**Genetic parameters of milk cholesterol content in Holstein cattle**

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<th>Journal:</th>
<th>Canadian Journal of Animal Science</th>
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<tr>
<td>Manuscript ID</td>
<td>CJAS-2018-0010.R2</td>
</tr>
<tr>
<td>Manuscript Type:</td>
<td>Article</td>
</tr>
<tr>
<td>Date Submitted by the Author:</td>
<td>14-Apr-2018</td>
</tr>
<tr>
<td>Complete List of Authors:</td>
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<tr>
<td>Keywords:</td>
<td>Milk cholesterol, Genetic parameters, Heritability, Holstein cows</td>
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Genetic parameters of milk cholesterol content in Holstein cattle

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**Abbreviation:** ABCA1, ATP-binding cassette sub-family A member 1; ABCG1, ATP-binding cassette sub-family G member 1; APOA-1, apolipoprotein A1; CVD, cardiovascular diseases; CHL, Cholesterol; DIM, Days in milk; HDL, high density lipoprotein; HMGCR, 3-hydroxy-methylglutaryl-coenzyme-A (HMG-CoA) reductase; HMGCS1, 3-hydroxy-methylglutaryl-coenzyme-A (HMG-CoA) synthase; FDFT, farnesylphosphatase-farnesyltransferase; LDL_CHL, low density lipoprotein cholesterol; SCC, somatic cell count; SCS, somatic cell score; SREBF2, sterol regulatory element binding transcription factor 2; SREBP1, sterol regulatory element binding protein 1; SREBP2, sterol regulatory element binding protein 2.

**ABSTRACT**

This study aimed to estimate heritability for milk cholesterol (CHL) and genetic correlations between milk CHL and other production traits (test-day milk, fat and protein yields, fat and protein percentages and somatic cell score (SCS)). Milk CHL content was determined by gas chromatography and expressed as mg of CHL in 100 g of fat (CHL_fat) or in 100 mg of milk (CHL_milk). Univariate models were used to estimate variances and heritability; whereas bivariate models were used to compute correlations using data from 1,793 cows. The average concentrations (SD) of CHL_fat and CHL_milk were 275.63 (75) mg and 11.16 (3.63) mg, respectively. Milk CHL content was significantly affected by days in milk and herd ($P<0.05$), but not by parity, regardless of the scale of expression. Heritability estimates for CHL_fat and CHL_milk were 0.06±0.04 and 0.17±0.06, respectively. Phenotypic and genetic correlations between CHL_fat and CHL_milk were 0.82 and 0.44±0.24, respectively. CHL_fat had non-significant genetic correlations with all production traits whereas CHL_milk had significant
(P<0.05) genetic correlations with milk yield (-0.47), fat yield (0.51), protein percentage (0.56) and fat percentage (0.88). This is the first study to estimate genetic parameters for milk CHL content. Further studies are required to assess the possibility of genetically selecting cows with lower milk CHL content.

**Keywords**: Milk cholesterol, Genetic parameters, Heritability, Holstein cows

Bovine milk is an important human dietary component, serving as a delivery medium for crucial molecules including cholesterol (CHL). Cholesterol is a metabolic precursor of bile acids, steroid hormones and vitamin D. It is required for the metabolic systems involved in DNA synthesis and cell division, and also plays an integral role in lipid transport. A normal adult human body contains around 150 g of CHL and synthesizes 700-1,500 mg of CHL daily (Sieber 1993). Since plant materials contain little or no CHL, humans derive most of their dietary CHL from animal sources, including meat, milk and eggs. High concentration of CHL in human blood and in particular high concentration of low-density lipoprotein CHL (LDL-CHL) are widely considered modifiable risk factors of cardiovascular diseases (CVD). In particular, guidelines for the management of dyslipidaemia (e.g. 2016 EAS/ESC guidelines, Catapano et al. 2016), stressed the need to lower LDL-CHL levels and address the important role that diet and lifestyle change can play in the prevention of CVD. Although it is still debated whether dietary CHL is a risk factor for CVD (Pencina et al. 2014; Peters et al. 2016; Ridker 2014; Saleheen et al. 2015) reducing the total and LDL-CHL concentrations in blood has received enormous attention in recent years. For example, a recent study indicated that medical practitioners and dieticians advised mildly to moderately hypercholesterolaemic individuals to change their dietary patterns.
in favor of foods with added plant sterols with the result that blood total and LDL-CHL levels were reduced (Sialvera et al. 2017). Consequently, dietary approaches proposed to lower human blood CHL levels include decreased intake of saturated fatty acids, increased intake of unsaturated fatty acids, plant fiber and sterols (Kris-Etherton et al. 2002; Ras et al. 2014; Siri-Tarino et al. 2010; Whitehead et al. 2014).

Cow milk is the second highest contributor to human dietary CHL intake (Royo-Bordonada et al. 2003) therefore, decreased milk CHL content may benefit human health. The CHL content of milk varies between species and breeds and is impacted by such factors as genetics, nutrition, stage of lactation and breed (Jensen, 2002; Reklewska et al., 2002; Strzałkowska et al., 2009a, b; Faye et al., 2015). Knowledge about the effects of physiological factors on milk CHL is scarce.

Analyzing the milk CHL content of 124 Polish Holstein-Friesian cows, Strzałkowska et al. (2009a) reported that the CHL content of milk (mg/dL of milk) and milk fat (mg/100 g of fat) were significantly affected by stage of lactation, season of year, somatic cell count and fat content, but not by parity. However, the authors observed that the amount of CHL produced by a cow in a day (mg/cow/day) was significantly increased when cows reached a higher parity. Precht (2001) indicated that CHL content varied with stage of lactation with a very high concentration during the colostrum phase (day 1 to 7 post-partum, 327.2 mg/100 g of fat) as compared to the population mean of 265.5 mg/100 g of fat. Strzalkowska et al. (2009) reported a positive correlation between milk CHL content and somatic cell count. Several gene expression/proteomics studies have reported genes with potential involvement in milk CHL concentration/metabolism (Altenhofer et al. 2015; Gross et al. 2015; Kessler et al. 2014; Mani et al. 2011; Ontsouka et al. 2017; Ontsouka et al. 2013; Schlegel et al. 2012; Viturro et al. 2009; Weber et al. 2013). For instance, Mani et al. (2011) reported the presence of \textit{ABCA1} and \textit{ABCG1}
proteins in milk fat globule membranes thus suggesting their potential involvement in CHL exchange between mammary epithelial cells and alveolar milk. Using cell culture study, Ontouka et al. (2017) indicated that CHL transport in mammary epithelial cells is mediated by APOA-1/ABCA1 and ABCG1/HDL dependent pathways. Studying the response of CHL metabolism to negative energy balance induced by feed restriction, Gross et al. (2015) observed that CHL metabolism is influenced by nutrient and energy deficiency according to stage of lactation in dairy cows.

Considering the possible impact of milk CHL on human health and the sparse information on the environmental and genetic factors that affect milk CHL content, the objectives of the current study were to estimate heritability of milk CHL, and to investigate genetic correlations between milk CHL and other production traits.

**MATERIALS AND METHODS**

**Animals and Milk Sampling**

A total of 1,848 Holstein cows from 29 herds in the province of Quebec, Canada (minimum: 33 cows/herd, maximum: 172 cows/herd) were sampled from November 2014 to June 2015. Within a herd, all milk sampling occurred in a single day. A total of 100 mL of milk was obtained from each animal during the morning milking and 50 mL was used for the routine determination of milk components by regular dairy herd improvement (DHI) milk testing, and 50 mL was used for the determination of milk CHL content. Test day milk fat and protein yields, fat and protein percentages, and somatic cell count (SCC) were provided by Valacta (Ste-Anne-de-Bellevue, QC) from regular DHI milk testing. The care of animals and use procedures were according to
the Canadian Council on Animal Care (CCAC 2009) and were approved by the Animal Care and Ethics Committee of Agriculture and Agri-Food Canada.

**Determination of Milk Cholesterol Content**

Milk CHL content was determined in milk fat samples by direct saponification and capillary gas chromatography according to Fletouris et al. (1998). Briefly, 0.2 mg milk fat was saponified in capped tubes with 0.5 M methanolic KOH solution by heating for 15 minutes. The unsaponifiable fraction was extracted with toluene and analyzed by capillary gas chromatography using Agilent HP 6890 Series Gas Chromatography System (Agilent Technologies, California, USA). A BPX50 capillary column (30 m long, 0.25 mm internal diameter) coated with a 0.25 mm thick film of 50% PH polysilphenylene siloxan (SGE, Melbourne, Australia) was used. Oven temperature was set at 285°C and holding time was 12.5 mins, injection port temperature was set at 250°C, and flame ionization detector temperature at 300 °C. The flow rates were 2 mL/min for helium, 30 mL/min for hydrogen, and 300 mL/min for air. The injection volume was 1 µL with a split ratio of 5:1. The makeup gas was helium, and the total of constant column and makeup flow was 40 mL/min. A calibration curve was generated by injecting 1 µL from (40, 60, 80, 120 and 160 µg/ml) of reference CHL standard (Sigma-Aldrich, Missouri, USA) and plotting the recorded peak area versus the corresponding mass of the analyte injected. The slope, intercept, and least squared fit of the standard curve were computed. Data for the slope and intercept of the calibration curve were used to compute the mass of the analyte in unknown sample extracts (1 µL) that were injected. The concentration C (CHL\_fat; mg/100g of fat) of CHL in analyzed samples was calculated according to the equation C = M × 2.5, where M = computed mass (ng) of the analyte in the injected extract (1 µL). The concentration of CHL in
milk (CHL_milk; mg/100g of milk) was calculated by multiplying the CHL concentration in fat by the milk fat percentage obtained through DHI milk testing.

**Data Editing**

Cows had records for cow registration number, dam and sire information, parity, days in milk (DIM), and test day milk component records. Since SCC is count data, it was log transformed to somatic cell score (SCS) using the formula \( SCS = \log_2 \left( \frac{SCC}{100,000} \right) + 3 \) proposed by Ali and Shook (1980) in order to normalize the distribution. Parity was classified as 1, 2 and 3 for cows in first, second and third or greater parities, respectively. Records from cows greater than 365 DIM were excluded. After data editing, records of 1,793 cows from 29 different herds were retained for analysis. A pedigree file, going back as many generations as available, containing 41,273 cows, 10,887 sires and 28,789 dam records was used. Pedigree data was provided by the Canadian Dairy Network (Guelph, ON).

**Statistical and Genetic Analyses**

The significance of the fixed effects of DIM, herd, and parity on CHL content in both fat and milk was first tested using the GLM procedure of SAS (SAS Institute, 2013). A significant linear effect of DIM \((P < 0.001)\) was found for both CHL_fat and CHL_milk. The fixed effect of herd was significant \((P < 0.001)\), while parity was not \((P = 0.47)\). However, the interaction between herd and parity was significant \((P < 0.001)\) and, therefore, it was included in the final mixed linear animal model.

Univariate and bivariate linear mixed animal models were used to estimate variance components using the average information-restricted maximum likelihood (AI-REML)
procedure in the DMU package (Madsen and Jensen, 2008). The following linear mixed animal model was applied to all traits:

\[ y_{ijk} = \mu + (H \times P)_{ij} + b_1 \text{DIM} + a_k + e_{ijk} \]

where \( y_{ijk} \) is the record of the trait of interest on the \( k \)th animal; \( \mu \) is the fixed overall mean; \((H \times P)_{ij}\) is the fixed effect of the interaction between the \( i \)th herd and \( j \)th parity group; \( b_1 \) is the slope of the fixed linear regression on DIM; \( a_k \) is the random additive genetic effect of the \( k \)th animal; and \( e_{ijk} \) is the random error term. The random animal and residual terms were assumed to be independent and distributed as follows: \( a \sim N(0, A\sigma_a^2) \) and \( e \sim N(0, I\sigma_e^2) \), where \( \sigma_a^2 \) and \( \sigma_e^2 \) are animal additive genetic and residual variances, respectively, and \( A \) and \( I \) are additive relationship and identity matrices, respectively. Bivariate models fitting the same effects as the univariate model described above were run for CHL_fat and CHL_milk, and for either CHL_fat or CHL_milk with one of the production traits at a time.

Heritability was calculated using the variance components estimated by the univariate analyses. Genetic correlations were estimated using (co)variances from the series of bivariate analyses of CHL content and production traits. The genetic correlation estimates were considered significantly different from 0 when the estimate deviated by more than 1.96 \( \times \) SE from 0 (\( P < 0.05 \)).

RESULTS

Descriptive Statistics

The descriptive statistics for milk CHL content and milk production traits are shown in Table 1. CHL content in milk was expressed in two ways: (i) mg of CHL per 100 g milk fat (CHL_fat) and (ii) mg of CHL in 100 mg milk (CHL_milk). CHL_fat ranged from 102.1 mg to 549.22 mg/100 g of fat with a mean (SD) of 275.63 (75) mg. On average (SD), 100 g of milk contained
11.16 (3.63) mg of CHL and the amount of CHL varied from 3.83 mg to 26.45 mg. Milk, fat, and protein yields varied highly among samples with maximum values about ten times higher than minimum values (Table 1). Less variation was noted for fat and protein percentages. CHL concentration increased linearly with increasing stage of lactation ($P < 0.05$) (Figure 1). The average concentrations (SD) of CHL by herd varied from 187.21 (30.65) to 350.45 (77.35) mg for CHL_fat and 7.59 (1.66) to 15.34 (4.25) mg for CHL_milk (Figure 2).

**Genetic Parameters**

The additive genetic and residual variances estimated for CHL_fat were 229.74 and 3,628.92, and for CHL_milk were 1.52 and 7.63, respectively. Heritability of CHL and estimated correlations between milk CHL content with other production traits are shown in Table 2. Heritability for CHL_milk ($0.17 \pm 0.06$) was higher than for CHL_fat ($0.06 \pm 0.04$); and low to moderate heritabilities were found for other production traits.

A strong and significant phenotypic correlation ($0.82$) was estimated between CHL_fat and CHL_milk ($P < 0.05$), while moderate and non-significant genetic correlation was reported between them ($0.44 \pm 0.24$) (Table 2). The estimated genetic correlations between CHL_fat and production traits (milk yield, fat yield and fat percentage, protein yield and protein percentage) were low and not significant. CHL_milk had negative but significant ($P < 0.05$) genetic correlation with milk yield ($-0.47 \pm 0.21$) and significant positive ($P < 0.05$) correlation with fat yield ($0.51 \pm 0.19$) and fat percentage ($0.88 \pm 0.08$). All CHL expressions had very low (non-significant) phenotypic correlations (and negative genetic correlations with SCS (Table 2).
DISCUSSION

Cholesterol Content in Milk and Effects of Environmental Factors on Cholesterol Content

The CHL content in milk can be measured using different methods and be expressed in various ways (mg in 100 g of fat, mg in 100 ml of milk, mmol/L of milk and so on) (Barlowska et al. 2011; Faye et al. 2015; Jensen 2002; Reklewska et al. 2002; Šterna and Jemeljanovs 2003; Strzałkowska et al. 2009a, b; Talpur et al. 2006). In this study, we used two different ways (CHL_fat and CHL_milk) to express the CHL content in milk in order to easily compare our data with previous studies.

The mean value of CHL_fat (275.63 mg/100 g fat) was higher than 265.6 mg/100 g fat reported for 1,142 German cows under diverse feeding systems (Precht 2001), but lower than 387 mg/100 g fat reported for 124 Polish cows fed ad libitum with a total mixed ration (TMR) system (Strzałkowska et al. 2010). Moreover, CHL_fat in this study had a larger range (from 102.1 mg to 549.22 mg/100 g fat) compared with 204.4 to 382.5 (mg/100 g fat) in German cows (n = 1,142) (Precht 2001). Sample variation generally increases with increase in sample size; this study was based on a larger sample size (n = 1,793) compared to previous studies (Altenhofer et al. 2015; Precht 2001a; Strzałkowska et al. 2009a). Moreover, samples in this study originated from 29 herds and milk CHL content varied significantly ($P < 0.001$) among herds. Average concentration of CHL_fat ranged from 187.21 mg to 350.45 (mg/100 g fat) and CHL_milk from 7.59 to 15.34 (mg/100 g milk) in the herds (Figure 2). A high variation in milk CHL content has been reported between cows in 13 European Union countries (Precht 2001), thus supporting our observations. However, it should be note that different management practices including nutritional regimes and genetic factors could be responsible for the variations in milk CHL.
content among herds. Several studies have shown that feeding systems (Barlowska et al. 2011) and the source of fat in the diet (Barlowska et al., 2011; Faye, 2015; Reklewska et al., 2002) significantly contributed to variation in milk CHL content. For instance, Barlowska et al. (2011) observed that feeding system had a significant effect on CHL content whereby, cows on a TMR system (constant composition of fodder throughout the year) had higher CHL content (by 2.18 mg/100 ml of milk) compared to cows on the traditional system (with pasture grazing in the summer season). The CHL_milk content was highly variable in this study since cows with the highest values (e.g. 26.45 mg/100 g milk) demonstrated about 6.9 times higher CHL_milk content than cows with the lowest values (e.g. 3.83 mg/100 g milk). This result is consistent with data obtained by Sharma and Singh (1996) who reported up to 300% variation in CHL_milk (CHL_milk varied from 8.7 to 25.4 mg/dL) in Indian cows.

Our results showed that CHL content was significantly affected by stage of lactation (Figure 1). Previously, several studies described variation in milk CHL content during different stages of lactation (Altenhofer et al. 2015; Precht 2001; Strzalkowska et al. 2009a). However, the effect of stage of lactation on milk CHL content was inconsistent among studies. For instance, Altenhofer et al. (2015) reported that milk CHL content decreased significantly from 20.26 ± 1.57 mg/dL in early lactation to 15.61 ± 0.81 mg/dL in mid-lactation, while Strzalkowska et al. (2009a) reported that milk CHL content increased consistently from early lactation (15.6 ± 0.21 mg/dL) to late lactation (16.9 ± 0.17 mg/dL). However, little is known about the mechanisms underlying the variations in milk CHL content by stage of lactation. Since the demands in energy varies between lactation stages, changes in milk CHL content might also be a consequence of metabolic adaptions in response to lactation stage dependent energy demands (Gross et al. 2015). Additionally, some studies suggest that changes in the expression of several genes might be
responsible for this variation, such as genes regulating CHL synthesis in the liver (\textit{HMGCS1, HMGCR} and \textit{FDFT}), genes regulating lipid metabolism (\textit{SREBP1} and 2) (Kessler et al. 2014; Viturro et al. 2009), and gene regulating cellular CHL homeostasis (\textit{ABCA1}) (Mani et al., 2010;Kessler et al., 2014). In addition, Mani et al. (2011) reported upregulation of \textit{ABCA1} mRNA in mammary tissue at the end of lactation as compared to early lactation, suggesting that \textit{ABCA1} might have a role in the regulation of CHL homeostasis in the mammary gland. Also, Kessler et al. (2014) reported upregulation of genes involved in the biosynthesis of CHL (\textit{SREBF2, HMGCS1,} and \textit{HMGCR}) as well as \textit{ABCA1} transporter at the onset of lactation, and suggested that increased expression of these genes might be due to a response by the liver to increased demand for CHL after parturition. It is important to note that the definition of stage of lactation was not consistent among studies. Altenhofer et al. (2015) divided lactation period into three stages: Early (< 100 DIM), Mid (100 - 200 DIM) and Late (>200 DIM), while (Strząłkowska et al. 2009a) partitioned lactation period into five stages with 60 DIM intervals.

Using Strząłkowska et al. (2009a) definition of lactation stage (60 DIM intervals), we observed that both milk CHL\_fat and CHL\_milk increased consistently from the first stage of lactation to the last stage of lactation (Figure 1), which is consistent with the observation by those authors. Parity was not an important factor for milk CHL content in this study, which is consistent with results obtained by Strząłkowska et al. (2009a).

\textbf{Genetic Parameters}

To the best of our knowledge, this is the first study to report on the heritability of milk CHL content. Most genetic studies on CHL have been done with blood from human and animal models (including pigs) due to their importance to human disease studies. In humans, several studies reported modest to high estimates of heritabilities (0.24-0.83) for plasma high density
lipoprotein CHL (Boes et al. 2009) or 0.52 ± 0.02 for total plasma CHL (Bielinski et al. 2006). In pigs, heritability estimate for plasma CHL was 0.45 ± 0.23 (Pond et al. 1986). In the current study, heritability estimates for milk CHL content (0.06 for CHL_fat and 0.17 for CHL_milk) were low, reflecting that many factors contribute to variation in bovine milk CHL content. According to Long et al. (1980), most of the CHL in ruminant milk (~80%) is derived from blood while around 20% is synthesized de novo in mammary tissues. Therefore, it is expected that milk CHL content is controlled not only by blood CHL concentration, but also by other factors that regulate de novo CHL synthesis in mammary gland tissue as well as CHL transport in to the mammary gland (Ontsouka and Albrecht 2014). For other production traits, lower heritabilities were observed in this study compared to previous studies on Canadian Holsteins (Miglior et al. 2007; Muir et al., 2004; ). Miglior et al. (2007) estimated heritabilities of 0.52, 0.37, 0.42, and 0.19 for test day milk, fat, and protein yields, and SCC, respectively. The differences in heritability estimates might be due to differences in sample size and statistical models. Miglior et al. (2007) used a larger data set with 60,645 test day records of 5,022 cows and the test-day animal model for estimation of parameters, while 1,793 records and single record animal models were used in this study.

High phenotypic (0.82) and moderate genetic (0.44 ± 0.24) correlations were estimated between CHL_fat and CHL_milk. The high estimated phenotypic correlation was expected since CHL_milk measures were derived from CHL_fat as described above. Although genetic correlation between CHL_fat and CHL_milk was significantly different from a value of 1, it is not clear whether they are genetically distinct traits. More studies using direct measure for each trait, as well as using a larger phenotypic data set is needed to clarify this point. Genetic correlations between CHL content and production traits in the current study depended on how
the CHL content was expressed. Except SCS, genetic correlations (either negative or positive) of CHL_milk with milk and milk components traits were stronger compared to the values for CHL_fat. Both CHL_fat and CHL_milk had negative phenotypic and genetic correlations with milk yield (Table 2). Larsen (2012) reported that CHL_milk had negative phenotypic correlation with milk yield in Danish cows, while Strzałkowska et al. (2010) observed that milk yield did not significantly affect milk CHL content regardless of its expression. In agreement with Strzałkowska et al. (2010), we also reported negative (-0.03) and positive correlations (0.88) of CHL_fat and CHL_milk with fat percentage, respectively. CHL_fat and CHL_milk had no significant phenotypic and genetic correlations with SCS which is in disagreement with Strzałkowska et al. (2010) who reported that SCC significantly affected CHL_fat. Finally, it is important to note that we measured the CHL in milk by direct saponification of the fat fraction and quantification using capillary gas chromatography. Although capillary gas chromatography yields accurate milk CHL concentration, it is labor intensive requiring a lengthy procedure which might hinder routine generation of large data for use in genetic improvement. Therefore, quantification of CHL contained in milk using infrared spectra based predictors might be a good alternative to gas chromatography. It was reported that both fourier transformed infrared (FTIR) spectroscopic and fourier transformed near-infrared (FT-NIR) methods can accurately predict milk CHL content (prediction errors less than 6% compared to a conventional method like o-phthalaldehyde) (Paradkar and Irudayaraj 2002a; Paradkar and Irudayaraj 2002b; Rudel and Morris 1973). However, these authors used pure cholesterol as starting material therefore it is not clear how these methods (FTIR and FT-NIR) will perform if the starting material is milk. Another study reported less accuracy for prediction of CHL content in cheese using near infrared transmittance spectroscopy due to low concentration of CHL in cheese.
(Manuelian et al. 2017). Further studies are therefore required to improve the accuracy and procedure for quantification of milk CHL as well as other dairy products with infrared spectra based methods.

**CONCLUSIONS**

To the best of our knowledge, this is the first study to estimate genetic parameters and heritability for milk CHL content. This study confirmed that milk CHL content varied highly among different herds and indicated that milk CHL content significantly increased with increasing lactation days. Heritability estimates for CHL_fat and CHL_milk were low (0.06 and 0.17), indicating important non-genetic effects contributing to the phenotypic variation of milk CHL content in Holsteins. The content of CHL in milk had a non-significant low genetic correlation with the content of CHL in fat. Further studies on selection responses and genomics are required to evaluate the possibility of selecting cows with lower milk CHL content.

**ACKNOWLEDGEMENTS**

Authors thank participating farmers for animal management and Anne-Marie Christen of Valacta for coordinating milk sampling by Valacta (www.valacta.com). The authors also thank Mr. Jasmin Brochu (AAFC) for help with fat saponification, Mr. Peir-Luc Dudemain, Ms Pamela Warburton and Mr. Adolf A. Ammah (all of AAFC) for help with milk processing and Dr. Khalida Bekri (AAFC) for help with gas chromatography. This research was supported by the DairyGen Council of the Canadian Dairy Network and the Natural Sciences and Engineering Research Council of Canada (NSERC).
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TABLE AND FIGURE TITLES

Table 1: Descriptive statistics for milk cholesterol content (CHL_fat and CHL_milk) and production traits for milk samples from 1,793 Canadian Holstein cows

Table 2: Heritability estimates of milk cholesterol content (CHL_fat and CHL_milk) and milk production traits, and genetic or phenotypic correlations between them

Figure 1: Cholesterol concentration (CHL_fat and CHL_milk) by stage of lactation. CHL_milk: mg of cholesterol in 100 g of milk, CHL_fat: mg of cholesterol in 100 g of milk fat. Stage of lactation was based on 60 DIM intervals.

Figure 2: Boxplot of milk cholesterol concentration (presented in ascending order from left to right) in different herds. CHL_milk (mg of cholesterol in 100 g of milk), CHL_fat (mg of cholesterol in 100 g of fat).
Figure 1

182x187mm (300 x 300 DPI)
Figure 2

254x190mm (300 x 300 DPI)
Table 1. Descriptive statistics for milk cholesterol content (CHL\textsubscript{fat} and CHL\textsubscript{milk}) and production traits for milk samples from 1,793 Canadian Holstein cows

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<td>3.35</td>
<td>0.34</td>
<td>2.50</td>
<td>4.67</td>
</tr>
<tr>
<td>SCS\textsuperscript{b}</td>
<td>2.14</td>
<td>1.92</td>
<td>-3.64</td>
<td>9.21</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Standard deviation of the mean

\textsuperscript{b}SCS: Log transformed somatic cell count (SCC) (SCS = \log_{2}(SCC/100,000) + 3)
Table 2: Heritability estimates of milk cholesterol content (CHL\_fat and CHL\_milk) and milk production traits, and genetic or phenotypic correlations between them

<table>
<thead>
<tr>
<th>Traits</th>
<th>CHL_fat</th>
<th></th>
<th></th>
<th>CHL_milk</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Heritability</td>
<td>Genetic correlation</td>
<td>Phenotypic correlation</td>
<td>Genetic correlation</td>
<td>Phenotypic correlation</td>
<td></td>
</tr>
<tr>
<td>CHL_fat(^a)</td>
<td>0.06 ± 0.04</td>
<td>0.44 ± 0.24</td>
<td>0.82*</td>
<td>0.44 ± 0.24</td>
<td>0.82*</td>
<td></td>
</tr>
<tr>
<td>CHL_milk(^b)</td>
<td>0.17 ± 0.06</td>
<td>0.44 ± 0.24</td>
<td>0.82*</td>
<td>0.44 ± 0.24</td>
<td>0.82*</td>
<td></td>
</tr>
<tr>
<td>Milk yield (kg)</td>
<td>0.26 ± 0.07</td>
<td>-0.14 ± 0.34</td>
<td>-0.03</td>
<td>-0.47 ± 0.21*</td>
<td>-0.19</td>
<td></td>
</tr>
<tr>
<td>Fat yield (kg)</td>
<td>0.28 ± 0.07</td>
<td>0.02 ± 0.34</td>
<td>-0.05</td>
<td>0.51 ± 0.19*</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>Protein yield (kg)</td>
<td>0.25 ± 0.07</td>
<td>-0.14 ± 0.34</td>
<td>-0.01</td>
<td>-0.19 ± 0.23</td>
<td>-0.06</td>
<td></td>
</tr>
<tr>
<td>Fat percentage (%)</td>
<td>0.46 ± 0.08</td>
<td>-0.03 ± 0.29</td>
<td>-0.03</td>
<td>0.88 ± 0.08*</td>
<td>0.53</td>
<td></td>
</tr>
<tr>
<td>Protein percentage (%)</td>
<td>0.32 ± 0.07</td>
<td>-0.15 ± 0.30</td>
<td>0.09</td>
<td>0.56 ± 0.17*</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>SCS(^c)</td>
<td>0.11 ± 0.05</td>
<td>-0.42 ± 0.40</td>
<td>0.02</td>
<td>-0.08 ± 0.29</td>
<td>0.05</td>
<td></td>
</tr>
</tbody>
</table>

**Note:** *The correlations differed significantly from zero at P < 0.05
\(^a\)CHL\_fat: mg of cholesterol in 100 g of fat. \(^b\)CHL\_milk: mg of cholesterol in 100 g of milk. \(^c\)SCS: Log transformed somatic cell count (SCC) (SCS = log₂(SCC/100,000) + 3).