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Association of *DGAT1* genotype, fatty acid composition, and concentration of copper in milk with spontaneous oxidized flavor

J. Juhlin,* W. F. Fikse,* J. Pickova,† and A. Lundén*[1]
*Department of Animal Breeding & Genetics, Swedish University of Agricultural Sciences, PO Box 7023, SE-750 07 Uppsala, Sweden
†Department of Food Science, Swedish University of Agricultural Sciences, PO Box 7051, SE-750 07 Uppsala, Sweden

ABSTRACT

In 136 cows with altogether 969 milk samples, we investigated the effect of the acyl-coenzyme A:diacylglycerol acyltransferase 1 (*DGAT1*) *K232A* polymorphism on milk fatty acid (FA) composition and how, in combination with copper concentration in milk, this influences the occurrence of spontaneous oxidized flavor. All milk samples were analyzed for concentrations of copper and individual FA and subjected to sensory analysis by trained judges. We found an effect of *DGAT1* genotype on FA composition where mainly the long-chain FA were affected. The *232A* allele was associated with larger proportions of the C18:2 *cis*-9,*trans*-11 conjugated linoleic acid and lower proportions of C16:0 FA. Milk concentrations of unsaturated FA and copper showed strong and unfavorable associations with spontaneous oxidized flavor (SOF) development. The interaction between FA and copper indicates that SOF will not develop as easily in milk with high copper content unless the substrate is available (i.e., in addition to the previously shown effect of copper in milk, unsaturated FA are required for the process of oxidation to progress). We observed a marked effect of the *DGAT1* genotype on SOF development where the *A* allele was associated with a higher risk of SOF. Moreover, our results suggest that the effects of the FA C18:3 n-3 and of the polyunsaturated index on SOF development are beyond the effect of the *DGAT1* genotype. Breed had an effect on FA composition but not on SOF development. Our results imply that copper, FA composition, and *DGAT1* genotype are risk factors for SOF and considerations to these factors might be necessary in future breeding decisions.

Key words: spontaneous oxidized flavor, fatty acid composition, copper, *DGAT1*

INTRODUCTION

Milk fat composition has a major influence on dairy products affecting shelf life and processing quality of the milk. A more unsaturated milk fat is preferred from human nutritional and health perspectives. The FA composition in milk varies due to factors such as feed, stage of lactation, parity, season, and genotype of the cow (see review by Palmquist, 2006). Several studies have identified genetic variation in the composition of milk fat (Karijord et al., 1982; Soyeurt et al., 2007; Bobe et al., 2008; Stoop et al., 2008; Garnsworthy et al., 2010), and efforts are being made to elucidate the genes contributing to this variation. Milk fat consists of approximately 98% triglycerides, and the acyl-CoA:diacylglycerol acyltransferase 1 (**DGAT1**) enzyme has an important function in milk fat synthesis, because it catalyzes the final step in the formation of triglycerides. A dinucleotide substitution in the gene coding for DGAT1, resulting in a replacement of the AA lysine (K) with alanine (A) in position 232 (*K232A*), has been shown to be associated with increased yields of protein and milk, and a decrease in yield of fat and concentrations of fat and protein (Grisart et al., 2002; Spelman et al., 2002; Winter et al., 2002; Thaller et al., 2003). In a study by Schennink et al. (2007), it was found that the *DGAT1 K* allele was associated with a larger fraction of C16:0 and smaller fractions of C14:0 and unsaturated C18. The same research group also reported evidence that the *DGAT1 A* allele is associated with a higher unsaturation index for the long-chain FA found in milk, and argued that selective breeding in favor of the *A* allele would give milk with a more desirable FA composition, from a public health perspective (Schennink et al., 2008).

A potential downside of such a breeding strategy is that unsaturated milk FA are more prone to oxidize (Sidhu et al., 1975; Barrefors et al., 1995; Granelli et al., 1998; Timmons et al., 2001), giving a carbon, metal, talcum, or fishy flavor to the milk (Shipe et al., 1978). This off-flavor results from volatile compounds that accumulate in the milk through the oxidation of the double bonds between the carbon atoms in unsatu-

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[1]Corresponding author: anne.lunden@slu.se

rated FA. Oxidative off-flavor can be either induced or spontaneous (**SOF**; i.e., develop without exposure to light and with no addition of prooxidants). Oxidation is often initiated by naturally occurring prooxidants such as copper and iron, but to a certain extent these can be balanced by antioxidative substances in the milk, such as β-carotene and α-tocopherol. According to the report by Lindberg et al. (2004), oxidative off-flavor is the second most prevalent off-flavor in bulk milk at individual farms, next to rancid flavor. Of the routine bulk milk samples collected monthly from each farm for sensory tests during year 2002, 1.81% had an off-flavor, of which 0.39% were judged to have an oxidative off-flavor, compared with 0.65% of samples with rancid flavor. Variation in oxidative stability of milk from individual cows has previously been reported by Corbett and Tracy (1943), Dunkley and Franke (1967), Barrefors et al. (1995), and Granelli et al. (1998). In a recent publication, Clausen et al. (2010) observed large individual variation in oxidative stability of milk and concluded that FA composition and antioxidants such as α-tocopherol only explained a limited part of the variation in milk oxidation. Comparable results were reported by Juhlin et al. (2010a) regarding α-tocopherol, whereas the latter study found that concentration of PUFA in milk was positively associated with increased risk of developing SOF. The most potent prooxidant in milk is copper (Haase and Dunkley, 1970; Bruhn et al., 1975). Copper occurs naturally in milk where its concentration varies between cows and also depends on the diet and level of mineral supplementation (Dunkley et al., 1968). We have previously shown that copper concentration in milk has a major effect on the development of SOF (Juhlin et al., 2010a,b).

The dairy herd at the Swedish University of Agricultural Sciences includes cows of the Swedish Red (**SR**) and the Swedish Holstein (**SH**) breeds. The SR cows belong to either of 2 selection lines producing milk with a high or low concentration of milk fat at equal total milk energy production, such that cows from both lines have similar nutritional requirements. This material offers an interesting opportunity to explore the biology and genetics behind variation in milk composition and especially fat content. The aim of the present study was to investigate the effect of a *DGAT1* polymorphism and selection toward high or low milk fat content on milk FA composition and how this, in combination with natural variation in milk copper content, influences the occurrence of SOF. Due to the proposed relationship between *DGAT1* genotype and the proportion of unsaturated FA in milk (Schennink et al., 2007) we hypothesize that the *DGAT1 K232A* polymorphism is associated with the tendency of milk to develop SOF.

MATERIALS AND METHODS

Animals

The experimental herd at the University of Agricultural Sciences in Uppsala, Sweden consists of pure bred dairy cows of the SR and the SH breeds. Since 1985, the SR cows have been selected for either high (**HF**) or low (**LF**) milk fat content, with the 2 lines having an equal total milk energy production. The cows in the 2 lines are inseminated with bulls with high or low breeding values for milk fat content, respectively, all of them belonging to the top bulls for total milk energy production. The bulls used for insemination of the SH cows rank among the top bulls, based on total merit index. A total of 136 cows from the experimental herd were available for this study of which 50 belonged to the SH breed, 38 belonged to the SR/HF line, and 48 to the SR/LF line. The cows were genotyped for the *DGAT1 K232A* polymorphism using the method of pyrosequencing (Ronaghi et al., 1998) according to Näslund et al. (2008).

Sampling and Analysis of Milk

Individual morning milk samples were collected monthly from all lactating cows in the herd from October 2000 until December 2001 (a total of 969 samples). Depending on the number of months in lactation during this period, the number of samples per cow varied between 1 and 11. Fat, protein, and lactose concentrations were determined by infrared spectroscopy (Dairy-Lab2, A7S; Foss Electric A/S, Hillerød, Denmark). Aliquots of morning milk were stored at −80°C for analysis of FA composition. Lipids were extracted according to the method described by Nourooz-Zadeh and Appelqvist (1988). Preparation of FA methyl esters was done using the protocol by Appelqvist (1968), and analysis was carried out with a HP5890 series II gas chromatograph (Hewlett Packard Co., Rolling Meadows, IL), fitted with a flame ionization detector and a capillary column DB-23 (Agilent Technologies, Stockholm, Sweden; 30-m length, 0.25-mm i.d., 0.25-μm film thickness). The column temperature was programmed at 2°C/min from 40 to 220°C. Injector and detector temperatures were 250°C. Identification of FA was performed by comparing the obtained peaks with those of standards (Larodan Fine Chemicals; Sigma Chemical Co., Malmö, Sweden). Peak areas were integrated using HP ChemStation software. The carrier gas was helium, and make-up gas was nitrogen. Values are shown for the major milk FA and those with particular relevance for fat oxidation (Table 1). The methylation step used for the FA analyses was