The protected canola seed (6% DM) diet did not have a major effect on muscle n-3 FA but resulted in an increase in n-6 and the n-6/n-3 ratio of meat [28]. Research published in the early 1990s in lambs reported only the dietary manipulation of polyunsaturated fatty acid content, especially linoleic acid, an n-6 FA, and  $\alpha$ -linolenic acid, an n-3 FA, in muscle and (or) subcutaneous fat. One study reported that feeding technologically protected lipid supplements containing high levels (8% and 12%) of fish oil in feedlot rations increased muscle EPA and DHA content. There were no data reported on muscle DHA and EPA content when lambs were fed oilseed meals and (or) oil seed as a supplement.  $\alpha$ -Linolenic acid is a precursor of long-chain n-3 FA such as EPA and DHA. Feeding canola seed in a natural form to lambs reduced the  $\alpha$ -linolenic acid content of longissimus muscle. An increase in muscle linoleic acid content in lambs with soy lecithin and in cattle with protected canola seed or protected sunflower seed has also been shown. Arachidonic acid is the metabolite of linoleic acid produced by an enzymatic desaturation and elongation process. Synthesis of arachidonic acid from linoleic acid involves a rate-limiting step at the  $\Delta$ -6 desaturase and there is competition between n-3 and n-6 polyunsaturated fatty acid for the  $\Delta$ -6 desaturase enzyme. The arachidonic acid content in muscle was not affected by the marginal decrease in muscle linoleic acid content [28]. Ponnampalam et.al (2002), investigate the Effects of dietary lipid type on muscle fatty acid composition, carcass leanness, and meat toughness in lambs and reported that Lamb meat with increased levels of long-chain omega-3 FA can be produced without altering the sensory quality (flavour or aroma) of the cooked meat [31]. Ponnampalam et.al (2002) investigate dietary manipulation of muscle omega-3 and omega-6 fatty acids and sensory properties of lamb meat and reported that the fish meal and barley/fish meal diets produced larger, lean carcasses with increased concentrations of long-chain n-3 FA and decreased ratios of n-6:n-3 FA in meat. In contrast, the lupin and barley/lupin diets produced large, fatter carcasses with increased concentrations of n-6 FA and a higher n-6:n-3 FA ratio in the meat. This indicates that the type of FA in the diet contributed to energy partition in the carcass of lambs and may have overridden or added to any effects of intestinally digested protein or carbohydrate (energy). We note also that with 2-d supplementary feeding, the potential for non-steady state in energy substrates and metabolic conditions may contribute to the partitioning outcomes for energy deposition [32]. Kim et.al. (2007), investigate the Effects of dietary n-6/n-3 fatty acid ratio on feed intake, digestibility, and fatty acid profiles of the ruminal contents, liver and muscle of growing lambs. In this study investigated the effect of modifying the n-6/n-3 fatty acid ratio (FAR) of diets using linseed, soybean, and cottonseed oils in finishing lamb diets.

Total fatty acid content was 6.2% of diet DM. The animals were fed in metabolism crates for 35 d, after which they were slaughtered. Whereas LNA in foreshank muscle was greatest with the low-ratio diet (2.1% of fatty acids, decreasing to 0.9% at the widest ratio), EPA did not differ across diets, and DPA and DHA increased with an increasing ratio. Although they referred to their samples as “muscle,” the total fatty acid content was 10% of DM, indicating that the samples were predominantly adipose tissue, rather than muscle tissue [33]. Demirel et al. (2004) reported that the lambs supplemented with fish oil plus linseed oil had greater concentrations of C14:0 in the polar lipid fraction of lamb musculus semimembranosus than lambs supplemented with a Ca salt of palm oil, but this was not the case for the neutral lipid fraction [34]. Demirel et al. (2006) reported that the concentration of C18:2n-6 in longissimus thoracis of lambs could be increased dramatically from 5.0 to 11.7% of identified FA when the dietary ratio of grass hay to concentrate was changed from 75:25 to 25:75 (DM basis). This increase in C18:2n-6 was likely due to a combination of greater intakes of C18:2n-6 and a lower extent of ruminal biohydrogenation of C18:2n-6 associated with a more acidic pH in the rumen of lambs fed the high concentrate diet [35]. Cooper et al. (2004) reported concentrations of 5.6 and 16.0% C18:2n-6 (expressed as a percentage of identified FA and corrected for proportions of neutral lipids and phospholipids) in longissimus muscle of lambs fed supplemental linseed oil or oilseeds encapsulated in formaldehyde-treated protein, respectively. Their work demonstrates that muscle tissue of lambs can accumulate C18:2n-6 when C18:2n-6 that is partially protected from ruminal biohydrogenation is fed [36]. Demirel et al. (2004) provided an in depth discussion of dietary effects on changes in all fatty acids among diets and tissues [34].

## **2- Milk fat**

### **2-1- Cow milk fat**

Very long chain (VLC) n-3 polyunsaturated fatty acids are essential for growth and development and are beneficial in the maintenance of human health and the prevention of chronic diseases including cardiovascular disease, inflammatory diseases, and neurological disorders. Milk fat levels of n-3 fatty acids are typically very low, less than 0.5% of total fatty acids, and this is mainly  $\alpha$ -linolenic acid (ALA; 18:3n-3).  $\alpha$ -Linolenic acid is an essential n-3 fatty acid for some development processes; however, conversion of ALA to VLC n-3 polyunsaturated fatty acid is necessary for other physiological functions and essential for optimum health and the prevention of chronic diseases [3].

Fat and proteinaceous feed supplements should be designed to achieve optimal microbial efficiency in the rumen and to maximize digestibility and absorption of long-chain FA and proteins from the small intestine. The degree of protection of fat supplements from ruminal metabolism should be as high as possible in order to minimize their deleterious impact on microbial metabolic activities. Fat supplements should also contain a FA profile that allows the composition of milk fat to satisfy the nutritional demands of consumers and to provide the special physicochemical properties that are required for manufacturing [37]. Bernal-Santos, et. al (2010) reported that the Stearidonic acid source was genetically modified soybeans, and our results demonstrated that abomasal infusion of Stearidonic acid-enhanced soybean oil resulted in a 5-fold increase in the n-3 fatty acid content of milk fat, specifically increasing ALA, Stearidonic acid, eicosatetraenoic acid, and eicosapentaenoic acid. Relative to meeting the human dietary requirements for n-3 fatty acids, an increase in Stearidonic acid has an advantage over ALA because humans can more efficiently convert Stearidonic acid to eicosapentaenoic acid.

However, also indicate that rumen protected formulations of Stearidonic acid -enriched soybean oil would be needed to achieve increases in the n-3 fatty acid content of milk fat. Nevertheless, results from the present study clearly demonstrate the impressive potential to utilize Stearidonic acid -enhanced soybeans to achieve increases in the n-3 fatty acid content of dairy products of benefit to human health [3]. Harvatine et al (2009) reported that the polyunsaturated fatty acids are biohydrogenated in the rumen, which often results in the production of fatty acid intermediates that inhibit mammary synthesis of milk fat. These effects are less pronounced or negligible when the intake of polyunsaturated fatty acid is reduced or meal frequency is increased [39]. Bauman et al. (2006), reported that the vaccenic acid originates as an intermediate in rumen biohydrogenation of 18-carbon polyunsaturated fatty acid and it is converted to cis-9, trans-11 18:2 (conjugated linoleic acid; CLA) by the mammary enzyme  $\Delta^9$ -desaturase [40]. Hagemeister et al. (1991) investigate the effect of  $\alpha$ -Linolenic acid transfer into milk fat and its elongation by cows and reported that abomasal infusion of linseed oil to dairy cows markedly increased the milk fat content of ALA, but the increase in the EPA content of milk fat was minimal, representing only 1.3% of that observed for ALA [41]. Lock and. Bauman (2004) reported that the concentrations of conjugated linoleic acid, and to a lesser extent EPA and DHA, can be significantly enhanced through the use of diet formulation and nutritional management of dairy cows [42]. Petit, (2003) reported that the formaldehyde treatment had limited effect on milk fatty acid composition, suggesting that formaldehyde was not very effective in protecting polyunsaturated fatty acids against ruminal biohydrogenation. Feeding flaxseed resulted in the lowest omega 6 to omega 3 fatty acid ratios, which would improve the nutritive value of milk from a human health point of view. Formaldehyde treatment of flaxseed and sunflower seed had no effect on milk fatty acid composition [43]. Shingfield et.al (2006) reported that the inclusion of Fish oil and sunflower oil in the diet decreased milk fat content, but had no effect on milk yield. Decreases in milk fat content to the fish and sunflower oil diet were associated with an increase in the secretion of several biohydrogenation intermediates in milk, including trans-10 18:1, trans-10, cis-12 conjugated linoleic acid, and trans- 9, cis-11 conjugated linoleic acid. Compared with the control, the fish and sunflower oil diet reduced milk fat 4:0 to 18:0 and cis 18:1 content and increased trans 18:1, trans 18:2, conjugated linoleic acid, 20:5 n-3, and 22:6 n-3 concentrations. Milk fat cis-9, trans-11 conjugated linoleic acid responses to the fish and sunflower oil treatment were extremely rapid, but the levels of enrichment declined after d 5, responses that were associated with concomitant increases in other trans fatty acids, predominantly trans-10 18:1 and decreases in trans-11 18:1 concentrations in milk. The combined use of Fish oil and sunflower oil in the diet is an effective strategy for increasing milk fat cis-9, trans-11 conjugated linoleic acid content, but the high level of enrichment declines because of time-dependent modifications in biohydrogenation, causing trans-10 to replace trans-11 as the major 18:1 intermediate leaving the rumen. Thus, concentrations of the potentially beneficial fatty acids cis-9, trans-11 conjugated linoleic acid and trans-11 18:1 decreased over time, while the levels in milk of other trans 18:1 and trans 18:2 fatty acids of unknown biological efficacy in humans increased. If the mechanisms underlying changes in ruminal lipid metabolism to Fish oil and sunflower oil in the diet can be identified and controlled, it could be possible to develop nutritional strategies for long-term production of milk enriched with high levels of cis-9, trans-11 conjugated linoleic acid and trans-11 18:1 [4]. Whitlock, et.al (2002) reported that the Cows consumed the fish oil or fish oil with extruded soybeans diets had similar concentrations of transvaccenic acid and conjugated linoleic acid in their milk, which was more than when fed the extruded soybeans diet. Concentrations of transvaccenic acid and conjugated linoleic acid in



milk from cows fed the fish oil with extruded soybeans diet were greater than predicted by the average of values from milk produced from the fish oil and extruded soybeans diets. But this was primarily a response from the Holsteins. Brown Swiss milk inherently contained more transvaccenic acid and conjugated linoleic acid than did Holstein milk when fed a control diet but Brown Swiss milk was less responsive to dietary manipulation. The similar transvaccenic acid and conjugated linoleic acid concentrations in cows fed the fish oil and fish oil with extruded soybeans diets, and the higher concentration of transvaccenic acid and conjugated linoleic acid in the fish oil with extruded soybeans diet compared with the average of the fish oil and extruded soybeans diets, suggests that the fish oil present in the diet stimulated the conversion of the linoleic and linolenic acids present from other feeds, such as in the extruded soybeans, into transvaccenic acid and conjugated linoleic acid. Further research needs to be conducted to find the lowest level of fish oil necessary to stimulate this conversion [44]. Jenkins, (2000) reported that the oleamide was successfully synthesized from readily available ingredients and equipment, but because of its lower purity, did not increase milk cis-C18:1 to the same extent as a commercial source of oleamide. Both oleamide supplements markedly enhanced the C18:1:C16:0 ratio in milk while reducing most fatty acids of carbon length C16 or lower. Feeding oleic acid in amide form to Jersey cows offers a nutritional approach to enhancing unsaturated fatty acids in milk fat that may have beneficial functional and nutritional properties. Negative effects of oleamide on dry matter intake and milk yield also were observed in Jerseys as reported previously for Holsteins [21]. Jenkins, (1999) reports that the oleamide fed to lactating dairy cows substantially increases monounsaturated fatty acid concentration in milk primarily at the expense of palmitic acid. Changes in milk fatty acid composition were not accompanied by the appearance of amide in milk. Oleamide between 0 and 5% of the diet DM linearly reduced dry matter intake, but milk yield was not numerically reduced until diet amide concentration exceeded 2%. Therefore, dairy cows fed diets containing 2 to 3% oleamide substantially increased the milk C18:1/C16:0 ratio without greatly affecting milk yield [22].

## **2-2- Sheep and goat milk fat**

Chilliard, et.al (2003) reported that the responses of milk yield and fat content to lipid supplementation differ widely between the goat and the cow, even though the response of milk FA composition is similar, at least for major FA, including *trans*-vaccenic rumenic acid. Although the physiological and nutritional regulation of milk lipoprotein lipase activity is similar between the goat and the cow, their lipolytic systems differ. This probably explains why the physiological regulation and the husbandry factors of spontaneous lipolysis differ significantly between the two species. Peculiarities of goat milk FA composition and lipolytic system play an important role in the development of goat flavor. Quantitative and qualitative aspects of milk quality cannot always be increased simultaneously. For example, lipid supplementation could improve the efficiency of goat cheese yield and its FA profile but decrease its sensorial quality. In other respects, the polymorphism of the casein  $\alpha$ -s1 gene in goats presents an interesting model to study the mechanistic links between mammary protein and fat secretions, as well as the opposite regulations of goat milk fat and lipoprotein lipase secretion and their relationships with the development of goat flavor. The present and future knowledge on genetic, physiological, and nutritional factors regulating goat milk FA composition and lipolytic system will be helpful in the management procedures of goat husbandry to optimize the quantitative and qualitative (nutritional, sensorial, technological, etc.) aspects of goat dairy products [45].



Reynolds et al. (2006) fed lactating ewes a high-DHA algae + soybean oil supplement (24 g LA and 5 g DHA/ kg diet) with alfalfa- or corn silage based diets. Milk fatty acid composition responses to supplemental vegetable and marine oils were affected by forage source. Milk trans-C18:1 concentration was higher when corn silage was fed in Studies 1 and 2, but the effect of forage species on conjugated linoleic acid concentration differed between studies, which may reflect differences in diet polyunsaturated fatty acid content and consumption, as well as amounts of dietary starch and fiber consumed. Despite large increases in trans-C18:1 concentration, milk fat content was not decreased by feeding unsaturated oils to ewes, even at diet levels of 45 g/kg of ration DM [46].

## CONCLUSION

Essential fatty acids are fatty acids that humans and other animals must ingest because the body requires them for good health but cannot synthesize them. The term "essential fatty acid" refers to fatty acids required for biological processes, and not those that only act as fuel. In humans, not all fatty acids can be produced endogenously due to the absence of certain desaturases. Thus, specific fatty acids termed essential (linoleic, alpha-linolenic) need to be taken from the diet. Other fatty acids whose synthesis depends on essential fatty acid intake include eicosapentaenoic acid and docosahexaenoic acid. Most abundant FA with a conjugated system of double bonds are isomers of LA (conjugated linoleic acid; CLA). These FA appear in red meat and dairy products; cows grazing pasture have a several times higher content of conjugated linoleic acid in meat and milk fat than cows fed typical dairy diets. With greater interest in increasing the n-3 fatty acid content of human diets, interest has also developed to increase these fatty acids in animal products.

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