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Genome-wide association study for cheese yield and curd nutrient recovery in dairy cows

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ABSTRACT

Cheese production and consumption are increasing in many countries worldwide. As a result, interest has increased in strategies for genetic selection of individuals for technological traits of milk related to cheese yield (CY) in dairy cattle breeding. However, little is known about the genetic background of a cow's ability to produce cheese. Recently, a relatively large panel (1,264 cows) of different measures of individual cow CY and milk nutrient and energy recoveries in the cheese (REC) became available. Genetic analyses showed considerable variation for CY and for aptitude to retain high proportions of fat, protein, and water in the coagulum. For the dairy industry, these characteristics are of major economic importance. Nevertheless, use of this knowledge in dairy breeding is hampered by high costs, intense labor requirement, and lack of appropriate technology. However, in the era of genomics, new possibilities are available for animal breeding and genetic improvement. For example, identification of genomic regions involved in cow CY might provide potential for marker-assisted selection. The objective of this study was to perform genome-wide association studies on different CY and REC measures. Milk and DNA samples from 1,152 Italian Brown Swiss cows were used. Three CY traits expressing the weight (wt) of fresh curd (%$\mathrm{CY_{CURD}}$), curd solids (%$\mathrm{CY_{SOLIDS}}$), and curd moisture (%$\mathrm{CY_{WATER}}$) as a percentage of weight of milk processed, and 4 REC ($\mathrm{REC_{FAT}}$, $\mathrm{REC_{PROTEIN}}$, $\mathrm{REC_{SOLIDS}}$, and $\mathrm{REC_{ENERGY}}$, calculated as the % ratio between the nutrient in curd and the corresponding nutrient in processed milk) were analyzed. Animals were genotyped with the Illumina BovineSNP50 Bead Chip v.2. Single marker regressions were fitted using the GenABEL R package (genome-wide association using mixed model and regression–genomic control). In total, 103 significant associations (88 single nucleotide polymorphisms) were identified in 10 chromosomes (2, 6, 9, 11, 12, 14, 18, 19, 27, 28). For $\mathrm{REC_{FAT}}$ and $\mathrm{REC_{PROTEIN}}$, high significance peaks were identified in *Bos taurus* autosome (BTA) 6 and BTA11, respectively. Marker ARS-BFGL-NGS-104610 (~104.3 Mbp) was highly associated with $\mathrm{REC_{PROTEIN}}$ and Hapmap52348-rs29024684 (~87.4 Mbp), closely located to the casein genes on BTA6, with $\mathrm{REC_{FAT}}$. Genomic regions identified may enhance marker-assisted selection in bovine cheese breeding beyond the use of protein (casein) and fat contents, whereas new knowledge will help to unravel the genomic background of a cow's ability for cheese production.

Key words: genome-wide association study, cheese yield, curd recovery, whey loss, dairy cattle

INTRODUCTION

Milk and dairy products are important components of the human diet and the proportion of milk used for manufactured products (e.g., cheese) is steadily increasing in many countries worldwide (Food and Agriculture Organization of the United Nations, 2015).

Milk characteristics (e.g., acidity and solid components, in particular casein and fat) are the cornerstone of cheese-making, and their role in this process has been previously investigated (Walstra et al., 2014). Moreover, milk coagulation properties (**MCP**) and curd firming modeling parameters (**$\mathbf{CF_t}$**), together with the phenomenon of syneresis, are considered crucial technological features for cheese production (Bittante et al., 2012). However, milk components and MCP-$\mathrm{CF_t}$ traits can

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only be used as indicators of the cheese-making process. On the other hand, traits such as the quantity of cheese obtained from a given amount of processed milk, or the recovery of milk components into the cheese, are direct measures of the cheese-making aptitude of milk and so are of great economic interest.

Although a considerable amount of literature on cheese-making is available, knowledge is mostly based on bulk milk. The importance of the percentage of cheese yield (**%CY**) at the individual level (i.e., based on individual milk and not on bulk milk) has been pointed out by Othmane et al. (2002). Moreover, Banks (2007) discussed the significance of the recovery of milk constituents into the curd, as well as their loss in the whey for improved cheese quantity and quality. Previous studies have explored the potential of individual %CY using bovine milk, albeit based on relative small numbers of individuals (Hurtaud et al., 1995; Wedholm et al., 2006). Recently, a large data set (n = 1,264) of different measures of individual cow %CY and milk nutrient and energy recovery in the cheese (**REC**) became available, using a cheese-making model approach assessed at the laboratory level (Cipolat-Gotet et al., 2013). Further analysis has shown important genetic variation in individual %CY that does not solely depend on milk components but also relies heavily on the recovery of milk components in the curd (Bittante et al., 2013). Nevertheless, integration of the new knowledge into breeding programs is hampered by high costs, intensive labor requirements, and lack of appropriate technology.

At present, few potential alternatives have been suggested to overcome this problem (e.g., prediction of the aforementioned traits through infrared spectroscopy). Determination of milk components using spectral data is routinely used in the dairy industry (ICAR, 2012) and ongoing research is focused on the precision of the technology for predicting detailed milk components (Rutten et al., 2009), technological traits of milk such as milk coagulation (Cecchinato et al., 2009; Chessa et al., 2014), or different cheese measures (Ferragina et al., 2013; Bittante et al., 2014). On the other hand, genomic information offers a unique potential for a better understanding of the genetics underlying cheese-making properties. A first step toward this direction is, for example, the application of genome-wide association studies (**GWAS**; McCarthy et al., 2008; Visscher et al., 2012) where thousands of DNA markers, in the form of SNP, are scanned throughout the entire genome (Bovine Genome Sequencing and Analysis Consortium et al., 2009) linking the phenotype of interest to specific regions on the genome (Goddard and Hayes, 2009). In addition, genomic information can be used in marker-assisted or genomic selection breeding programs (Goddard and Hayes, 2009; de Los Campos et al., 2013; Van Eenennaam et al., 2014).

Genomic regions associated with bovine milk quality traits have already been identified in a variety of studies, either using a small number of preselected DNA markers or a whole genome scan, and candidate genes have been detected. In a GWAS study using Holstein-Friesian cattle, different protein variants (especially casein variants) showed high peaks on BTA6 and BTA11 (Schopen et al., 2011). Concerning MCP traits, milk coagulation has been associated with chromosomes 2, 18, and 24 in Finnish Ayrshire cattle (Tyrisevä et al., 2008), whereas for rennet coagulation time, β-casein (*CSN2*), β-lactoglobulin (*LGB*), and growth hormone 1 (*GH1*) have been identified as candidate genes (Bonfatti et al., 2010; Cecchinato et al., 2012, 2015). Moreover, MCP and some cheese characteristics have also been associated with κ-casein (*CSN3*), leptin (*LEP*), and leptin receptor (*LEPR*; Glantz et al., 2011). Recently, 2 GWAS studies identified other chromosomal regions associated with different MCP, CF_t, and syneresis traits (Gregersen et al., 2015; Dadousis et al., 2016). Nevertheless, these studies have identified genomic regions associated with indicators and not with direct measures of the cheese-making aptitude of milk.

The objective of our study was to conduct a GWAS analysis using individual cheese yield (**%CY$_{\textbf{CURD}}$**, **%CY$_{\textbf{SOLIDS}}$**, and **%CY$_{\textbf{WATER}}$**) and milk nutrient and energy recovery into the curd measures (**REC$_{\textbf{FAT}}$**, **REC$_{\textbf{PROTEIN}}$**, **REC$_{\textbf{SOLIDS}}$**, and **REC$_{\textbf{ENERGY}}$**) to shed light on the genetics underlying a cow's cheese-making ability. An Italian Brown Swiss dairy cattle sample genotyped with a 50k SNP chip and with all cheese-making traits measured through individual model-cheese manufacture was used.

MATERIALS AND METHODS

Field Data

Milk and blood samples were collected from 1,264 Italian Brown Swiss cows reared in 85 herds located in Trento Province (Italy). A full description of the sampling procedure can be found in Cecchinato et al. (2013). In brief, 15 cows per herd were individually sampled once (evening milking) and all samples were processed within 20 h after collection. Information on cows and herds was supplied by the Breeders Association of Trento Province.

Definition of Phenotypes

The phenotypes were obtained through a model cheese-making procedure on 1,500 mL of milk for each

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