Journal of Food and Nutrition Research, 2014, Vol. 2, No. 8, 506-509
Available online at http://pubs.sciepub.com/jfnr/2/8/12

DOI:10.12691/jfnr-2-8-12

Cheddar Cheese from Cow Milk with Elevated Conjugated Linoleic Acid Levels

Mian Anjum Murtaza[1,*], Nuzhat Huma[2], Zafar Hayat[3], Mian Shamas Murtaza[2], Asif Meraj[1]

[1]Institute of Food Science and Nutrition, University of Sargodha, Sargodha, Pakistan
[2]National Institute of Food Science and Technology, University of Agriculture, Faisalabad, Pakistan
[3]Department of Animal Sciences, University of Sargodha, Sargodha, Pakistan
*Corresponding author: anjum_ft@yahoo.com

Received August 04, 2014; Revised August 10, 2014; Accepted August 14, 2014

Abstract The aim of the study was to evaluate the effect of feed management on conjugated linoleic acid (CLA) level in milk and subsequently on fatty acid profile of cheese. Twenty cows were divided into 2 groups, fed on grass silage and pasture feed with sunflower supplementation for 14 days. Milk samples were collected and analyzed for composition and fatty acid profile using gas chromatograph. Cheddar cheese was manufactured from both milk types and ripened for 120 days. During ripening, it was analyzed for composition and fatty acid quantification. The supplemented diet significantly increased the CLA content in milk. Similarly the cis-9, trans-11 CLA content in Cheddar cheese were more than twofold in case of supplemented diet as compared to grass silage. During ripening, CLA concentration remained stable. The sunflower supplementation showed no effect on cheese composition. Hence, supplemented pasture resulted in elevated CLA content in milk compared to grass silage.

Keywords: *milk, fatty acids, cheddar cheese, conjugated linoleic acid*

Cite This Article: Mian Anjum Murtaza, Nuzhat Huma, Zafar Hayat, Mian Shamas Murtaza, and Asif Meraj, "Cheddar Cheese from Cow Milk with Elevated Conjugated Linoleic Acid Levels." *Journal of Food and Nutrition Research* vol. 2, no. 8 (2014): 506-509. doi: 10.12691/jfnr-2-8-12.

1. Introduction

Cheddar is a hard type of cheese having high nutritional value owing to the concentration of caseins, fat, minerals and vitamins [1,2]. It is a biochemically active product that experiences momentous changes during ripening[3].It undergoes a multifarious chain of microbial and enzymatic reactions responsible for the characteristic flavor and texture [4,5].

Milk composition is an imperative parameter that influences the quality of Cheddar cheese and affects the development of discrete flavor and texture properties all through manufacturing and ripening [6,7]. It is influenced by individuality, breed, species, and stage of lactation, health, nutritional status and diet management of producing animals [8,9]. Diet management plays a key role in modulating the milk composition particularly fatty acid profile of ruminant milk [10].

Milk fat is accountable for hypercholesterolemia since it comprises the cholesterol and saturated fatty acids [11]. The consumption of saturated fatty acids sources the elevation in low density lipoprotein. It is obvious that there is a positive correlation between the high cholesterol and the arteriosclerosis sickness [12].

A recent inclination of the food market is to offer products with lower or modified fat profile [13]. Milk fatty acid profile can be tailored by directly adding fatty acid supplements to milk and its products, by manipulating the genetic, or by providing particular diets to animals [14]. Directly adding supplements is the simplest technique to get desired level of fatty acids however; its shortcoming is odd flavors associated with sources [8,14].

Conjugated linoleic acid (CLA) refers to the set of positional and geometric isomers of linoleic acid having conjugated double bonds in the chain. Dairy products and meat from ruminants are the richest natural sources of CLA [15]. It is a natural component of milk fat. The hydrogenation of linoleic and linolenic acids by microbes is mainly responsible for its production in the rumen during the formation of stearic acid [16]. CLA shows positive impacts on human health like improved immunity, anticancer and antiatherogenic actions and lessening the body fat [17]. Its existence in human body is mostly coupled with ingested foods rich in CLA as compared to its endogenous synthesis [18]. Hence, CLA concentration should initially be elevated in rumen milk through diet management in order to naturally supplement the dairy products with CLA.

Keeping the above facts in view, the study was designed with the objective to assess the influence of feeding sunflower oil on CLA content in milk fat and its subsequent impact on fatty acid profile of Cheddar cheese during ripening.

2. Materials and Methods

2.1. Diet Management and Milk Collection

Twenty dairy cows of almost parallel calving dates and milk yield were fed full time on pasture for 96 days and had received no supplement. Then these were divided into 2 groups (10 cows in each group), one was fed on grass silage and typical concentrate (control) and other on pasture feed and supplement containing sunflower oil @ 100 g/kg (Suppl.) for next 14 days. Milk samples for analysis and cheese manufacturing were collected on 14th day from each cow. The milk was pooled and representative (for analysis) as well as bulk (for cheese) samples were taken in triplicate.

2.2. Milk Composition

Basic composition of milk was measured following the standard procedures given by AOAC [19]. Moisture content was determined by oven drying, protein by Kjeldahl method, fat content using Gerber method, pH was measured with a pH meter (inoLab WTW Series 720)after calibrating with fresh pH 4.0 and 7.0 standard buffers and lactose contents using Fehling solutions.

2.3. Milk Fatty Acid Analysis

Milk fat samples were obtained as described by Murphy et al [20]. These samples were methylated by acid-catalyzed methylation to make fatty acid methyl esters (FAME) following the protocol given by Stanton et al [21]. Fatty acids were quantified using a gas chromatograph (Varian, Harbor City, CA), having a flame-ionization detector. The data was analyzed using Varian software system 4.5. Fatty acids were identified with reference to retention times of standards (Sigma-Aldrich, St. Louis, MO) and quantified with regard to the internal standards. The results were calculated in g/100 g of total FAME.

2.4. Cheddar Cheese Manufacturing

Milk was standardized (fat to protein ratio 0.97:1.00) and used for Cheddar cheese manufacturing with mesophilic starter cultures and rennet (Chr. Hansen Ireland Ltd., Cork, Ireland) following the standard protocol described by Scott [22]. The samples were stored for ripening at 6-8°C for 120 days.

2.5. Cheese Composition

Fresh cheese was analyzed for moisture, protein, fat, salt and pH value following the protocols of AOAC [19]. Moisture content was determined by oven drying, protein by Kjeldahl method, fat content using Babcock method and salt content by Vol hard method. The pH of cheese slurry prepared by blending 20 g of grated and shredded cheese sample with 12 ml of water, was measured with a pH meter (inoLab WTW Series 720)after calibrating with fresh pH 4.0 and 7.0 standard buffers.

2.6. Cheese Fatty Acid Profile

Cheese was sampled for fatty acid profile at 0, 60 and 120 days of ripening. 15 g of grated cheese was mixed with 25 g freshly prepared of ammonium thiocyanate solution (30% wt/volume). Then it was placed at at 60°C for 60 minutes in water bath and centrifuged at 538 g for 20 min. The fat layer was separated and stored at 4°C. Methyl esters of fatty acid were prepared and quantified as described above [21].

2.7. Statistical Analysis

The results obtained for composition and fatty acid quantification of milk and cheese were analyzed statistically using SAS and procedures described by Steel et al [23].

3. Results and Discussion

3.1. Milk Composition

Compositional analysis of raw milk revealed that supplementation of sunflower significantly decreased the fat and subsequently increased the protein and lactose concentrations (Table 1). These findings are in accordance with the results reported by Kay et al [24].

Table 1. Composition of raw milk and cheese

Parameters	Milk		Cheese	
	Control	Suppl.	Control	Suppl.
Moisture %	87.4	87.5	40.28	39.84
Protein %	3.20	3.45	25.88	26.04
Fat %	3.70	3.05	29.12	29.28
pH	6.67	6.66	5.12	5.10
Lactose	4.60	4.68	-	-
Salt %	-	-	1.28	1.30

3.2. Milk Fatty Acids

Table 2. Fatty acid profile of raw milk

Fatty acid (g/100 g)	Control	Supplemented
C4:0	1.51	1.53
C6:0	1.56a	1.20b
C8:0	1.22a	0.79b
C10:0	2.89a	1.78b
C12:0	3.61a	2.22b
C14:0	12.8a	9.35b
C14:1	1.42	1.37
C15:0	1.72a	1.10b
C16:0	34.9a	20.54b
C16:1	2.24a	2.02b
C17:0	0.58	0.60
C17:1	0.57a	0.38b
C18:0	7.45b	12.72a
C18:1 (trans-9) & C18:1 (trans-11)	1.32b	7.95a
C18:1 (trans-13) & C18:1 (cis-9)	17.8b	26.24a
C18:2 (linoleic acid)	1.08b	2.68a
C20:0	0.13a	0.09b
C18:3 (linolenic acid)	0.50	0.51
C18:2(CLA)	0.52b	2.35a
additional fatty acids	4.48	4.62

Fatty acid profile of cow milk under trials is given in Table 2. There was a significant increase in proportion of linoleic acid content in milk from animals fed on supplemented diets. It might be owing to the high levels of linoleic acid in sunflower oil. Most of other fatty acids were also considerably influenced from supplemented diet. As a whole the significant decrease in the quantities of short chain fatty acids and increase in long chain fatty acids was observed. However, butyric acid and linolenic acid were the exceptions, which remained constant. The significant decrease in palmitic acid and increase in stearic acid concentrations were recorded in supplemented diet milk as compared to control (grass silage and concentrate).

These variations in fatty acid concentrations are comparable to those reported by Murphy [25].

A highly significant increase in the quantities of C18:1 and C18:2 were found. These results undoubtedly reveal that sunflower supplemented diet effectively increased the concentration of CLA in cow milk within 2 weeks only. This shows the valuable impact of sunflower oil supplementation in diet to increase CLA contents as reported by Coakleyet al [16] and Kay et al [24].

3.3. Cheese Composition

As a result of elevated CLA level in milk, no significant variation in cheese composition was found compared with control treatment (Table 1).

3.4. Cheese Fatty Acid Profile

Fatty acid profile of Cheddar cheese during ripening of 120 days in summarized in Table 3. The variations in fatty acid concentrations in cheese owing to the use of supplemented diets and subsequent increase in CLA levels were comparable to those found in raw milk from which it was manufactured as were established by Dhimanet al [26]. Most of the fatty acids particularly the CLA concentrations remained stable during 120 days of ripening. Due to supplemented diet, vaccenic acid (C18:1 *trans*-11 vaccenic acid; TVA) concentration increased significantly in fresh cheese and sustained during whole ripening period. This fatty acid is also produced intermediary and takes part as substrate for bovine mammary desaturase enzyme [27].

Table 3. Fatty acid profile of Cheddar cheese during ripening

Ripening period	0 day		60 days		120 days	
Fatty acid (g/100 g)	Control	Supplemented	Control	Supplemented	Control	Supplemented
C4:0	1.42	1.51	1.26	1.18	1.67b	1.80a
C6:0	1.38	1.34	1.44	1.20	1.50	1.54
C8:0	1.07	1.09	1.14	0.89	1.10	1.18
C10:0	2.57	2.62	2.62	2.44	2.75	2.82
C12:0	3.18	3.25	3.38	3.12	3.52	3.17
C14:0	11.98	11.41	12.12a	10.86b	12.46a	10.92b
C14:1	1.07	0.97	1.14	0.99	1.08	0.89
C15:0	1.57a	1.26b	1.74a	1.18b	1.71a	1.24b
C16:0	33.02a	23.37b	33.06a	22.56b	33.04a	24.10b
C16:1 (trans)	0.21b	0.62a	0.19b	0.62a	0.23b	0.52a
C16:1 (cis)	2.13a	1.29b	2.16a	1.61b	2.04a	1.50b
C17:0	0.72	0.58	0.65	0.59	0.59	0.55
C17:1	0.53a	0.29b	0.53a	0.29b	0.50	0.38
C18:0	8.73	9.89	9.15b	11.65a	8.14b	10.66a
C18:1 (trans-9)	0.30b	0.73a	0.30	0.62	0.39b	0.91a
C18:1 (vaccenic acid)	2.61b	6.98a	2.43b	6.96a	2.55b	6.68a
C18:1 (trans-13)	0.92	1.28	0.68b	1.74a	0.81	1.43
C18:1 (oleic acid)	18.88	20.75	20.02b	21.90a	19.02b	20.89a
C18:2 (linoleic acid)	1.18b	1.92a	1.26b	1.91a	1.18b	1.90a
C20:0	0.11	0.10	0.11	0.10	0.10	0.10
C18:3 (linolenic acid)	0.56	0.58	0.57	0.60	0.57	0.57
C18:2 (CLA)	0.72b	2.10a	0.79b	2.04a	0.71b	1.90a
C18:2 (trans-10 &cis-12)	0.16	0.10	0.11	0.08	0.10	0.08
additional fatty acids	5.20	5.94	4.44	5.48	4.96	5.72

The concentration of cis 9, *trans*-11 CLA in cheese even at the end of 120 days ripening, was appreciable. This much quantity of CLA in combination with TVA, a few of which might be changed to CLA, can considerably contribute to the *cis*-9, *trans*-11 CLA level in human beings to have better health.

4. Conclusion

It was concluded that the concentration of CLA can be increased in milk and milk products through supplementation of pasture with oil containing high levels of linoleic acid. Moreover, the elevated CLA level in milk is transferred to the products resulting in increased health benefits.

References

[1] Hughes, K. V.-Willenberg, B. J.: Using and Storing Cheddar Cheese. Human Environmental Science Publication, GH1287. Department of Food Science and Human Nutrition, University of Missouri, Columbia, 1993.

[2] Murtaza, M. A.-Rehman, S. U.-Anjum, F. M.-Nawaz, H.: Nutritional comparison of cow and buffalo milk Cheddar cheese. Pakistan Journal of Nutrition, *7*, 2008, pp. 509-512.

[3] Murtaza, M. A.-Rehman, S. U.-Anjum, F. M.-Huma, N.-Hafiz, I.: Cheddar Cheese Ripening and Flavor Characterization: A Review. Critical Reviews in Food Science and Nutrition, *54*, 2014, pp. 1309-1321.

[4] Smit, G.-Smit, B. A.-Wim, J. M.-Engels, J. M.: Flavour formation by lactic acid bacteria and biochemical flavour profiling of cheese products. FEMS Microbiology Reviews, *29*, 2005, pp. 591-610.

[5] Azarnia, S.-Robert, N.-Lee, B.: Biotechnological methods to accelerate Cheddar cheese ripening. Critical Reviews in Biotechnology, *26*, 2006, pp. 121-143.

[6] McSweeney, P. L. H.-Sousa, M. J.: Biochemical pathways for the production of flavour compounds in cheese during ripening: A review. Lait, *80*, 2000, pp. 293-324.

[7] Murtaza, M. A.-Rehman, S. U.-Anjum, F. M.-Huma, N.: Descriptive sensory profile of cow and buffalo milk Cheddar cheese prepared using indigenous cultures. Journal of Dairy Science, *96*, 2013, pp. 1380-1386.

[8] Fox, P. F.-Guinee, T. P.-Cogan, T. M.-McSweeney, P. L. H.: Fundamentals of Cheese Science. Gaithersburg, M.D., USA: Aspen Publishers, Inc., 2000.

[9] Murtaza, M. A.-Rehman, S. U.-Anjum, F. M.-Huma, N.-Tarar, O. M.-Mueen-ud-Din, G.: Organic acids contents of buffalo milk Cheddar cheese as influenced by accelerated ripening and sodium salt. Journal of Food Biochemistry, *36*, 2012, pp. 99-106.

[10] Addis, M.-Cabiddu, A.-Pinna, G.-Decandia, M.-Piredda, G.-Pirisi, A.-Molle, G.: Milk and Cheese Fatty Acid Composition in Sheep Fed Mediterranean Forages with Reference to Conjugated Linoleic Acid *cis-9*, *trans-11*. Journal of Dairy Science, *88*, 2005, pp. 3443-3454.

[11] Arslan, S.-Topcu, A.-Saldamli, I.-Koksal, G.: Utilization of Interesterified Fat in the Production of Turkish White Cheese. Food Science and Biotechnology, *19*, 2010, pp. 89-98.

[12] Moon, S. S.-Jin, S. K.-Hah, K. H.-Kim, I. S.: Effects of replacing back fat with fat replacers and olive oil on the quality characteristics and lipid oxidation of low-fat sausage during storage. Food Science and Biotechnology, *17*, 2008, pp. 396-401.

[13] Ashrafie, N. T.-Azizi, M. H.-Taslimi, A.-Mohammadi, M.-Neyestani, T. R.-Mohammadifar, M. A.: Development of reduced-fat and reduced-energy dark chocolate using collagen hydrolysate as cocoa butter replacement agent. Journal of Food and Nutrition Research, *53*, 2004, pp. 13-21.

[14] Avramis, C. A.-Wang, H.-McBride, B. W.-Wright, T. C.-Hill, A. R.: Physical and Processing Properties of Milk, Butter, and Cheddar Cheese from Cows Fed Supplemental Fish Meal. Journal of Dairy Science, *86*, 2003, pp. 2568-2576.

[15] Bialek, A.-Tokarz, A.-Zagrodzki, P.: Conjugated linoleic acids in diet of female rats inhibit the breast cancer formation in their offspring. Journal of Food and Nutrition Research, *53*, 2014, pp. 39-50.

[16] Coakley, M.-Barrett, E.-Murphy, J. J.-Ross, R. P.-Devery, R.-Stanton, C.: Cheese Manufacture with Milk with Elevated Conjugated Linoleic Acid Levels Caused by Dietary Manipulation. Journal of Dairy Science, *90*, 2007, pp. 2919-2927.

[17] Wahle, K. W.-Heys, S. D.-Rotondo, D.: Conjugated linoleic acids: Are they beneficial or detrimental to health? Progress in Lipid Research, *43*, 2004, pp. 553-587.

[18] Parodi, P. W.: Conjugated linoleic acid in food, In: Sebedio, J. L.-Christie, W. W.-Adlof, R. (Eds): Advances in Conjugated Linoleic Acid Research, Vol. 2, AOCS Press, Champaign, IL, 2003, pp. 101-122.

[19] AOAC: Official methods of analysis of AOAC international 18th Ed., AOAC International, 481 North Frederick Avenue, Gaithersburg, Maryland, USA, 2005.

[20] Murphy, J. J.-McNeill, G. P.-Connolly, J. F.-Gleeson, P. A.: Effect on cow performance and milk fat composition of including full fat soybean and rapeseed in the concentrate mixture for lactating dairy cows. Journal of Dairy Research, *57*, 1990, pp. 295-306.

[21] Stanton, C.-Lawless, F.-Kjellmer, G.-Harrington, D.-Devery, R.-Connolly, J. F.-Murphy, J.: Dietary influences on bovine milk cis-9, trans-11-conjugated linoleic acid content. Journal of Food Science, *62*, 1997, pp. 1083-1086.

[22] Scott, R.: Cheese Making Practice. London, UK: Applied Science Publishers Ltd., 1981, pp. 367-369.

[23] Steel, R. G. D.-Torrie, J. H.-Dickey, D. A.: Principles and Procedures of Statistics. A Bio-Metrical Approach 3rd Ed. New York, USA: McGraw Hill Book Co., Inc., 1997.

[24] Kay, J. K.-Mackle, T. R.-Auldist, M. J.-Thomson, N. A.-Bauman, D. E.: Endogenous synthesis of cis-9, trans-11 conjugated linoleic acid in dairy cows fed fresh pasture. Journal of Dairy Science, *87*, 2004, pp. 369-378.

[25] Murphy, J. J.: Synthesis of milk fat and opportunities for nutritional manipulation. British Society of Animal Science, *25*, 2000, pp. 201-222.

[26] Dhiman, T. R.-Helmink, E. D.-McMahon, D. J.-Fife, R. L.-Pariza, M. W.: Conjugated linoleic acid content of milk and cheese from cows fed extruded oilseeds. Journal of Dairy Science, *82*, 1999, pp. 412-419.

[27] Griinari, J. M.-Corl, B. A.-Lacy, S. H.-Chouinard, P. Y.-Nurmela, K. V. V.-Bauman, D. E.: Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by 9-desaturase. Journal of Nutrition, *130*, 2000, pp. 2285-2291.