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Starter cultures and cattle feed manipulation enhance conjugated linoleic acid concentrations in Cheddar cheese

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ABSTRACT

Conjugated linoleic acid (CLA) is a fatty acid (FA) that provides several health benefits to humans. The feeding of fish oil-supplemented diets to dairy cows has been extensively studied as a means to improve the CLA content in milk. Several studies have also been conducted on the ability of many microorganisms to produce CLA by utilizing substrates containing linoleic acid. In the present study, the dietary manipulated milk was used in combination with the CLA-producing culture to manufacture Cheddar cheese. The two diets fed to cattle were control and treatment diets to obtain control and treatment milk, respectively. The treatment diet containing fish oil (0.75% of dry matter) was fed to 32 dairy cows grouped in a pen for 18 d to increase the total CLA content in milk. Treatment milk had a CLA content of 1.60 g/100 g of FA compared with 0.58 g/100 g of FA in control milk obtained by feeding the control diet. A 2 × 2 factorial design with 3 replicates was used to test the combined effect of the CLA-producing starter culture of *Lactococcus lactis* (CI4b) versus a commercial CLA nonproducing cheese starter as the control culture, and type of milk (control vs. treatment milk) on CLA content in Cheddar cheese. Chemical composition (moisture, salt, fat, and protein) was not affected by the type of culture used. However, the age of the cheese affected the sensory properties and microbiological counts in the different treatments. Ripening with the CI4b culture was found to be effective in further enhancing the CLA content. The CI4b cheeses made from control milk and treatment milk contained 1.09 and 2.41 (±0.18) g of total CLA/100 g of FA after 1 mo of ripening, which increased to 1.44 and 2.61 (±0.18) g of total CLA/100 g of FA after 6 mo of ripening, respectively. The use of treatment milk resulted in an increase in the CLA isomers (*trans*-7,*cis*-9 + *cis*-9,*trans*-11, *trans*-9,*cis*-11 + *cis*-10,*trans*-12, *trans*-10,*cis*-12, *cis*-9,*cis*-11, *trans*-11,*cis*-13, *cis*-11,*cis*-13, *trans*-11,*trans*-13, and *trans*-9,*trans*-11). The CI4b culture specifically increased *cis*-11,*cis*-13 and *trans*-10,*cis*-12 isomers in cheese. The total CLA content in cheese was significantly higher when the CI4b culture was used compared with CLA nonproducing culture cheeses made from control milk and treatment milk after 1 mo [1.09 and 2.14 (±0.18) g of total CLA/100 g of FA] and 6 mo [0.99 and 2.05 (±0.18) g of total CLA/100 g of FA] of ripening, respectively. The results indicated that the combination of a CLA-producing starter culture and milk from cattle fed fish oil-supplemented diets (0.99 g of CLA/100 g of FA) could enhance levels of total CLA in Cheddar cheese by up to 2.6 times compared with cheese made from control milk with CLA nonproducing starter culture (2.61 g of CLA/100 g of FA) after 6 mo.
Key words: fish oil, *Lactococcus*, conjugated linoleic acid, cheese

INTRODUCTION

Conjugated linoleic acid (**CLA**) is a collective term for all the positional and geometric isomers of linoleic acid. The different CLA isomers are associated with several health benefits, such as anticarcinogenic, antioxidant, antidiabetic, antiatherosclerosis, and immune modulation benefits (Benjamin and Spener, 2009). Isomers *cis*-9,*trans*-11 CLA and *trans*-10,*cis*-12 CLA are considered to be the most important isomers of CLA associated with health benefits. Major sources of CLA in the human diet are ruminant meat and dairy products. Milk, cheese, and fermented dairy products contain total CLA of about 0.41 to 0.55, 0.40 to 1.70, and 0.30 to 0.50 g/100 g of FA, respectively (Chin et al., 1992; Shantha et al., 1995; Mushtaq et al., 2010).

Conjugated linoleic acid in milk is preformed in the rumen of cattle as an intermediary in the microbial hydrogenation of PUFA. Several studies have shown that feeding fish oil to cattle increases the CLA content in milk (Donovan et al., 2000; Shingfield et al., 2012). Addition of fish oil (2% DM) to cattle diets also increased the CLA content in milk up to 2.07 g/100 g of FA compared with 0.60 g/100 g of FA in control milk

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(Whitlock et al., 2002). Donovan et al. (2000) proposed that fish oil acts as a rumen modifier to stimulate the production of additional CLA from linoleic acid present in the other feed materials. Cheddar cheese manufactured from enhanced CLA milk (4.8 times increase in CLA compared with control milk) obtained by dietary manipulation of cattle was found to have higher CLA content of 1.93 g/100 g of FA compared with control cheese containing 0.78 g/100 g of FA (Coakley et al., 2007).

Apart from rumen bacteria, several lactic acid bacteria have also been shown to produce CLA (Ogawa et al., 2005; Rodríguez-Alcalá et al., 2011). Most of the studies on production of CLA by food-grade bacteria utilize linoleic acid-rich oils as substrates (Andrade et al., 2012). In a previous study in our laboratory, a CLA-producing starter culture of *Lactococcus lactis* (**CI4b**) was identified, which increased the total CLA content from 0.41 to 1.21 g/100 g of FA in fermented milk without the addition of any substrates (Pandit et al., 2012). The CI4b culture was also found to possess good starter culture activity for cheese manufacture. The CI4b starter increased the CLA content of Cheddar cheese from 0.61 to 0.97 g/100 g of FA (Pandit, 2008).

The health benefits of CLA have been known for more than 3 decades. Consequently, efforts have been made to increase the CLA content in milk and milk products. Despite these efforts, the estimated dietary intake of CLA in human diet remains very low compared with the dosage required for rendering the health benefits. Huth and group (2006) have quoted up to 0.42 g of CLA/d as the effective dosage for anticarcinogenic effects in human beings. Fortification with commercially available CLA isomers, which could have been the most efficient method of enhancing CLA content in dairy products, however, is associated with many disadvantages. Fortification techniques have been associated with off-flavors in the final products (Campbell et al., 2003). The CLA isomer composition in such fortified products is different from the naturally available isomer compositions in food products. Dietary manipulation and use of CLA-producing cultures increases the CLA content in milk and milk products considerably (Donovan et al., 2000; Ogawa et al., 2005). Although these methods have been studied individually, to our knowledge no studies have been reported combining these 2 methods to further enhance the CLA content in dairy products. We, therefore, hypothesized that the use of CLA-enhanced milk produced through dietary manipulation in combination with a starter culture producing CLA to manufacture Cheddar cheese would further enhance the CLA content of cheese compared with the individual treatments. This could, in turn, provide the amount of CLA in the diet required for conferring health benefits. Our specific objective was to further enhance the CLA content in Cheddar cheese by using milk obtained by dietary manipulation by feeding fish oil to dairy cows and by using CI4b culture, in combination.

MATERIALS AND METHODS

Dietary Manipulation of Cattle for Enhancement of CLA in Milk

Dietary manipulation of cattle was done by feeding the cattle with diets supplemented with fish oil at the Dairy Research and Training Facility at South Dakota State University (Brookings). The control diet consisted of concentrates and forage in the ratio of 47:53. The concentrate portion consisted of 13.1% soybean meal, 14.7% corn (high-moisture shelled), 3.2% dried distillers grains with solubles, 4.8% fuzzy cottonseed, 3.8% beet pulp, 2.2% sugar, 1.1% rumen-inert fat, and 3.6% vitamin and mineral supplements, whereas the forage portion consisted of 11.4% alfalfa hay, 4.9% alfalfa haylage, 4.6% ground shell corn, and 32.5% corn silage. The treatment diet was the control diet supplemented with Menhaden fish oil (Omega Protein Inc., Hammond, LA) at 0.75% of the diet DM. The control and treatment diets were fed to 32 dairy cows grouped in a pen. The control diet feeding was started 1 mo before the feeding of the treatment diet. The treatment diet was fed for a period of 18 d, as Whitlock et al. (2002) observed that the maximum yield of CLA in milk occurs during this period. The feed samples for both control and treatment diets were collected during this period on d 7, 10, and 17 and stored at −20°C until further analysis. The chemical composition (DM, CP, and NDF) of the 2 diets was similar. The supplementation of 0.75% (by DM) fish oil to the control diet increased the DM to 56.9% in the treatment diet compared with 55.6% in the control diet. The treatment diet had CP and NDF contents of 18.3 and 27.7% of DM, respectively. This was similar to the control diet with CP being 18.3% of DM and NDF being 30.4% of DM.

The milk obtained after feeding fish oil (treatment diet) was termed treatment milk. Control milk was obtained from feeding the control diet. Both types of milk were collected on the respective day of cheese making.

Feed Composition Analysis

The frozen feed samples were thawed and dried at 55°C in an oven (style V-23; Despatch Oven Co., Minneapolis, MN) for 48 h. The dried samples were then ground to pass through a 2-mm screen of a Wiley mill