Available online at www.dergipark.gov.tr

INTERNATIONAL ADVANCED RESEARCHES
and
ENGINEERING JOURNAL

e-ISSN: 2618-575X

Journal homepage: www.dergipark.gov.tr/iarej

International Open Access

Volume 03
Issue 01

April, 2019

Review Article

The effect of dairy cow feeding regime on functional milk production

Habip Muruz [a,*] ***and Nurcan Çetinkaya*** [a]

[a] *Faculty of Veterinary Medicine, Ondokuz Mayıs University, 55139, Samsun, Turkey*

ARTICLE INFO

Article history:
Received 21 March 2018
Revised 21 October 2018
Accepted 13 January 2019

Keywords:
Functional milk
Conjugated linoleic acid
Nutrition
Dairy cow

ABSTRACT

The purpose of this paper is to evaluate nutritional strategies that will increase the concentration of the conjugated linoleic acid in milk to produce functional milk with regard to health benefits in dairy cow. Conjugated linoleic acid isomers are naturel fatty acids in foods obtained from ruminants.The main substrates for biohydrogenation are linoleic acid and linolenic acid, an essential fatty acid. It has been identified 54 different conjugated linoleic acid isomers that have beneficial biological activity. *cis*-9, *trans*-11 18:2 and *trans*-10, *cis*-12 18: are most bioactive isomers of CLA. In milk fat, the *cis*-9, *trans*-11 conjugated linoleic acid is found in major amounts more than *trans*-10, *cis*-12 conjugated linoleic acid. Conjugated linoleic acid is first produced as an intermediate product during the biohydrogention in the rumen of dietary linoleic acid and linolenic acid. Another major pathway of conjugated linoleic acid synthesis in dairy cows is endogenous synthesis in the mammary gland. Dietary *cis*-9, *trans*-11 conjugated linoleic acid is of great interest due to its health benefits known to a cancer chemopreventive and antiatherogenic. Therefore, many researchers have looked for ways of increasing the amount of conjugated linoleic acid in cow milk. For this pupurpose, researchers have adopted two approaches. The first approach is to make dietary changes to increased the natural conjugated linoleic acid production of cow. The second approach is to feed with mixtures of conjugated linoleic acid isomers protected against microbial biological hydrogenation in the rumen. As a result, as consumers continue to be aware of the relationship between diet and health, increased conjugated linoleic acid level of milk may provide new market opportunities for milk and milk products as functional food.

1. Introduction

Dairy products from ruminants are the most important sources of nutrient quality protein, energy, minerals and vitamins. Recent studies have shown that dairy products have many bioactive compounds with associated health effects for the consumer beyond simple nutrition [1]. The Conjugated linoleic acid (CLA), which cannot be synthesized by the human body, is mostly found in the raw milk derived from cows. The discovery of a "functional food" role for CLA occurred over the past decade. CLA is considered an essential nutrient for human. Therefore, this fatty acid, human can only obtain from ruminant products [2]. Data from several studies in both animal and human models suggest that CLA has several the physiological properties such as anticarcinogenic, antilipogenic, antidiabetic, modulate immune function and inflammatory response, antihypertensive, antiatherosclerotic [3].

The term CLA refers to a group mixture of positional and geometric isomers (*cis*, *trans*) of linoleic acid or linolenic acid that have conjugated double bonds. It has been determined that great numbers of isomers of CLA found in milk and meat products [4], but *cis*-9, *trans*-11 18:2 (*c*9, *t*11 CLA), also called rumenic acid, and *trans*-10, *cis*-12 18:2 (*t*10, *c*12 CLA) are most bioactive isomers of CLA [5]. It reported that physiological effect of *c*9, *t*11 CLA is principal anticarcinogenic, while *t*10, *c*12 CLA isomer is antidiabetic, antiobese and anticarcinogenic [6].

A portion of CLA is produced uncompleted ruminal biohydrogenation of linoleic acid and another portion derives from Δ-9 desaturase on vaccenic acid (C18:1 *trans*-11) (occurs during biohydrogenation linoleic acid, linolenic acid and oleic acid) within mammary gland and

* Corresponding author. Tel.: *+90 362 312 1919*; Fax: *+90 362 457 69 22*
E-mail addresses: habip.muruz@omu.edu.tr *(H. Muruz)*, nurcanc@omu.edu.tr *(N. Çetinkaya)*
ORCID: *0000-0002-1975-4545 (H. Muruz), 0000-0002-9977-2937 (N. Çetinkaya)*

in the other body tissues [7,8]. McCrorie et al. [9] demonstrated that the CLA contentent in milk fat ranged from 2 to 37 mg/g fat due to breed, species, lactation period, age, feeding strategy and the fatty acid composition of diet [10]. However, among these factor, diet plays central role on CLA composition in milk fat [11].

In this review, dietary change to increased the natural CLA production in dairy cows was be discussed here.

2. The Fatty Acids in Cow Milk Fat

Whole cow's milk contents 3.2-4.7% fat depending on particularly feeding strategies (generally increased with increasing fiber content) and lactation stage. Approximately 98% of the total milk fat consist of triglycerides. Other milk lipids include: diacylglycerides (0.25-0.48%); monoacylglycerides (0.02-0.04%); phospholipids (0.6-1.0%); cholesterol (0.2-0.4%); glycolipids (0.006%); and FAs in milk (0.1-0.4%). Milk fat contains saturated FA (SFA), monounsaturated FA (MUFA), and polyunsaturated FA (PUFA), approximately 70% , 25 and 5, respecitively, but this can be modified bu changing the animal diet [12]. PUFA includes linolenic acid (C18:3 n-3) and rumenic acid (C18:2 *cis*-9, *trans*-11). Milk MUFA consist mainly of oleic acid (C18:1 *cis*-9) and also trans vaccenic acid (C18:1 *trans*-11). Both rumenic and vaccenic acid are *trans*-11 FA produced by rumen microorganisms. There are approximately 400 different types of FA [13].The milk FAs, which range from 4 to 20C chain length, are derived from the feed and the microbial biohydrogenation in the rumen. The FAs in ruminant milk are synthesized either mammary gland (carbon chains: <C15 and a portion of C16) from acetate and to a lesser extent from β-hydroxybutyrate or approximately one half of the FAs (a portion of C16 and >C17) from dietary lipids and adipose tissue reserves [14].

CLA, which is unsaturated FAs and constitute up to 5% of all FAs [15]. Up to now, total of 54 different isomers of CLA was identified [16]. Within this group, *c*9, *t*11 CLA and t10, *c*12 CLA are believed to be the most common natural two isoforms of the group of CLAs [17]. It has been shown that CLAs are formed both during the biohydrogenation of PUFA originated from the diet in the rumen and mammary synthesis from vaccenic acid [18]. The *c*9, *t*11 CLA isomer is found in more abundant (72 to 94% of total CLA) than the *t*10, *c*12 CLA in milk and meat products [19, 20].

3. The Fatty Acid Composition of Plants

Unlike short and medium-chain FA, long chain C18 FA can not be endogenously synthesized by ruminants desired in meat and milk. For this reason, these FA have to be ingested by feed ruminant.

The main substrates for ruminal biohydrogenation is C18 linoleic acid and linolenic acid. The lipids in the ruminant feeds are mainly trigliserides as well as in lower proportion phospholipids and galactolipids. The lipid composition of forage is amply composed of glycolipids and phospholipids, and the main FA is linolenic acid which is unsaturated fatty acids, while seed oils is largely triglycerides containing linoleic and [21]. It has been shown that the lipid fraction ranged from 30–100 g kg^{-1} DM in the leaves of grasses and grasses. [22]. However, the lipids present in plants are not static structures, but are constantly expose to turnover due to the lipid degradation in living plant by normally present lipases [22].

As shown in Table 1 [23], the FA profile of lipids in feeds is highly variable. Linolenic acid levels generally depen on plant and environmental factors such as wilting prior to ensiling, hay and haylage making, stage of maturity and light intensity. The pre-wilting process of ensiling causes about 40% reduction in total fatty acid level, 40% loss even for linolenic acid. An ensiling process and silage additives (formic acid) led to smaller losses. In contrast, the use of hay and haylage reduced total FA by 50% and 70%, respectively. [22]. It reported that nitrogen fertilization was significantly increased of palmitic acid (18%), linoleic acid (12%) and linolenic acid (40%) in the herbage [24].

Many of the oils seeds are rich in linoleic, accounting for 53 to 69% of total FA, and but its composition is considerably variable. For example, in ground nuts, rape seed and sesame seed: high in oleic acid; cotton seed, soybeans and sunflower seeds: high in linoleic acid and linseed: high in linolenic acid. Fish oil, the richest source FA of 20 or 22 C, contains relatively low amounts of linoleic and linolenic [25].

Table 1. Fatty acid content of common dairy cow feeds [25]

Feed	Oleic	Linoleic	Linolenic	Other
	-fatty acid, % of total reported FAs-			
Pasture				
Grass	2.2	20.4	55.9	0.0
Clover	3.6	21.1	48.2	0.0
Grass+legume	4.2	18.9	51.6	0.0
Silage				
Grass	6.3	14.5	46.2	0.0
Corn	18.9	40.9	6.1	13.8
Hay alfalfa	4.9	18.1	23.5	25.0
Concentrates				
Barley	20.5	43.3	4.3	1.9
Corn	30.9	47.8	2.3	0.0
Oats	38.1	34.9	2.1	0.5
Wheat	17.5	55.8	4.5	0.2
Byproduct				
Gluten meal	26.7	53.0	1.4	0.0
Distillers grains	24.2	54.5	1.8	1.2
Plantseed/oils				
Soybean	23.3	54.5	5.9	1.5
Extruded soybean	19.5	53.2	9.1	0.0
Extruded cottonseed	16.5	57.4	0.0	0.0
Sunflower	21.2	69.4	0.0	0.0
Peanut	51.5	30.2	0.0	2.8
Linseed	22.7	15.4	51.4	0.0
Fish oil	21.0	2.0	1.0	32.0
Animal tallow	45.9	5.9	0.3	0.0

4. Synthesis of CLA in the Dairy Cows

In the ruminal biohydrogenation of lipid, ruminal bacteria are play a key role and the formation lipid in the rumen occurs in two important step: The initial step is lipolysis, relaesing free FAs with bacterial lipases of dietary lipids entering the rumen [26]. Another step is biohydrogenation of FAs by rumen bacteria to produce saturated [1]. The main substrates for biohydrogenation are linoleic acid and linolenic acid. The first linoleic is rapidly converted to *c*9, *t*11 CLA in the rumen, except for linolenic acid, afterwards *c*9, *t*11 is converted to C18:1, *t*11 vaccenic acid during biohydrogenation process. C18:1, *t*11 vaccenic acid is then reduced to stearic acid as the end product. As for linolenic acid, this is transformed to C18:2 *t*11, *c*15 after that C18:2 *t*11, *c*15 is converted to C18:1, t11 vaccenic acid. Similarly, vaccenic acid is reduced to stearic acid (Figure 1). Both linoleic acid and linolenic acid are converted to vaccenic acid which is the common intermediate product in the rumen during biohydrogenation. Generated vaccenic acid is leaving the rumen and is absorbed across the small intestine, and incorporated into milk fat. In the mamary gland, vaccenic acid is converted to *cis*-9, *trans*-11CLA by action of the Δ-9 desaturase enzyme [27]. According to Corl et al. [28], endogenously synthesized CLA in mammary gland is about 78% of the CLA in milk fat. Therefore, the key to increasing CLA concentration in milk is to increase vaccenic acid production in the rumen [1].

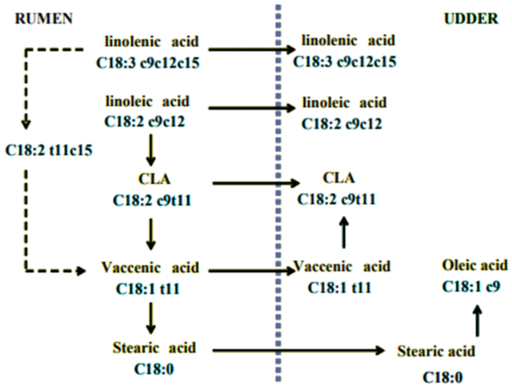

Figure 1. Simplified scheme of biohydrogenation and desutaration pathways of C18 fatty acids in rumen and udder of dairy cow [27]

5. The Feeding Regime to Increase the Content of CLA in Milk

A number of of factors are known to affect the level of milk fat CLA; these are presented in Table 2. However, the dietary factors affecting the content of CLA in milk could be grouped into two categories [29]. (a) The first approach is to make dietary changes: the use of lipid sources as pasture (rich in linoleic) and and vegetable fats (rich in linoleic and linolenic acid). (b) The secund approach is to feed with mixtures of CLA isomers in the rumen protected. In Table 2 are shown the nutritional factors that affect CLA amount in milk fat. Dietary supplementation of plant oils (sunflower, soybean, corn, canola, linseed, and peanut) have been shown to increase CLA in milk fat due to give the greatest response plant oils high in linoleic acid [31], and this response is a dose-dependent.

In generally, ruminant diets no include the plant oils because they has potentially toxic effects on rumen microbial growth. To minimize this effect, Ca salts of the FAs is added to diet so as to bypass from rumen and only a small part are exposed to biohydrogenation. Plant oils, which is unsaturated, have been found to increase CLA in milk fat more than saturated animal fat due to the available lipid substrate for biohydrogenation [30].

Table 2. Dietary factors that affect content of CLAs in milk fat [18, 23, 30]

Dietary factor	Content of CLA
I. Lipid substrate	
Unsaturated	Increased
Plant oils	type, level and dose-dependent increase
High-oil plant seeds	
Raw seeds	No effect
Processed seeds	Increased over raw seeds
Calcium salts of plant oils	Increased with increasing amounts
High-oil corn grain and silage	Minimal effect
Animal fat by-products	Minimal effect
Fish oil	Increased in relation to level fed in the diet
II.Forage:concentrate ratıo	Increased with high forage diet
III.Hıgh forage diet	Increased
VI.Nonstrustural carbohydrate level	Minor effect
V.Low energ diets	Probably positive
VI.Pasture related	
Pasture versus TMR	Increased with consumption of pasture
Fresh cut pasture	Higher than conserved forages
Fresh/Green	Hıghly positive
Pasture+ full fat extruted soybean	No effect
Pasture+Soy oil	No effect
Pasture+Fish oil	Increased
Diversity in plant species	Increased
Maturity of forage	Increased with less mature forage
Elevation of pasture	Highland>mountain>lowland
VII.CLA supplement	Dose-dependent increase

As another method, fat seeds are added to diet [21]. However, the result of researches have showed that feeding full-fed seeds have not effect on the concentration of CLA in milk fat due to unavialable by rumen bacteria responsible for biohydrogenation of the PUFA in these seeds [32].

Fish oils, containing mainly FA of 20 or 22 C as the main FA, produced to a higher CLA than an evenly of plant oils [22, 33]. A meta-analysis of comparison of averages of dry matter intake (DMI), milk yield and composition, and CLA in milk of different fat sources and pressessing method suggested that the best strategy to enrich milk with CLA were the combination of fish oil and vegetable fats (395 mg of cis-9, trans-11CLA/l vs. 188 mg of cis-9, trans-11 CLA/l; increase of 2.1 times) (Table 3) [34].

Data from several studies suggest that milk CLA content significantly increased in dairy cows feeding with processed seeds than feeding unprocessed seeds [33], but for extruded seeds [37]. Recent evidence suggests that

Table 3. The effectt on milk yield and composition of dietary ingredient [34]

	Treatments										
	Control	Fresh Pasture	Rapeseed	Corn	Soybean	Sunflower	Linseed	Fish Oil	Fish Oil+Vegetable Fats	SEM	P-Value
Total fatty acids, g/kg DM	30.0d	25.9d	54.1ab	44.1bc	49.0ab	59.3a	55.2ab	37.1cd	45.5abc	1.78	<0.01
DMI, Kg/d	21.3ab	19.0	21.1	21.8	20.4	19.6	20.8	21.0	20.9	0.51	<0.01
Milk yield, Kg/kg	31.1a	27.0c	29.6ab	32.4a	30.6ab	28.8bc	29.2b	31.0ab	31.4ab	0.87	<0.01
Fat, g/Kg	36.1a	38.0a	34.2ab	34.4ab	33.2b	33.6b	36.5a	32.2b	31.2b	00.63	<0.01
Fat yield, Kg/d	1.11c	0.94ab	1.04ab	1.13a	1.02ab	0.94b	1.07a	1.03ab	1.01ab	0.039	<0.01
cis-9,*trans*-11CLA,	0.61c	1.13ab	0.83bc	0.84bc	1.00ab	1.04ab	0.90b	0.67bc	1.34a	0.044	<0.01
cis-9,*trans*-11CLA,g/d	5.67c	8.56abc	7.78bc	8.75ab	9.24ab	10.1ab	8.50bc	5.91c	12.4a	0.399	<0.01

Menas within a row differ with treatment (p<0.05)

extrusion of oil seeds (0.57 vs. 1.11 g of CLA/100 g of FA) and oils (0.57 vs. 1.10 g of CLA/100 g of FA) are the best processing method to increase milk CLA, and but extruded seeds and oils decreased milk yield (30.4 vs. 28.9 kg/d) and milk fat (36.1 vs. 33.1 g/kg), respectively [34].

In general, high consumption of pasture shows a higher effect on CLA amount in milk fat compared with diets rich in silage, hay, concentrate feeds [14] and total mixed rations (TMR) based on conserved forage and grain [38].

Table 4. Feeding pasture versus total mixed ration (TMR) on the level of CLA in milk fat [30]

Research Location	TMR mg CLA/g fat	Pasture mg CLA/g fat
Penn State	5.4	10.9
Wisconsin	5.6	22.7

A study at Penn State and Wisconsin demonstrated that a two-fold and four-fold, respectively increase in CLA with pasture (Table 4) [30]. Previous studies have reported that grazing has contributed to the increase in CLA due to probably enables selection of leafy plant parts with higher lipid concentration.

This view is supported by Dhiman et al. [25] who writes that milk CLA content of grazing animals presented 500% greater than animals with fed with 50:50 concentrate and conserved forage diet. Previous research comparing total mixed ration (TMR), pasture + TMR and pasture plus concentrate has found that feeding pasture plus TMR and pasture plus concentrate were enhanced the CLA [30]. Similarly, a recent study by Marin et al. [39] observed that cows with more intensive grazing and low levels of concentrate presented higher levels of CLA and omega-3 fatty acids than cows with low grazing and higher concentrate. Forage maturity is also a significant impact on CLA content. Previous research has establish that diets containing forage at less mature forage was increased milk fat CLA compared to late-growth [40].

Dietary supplements of CLA can also increase the CLA content. Chouinard et al [36] suggested that supplements of CLA isomers to dairy cow diet, mainly consisting of *cis*/*trans* 9, 11; *cis*/*trans* 10,12, and *cis*/*trans* 11,13, were transferred to milk fat. However, these supplements also caused a reduction in the milk fat content [14]. Therefore, it recommended that CLA isomers are protected from ruminal biohidrogenation. In other hand, as plant oils increased in diets, milk fat tended to decrease, generally so called as milk fat depression [21]. In a study on the effect of different fatty acids on the CLA content of milk, *cis*-9, *trans*-11 CLA was higher in diets rich in linoleic acid compared with control and diets rich in linolenic acid, and lower in control compared with other diets (Table5) [34].

6. Conclusion

Cows' milk fat is the richest natural source of CLA, which is produced in the rumen and mammary gland. Milk fat is naturally included many isomers of CLA, but *c*9, *t*11 is predominant. Diet is a key factor that could be enhanced CLA content in milk fat. Allowing dairy cows to graze pasture, vegetable fats, dietary fish oil supplementation, feeding the fat as a calcium salt and encapsulation of the CLA isomers is the best strategy to increase the CLA content in milk

Table 5. The Effect on milk yield and composition of different fatty acids. [34]

	Treatments					
	Control	Oleic acid	Linoleic acid	Linolenic acid	SEM	P-Value
Total fatty acids, g/kg DM	28.8	52.6	50.1	50.3	2.03	<0.01
DMI, Kg/d	21.1	21.0	20.2	20.7	0.55	<0.01
Milk yield, Kg/kg	30.0	30.1	30.1	28.8	0.92	<0.01
Fat, g/Kg	36.3	34.4	33.9	36.8	00.64	<0.01
Fat yield, Kg/d	1.11	1.06	1.02	1.05	0.040	<0.01
cis-9,*trans*-11CLA, g/100g fatty acids	0.62	0.82	1.00	0.83	0.044	<0.01
cis-9,*trans*-11CLA,g/d	5.79	7.82	9.66	7.54	0.390	<0.01

References

1. Badinga, L. and Miles R.D. *Adding value to milk by increasing its conjugated linoleic acid content.* AN265, one of a series of the Animal Sciences Department, UF/IFAS Extension. Original publication date July 2011. Reviewed October.
2. Benjamin, S., Prakasan, P., Sreedharan, S., Wright, A.D. and Spener, F. *Pros and cons of CLA consumption: an insight from clinical evidences.* Nutr Metab. 2015. **12**: p.4–23.
3. Du, R., Zhong, T., Zhang, W.Q., Song, P., Song, W.D., Zhao, Y., C. Wang, Tang, Y.Q., Zhang, X. and Zhang, Q. *Antitumor effect of iRGD-modified liposomes containing conjugated linoleic acid-paclitaxel (CLA-PTX) on B16–F10 melanoma*. Int J Nanomedicine. 2014. **9**: p. 3091–3105.
4. Delmonte, P., Roach, J., Mossoba, M., Losi, G. and Yurawecz, M. *Synthesis, isolation, and GC analysis of all the 6, 8-to 13, 15-cis/trans conjugated linoleic acid isomers*. Lipids. 2004.39: p. 185–191.
5. Pariza, M. W., Park, Y. and Cook, M. E. *The biologically active isomers of conjugated linoleic acid. Prog*. Lipid Res. 2001. **40**: p. 283–298.
6. Koba, K., Yanagita, T. *Health benefits of conjugated linoleic acid (CLA*). Obes Res Clin Pract. 2014. **8**(6). (Abstr).
7. Mosley, E.E., Powell, G.L., Riley, M.B,. Jenkins, T.C. *Microbial biohydrogenation of oleic acid to trans isomers in vitro*. Journal of Lipid Research. 2002. **43**: p. 290–296.
8. Rodriıguez-Alcala, L. M., Braga, T., Xavier Malcata, F., Gomes, A., Fontecha, J. *Quantitative and qualitative determination of CLA produced by Bifidobacterium and lactic acid bacteria by combining spectrophotometric and AgC -HPLC techniques*. Food Chem. 2011. **125**: p. 1373–1378.
9. McCrorie, T.A., Keaveney, E.M., Wallace, J.M.W., Binns, N., Livingstone, M.B.E. *Human health effects of conjugated linoleic acid from milk and supplements*. NutrResRev. 2011. **24**: p. 206–227.
10. Nagpal, R., Yadav, H., Puniya A.K., Singh, K., Jain, S. and Marotta, F. *Conjugated lınoleıc acıd: sources, synthesıs and potentıal health benefıts- an overvıew.* Curr Top Nutraceutical Res. 2007. **5**: p. 55-66.
11. Khanal, R.C and Olsen, K.C. *Factors affecting conjugated linoleic acid (CLA) content in milk, meat and egg: A review*. Pakistan Journal of Nutrition. 2004. **3(2):** p**.** 82-98.
12. Grummer, R.R. *Effect of feed on the composition of milk fat*. J Dairy Sci. 1991. **74**: p.3244–3257.
13. Gottardoa, P., Penasab, M., Righic, F., Lopez-Villalobosd, N., Cassandrob, M., De Marchi, M. *Fatty acid composition of milk from Holstein-Friesian, Brown Swiss, Simmental and Alpine Grey cows predicted by mid-infrared spectroscopy*. Italy J AnimSci. 2017. **16**(3): p. 380–389.
14. Harvatine, K.J., Boisclair, Y.R., Bauman, D.E. *Recent advances in the regulation of milk fat synthesis*. Animal. 2009. **3**: p. 40-54.
15. Glasser, F., Schmidely, R., Sauvant, D., Doreau, M. *Digestion of fatty acids in ruminants: a metaanalysis of flows and variation factors: 2. C18 fatty acids*. Animal. 2008. **2**: p. 691-704.
16. Delmonte, P., Roach, J., Mossoba, M., Losi, G. and Yurawecz, M. Synthesis, isolation, and GC analysis of all the 6, 8-to 13, 15-cis/trans conjugated linoleic acid isomers. Lipids. 2004. **39**: p. 185–191.
17. SamkováI, E., SpickaI, J., Pesek, M., PelikánováI, T., Hanus, O. *Animal factors affecting fatty acid composition of cow milk fat: a review.* S Afr J Anim Sci. 2012.**42**: p. 83-100.
18. Bauman, D.E., Perfield, J.W.I., Harvatine, K.J. and. Baumgard, L.H. *Regulation of fat synthesis by conjugated linoleic acid: lactation and the ruminant model*. J Nutr. 2008. **138**: p. 403–409.
19. Bhattacharya, A., Banu, J., Rahman, M. *Biological effects of conjugated linoleic acid in health and disease.* J Nutr Biochem. 2006. **17**: p. 789-810.
20. Park, Y. and Pariza, M.W. *Mechanisms of body fat modulation by conjugated linoleic acid (CLA)*. Food Res Int. 2007. **40**: p. 311-323.
21. Bauman, D.E., Baumgard, L.H., Corl, B.A., Griinari, J.M**.** *Biosynthesis of conjugated linoleic acid in ruminants*. J Anim Sci. 1999. p. 1-15.
22. Elgersma, A., Wever, A.C., Nałęcz-Tarwacka, T. *Grazing versus indoor feeding: effects on milk quality*. Grassland Science in Europe. 2006. **11**: p.419-427.
23. Kalac, P. and Samková E.*The effects of feeding various forages on fatty acid composition of bovine milk fat: A review*. Czech J Anim Sci. 2010. **12**: p. 521–537.
24. Boufaied, H., Chouinard P.Y., Tremblay, G.F., Petit, H.V., Michaud, R., Belanger G. Fatty acids in forages. I. Factors affecting concentrations. Can J AnimSci. 2003. **83**: p. 501-511.
25. Dhiman, T., Nam, S. and Ure, A. 2005. *Factors affecting conjugated linoleic acid content in milk and meat.* Crit Rev Food Sci Nutr. 2005. **45**(6): p. 463–482.
26. Dawson, R.M., Hemington, N. and Hazlewood, G.P. *On the role of higher plant and microbial lipases in the ruminal hydrolysis of grass lipids*. Br J Nutr. 1977. **38**:p. 225-232.
27. Bauman, D. and Griinari, J. *Nutritional regulation of milk fat synthesis*. Annu Rev Nutr. 2003. **23**: p. 203–227.
28. Corl, B.A., Baumgard, L.H., Dwyer, D.A., Griinari, J.M., Phillips, B.S., and Bauman, D.E. *The role of delta(9)-destaurase in the production of cis-9, trans-11 CLA*. J Nutr Biochem. 2011.**12**: p. 622–630.
29. Whitlock, L.A., Schingoethe, D.J., AbuGhazaleh, A.A., Hippen, A.R. and Kalscheur, K.F. *Milk production and composition from cows fed smallamounts of fish oil with extruded soybeans*. J Dairy Sci. 2006. **89**: p. 3972–3980.
30. Muller, L., Delahoy. *Conjugated linoleic acid (CLA) in animal production and human health. Penn State Extension*. [cited 2018 05 March]; Available from: https://extension.psu.edu/conjugated-linoleic-acid-cla-in-animal-production-and-human-health.

31. Kelly, M.L., Berry J.R., Dwyer, D.A., Griinari, J.M., Chouinard, P.Y., Van Amburgh, M. E. and Bauman D. E. *Dietary fatty acid sources affect conjugated linoleic acid concentrations in milk from lactating dairy cows*. J Nutr. 1998. **128**: p. 881-885.
32. Khanal, R.C and Olsen, K.C. *Factors affecting conjugated linoleic acid (CLA) content in milk, meat and egg: A review*. Pak J Nutr. 2004. **3(2):** p**.** 82-98.
33. Chouinard, P.Y., Corneau, L., Bauman, D.E., Butler, W.R., Chilliard, Y. and Drackley, J.K. *Conjugated linoleic acid content of milk from cows fed different sources of dietary fat*. J Dairy Sci. 1998. **81**(Suppl. 1): p. 233 (Abstr.).
34. Siurana, A., Calsamiglia, S. *A meta analysis of feding strategies to increase the content of conjugated linoleic acid (CLA) in dairy cattle milk and the impact on daily human consumption.* Anim Feed Sci Technol.2016. **217**:p 13–26.
35. Ward, A.T., Witlenberg, K.M., Froebe, H.M., Pryzbyiski, R and Malcolmson, L. *Fresh forage and solin supplement on conjugated linoleic acid levels in plasma and milk*. J Dairy Sci. 2003. **86**: p. 1742-1750.
36. Siurana, A. and Calsamigli, A. *Metaanalysis of feeding strategies to increase the content of conjugated linoleic acid (CLA) in dairy cow milk and the impact on daily human consumption.*Anim Feed Sci Technol. 2016**. 217**: p. 13-26.
37. Chouinord, P.Y., Corneau, L., Barbano, D.M., Metzger, L.E. and Bauman, D. E. *Conjugated linoleic acids alter milk fatty acid composition and inhibit milk fat secretion in dairy cows*. J Nutr. 1999. 129:p. 1579-1584.
38. Dhiman, T.R., Anand, G.R., Satter, L.D. and Pariza, M.W. *Conjugated linoleic acid content of milk from cows fed different dietas.* J Dairy Sci. 1999. **82**: p. 2146-2156.
39. Marína, M. P.,Meléndez, P. G., Aranda, P. and Ríos, C. *Conjugated linoleic acid content and fatty acids profile of milk from grazing dairy cows in southern Chile fed varying amounts of concentrate.* J Appl Anim Res. 2018. 46(1): p. 150–154.
40. Chouinard, P.Y., Corneau, L., Kelly, M.L., Griinari, J.M. and Bauman D.E. *Effect of dietary manipulation on milk conjugated linoleic acid concentrations.* J Dairy Sci. 1998. **81**(Suppl. 1): p. 233 (Abstr.).