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VARIABILIDAD GENÉTICA DE ÁCIDOS GRASOS Y FRACCIONES NITROGENADAS EN LECHE DE BOVINOS

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Tesis realizada por **LUIS ANTONIO SAAVEDRA JIMÉNEZ**, bajo la supervisión del Comité Asesor indicado, aprobada por el mismo y aceptada como requisito parcial para obtener el grado de:

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Dedicatorias

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Resumen general

VARIABILIDAD GENÉTICA DE ÁCIDOS GRASOS Y FRACCIONES NITROGENADAS EN LECHE DE BOVINOS

Dada la necesidad de satisfacer la demanda social de alimentos de origen animal, mejorar las características funcionales de estos alimentos y reducir el impacto ambiental del ganado lechero, en años recientes, en los programas de selección de ganado bovino lechero se han propuesto nuevos objetivos. Los parámetros genéticos (PG) son importantes para diseñar programas de mejoramiento genético animal y estimar la respuesta a la selección. En este estudio se analizó el componente genético aditivo para características de ácidos grasos (AG) y fracciones nitrogenadas (FN) en leche de bovinos Suizo Americano de México (BSAM). Se presenta una revisión de literatura sobre factores que podrían afectar la estimación de PG para AG en leche de bovinos, así como interacciones entre dichos factores. Además, se realizó un meta-análisis para obtener estimaciones ponderadas de heredabilidad (h^2) y correlación genética (r_g) para AG. Tanto el método de cuantificación como la ruta metabólica de producción de los AG, son factores clave en la estimación de PG. El meta-análisis mostró h^2 <0.50 y r_g, entre AG y componentes de la leche, >0.40. Es posible modificar la composición de la grasa de la leche utilizando el componente genético. Finalmente, se estimó h^2 para AG, componentes de la leche, FN, y para la proporción caseína:proteína en leche de BSAM, y r_g entre AG individuales, entre AG y componentes de la leche, entre FN, y entre FN y la proporción caseína:proteína. La h^2 estimada, en general, fue moderada; para los componentes de la leche, AG, FN y la proporción caseína:proteina fue <0.70, <0.40, <0.30 y <0.20, respectivamente; sin embargo, para FN algunas estimaciones fueron >0.70. Las r_g estimadas abarcaron el rango -0.90 a 0.90, algunas incluso cercanas a 1.00 Existe variabilidad genética aditiva suficiente para lograr una mejora genética de las características estudiadas; por tanto, podrían ser consideradas en programas de selección para la población estudiada.

Palabras clave: bovinos Suizo Americano, calidad de la leche, parámetros genéticos, programas de selección.

Tesis de Doctorado en Ciencias, Programa de Posgrado en Producción Animal,
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Abstract

GENETIC VARIABILITY OF FATTY ACIDS AND NITROGENOUS FRACTIONS IN MILK OF DAIRY CATTLE

Given the needs to meet social demands for products of animal origin, to improve their functional traits, and to reduce the environmental impact of dairy cattle, in recent years, new breeding objectives have been proposed in selection programs of dairy cattle. Genetic parameters are important to design animal breeding programs and to estimate response to selection. The additive genetic component for fatty acids (FA) and nitrogenous fractions (NF) traits in milk of Mexican Brown Swiss cattle (MBSC) was analyzed. A literature review is presented on the factors that could affect the estimation of genetic parameters for milk FA in dairy cattle, as well as interactions between these factors. In addition, a meta-analysis was performed to obtain weighted estimates of heritability (h^2) and genetic correlation (r_g) for milk FA in dairy cattle. Both the quantification method and the FA metabolic pathway are key in the estimation of genetic parameters. The meta-analysis showed h^2 <0.50, and r_g between FA and milk components, >0.40. It is possible to modify the milk fat composition of dairy cattle using the genetic component. Finally, h^2 was estimated for FA, milk components, NF, and for the casein:protein ratio in milk of MBSC, as well as r_g between individual FA, between FA and milk components, between NF, and between NF and casein:protein ratio. Estimates of h^2, in general, were moderate; for milk components, FA, NF and casein:protein ratio: <0.70, <0.40, <0.30 and <0.20, respectively. However, some h^2 estimates, within NF, were >0.70. The estimated r_g ranged from -0.90 to 0.90, some estimates were close to 1.00. There is enough additive genetic variability to achieve genetic improvement on the traits studied; therefore, they could be considered in selection programs for the population studied.

Key words: breeding programs, Brown Swiss cattle, genetic parameters, milk quality.

Doctoral Thesis in Livestock Innovation, Graduate Program in Animal Production, Universidad Autónoma Chapingo
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1. Introducción general

La leche y sus derivados son fuentes de nutrientes en la dieta; aportan energía, proteínas, vitaminas y minerales (Huth, DiRienzo, & Miller, 2006), por lo que representan una valiosa alternativa para acceder a nutrientes. Sin embargo, el incremento de enfermedades cardiovasculares y algunos tipos de cáncer relacionados con la composición de la leche, han contribuido al aumento en la preocupación de consumidores y profesionales de la salud (Soyeurt et al., 2011).

Los componentes (%) de la leche son: lactosa (4.8), grasa (3.7), proteína (3.4), cenizas (0.7), el resto es agua. Además, contiene vitaminas, iones y saborizantes (Fox & Mcsweeney, 1998). La grasa de la leche está constituida por triacilgliceroles (aproximadamente 98%), diacilglicerol (2% de la fracción lipídica), colesterol (<0.5%), fosfolípidos (alrededor de 1%) y ácidos grasos (AG) libres (alrededor de 0.1%) (Jensen & Newburg, 1995). Contiene también trazas de éter, lipo-vitaminas y otros compuestos (Parodi, 2004). Dentro de estos últimos, los AG desempeñan un papel importante, no sólo por los beneficios a la salud humana (Hu et al., 1999; Tanaka, 2005; Rasmussen et al., 2006), sino también por su relación con los gases de efecto invernadero (Dijkstra et al., 2011; Kandel, Soyeurt, & Gengler, 2012; Vanrobays et al., 2015), y por su contribución a mejorar la calidad y propiedades tecnológicas de distintos derivados lácteos (Bobe, Hammond, Freeman, Lindberg, & Beitz, 2003; Bobe et al., 2007; Glantz et al., 2009). Se han identificado cerca de 400 AG en la leche, presentes en cantidades traza, aunque alrededor de 15 (**Cuadro 1**) se han determinado en concentraciones mayores que 1% (MacGibbon & Taylor, 2006).

Por décadas, el ganado lechero se ha seleccionado con enfoque similar alrededor del mundo; características relacionadas con producción y composición

de leche, tipo, reproducción y salud, son registradas y usadas comúnmente en esquemas de selección (Banos, 2010). Sin embargo, se deben definir nuevos objetivos para enfrentar los retos de una producción sustentable, restaurar las características funcionales del ganado lechero y atender las demandas sociales de los consumidores por los distintos derivados lácteos (Boichard & Brochard, 2012). Algunos de estos objetivos podrían ser enfocados al perfil de AG, ya que estas características tienen efecto económico (calidad de productos lácteos; Palmquist, Denise Beaulieu, & Barbano, 1993), social (los AG insaturados son benéficos para la salud humana; Soyeurt & Gengler, 2008) y ambiental (relacionado con la producción de metano; Dijkstra et al., 2011). Para este propósito, es conveniente desarrollar un índice de selección, y el primer paso es la estimación de parámetros genéticos para el perfil de AG (Arnould & Soyeurt, 2009). Diferentes investigaciones (Pegolo et al., 2016; Petrini et al., 2016; Narayana et al., 2017) del componente genético aditivo de los AG han estimado parámetros genéticos y sugieren que existe variación suficiente para considerarse en programas de mejoramiento genético, basados en el perfil de AG. Por lo anterior, su inclusión en programas de mejora genética de bovinos lecheros podría ayudar a enfrentar los retos mencionados previamente.

Cuadro 1. Concentración (%) de los principales ácidos grasos en leche de bovino.

Nombre, No.C[Z]	Conc[Y]	Nombre, No.C[Z]	Conc[Y]
Butírico, 4	3.9	Palmítico, 16	27.9
Caproíco, 6	2.5	Palmitoleio, 16:1	1.5
Caprílico, 8	1.5	Esteárico, 18	12.2
Cáprico, 10	3.2	Oleico, 18:1	17.2
Laúrico, 12	3.6	18:1trans	3.9
Míristico, 14	11.1	Linoleico, 18:2	1.4
Miristoleico, 14:1	0.8	Lonoleico conjugado	1.1
Pentadecanoico, 15	1.2	Linolenico, 18:3	1.0

Fuente: Creamer & Macgibbon, 1996. [z] Nombre, No.C = Nombre del ácido graso, número de carbonos; [Y] Conc= Concentración de ácido graso.

Algunos esfuerzos para implementar evaluaciones genéticas enfocados en la modificación del perfil de AG han sido desarrollados en países europeos (Gengler, Troch, Vanderick, Bastin, & Soyeurt, 2012). Sin embargo, aunque en México se ha llevado a cabo la evaluación genética del ganado Suizo Americano desde el año 2004 (Núñez, Ramírez, García, & Hidalgo, 2018), sólo se ha realizado para producción de leche. Dadas las nuevas tendencias en la alimentación humana de consumir alimentos nutritivos y funcionales, existe el interés por parte de criadores de ganado Suizo Americano, de ofrecer leche con mejores características y así aprovechar los nuevos nichos de mercado a nivel nacional e internacional, por lo que la inclusión de características de AG, como características alternativas y complementarias a la evaluación genética actual, permitiría a los ganaderos mexicanos participar con mayores oportunidades en el mercado.

Hipótesis

Existe suficiente variabilidad genética aditiva para características de ácidos grasos en leche de bovinos Suizo Americano de la población mexicana, por lo que la evaluación genética de estas características podría ser implementada en la población estudiada.

Objetivos

- Investigar la variación fenotípica y genética de los ácidos grasos en leche de bovinos Suizo Americano de la población mexicana, cuantificados mediante cromatografía de gases.
- Investigar la variación fenotípica y genética de las fracciones nitrogenadas y la proporción caseína:proteína en leche de bovinos Suizo Americano mexicanos.
- Estimar parámetros genéticos para ácidos grasos en leche de bovinos Suizo Americano mexicanos.

- Estimar parámetros genéticos para fracciones nitrogenadas y para la proporción caseína:proteína en leche de bovinos Suizo Americano mexicanos.

Estructura de la tesis

En el **Capítulo 2** de esta tesis se presenta una revisión de literatura, donde se resumen estimaciones de parámetros genéticos (heredabilidad y correlación genética) para características de AG; se describen los posibles factores que pudieran generar diferencias en las estimaciones, así como interacciones entre los factores identificados. Finalmente, se hace una revisión de los avances en selección genómica para estas características.

Debido a los diferentes factores que afectan las estimaciones de parámetros genéticos para AG, se dificulta su comparación directa. En el **Capítulo 3** se presenta un meta-análisis, basado en un modelo de efectos aleatorios, que consideró la varianza dentro y entre estudios, para obtener estimaciones ponderadas de h^2 y r_g para AG.

Previo a la evaluación genética del contenido de AG en leche de bovinos Suizo Americano en la población mexicana, es necesario estimar los parámetros genéticos que caracterizan a dicha población. En el **Capítulo 4** se estimaron dichos parámetros para nueve AG (g/100 g de leche) y los componentes (%) de la leche en ganado Suizo Americano, cuantificados por cromatografía de gases y espectroscopia.

Como un trabajo colateral relacionado con el objetivo central de la tesis, en el **Capítulo 5** se muestra un estudio que tuvo como propósito estimar parámetros genéticos para fracciones nitrogenadas y la proporción caseína:proteína en leche de la población mexicana de bovinos Suizo Americano. Este estudio complementa la posibilidad de incluir características relacionadas con la calidad de leche, como una alternativa a sólo considerar la producción de leche en la evaluación genética de la población estudiada.

Finalmente, como resultado de trabajo desarrollado en la estancia de investigación, en la Universidad de Otago, en Nueva Zelanda, en el **Capítulo 6** se presenta un estudio que tuvo como propósito comparar el efecto de diferentes estrategias de agrupar progenitores desconocidos ("fantasmas"), en la evaluación genética de características de crecimiento para la población mexicana de bovinos Suizo Europeo. Este trabajo contribuye al mejoramiento general de la calidad de las evaluaciones genéticas que la Universidad Autónoma Chapingo realiza de forma anual y rutinaria del ganado Suizo Europeo de México.

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2. Heritability estimates for milk fatty acid traits in dairy cattle: A review

Abstract

There is growing interest both by industry and dairy producers in changing the milk fatty acid (FA) profile of dairy cattle using genetic selection. Heritability (h^2) and genetic correlation (r_g) are required to estimate the response to selection and to design breeding programs. This review summarizes h^2 estimates for FA, describes patterns of factors affecting them; describes r_g among FA, and FA and dairy traits; recent advances in genomic selection for FA are also reviewed. From literature, there were h^2 estimates for 59 individual FA and for nine groups of FA. In general, h^2 estimates were from low to moderate for both individual and FA grouped. Heritability estimates might vary by breed, method of FA determination and units to express them, the way h^2 is estimated, paternity and connectedness structure, and statistical model used to analyze the data. Estimates of r_g among FA were positive and influenced by their origin, and those between FA and dairy traits were affected by stage of lactation. Candidate genes affecting milk fat composition have been proposed. Information from molecular markers, SNPs, has been used to estimate h^2 for FA, but the scarce phenotypic and genotypic data limit the application of genomic selection for FA. There is enough additive genetic variability for FA to implement breeding programs. Pathway of FA synthesis have great influence on h^2 estimates, and FA quantification methodology on their accuracy. Genetic correlations estimates are influenced by both FA origin and stage of lactation. At present, the available data do not allow the application of genomic selection to improve the FA profile of dairy cattle.

Keywords: breeding program, fat composition, genetic parameters, lactation.

Introduction

Livestock production faces new challenges to restore functional traits, address social demands, and decrease its footprint (Boichard & Brochard, 2012). The new breeding goals should consider supplying high quality animal products at a reasonable price (Berry, 2013). On this regard, some efforts have been developed

to implement genetic evaluations for milk fatty acids (FA) profile (Gengler, Troch, Vanderick, Bastin, & Soyeurt, 2012), because their inclusion into breeding programs may support some of these new challenges. In addition, FA fulfill the criteria proposed by Berry (2013) to be considered as goal traits: they are important (economically, environmentally, and socially), they are under genetic control and, with the advance of technology, they will be easily measurable at an affordable cost.

Among the natural components of milk, the milk fat is one of the most complex (MacGibbon & Taylor, 2006). Around 96% of milk fat are triglycerides, each one made up of glycerol esterified with three FA, which are carboxylic acids with aliphatic chains of different length and saturation degree (Jensen, 1995). The variation in FA profile is attributed to genetic and non-genetics factors. Some of these factors have been reviewed but focused on non-genetic factors, such as lactation stage, cow health and type of feed (Jensen, 2002; Kay, Weber, Moore, Bauman, & Hansen, 2005), insofar as animal variation and breed (Palmquist, Beaulieu, & Barbano, 1993; Samková, Spicka, Pesek, Pelikánová, & Hanus, 2012) have been studied as genetic factors as well.

Bovine milk fat composition has been studied for some years, but genetic studies on FA are limited and most of them are recent. Nowadays, there is increasing interest in changing the FA profile using animal selection because those changes are permanent and give additional value to dairy products. Therefore, heritability (h^2) estimates and genetic correlations (r_g) are required to estimate the response to selection and to design breeding programs. Recent h^2 estimates (Pegolo et al., 2016; Petrini et al., 2016; Narayana et al., 2017) suggest an important genetic variation that could be used to develop genetic programs. Thus, this review aims to give an overview of advances in h^2 estimates for FA in dairy cattle and to identify some of the main factors that could influence them. Additionally, it presents r_g among FA, and FA with dairy traits; finally, it summarizes advances in genomic selection for FA.

Implications of milk fatty acids for the dairy industry

The FA profile influences the nutritional value (Mensink, Zock, Kester, & Katan, 2003), quality and technological properties of milk products (Bobe et al., 2007). For example, it plays a key role in cheese texture (Bugaud et al., 2001; Coppa et al., 2011), cheese flavor (Collins, McSweeney, & Wilkinson, 2003; Haug, Høstmark, & Harstad, 2007), and coagulation attributes (Auldist, Johnston, White, Fitzsimons, & Boland, 2004). In addition, FA profile affects physical butter characteristics such as spreadability, texture and hardness (Bobe, Hammond, Freeman, Lindberg, & Beitz, 2003; Coppa et al. 2011; Smith, Coffey, Johnstone, & Wall, 2014). Therefore, modification of the FA profile could have additional value for the dairy industry and could allow customer a greater intake of nutrients from dairy products.

Implications of fatty acids for human health

Theoretically, milk contains at least 400 different FA (Jensen, 2002). The effects on consumer health of every one of them have not been studied yet, maybe, because almost all of them are in low concentrations. In nutritional terms, some FA may have negative effects on human health; for example, the C12:0, C14:0 and C16:0 have been associated with adverse health effects because their intake could contribute to elevate the blood levels of low-density lipoproteins (Mensink et al., 2003). On the contrary, other FA could be beneficial for human health; for instance, unsaturated FA have been found to have a negative relationship with the incidence of coronary heart disease (Connor, 2000), they could improve insulin sensitivity and glucose tolerance (Parodi, 2004), and some long chain FA have shown anti-inflammatory effects (Haug et al., 2007). This summarized evidence emphasizes the importance of FA for human health.

Factors that could affect estimates of heritability

Genetic parameter estimates are specific for the population and time where they are estimated; h^2 estimates of individual FA have ranged between 0.00 and 0.74, higher than those summarized by Arnould and Soyeurt in 2009 (0.00-0.54), who only include the major FA. For FA grouped by their degree of saturation, h^2 has oscillated between 0.00 and 0.69, and between 0.07 and 0.68, when they are grouped by their chain length.

Some factors have been proposed (Mele et al., 2009; Garnsworthy, Feng, Lock, & Royal, 2010; Tullo et al., 2014; Pegolo et al., 2016; Petrini et al., 2016) to affect h^2 estimates of FA. Some of the factors are the method used to measure FA, the differences in data editing and structure, the inclusion or not of repeated records, the stage of lactation, the units used to express FA, the statistical models used to analyze the data, the breed and number of available samples, and the way in which h^2 is expressed.

Way in which h^2 is expressed

Heritability of FA has been estimated mainly from: a) analysis of variance of a trial having a large number of genotypes, b) genetic and environmental variances across generations, and c) parent–offspring regression. Comparison of h^2 estimates among studies might be complicated, due to differences in which phenotypic variance was decomposed. For example, Heringstad, Gianola, Chang, Ødegård, and Klemetsdal (2006) refer to intraherd h^2, as the one required to predict response to selection of alternative breeding programs, and allows comparisons with studies that consider the effect of herd as a fixed effect (Duchemin et al., 2013). Another example is the way of estimating h^2 (Krag, Janss, Shariatia, Berg, & Buitenhuis, 2013a). These authors, showed that SNP information could be used, as an alternative to traditional pedigree-based

methods, to obtain genomic h^2. This way to estimate h^2 was used by Poulsen, Eskildsen, Skov, Larsen, and Buitenhuis (2014).

Breed

The most recent studies focus their efforts in few breeds, especially European breeds, as Brown Swiss (BS), Montbeliarde (MO), Normande (NO), and Holstein (HO) cattle. The HO breed, in addition to be the most studied (Boichard et al., 2014; Poulsen et al., 2014; Penasa, Tiezzi, Gottardo, Cassandro, & De Marchi, 2015), have had the broadest range of h^2 for individual FA (0.0 to 0.74), for FA grouped by their degree of saturation (0.07 to 0.69), and for FA grouped by their chain length (0.17 to 0.68). Regarding the MO and NO breeds (Gion, Larroque, Brochard, Lahalle, & Boichard, 2011; Boichard et al., 2014), the h^2 estimates for individual FA and FA grouped by their degree of saturation were similar between them but lower than for HO. They were inside the range of 0.10 and 0.48 for individual FA, and between 0.11 and 0.36 for FA grouped by their degree of saturation. In BS, Tullo et al. (2014) and Pegolo et al. (2016) have reported h^2 for individual FA between 0.02 and 0.36; from 0.06 to 0.18 for their degree of saturation, and from 0.05 to 0.15 according to their chain length. This scenario opens the possibility of investigating a larger number of breeds under different production and environmental conditions, especially those for breeds locally adapted.

According to Garnsworthy et al. (2010), the magnitude of the h^2 estimate is likely to contain greater genetic variance if the studies include multiple breeds. In this regard, some studies combined information from two or more breeds. Soyeurt et al. (2007; 2008a) combined information of BS, Belgian Blue, HO, Jersey, MO, NO, and Meuse-Rhine-Yssel. Nonetheless, almost all breeding programs are focused for a specific population into a breed, so that the h^2 estimates obtained in these studies should be taken with caution. Breed differences may be important to get estimates of h^2 and heterosis effects for FA.

Lopez-Villalobos et al. (2014) used information of HO×Jersey cows, and they got moderate estimates of h^2, so much for individual FA (0.05 to 0.45) as for FA grouped by their degree of saturation (0.14 to 0.48). These estimates were similar to those reported for MO and NO breeds, but lower than HO when FA were grouped by chain length (0.30–0.50). These authors also estimated effects of heterosis, and although the results indicated significant effects only for some FA, their research may serve to guide the crosses and management practices to assure the use of animals with production of FA according to the final consumer needs.

Source and units to measure milk fatty acid profile

Units to express the quantities of FA vary among studies making difficult their comparison and perhaps interpretation as well. Some of the more common units used are FA weight as a proportion of total fat weight, g of FA 100 g^{-1} of fat and g of FA 100 g^{-1} of milk. Other units less frequently used include percentages of a FA of total FA and g of FA L^{-1}.

Based on the literature reviewed, it has been observed that expressing FA in milk results in higher h^2 than when they are measured in milk fat or when they are expressed with FA. In this sense, it was observed that individual FA follows this trend (0.34 in milk, 0.31 in milk fat or 0.16 in FA proportion), as well as considering the chain length, the average h^2 for FA in milk was higher (0.40) than that for milk fat (0.25). When FA are grouped by their degree of saturation, average h^2 was similar to when they were measured in milk or milk fat (0.24 and 0.23). According to these findings, measuring FA in milk could be the best option to get a greater response to selection. In addition, quantifying FA in milk could represent less man-hours in the laboratory as well as fewer reagents than when FA are quantified by gas chromatography (GC).

Analytical methodology

Mele et al. (2009) indicated that differences in h^2 estimates among studies could be due to differences in the analytical method used to measure FA profile, because of the error variance, which is influenced by the sample size. Two methods have been used to quantify FA: GC and mid-infrared spectrometry (MIR). Gas chromatography analysis has the advantage of being efficient but requires a lengthy analysis, expensive reagents, and highly skilled staff (Soyeurt et al., 2006a; Narayana et al., 2017). In contrast, MIR has the advantage of high throughput, ease of use, and high availability (Soyeurt et al., 2006b). In this context, studies based on GC were generally based on a limited number of records, compared to studies based on MIR, that in some cases have had more than 100,000 FA measures available, confirming the potential use of MIR to quantify FA profile, particularly given its low cost of analysis.

Until a few years ago, GC was the most common analytical method used to quantify FA. However, the number of studies that use GC (Krag et al., 2013b; Bilal, Cue, Mustafa, & Hayes, 2014; Poulsen et al., 2014) is comparable to those that use MIR (Bastin, Soyeurt, & Gengler, 2013; Boichard et al., 2014; Vanrobays et al., 2015). This new trend could be attributed to Soyeurt et al. (2006a), who promoted research aimed at developing prediction equations to measure FA content using MIR. Mid-infrared spectroscopy offers an opportunity to use larger number of records for genetic analysis and to provide more accuracy than GC. It is important to recognize that standard errors were not reported in all the reviewed papers; however, so far MIR studies seem to result in smaller standard errors than GC studies.

Population structure and statistical model

The number of individuals making up the sampled population is crucial to determine the genetic variability. A small number of individuals sampled into a

population leads to a decrease in additive genetic variance, resulting in an increase of probable adverse effects of inbreeding and smaller genetic gain, that is why, population structure should be considered to get more precision of h^2 estimates (Krag et al., 2013a).

Bastin, Gengler, and Soyeurt (2011a) pointed out that another important source of differences in h^2 estimates may lie in the genetic model used to analyze the data and estimate genetic parameters. Selection of models includes considerations from different nature, factors such as experimental design and objectives of research, collection and distribution of data, the relationship between explanatory and dependent variables, the capacity of the model for describing the phenomenon to increase the determination coefficient, and the number of parameters the analyst wants to explain.

Considering the literature reviewed, it was revealed that animal, random regression and repeatability models were the most common models fitted to analyze FA profile. The h^2 estimates for individual FA with animal models showed the highest values, although similar to those maximum values obtained with the random regression and sire models. The narrowest range of h^2 for individual FA was reported with the repeatability model, and the repeatability and sire models were associated with a narrower h^2 range than the animal or random regression models.

Heritability for individual milk fatty acids

Although milk contains a wide range of FA, estimates of h^2 were found for 59 individual FA (**Tables 1** and **2**); most of these studies included less than 20 FA and some of them considered more than 30 FA (for example, Bilal et al., 2014; Pegolo et al., 2016). The FA C4:0, C6:0, C8:0, C10:0, C12:0, C14:0, C14:1cis9, C16:0, C18:0, C18:1cis9, and C18:2cis9trans11 are among the most individually

studied. There is an observed trend to study individual together with grouped FA into the same research.

The major FA (**Table 1**), considered to be those present at or above 1% concentration (MacGibbon & Taylor, 2006), had moderate h^2 (0.17-0.41). In general, individual saturated FA showed higher h^2 than unsaturated FA (0.32 *vs* 0.21); this trend was pointed out by Soyeurt, Dardenne, Dehareng, Bastin, and Gengler (2008b), Krag et al. (2013b) and Boichard et al. (2014). However, individual unsaturated FA have been less studied than saturated, so the above mentioned difference should not be taken as definitive. Among individual saturated FA, C10:0 had the highest h^2 (0.41) and C23:0 the lowest (0.03). The individual unsaturated C12:1, and C22:5 (n-3) joint with C18:2trans10cis12 showed the highest and the lowest h^2, 0.41 and 0.01, respectively. In general, FA with an even-numbered carbon chain had higher h^2 than FA with an odd-numbered carbon chain. Also, h^2 decreased with increasing number of carbons in the chain (**Tables 1** and **2**).

Heritability for groups of milk fatty acids

Fatty acids could be grouped by their degree of saturation in saturated (SFA) and unsaturated (UFA). Within the UFA, they have been classified in monounsaturated (MUFA) and polyunsaturated (PUFA). Also, FA could be grouped by the carbon chain length in short (C4 to C10, SCFA), medium (C12 to C16, MCFA), and long (≥ C17, LCFA) FA. Pegolo et al. (2016) used another classification. These authors grouped the FA in branched-chain (BCFA) and odd-chain FA (OCFA),

Milk fatty acids grouped by their degree of saturation are more studied than grouped by their chain length. Most published SFA have shown higher h^2 than MUFA, PUFA or UFA (**Table 3**), but PUFA and UFA showed higher h^2 estimates than MUFA. However, Bilal et al. (2014) reported that SFA and MUFA had higher

h^2 than PUFA within Canadian HO, but in this summary (**Table 3**) MUFA had the lowest average h^2. Higher h^2 for SFA could be due to the low activity of the Δ^9 desaturase enzyme on FA shorter than 18 carbons (Chilliard, Ferlay, Mansbridge, & Doreau, 2000).

In **Table 3**, similar h^2 could be observed for SCFA and MCFA, and these h^2 estimates being larger than those for LCFA, supporting conclusions derived by Bastin et al. (2011a) and Krag et al. (2013b). In addition, h^2 estimates (individually or in groups) also were higher for SCFA than for LCFA, and this was associated with the biosynthesis path of FA (Garnsworthy et al., 2010; Bouwman et al., 2011; Gion et al., 2011). Bovine FA originate from two main sources: *de novo* synthesis and dietary uptake of preformed FA. Almost all of the FA C4:0 to C14:0 and approximately half of the C16:0 (Bauman & Griinari, 2003) are synthesized *de novo*, involving various enzymes in the mammary gland (Garnsworthy et al., 2010), such as acetyl-coenzyme A carboxylase and FA synthetase, which are under genetic control, whereas the remaining C16:0, and the higher carbon chain FA are mainly derived from the diet (Bauman & Griinari, 2003).

According to Stoop et al. (2008), Garnsworthy et al. (2010), and Bastin et al. (2011a), *de novo* synthesized FA (short and medium chain) have a stronger genetic control (expected to have a higher h^2) than FA derived from the cow's diet or body fat mobilization. The low h^2 estimates for LCFA indicates that processes involved in the inclusion of these FA into milk, such as biohydrogenation in the rumen, absorption in the intestine, or mobilization of FA from adipose tissue, may have small influence from the genetic side (Bastin et al., 2011a).

Table 1. Minimum, mean and maximum h^2 estimates reported for the major milk fatty acids in dairy cattle, expressed in relation to fatty acids (FA), fat, and milk.

Individual FA	FA			Fat			Milk			Avg[1]
	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	
C4:0	0.03	0.15	0.38	0.31	0.41	0.48	0.00	0.31	0.63	0.33
C6:0	0.04	0.20	0.32	0.12	0.36	0.48	0.00	0.37	0.67	0.34
C8:0	0.12	0.25	0.32	0.12	0.41	0.62	0.18	0.42	0.68	0.39
C10:0	0.17	0.24	0.34	0.09	0.46	0.74	0.22	0.45	0.71	0.41
C12:0	0.13	0.23	0.31	0.09	0.42	0.65	0.18	0.43	0.69	0.39
C14:0	0.09	0.15	0.19	0.07	0.36	0.62	0.00	0.40	0.68	0.35
C14:1cis9	0.28	0.34	0.39	0.19	0.35	0.60	0.32	0.37	0.40	0.35
C15:0	0.07	0.21	0.52	0.13	0.35	0.60	-	-	-	0.27
C16:0	0.00	0.21	0.36	0.03	0.25	0.43	0.09	0.35	0.67	0.28
C16:1cis9	0.06	0.16	0.30	0.14	0.33	0.51	0.23	0.35	0.49	0.29
C18:0	0.04	0.20	0.33	0.08	0.22	0.52	0.13	0.26	0.60	0.23
C18:1cis9	0.00	0.12	0.22	0.02	0.19	0.37	0.05	0.20	0.52	0.18
C18:2cis9,12	0.06	0.19	0.45	0.10	0.17	0.27	0.08	0.16	0.26	0.17
C18:2cis9trans11	0.02	0.14	0.41	0.12	0.27	0.44	0.11	0.21	0.42	0.23
C18:3cis9,12,15	0.05	0.15	0.41	0.22	0.25	0.26	0.09	0.18	0.26	0.19

[1]Avg = overall average. Elaborated with data from: Schennink et al. 2007; Soyeurt et al. 2007; Bobe, Minick Bormann, Lindberg, Freeman & Beitz, 2008; Soyeurt et al. 2008a; Stoop, van Arendonk, Heck, van Valenberg & Bovenhuis, 2008; Mele et al. 2009; Schennink et al. 2009b; Stoop et al. 2009; Garnsworthy et al. 2010; Pintana, Bouwman, Rutten, Bovenhuis & Söelkner, 2010; Rutten, Bovenhuis & van Arendonk, 2010; Bastin et al. 2011a; Bastin, Soyeurt, Vanderick & Gengler, 2011b; Bouwman, Bovenhuis, Visker & van Arendonk, 2011; Gion et al. 2011; Bastin, Berry, Soyeurt & Gengler, 2012; Bouwman, Visker, van Arendonk & Bovenhuis, 2012; Bastin et al. 2013; Duchemin et al. 2013; Krag et al. 2013b; Bilal et al. 2014; Boichard et al. 2014; Lopez-Villalobos et al. 2014; Smith et al. 2014; Poulsen et al. 2014; Lassen, Poulsen, Larsen & Buitenhuis, 2016; Pegolo et al. 2016; and Petrini et al. 2016.

Table 2. Minimum, mean and maximum h^2 estimates reported for the minor milk fatty acids in dairy cattle, expressed in relation to fatty acids (FA), fat, and milk.

Individual FA	FA			Fat			Milk			Avg[1]
	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	
Saturated Fatty Acids										
C5:0	-	-	-	0.14	0.14	0.14	-	-	-	0.14
C7:0	-	-	-	0.17	0.17	0.17	-	-	-	0.17
C9:0	-	-	-	0.25	0.25	0.25	-	-	-	0.25
C11:0	0.13	0.19	0.25	0.34	0.34	0.34	-	-	-	0.24
C13:0	0.02	0.09	0.18	0.19	0.19	0.20	-	-	-	0.15
C17:0	0.09	0.24	0.41	0.07	0.20	0.33	0.35	0.47	0.70	0.34
C19:0	-	-	-	0.23	0.23	0.23	-	-	-	0.23
C20:0	0.00	0.14	0.38	0.24	0.24	0.24	-	-	-	0.16
C22:0	0.03	0.15	0.35	-	-	-	-	-	-	0.15
C23:0	0.03	0.03	0.03	-	-	-	-	-	-	0.03
C24:0	0.03	0.05	0.06	-	-	-	-	-	-	0.05
Monounsaturated Fatty Acids										
C10:1cis9	0.12	0.21	0.3	0.33	0.38	0.48	-	-	-	0.34
C12:1	0.41	0.41	0.41	0.37	0.41	0.48	-	-	-	0.41
C14:1trans9	0.02	0.02	0.02	-	-	-	-	-	-	0.02
C16:1trans9	0.03	0.04	0.05	-	-	-	-	-	-	0.04
C17:1cis9	0.07	0.11	0.14	0.43	0.43	0.43	-	-	-	0.21
C18:1cis11	-	-	-	0.21	0.21	0.21	0.12	0.17	0.22	0.18
C18:1cis12	0.08	0.08	0.08	0.21	0.21	0.21	-	-	-	0.15
C18:1trans4	0.02	0.02	0.02	-	-	-	-	-	-	0.02
C18:1trans9	0.01	0.05	0.08	0.22	0.22	0.22	0.11	0.17	0.22	0.13
C18:1trans10	0.06	0.09	0.13	0.10	0.10	0.10	-	-	-	0.10
C18:1trans11	0.00	0.09	0.27	0.06	0.16	0.29	0.12	0.20	0.28	0.15
C18:1trans16	0.06	0.06	0.06	-	-	-	-	-	-	0.06

Table 2. Minimum, mean and maximum h^2 estimates reported for the minor milk fatty acids in dairy cattle, expressed in relation to fatty acids (FA), fat, and milk (Continuation…)

Individual FA	FA			Fat			Milk			Avg[1]
	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	
C20:1cis9	0.06	0.06	0.06	-	-	-	-	-	-	0.06
C20:1cis11	0.37	0.37	0.37	-	-	-	-	-	-	0.37
Poly-unsaturated Fatty Acids										
C18:2(n-6)	0.16	0.17	0.18	0.12	0.15	0.17	-	-	-	0.16
C18:2trans4,8	-	-	-	0.36	0.36	0.36	0.18	0.27	0.35	0.30
C18:2trans6,8	0.06	0.06	0.06	-	-	-	-	-	-	0.06
C18:2trans9,12	0.02	0.03	0.03	-	-	-	-	-	-	0.03
C18:2trans10cis12	0.01	0.01	0.01	-	-	-	-	-	-	0.01
C18:2trans11cis15	0.03	0.03	0.03	-	-	-	-	-	-	0.03
C18:3cis9,11,15	0.03	0.03	0.03	-	-	-	-	-	-	0.03
C18:3(n-3)	-	-	-	0.03	0.03	0.03	-	-	-	0.03
C18:3(n-6)	-	-	-	0.12	0.14	0.15	-	-	-	0.14
C18:3trans9,cis12,15	0.17	0.17	0.17	-	-	-	-	-	-	0.17
C20:3cis8,11,14	0.11	0.11	0.11	-	-	-	-	-	-	0.11
C20:3(n-6)	0.21	0.21	0.21	-	-	-	-	-	-	0.21
C20:4cis5,8,11,14	0.08	0.08	0.08	-	-	-	-	-	-	0.08
C20:4(n-6)	0.09	0.09	0.09	-	-	-	-	-	-	0.09
C20:5cis5,8,11,14,17	0.05	0.05	0.05	-	-	-	-	-	-	0.05
C20:5(n-3)	0.04	0.04	0.04	-	-	-	-	-	-	0.04
C22:4cis7,10,13,16	0.05	0.05	0.05	-	-	-	-	-	-	0.05
C22:5cis7,10,13,16,19	0.04	0.04	0.04	-	-	-	-	-	-	0.04
C22:5(n-3)	0.01	0.01	0.01	-	-	-	-	-	-	0.01

[1]Avg = overall average. Elaborated with data from: Schennink et al. 2007; Soyeurt et al. 2008a; Stoop et al. 2008; Mele et al. 2009; Schennink et al. 2009b; Stoop et al. 2009; Garnsworthy et al. 2010; Bouwman et al. 2011; Gion et al. 2011; Bouwman et al. 2012; Duchemin et al. 2013; Krag et al. 2013b; Bilal et al. 2014; Lopez-Villalobos et al. 2014; Poulsen et al. 2014; Lassen et al. 2016; and Pegolo et al. 2016.

Table 3. Minimum, mean and maximum h^2 estimates reported for groups of milk fatty acids in dairy cattle, expressed in relation to fatty acids (FA), fat and milk.

FA group[1]	FA			Fat			Milk			Avg[2]
	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	
SFA	0.14	0.25	0.46	0.09	0.22	0.34	0.05	0.34	0.68	0.30
MUFA	0.09	0.17	0.21	0.14	0.21	0.34	0.06	0.18	0.58	0.19
PUFA	0.03	0.18	0.42	0.12	0.22	0.28	0.00	0.23	0.69	0.22
UFA	0.48	0.48	0.48	0.13	0.24	0.33	0.07	0.20	0.60	0.23
SCFA	0.05	0.23	0.39	0.20	0.20	0.20	0.24	0.45	0.68	0.38
MCFA	0.15	0.23	0.30	0.20	0.20	0.20	0.32	0.47	0.68	0.39
LCFA	0.13	0.26	0.50	0.09	0.09	0.09	0.17	0.29	0.56	0.26
BCFA	0.08	0.08	0.08	-	-	-	-	-	-	0.08
OCFA	0.12	0.12	0.12	-	-	-	-	-	-	0.10

[1] SFA = saturated FA; MUFA = monounsaturated FA; PUFA = polyunsaturated FA; UFA = unsaturated FA; SCFA = short chain fatty acids; MCFA = medium chain fatty acids; LCFA = long chain fatty acids; BCFA = branched-chain fatty acids; OCFA = odd-chain fatty acids. [2] Avg = overall average. Elaborated with data from: Soyeurt et al. 2007; Bobe et al. 2008; Soyeurt et al. 2008a; Mele et al. 2009; Schennink et al. 2009b; Garnsworthy et al. 2010; Bastin et al. 2011a; 2011b; Gion et al. 2011; Bastin et al. 2012; Bastin et al. 2013; Duchemin et al. 2013; Krag et al. 2013b; Bilal et al. 2014; Boichard et al. 2014; Tullo et al. 2014; Lopez-Villalobos et al. 2014; Smith et al. 2014; Penasa et al. 2015; Vanrobays et al. 2015; Pegolo et al. 2016; Petrini et al. 2016; and Narayana et al. 2017.

Genetic correlations among milk FA

Bastin et al. (2012) showed that genetic correlations (r_g) among FA tend to be stronger when they have similar chain length, reflecting their common biosynthesis pathway (Soyeurt et al., 2007; Narayana et al., 2017). In general, r_g were high and positive among C4:0 to C14:0 (0.27 to 1.00; Stoop et al., 2008; Bastin et al., 2013; Duchemin et al., 2013), as well as among unsaturated C18, but correlations of C4:0 to C14:0 with unsaturated C18 were generally weak (Stoop et al., 2008). Some specific combinations have been found close to zero or negative (C14:0 and C18:0, 0.09; C16:0 and C18:0, -0.84; Mele et al., 2009). Some groups of FA that share a common pathway, *de novo* synthesis, have

shown associations due to unknown environmental factors and it suggests the existence of micro-environmental genetic variance (Pegolo et al., 2016).

Genetic correlations among all FA groups illustrates similarities and differences in their composition based on chain length and saturation (Narayana et al., 2017). The r_g between SCFA and MCFA may reflect the activity of the FA synthase mammary enzyme; insofar as the r_g among PUFA are likely to reflect the activity of the mammary elongase and desaturase enzymes (Pegolo et al., 2016). Estimates of r_g between FA groups are variable, ranging from 0.41, SFA with PUFA, to 0.95, MUFA with UFA (Penasa et al., 2015). Similar results were found by Bilal et al. (2014), who observed positive r_g between MUFA and PUFA, but SFA were negatively correlated with MUFA and UFA. These findings suggest that an index including FA with similar metabolic processes of production in the mammary gland could be used to modify FA genetically.

Genetic correlations among milk fatty acids and milk production traits

Milk fatty acid profile has been associated with traits traditional measured in dairy cattle. The magnitude of r_g depends on specific conditions of each study, however, especially individual FA seem to vary through lactation. This variability could reflect changes in the energy status of the cows; thus, changes in FA profile. Several studies concluded that r_g between milk yield and individual or grouped FA are moderate in magnitude but mainly negative.

Genetic correlations among milk yield and individual FA have not shown large variation among FA, but they are strongly affected by the day in milk (Bastin et al., 2011a), being weaker in early lactation, due to dilution effects, and ranging from -0.43 to -0.25 (Soyeurt et al., 2007; Bastin et al., 2013; Petrini et al., 2016). A similar trend of moderate and negative correlations has been observed for the different groups of FA, ranging from -0.47 to -0.05 (Bastin et al., 2013, Penasa et al., 2015; Petrini et al., 2016), depending strongly on the specific individual FA. According to Bastin et al. (2013), selection for milk yield in early lactation would

decrease contents of all FA groups but with different intensity, however, selection for milk yield throughout lactation would affect all FA profile almost equally.

The r_g between milk fat with milk and protein with milk could be a key factor in the relation with FA because when milk yield increases, the milk components concentration decrease. Milk fat content in milk has been used as a selection criterion over time, bringing changes in FA profile, apparently without any direction and in different magnitude, causing a wide range in the values of the reported r_g.

Opposite to the observed r_g with milk yield, the r_g between milk fat and FA does not have a clear tendency, and it seems to be more dependent on the specific combination considered. For example, the r_g between milk fat percentage and C14:0 is not well defined, because the range of r_g has been found broad in both signs (-0.40 to 0.43; Schennink et al., 2007; Soyeurt et al., 2007; Pintana et al., 2010). The r_g between milk fat percentage and C16:0 has been found generally positive (0.30 and 0.74; Stoop et al., 2008; Pintana et al., 2010) and even close to one (0.98; Petrini et al., 2016). Another example of r_g in both directions was the one obtained for milk fat percentage and C18:0, ranging from positive (0.86; Petrini et al., 2016) to negative (-0.74; Stoop et al., 2008; Pintana et al., 2010) values. In general, the r_g between milk fat percentage with grouped FA has been high and positive (Soyeurt et al., 2008b; Bastin et al., 2011a; Petrini et al., 2016). Since milk fat is formed by a big number of components, and although many of them have not been thoroughly investigated, some of them would have a key role in the dairy industry. Thus, their inclusion in a selection index for future breeding programs should ensure that the farmer offers supplies with enough milk fat to support the dairy industry, but with beneficial properties for human health, and in this way, to reduce the negative perception that milk fat has.

Similar to milk fat, there is no clear association between protein percentage and individual FA, because their r_g estimates have had a wide range of values, from positive to negative (Bastin et al., 2011a; 2013; Petrini et al., 2016). However,

some exceptions have been observed; for example, individual saturated FA have been found positively correlated with protein percentage (up to 0.6; Bastin et al., 2011a; 2013; Petrini et al., 2016), insofar as unsaturated individual FA have had negative r_g, especially with unsaturated FA of the family of C18 (up to -0.76; Stoop et al., 2008; Soyeurt et al., 2008b; Mele et al., 2009). Regarding to r_g between percentage of protein and FA grouped, except for the negative value for MUFA and percentage of protein (Soyeurt et al., 2007; Mele et al., 2009), most of the studies have obtained positive values (up to 0.6; Soyeurt et al., 2007, Tullo et al., 2014; Petrini et al., 2016). Results above mentioned show that an increase of milk fat or protein will modify the FA profile of milk; however, these changes are not directly related with the FA profile beneficial for consumer health.

Genetic correlations among milk FA and other traits

The r_g between reproductive traits and FA have not been widely studied. Genetic correlations between FA and days open widely varied throughout lactation (Bastin et al., 2011b; 2012). The r_g for UFA, MUFA, LCFA, C18:0 and C18:1 cis-9 with days open were positive at the beginning of lactation but changed sign after 100 days in milk. However, for other groups and individual FA, the r_g with days open has been negative throughout lactation (Bastin et al., 2012). These results could be related to the energetic balance of the cow (Bastin et al., 2011b), especially at the beginning of lactation when the cow is in negative balance, because during this stage the amount of energy required for maintenance and milk production exceeds the amount of energy that the cow can get from the diet. To reverse this situation, the cow uses its body reserves, that increase the release of LCFA and inhibit the de novo synthesis, and the diets offered are high in energy that could contribute to LCFA. This suggests that some FA could be indicators of body fat mobilization (Bastin et al., 2012), and they would be useful to design feeding programs of dairy cattle.

Recent studies suggest a relationship between some FA and CH_4 production, because both products have common precursors. This association has been determined, in general, as moderate and dependent on the lactation stage (Chilliard, Martin, Rouel, & Doreau, 2009; Dijkstra et al., 2011). Dijkstra et al. (2011) pointed out that FA from C8:0 to C16:0 and C17:0 anteiso had positive r_g with CH_4 emissions, whereas C17:0 iso and FA with more than 17 carbons were negatively related. Vanrobays et al. (2015) reported negative r_g between CH_4 and some groups of FA, as LCFA and UFA in early lactation, but positive in late lactation, with values into a wide range depending on the group of FA considered. These relationships support the possibilities to develop research focused on decreasing CH_4 production in dairy cattle, key piece into global warming, while the dairy industry could have supplies to offer milk products with better nutritional quality.

Genomic approach

Recently, some studies have been shown that single nucleotide polymorphism (SNP) could be used to estimate h^2. This methodology showed that SNP information captures the population structure and could be used as an alternative to traditional pedigree methods (Lassen et al., 2016). This methodology has been applied for FA and, the h^2 estimates based on genomic or pedigree data were similar, but genomic h^2 have shown lower standard errors (Poulsen et al., 2014). Thus, one initial step to develop a genomic selection is to identify those genes, and their location on the chromosome, responsible for the FA genetic variation. Several authors (Bouwman et al., 2011; Marchitelli et al., 2013; Li et al., 2014) have proposed lists of candidate genes affecting milk fat composition.

The Diacylglycerol acyltransferase (DGAT) gene is implied in the FA composition (Schennink et al., 2007; Conte et al., 2010). When FA are attached to the sn-3, on the glycerol molecule, the enzyme acyl-CoA:diacylglycerol acyltransferase acts as a catalyst (Schennink et al., 2007). A lysine/alanine

substitution on the protein encoded by bovine DGAT gene was reported (Grisart et al., 2002); this polymorphism has been found with a strong association with FA profile (Conte et al., 2010). The DGAT lysine variant has been associated with a higher content of C16:0 and lower contents of C14:0, unsaturated C18, and CLA (Schennink et al., 2007; 2008), and this has also been associated with more saturated fat, which led to increasing the ratio SFA:UFA (Schennink et al., 2008). Finally, the DGAT alanine variant has been found associated with lower C10:0, C12:0, C14:0, C16:0, C18:0 values (Schennink et al., 2008).

The Stearoyl-CoA desaturase (SCD) gene may change the proportion of saturated:unsaturated FA. It promotes the conversion of C10:0–C18:0 SFA into their MUFA counterparts and it is involved in the synthesis of CLA (Corl et al., 2001). Also, it is related to the concentration of the conjugated linoleic FA (Garnsworthy et al., 2010). There has been a report of a non-conservative substitution of valine with alanine in the 293rd residue (Mele et al., 2007; Mao et al., 2012). The valine allele has been associated with lower C10:0, C12:0, and C14:0 values, and with higher C16:0, C18:0 than the alanine allele (Schennink et al., 2008; Stoop et al., 2009).

The FA synthase (FASN) gene catalyzes *de novo* FA synthesis (Schennink et al., 2009a) and it has been associated with fat content in milk (Roy et al., 2006; Morris et al., 2007). There is evidence that FASN affects C4:0, C8:0, C10:0, C12:0, C14:0, C18:1, and C18:2 FA (Morris et al., 2007; Bouwman et al., 2011).

Other candidate genes in various pathways involved in FA synthesis are: the sterol regulatory element binding protein gene (SREBP), that regulates expression of SCD and other genes to lipid and FA metabolism (Harvatine & Bauman, 2006); the opioid receptor-like gene (OLR1), proposed by Khatib, Zaitoun, Wiebelhaus-Finger, Chang, and Rosa (2007), which has been found related to FA transport; the PPARGC1A gene has been observed related to FASN (Smith, 1994) and others genes of the acetyl-CoA cycle (Mao et al., 2012), and it

shows association with C16:1 (Bouwman et al., 2011). These genes could explain partially the genetic variation in FA composition, but, it is expected that more genes are involved.

The addition of genomic information to the estimation of genetic parameters based on pedigree and phenotypic data would allow a more accurate estimation of genetic parameters for FA. However, implementation of genomic selection on FA still faces some limitations, i. e. the number of animals with phenotype is small, and the number of animals genotyped is even smaller. When quantification of FA be a routine procedure, the genomic selection for these traits will be an alternative for genetic improvement.

Conclusions

For this review, it seems that there is enough genetic variability, supported by genetic parameter estimates, to develop animal selection programs focused on obtaining a FA profile according to what the dairy industry and society will demand in the future, without neglecting the health of animals. However, more research is necessary, which should consider a wide range of environments and breeds. Although the pathways of FA synthesis seem to have a greater influence on the estimates of h^2, the method of FA quantification is the most important factor for better accuracy, because it determines the number of possible samples to analyze and consequently, the data structure. The r_g between individual or grouped FA is strongly influenced by FA origin, whereas the r_g between FA and dairy traits are mainly influenced by the stage of lactation. Finally, when quantification of FA become a routine, genomic selection would be closer to its implementation for FA traits.

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3. A meta-analysis of genetic-parameter estimates for milk fatty acid traits in dairy cattle

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Abstract

A meta-analysis is defined as statistical analysis with the purpose of integrating findings of previous research. Over the years, genetic-parameter estimates (heritability and genetic correlation, h^2 and r_g) have been published for milk fatty acids (FA). Genetic-parameter estimates are required to calculate the response to selection and to design breeding programs. This study aimed to perform a meta-analysis based on random effects, combining different published genetic-parameter estimates for FA traits in dairy cattle. A literature search was undertaken based on the keywords: "genetic parameters", "heritability", "fatty acids" and "dairy cattle", and identification of other studies from references lists in published articles. Thirty-seven papers and estimates of genetic-parameters for 83 FA were identified. Weighted h^2 and r_g estimates for FA traits were based on few studies. Heritability estimates for saturated, unsaturated, and grouped FA traits ranged from 0.05 to 0.49, 0.08 to 0.42, and 0.08 to 0.45, respectively. Weighted h^2 had standard errors lower than 0.15, in all the traits. The lowest h^2 (0.05) was observed for C8 (g/dL), and the highest (0.49) for C20, expressed as g/100 g FA. Genetic correlation estimates ranged from -0.48 to 0.98, and their standard errors from 0.05 to 0.33; however, these results correspond to r_g that consider FA traits grouped by their degree of saturation, length of their carbon chain, milk production and quality traits. Genetic correlations were negative between FA and milk yield (kg), and positive between FA and protein (%) or fat (%). Genetic improvement for milk FA concentration is possible, as it is supported by low to medium magnitude heritability estimates (0.05 to 0.45). Specific correlations must be considered if these traits are included in breeding programs. More studies related with genetic factor for milk FA are required.

Keywords: animal resources, heritability, literature review, milk quality, random effects
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Introduction

Livestock production faces every time more complex challenges regarding sustainability. New breeding goals should be considered to meet these challenges (to restore functional traits, attend social demands and decrease livestock carbon footprint) (Boichard & Brochard, 2012). Over time, milk fatty acids (FA) have proven to be traits that could support to meet these

challenges; therefore, genetic-parameters have been estimated for these traits. Heritability (h^2) and genetic correlation (r_g) estimates are required to calculate the response to selection and design breeding programs. Recent studies (Pegolo *et al*., 2016; Petrini *et al*., 2016; Narayana *et al*., 2017) suggest a substantial genetic variation in FA that could be used to develop genetic improvement programs. Nonetheless, these genetic-parameters estimates have been derived from studies based on populations with different sources of variation. This factor could lead variability among estimates. To summarize those estimates, a meta-analysis (MA) could be an alternative to use them in practical situations.

The MA is defined as a statistical analysis that uses results from individual studies with the purpose of integrating findings (Glas, 1976). It is used to assess the results of previous research to derive more precise estimates, and its benefits include consolidating a quantitative review of a large body of literature (Lean *et al*., 2009). For FA, it has been proposed that source, units and method used to measure FA, breed, population structure and model for data analysis could affect the magnitude of estimates (Mele *et al*., 2009; Garnsworthy *et al*., 2010; Tullo *et al*., 2014; Pegolo *et al*., 2016; Petrini *et al*., 2016). Therefore, given the different conditions in which the genetic-parameters have been estimated, direct comparisons among studies can be complicated and could lead to observations and conclusions of limited applicability.

In animal breeding, some MA summarizing genetic-parameters have been developed for different traits; for example, carcass, feeding, growth, reproduction traits (Giannotti *et al*., 2005; Del Claro *et al*., 2012; Diaz *et al*., 2014; Lean *et al*., 2014; McEwin *et al*., 2018, Rojas *et al*., 2018), among others. However, a MA of genetic-parameters for FA in dairy cattle was not found in the literature. Therefore, the aim of this study was to perform a meta-analysis based on random effects including between and within study variance components, to combine various published genetic-parameter estimates of FA in dairy cattle.

Materials and methods

With the purpose of identifying original studies that publish genetic-parameters (h^2 or r_g) for individual or grouped FA traits (defined as a combination of *FA*×*unit of measure*), a literature search was undertaken using free search engines, and identification of other studies from references listed in published articles. Searches were based on the keywords: “genetic parameters”, “heritability”, “milk fatty acids”, and “dairy cattle”.

Publications were included or excluded from this study based on a series of criteria:

1) Studies published after 2000, which reported h^2 or r_g with their respective standard error (SE) for individual or grouped FA in dairy cattle. For studies in which SE were not reported, approximated SE were derived by using the combined-variance method (Sutton *et al*., 2000), given by

$$\mathrm{SE}_{ij} = \sqrt{\left(\sum_{k=1}^{K} s_{ik}^2 n_{ik}^2 \Big/ \sum_{k=1}^{K} n_{ik}\right) \Big/ n'_{ij}}$$

Where SE_{ij} was the predicted SE for the published parameter estimate for the *i*th trait on the *j*th article that did not have a SE reported, s_{ik} was the published SE for the parameter estimated for the *i*th trait on the *k*th article that did have a reported SE, n_{ik} was the number of records used to predict the published parameter estimate for the *i*th trait on the *k*th article that did have a reported SE, and n'_{ij} was the number of records to predict published parameters estimated for the *i*th trait on the *j*th article that did not have a reported SE.

2) Articles must indicate breed, number of samples, FA source, method for FA quantification, units used to express FA, and statistical model.

3) Only traits (*FA*×*unit of measure*) with three or more estimates (h^2 or r_g) were considered.

A MA based on the random-effects model was performed in which the parameter estimates for all traits were assumed independent and normally distributed; it was investigated using Box-Pierce and Shapiro-Wilk test in R software (R Core Team, 2018). Only traits meeting normality and independence ($\alpha = 0.05$) assumptions were included. The *metafor* package (Viechtbauer,

2010) was used to fit the model. According to Rojas *et al*. (2018), the random-effect model could be written as:

$$\widehat{\theta_i} = \bar{\theta} + u_i + e_i$$

where $\widehat{\theta_i}$ was the published parameter estimated on the *i*th article, $\bar{\theta}$ was the weighted population parameters mean, u_i was the among-study component of the deviation from the mean, assumed as $u_i \sim N(0, \tau^2)$, where τ^2 was the between-studies variance, e_i was the within-study component due to sampling error in the parameter estimates on the *i*th article, assumed as $e_i \sim N(0, \sigma^2_e)$, where $\hat{\sigma}^2_e$ was the within-study variance.

Genetic correlations were transformed to an approximate normal scale to remove the dependency of the variance on the estimates, using Fisher´s transformation (Steel & Torrie, 1960) as follows:

$$Z = 0.5\log\left[\frac{1 + r}{1\text{-}r}\right]$$

where *r* was the r_g, with the standard error (se_z) from:

$$se_z = (n\text{-}3)^{-0.5}$$

where *n* was the number of records.

Correlation analyses were performed using the transformed values. The estimated parameters were back-transformed using:

$$r_w = \frac{e^{2z}\text{-}1}{e^{2z} + 1}$$

where r_w was the weighted mean r_g, and z was the weighted mean for the *Z* transformed correlation.

Heterogeneity of results among trials was quantified using I^2 (Higgins &Thompson, 2002). It is defined as the proportion of total variance between studies that is due to real differences in size effect as opposed to chance. I^2 was calculated as:

$$I^2(\%) = \frac{\hat{\tau}^2}{\hat{\tau}^2 + \hat{\sigma}^2_e} \times 100$$

with τ^2 and σ^2_e as previously defined. I^2 lies between 0 and 100%, and negative values were fixed equal to zero. An I^2 value greater than 50% indicates a substantial heterogeneity.

Results

Thirty-seven papers were identified; two of them were out of the proposed period, and five did not have complete information, regarding the number of samples to be considered into the MA. The original estimates derived from four breeds (Holstein, Brown Swiss, Montebéliarde and Normande) and one cross (Holstein×Jersey). Results considered included five units, *g/100 g fat* (g of FA/100 g of total fat), *g/100 g milk* (g of FA/100 g milk), *g/100 g FA* (g of FA/100 g of total FA) *g/dL* (g of FA/dL of milk), *wt/wt %* (FA as a weight proportion of the total fat fraction). Published estimates were obtained either from restricted maximum likelihood (REML) or Bayesian inference.

Heritabilities and r_g estimates were identified for 83 FA; they included individual FA until C24, grouped FA by chain length, degree of saturation, and branched chain. The h^2 results were clustered in saturated, unsaturated and grouped FA. The combination *FA×unit of measure* gave 205 possible traits to be included. The frequency of FA traits ranged from one to 17 and from one to four, for h^2 and r_g, respectively. In addition, for r_g of individual and grouped FA, the r_g included milk production (kg), fat (%), protein (%), lactose (%), milk somatic cell count (SCC, ×1000), and

methane production (various units). Most of the analysed traits and correlations covered the requirements of normality and independence.

The number of articles used, weighted h^2 ± SE, and the I^2 index of heterogeneity for each trait (*FA*×*unit of measure*) are shown in **Table 1**. Weighted h^2 for saturated, unsaturated and grouped FA traits ranged from 0.05 to 0.49, 0.08 to 0.42, and 0.08 to 0.45, respectively, which could be considered from low to medium magnitude. These estimates had low SE in range 0.01 to 0.14, 0.02 to 0.12, and 0.01 to 0.09 for the same classification, respectively. In general, saturated traits had slightly higher h^2 than unsaturated or grouped FA traits (0.22 *vs* 0.20 or 0.21). Saturated FA traits, C8 (g/dL) and C20 (g/100 g FA) had the lowest and the highest weighted h^2 estimates; while C18:3n3 (g/100 g fat) and C16:1 (wt/wt %) were the traits with the lowest and the highest h^2 for unsaturated traits. For the grouped FA traits, unsaturated FA (g/100 g milk) had the lowest h^2 and short FA (g/dL) the highest. Heterogeneity among studies did not have a clear trend as a function of the proposed classification. However, more than 50% of the analyzed saturated traits, showed evidence of moderate to high heterogeneity (I^2 > 50%), while for unsaturated and grouped FA, most traits did not show evidence of heterogeneity among studies, being more remarkable for unsaturated FA traits.

The number of articles used, weighted r_g ± SE, and the I^2 indexes of heterogeneity for each r_g included are shown in **Table 2**. Unfortunately, r_g between individual FA did not cover the criteria considered to be included, so the findings obtained in this study correspond, mainly, to the r_g between grouped FA, and between grouped FA and milk production and milk quality traits. Genetic correlations were from medium to high magnitude, -0.48 to 0.98. The SE had a more extensive variation than those obtained for h^2, oscillating between 0.05 and 0.33. Genetic correlation with milk yield (milk, kg) were negative (< 0.30); while, r_g with milk quality traits, such as fat (%) or protein (%), were strong and positive (> 0.50). In all cases, there was enough evidence for heterogeneity (I^2 > 50%) among studies.

Discussion

Theoretically, milk contains at least 400 different FA (Jensen, 2002). Most of the identified studies included 20 or less FA, and a few of them included a greater amount of FA, up to a maximum of 47. Among these, the major FA (those present at or above 1% concentration) were the most studied, this fact could be function of the capacity in each study to identify and quantify FA. In addition, the large number of units used to measure FA traits was an obstacle to cover the criterion of number of estimates per trait to be included in the present study; therefore, most of the discarded traits were due to the low frequency of *FA*×*unit of measure*. Probably, this is because FA are novel traits within the field of genetic improvement or due to difficulties for their determination; thus, there is a wide area of study for these traits. Likewise, it is also a signal of the different points of view and methodologies with which these traits have been studied. That is the reason why the weighted h^2 for FA traits were based on a few number of estimates. Unfortunately, the number of studies that reported r_g decreased substantially compared with studies that reported h^2, giving as a consequence that the weighted r_g were based on a smaller number of studies than those for h^2.

There is little evidence that expressing FA traits as g/dL offers the highest h^2 estimates compared with the same trait expressed in other units. The result obtained here, indicate that it is possible to obtain responses to selection of sufficient magnitude to be included in breeding programs.

Fatty acids are traits that have shown their importance in technological properties of milk products (Bobe *et al*., 2007) or on consumer health (Connor, 2000). However, few attempts to include them in selection programs of dairy cattle have been developed (Gengler *et al*., 2012), among some factors, due to the difficulty to quantify them. There are some literature reviews related with FA (Jensen, 2002; Kay *et al*., 2005; Samková *et al*., 2012), but they focused on non-genetic factors. Since 2006, there was an increase in studies related with genetic-parameters, probably due to the Soyeurt *et al*. (2006) proposal to use infrared technology to quantify these traits, and in this way, to include FA in selection programs. Arnould & Soyeurt (2009) published a literature review including the genetic component of FA, and whose results (h^2 ranging between

0.00 and 0.54) were similar to results obtained here with differences in maximum value for h^2 estimate, but they only focused on the major FA.

In addition to non-genetic factors, Samková *et al*. (2012) included a summary of genetic-parameters for FA, and their results were more similar to those presented by Arnould & Soyeurt (2009) than results obtained here. Likewise, Samková *et al*. (2012) pointed out that the application of the animal factor for changing the composition of fat is a function of knowledge of the genetic variability of individual and grouped FA. A common conclusion in the studies related to the estimation of genetic-parameters is that it is possible to improve FA traits through genetic selection. Nevertheless, the need to carry out a higher number of studies related with the genetic factor of the content of FA should be considered.

Thus, genetic variability of FA is essential to achieve genetic improvement; likewise, it is of particular interest to know the r_g between FA (individual or grouped), and between FA and milk yield and quality traits. The relationship between FA and reproductive traits, such as days open, or traits related with environmental footprints, such as methane production, or enzymatic indices begin to be studied; unfortunately, the number of studies was still limited to be included in this MA. In general, the number of studies that reported r_g was reduced compared with those that reported h^2. Probably, as it was previously mentioned, because r_g was not the main objective of the similar research. This fact highlights the need for a more significant number of studies aimed at obtaining r_g estimates between FA and dairy traits with the aim to achieve a deeper understanding of the relationship among FA and milk yield and milk quality traits. Samková *et al*. (2012) presented an analytical review of r_g, mainly between individual FA (g/100g g fat) and milk yield (kg/day) and milk fat (%, kg/day) with variable results, as a function of the considered combination of traits.

Indexes of heterogeneity ranged between zero (all variation due to sampling error within trait) and 100% (reflects real differences between studies). These indexes do not depend only on the number of studies; however, there is a slight bias for a small number of studies (less than eight) (Higgins & Thompson, 2002). The I^2 interpretation mainly depends on how substantial is the heterogeneity. Some authors who used a random-effects model considered $I^2 > 50\%$ to define moderate to high heterogeneity (Vesterinen *et al*., 2014; Rojas *et al*., 2018). Higgins *et al*. (2003) proposed a guide for the interpretation of the I^2 value; however, there is no a universal rule that covers definitions for “moderate” or “severe”, these depend on the size and direction of the effect (Higgins & Thompson, 2002).

Results obtained with the MA procedure support main conclusions of the individual studies, which propose that there is enough genetic variability to develop breeding programs aimed at modifying the milk fat composition. However, large number of traits did not fulfill the proposed criteria to be included in the present analysis. It would be desirable to increase the number of studies focused on the estimation of genetic-parameters for FA traits.

Conclusions

Heritability estimates obtained in this study suggest that there is enough genetic variability for FA traits in dairy cattle, so genetic improvement is possible for traits considered in this study. If some of those traits are included into breeding programs, it is necessary to consider specific genetic correlations among them, and with milk yield and milk quality traits. It is expected that milk FA will be an integral component of dairy breeding programs in the near future. More studies are necessary in order to deeply explore this type of traits.

Table 1 Trait (*FA×unit of measure*), number of articles used (k), weighted heritability (h^2) ± standard error (SE), and index of heterogeneity (I^2) for each trait

Trait	*k*	h^2 ± SE	I^2 (%)	Trait	*k*	h^2 ± SE	I^2 (%)	Trait	*k*	h^2 ± SE	I^2 (%)
C4 (g/100 g fat)	3	0.39 ± 0.04	48.18	C14:1c9 (g/100 g fat)	6	0.24 ± 0.03	53.94	SAT (g/100 g fat)	7	0.15 ± 0.02	10.41
C4 (g/100 g FA)	5	0.12 ± 0.07	95.37	C14:1c9 (g/100 g milk)	3	0.37 ± 0.03	0.00	SAT (g/100 g milk)	8	0.30 ± 0.02	58.21
C4 (wt/wt %)	8	0.41 ± 0.03	0.00	C14:1c9 (g/100 g FA)	3	0.36 ± 0.04	0.00	SAT (g/100 g FA)	5	0.25 ± 0.07	94.17
C6 (g/100 g fat)	3	0.17 ± 0.07	0.00	C16:1c9 (g/100 g fat)	3	0.19 ± 0.03	36.69	MUFA (g/100 g fat)	9	0.19 ± 0.02	35.66
C6 (g/dL)	7	0.47 ± 0.05	95.63	C16:1c9 (g/100 g FA)	5	0.18 ± 0.05	54.29	MUFA (g/100 g milk)	8	0.11 ± 0.02	72.54
C6 (g/100 g FA)	7	0.16 ± 0.05	85.48	C16:1 (wt/wt %)	4	0.42 ± 0.04	0.00	MUFA (g/100 g FA)	4	0.20 ± 0.01	0.17
C8 (g/100 g fat)	3	0.19 ± 0.07	0.00	C18:1 (g/100 g fat)	3	0.14 ± 0.02	82.28	PUFA (g/100 g fat)	5	0.23 ± 0.03	0.00
C8 (g/dL)	7	0.49 ± 0.05	97.54	C18:1 (g/100 g FA)	3	0.21 ± 0.12	97.53	PUFA (g/100 g milk)	6	0.15 ± 0.03	84.71
C8 (g/100 g FA)	5	0.24 ± 0.03	40.06	C18:1 (wt/wt %)	3	0.30 ± 0.05	0.00	PUFA (g/100 g FA)	3	0.18 ± 0.09	96.71
C10 (g/100 g fat)	3	0.14 ± 0.07	0.00	C18:1c9 (g/100 g fat)	7	0.17 ± 0.02	0.00	UFA (g/100 g fat)	4	0.17 ± 0.03	14.52
C10 (g/100 g FA)	5	0.26 ± 0.04	24.63	C18:1c9 (g/100 g milk)	3	0.13 ± 0.03	0.00	UFA (g/100 g milk)	6	0.08 ± 0.01	0.00
C12 (g/100 g fat)	4	0.09 ± 0.01	0.00	C18:1c9 (g/100 g FA)	6	0.16 ± 0.03	53.98	UFA (wt/wt %)	3	0.31 ± 0.04	0.00
C12 (g/100 g FA)	7	0.21 ± 0.03	44.97	C18:1c9 (wt/wt %)	5	0.28 ± 0.04	0.00	SHORT (g/dL)	9	0.45 ± 0.05	99.04
C13 (g/100 g FA)	3	0.09 ± 0.05	91.64	C18:1c11 (wt/wt %)	3	0.18 ± 0.05	0.00				
C14 (g/100 g fat)	5	0.14 ± 0.03	72.39	C18:1t11 (g/100 g fat)	4	0.11 ± 0.03	0.00				
C14 (g/100 g FA)	7	0.14 ± 0.03	40.54	C18:1t11 (g/100 g FA)	4	0.09 ± 0.06	96.04				
C14 (wt/wt %)	7	0.45 ± 0.04	67.82	C18:1t11 (wt/wt %)	5	0.24 ± 0.04	0.00				
C15 (g/100 g fat)	3	0.32 ± 0.14	55.54	C18:2c9c12 (g/100 g FA)	4	0.19 ± 0.09	96.35				
C15 (g/100 g FA)	5	0.17 ± 0.03	55.54	C18:2c9c12 (wt/wt %)	5	0.22 ± 0.03	0.00				
C16 (g/100 g fat)	10	0.17 ± 0.03	78.19	C18:2c9t11 (g/100 g fat)	7	0.13 ± 0.02	0.00				
C16 (g/100 g milk)	5	0.32 ± 0.03	69.58	C18:2c9t11 (g/100 g milk)	3	0.14 ± 0.03	0.00				
C16 (g/100 g FA)	8	0.21 ± 0.04	86.50	C18:2n6 (g/100 g fat)	3	0.15 ± 0.07	0.00				
C17 (g/100 g fat)	3	0.10 ± 0.05	0.00	C18:3n3 (g/100 g fat)	3	0.08 ± 0.08	21.12				
C17 (g/100 g FA)	3	0.24 ± 0.09	95.56								
C18 (g/100 g fat)	7	0.17 ± 0.04	75.69								
C18 (g/100 g FA)	7	0.19 ± 0.03	77.94								
C20 (g/100 g total FA)	3	0.05 ± 0.03	78.78								
C22 (g/100 g total FA)	3	0.15 ± 0.10	97.75								

SAT = saturated FA, MUFA = mono-unsaturated FA; PUFA = poly-unsaturated FA, UFA = unsaturated FA, SHORT = short carbon chain FA

Table 2 Weighted genetic correlations (r_g) ± standard error (SE) between traits (FA×unit of measure), between traits and milk production traits, number of articles used (*k*), and index of heterogeneity (I^2) for each correlation analysed

Trait	*K*	r_g ± SE	I^2 (%)
SAT (g/100 g milk) - MUFA (g/100 g milk)	3	0.70 ± 0.06	99.62
SAT (g/100 g milk) - UFA (g/100 g milk)	3	0.65 ± 0.15	99.98
SAT (g/dL) - UFA (g/dL)	3	0.65 ± 0.05	99.90
SAT (g/dL) - SHORT (g/dL)	3	0.91 ± 0.06	99.92
SAT (g/dL) - MED (g/dL)	3	0.98 ± 0.20	99.99
SAT (g/dL) - LONG (g/dL)	3	0.70 ± 0.08	99.96
SAT (g/100 g milk) - Milk (kg)	4	-0.34 ± 0.06	99.84
SAT (g/100 g milk) - Fat (%)	4	0.97 ± 0.33	100.00
SAT (g/100 g milk) - Protein (%)	3	0.57 ± 0.07	99.72
MUFA (g/100 g milk) - Milk (kg)	3	-0.31 ± 0.06	99.65
MUFA (g/100 g milk) - Fat (%)	3	0.78 ± 0.05	99.56
MUFA (g/100 g milk) - Protein (%)	3	0.50 ± 0.05	99.48
UFA (g/dL) - SHORT (g/dL)	3	0.56 ± 0.06	99.92
UFA (g/dL) - MED (g/dL)	3	0.62 ± 0.06	99.92
UFA (g/dL) - LONG (g/dL)	3	0.91 ± 0.12	99.98
UFA (g/100 g milk) - Milk (kg)	3	-0.48 ± 0.13	99.97
UFA (g/100 g milk) - Fat (%)	3	0.74 ± 0.20	99.99
SHORT (g/dL) - MED (g/dL)	3	0.90 ± 0.08	99.96
SHORT (g/dL) - LONG (g/dL)	3	0.58 ± 0.10	99.98
MED (g/dL) - LONG (g/dL)	3	0.64 ± 0.09	99.97

SAT = saturated FA, MUFA = mono-unsaturated FA, UFA = unsaturated FA, SHORT = short carbon chain FA, MED = medium carbon chain FA, LONG = long carbon chain FA

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Author contribution

All the authors were involved in the design and writing of the manuscript. Luis Antonio Saavedra-Jiménez collected and analyzed the data; Rodolfo Ramírez-Valverde edited the manuscript until it was submitted to the journal for its publication. All authors read and approved the final manuscript.

Conflict of interest

The authors declare that there was no conflict of interest.

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4. Genetic parameters for milk fatty acids and milk components traits of Mexican Brown Swiss cattle

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Abstract

Milk quality includes fat composition. This study aimed to estimate genetic parameters for milk fatty acids (FA) and milk component traits in Mexican Brown Swiss cattle (BS). Morning milk samples were collected from 317 BS cows reared in eight commercial farms. Each sample was analyzed by mid-infrared spectrometry for total percentage of casein, fat, lactose, protein, and total solids, and by gas chromatography for C4, C6, C8, C10, C14, C16, C18, C18:1cis9 and, C18:2cis9cis12 FA (g/100 g milk). (Co)variance components and genetic parameters were estimated using an animal model. The pedigree included 2616 animals. Heritability estimates (h^2) for milk components were high, ranging from 0.15 for lactose percent to 0.64 for protein percent; while h^2 for FA were moderate, ranging from 0.20 for C18:1cis9 to 0.37 for C6 and C8. Standard errors of h^2 for milk components and FA, ranged from 0.12 to 0.26 and from 0.11 to 0.32, respectively. Casein percent and lactose percent showed low to moderate genetic correlation (r_g) with FA; conversely, fat percent, protein percent and total solids percent showed correlation from moderate to high with FA, in some cases higher than 0.90. Genetic correlation between FA with a carbon chain of similar size was high and positive (>0.7). There is enough genetic variability for milk components and FA, suggesting that milk composition could be improved in a short time by selection in the studied population.

Keywords: Gas chromatography, Heritability, Milk quality, Dairy cattle

Introduction

Cow's milk is a source of lipids, protein, amino acids, vitamins, and minerals (Haug et al. 2007), and other constituents dispersed in water (Ozrenk and Inci 2008). Recently, milk quality has taken a key role in consumers´ attention due to their effort to consume healthy and functional foods. Bovine milk contains around 3 to 5% fat, of which 95 to 98% are triglycerides composed of glycerol and fatty acids (FA); of the total FA, 50 to 70% are saturated FA (SFA), 20 to 40% are mono-unsaturated FA (MUFA), and 1 to 5% are poly-unsaturated FA (PUFA) (Jensen 2002). Research studies with humans have reported that SFA are related to heart diseases, weight gain, and obesity (Haug et al. 2007). Conversely, unsaturated FA (MUFA and PUFA) could improve insulin sensitivity and glucose tolerance (Parodi 2004), and some FA have shown anti-inflammatory effects (Haug et al. 2007).

Available studies point out the possibility of improving milk quality in terms of FA through genetic selection, supported by heritability (h^2) and genetic correlation (r_g) estimates. Heritabilities for individual FA, up to 24 carbons, have ranged from 0.03 to 0.36 (Pegolo et al. 2016), and from 0.05 to 0.18 for grouped FA (Tullo et al. 2014; Pegolo et al. 2016). According to Tullo et al. (2014), the r_g between grouped FA and milk quality traits, such as fat, protein, casein, and lactose percent, is positive. Conversely, the increase of grouped FA would be reflected in the decrease of somatic cell count and unfortunately in milk yield (negative r_g).

A deeper understanding about the additive genetic component of FA traits will provide useful information to identify strategies for breeding programs. As for other countries, Brown Swiss (BS) cattle is a breed of economic importance for Mexico. This breed represents opportunities to produce milk with a high content of fat and protein in dual-purpose systems in tropical environments; unfortunately, Mexican genetic evaluation is limited to milk yield only (Núñez et al. 2018). Nowadays, there is interest by some Mexican BS breeders to produce special milk for niche markets. Mexican BS breeders have identified the need to increase the knowledge about the genetic component on the milk's FA profile in the Mexican BS cattle population. They are enthusiastic so as to take the opportunity to improve the concentration of FA as a possible complementary alternative into a breeding program of their breed. This study was aimed to estimate genetic parameters for milk fatty acids and milk production traits in Mexican Brown Swiss cattle.

Materials and Methods

Collection of milk samples

Individual milk samples (60 mL) of 317 BS cows reared in eight herds of Mexico (with averages of lactation number 3.3 ± 2.0 and days in milk 154.8 ± 99.8; **Table 1**) were taken from June to August 2016. Milk samples occurred once per animal during the morning milking. These farms had differences in management. For example, 70% of the herds had one milking, and the rest had two. Only one herd had manual milking. Around 25% were confined herds with feeding based on corn silage, another 25% were grazing herds using mainly native grass, and in the remaining herds, the feeding of the herds was based on grazing and supplementation with commercial food during milking routine.

Table 1 Number of cows (n), mean ± standard deviation of lactation number and days in milk (DIM) by herd involved in the study

Herd	N	Lactation	DIM
1	26	3.2 ± 1.5	181.9 ± 102.8
2	47	3.4 ± 1.9	161.8 ± 90.7
3	19	2.8 ± 2.1	147.5 ± 89.7
4	39	4.0 ± 2.5	144.8 ± 85.4
5	63	4.1 ± 2.1	150.3 ± 95.8
6	18	2.9 ± 1.7	142.2 ± 126.5
7	69	2.8 ± 1.6	153.5 ± 113.0
8	36	2.3 ± 1.4	156.7 ± 99.9

All collected milk samples were labeled and stored at 4 °C during their transportation until they reached the university´s laboratories. Individual milk subsamples were analyzed with a Milkoscan FT120 (Foss Analytical Foss Electric, Denmark, infrared by Fourier transform) to obtain concentration (%) of casein, fat, lactose, protein, and total solids (TS). Another subsample was analyzed to determine the content of C4 (butyric acid), C6 (hexanoic acid), C8 (octanoic acid), C10 (decanoic acid), C14 (myristic acid), C16 (palmitic acid), C18 (stearic acid), C18:1*cis9* (oleic acid), and C18:2*cis9cis12* (linoleic acid) FA.

Determination of milk fatty acid profile

The FA profile was determined by using the methylation technique proposed by Sukhija and Palmquist (1988) and modified by Palmquist and Jenkins (2003) and Jenkins (2010), in which FA is presented as methyl-esters. About 0.5 g of sample was placed in polypropylene tubes, to which 3 mL of sodium methoxide (0.5 M in methanol) were added. The tubes were shaken for 1 min with a vortex. Once shaken, the tubes were placed in a bain Marie (50 °C) for 10 min, after which time the tubes were left to cool for 5 min. Subsequently, 3 mL of 5% methanolic hydrochloric acid was added to the tubes, and they were shaken for 1 min with a vortex. The tubes were again placed in a bain Marie (75 °C) for 10 min. After this time, the tubes were left to cool for 10 min. Then 3.5 mL of hexane and 5 mL of 6% potassium carbonate were added, these were shaken for 1 minute with a vortex, after this, the tubes were centrifuged for 5 min

at 2500 x g. Subsequently, the supernatant was recovered and deposited in other polypropylene tubes, which were previously added 0.5 g of potassium carbonate and 0.1 g of activated carbon, and centrifuged for 5 min at 1500 x g. Finally, the supernatant was recovered, and it was filtered through an acrodisc (Thermo Scientific, titan 44513-NN, a green filter of 17 mm and nylon membrane of 0.45 µm); it was then placed in a vial and stored (-5 °C) until its analysis by gas chromatography.

For the identification and quantification of the fatty acid methyl-ester, a gas chromatograph (Gas chromatograph, Perking Elmer, Clarus 680, USA) coupled to a mass spectrometer (Mass spectrometer, Clarus SQ 8 T, USA) was used, equipped with a capillary column of silica (100 m x 0.25 mm x 0.20 µm thick, Sp-2560, Supelco). Results were interpreted by the WinLab software and its fragmetogram library. The working conditions were: injector temperature 250 °C; oven temperature 100 °C for 2 min, then it was brought up to 300 °C with a ratio of 7 °C min^{-1}, and keep there for 5 min; injection volume 2 mL; 4:1 split injection mode; linear velocity flow control mode; column flow 1.0 mL min^{-1}; carrier gas helium, gas linear velocity 37.2 cm s^{-1}; ion source temperature 260 °C; interface temperature 280 °C; start time 10 minutes, with end time 50.54 min; initial m/z 30, with final m/z 500. The methyl ester peaks were identified based on the comparison of their retention times to those of pure standards.

Statistical analysis

Environmental factors included in the statistical model for the variance component estimation were previously tested for their significance with the GLM procedure of SAS software (SAS 2013). Genetic parameters and (co)variance components were estimated for each trait with an animal model using ASReml software (Gilmour et al. 2002). Heritability were estimated using a single-trait mixed model:

$$y = X\beta + Zu + e$$

where $\boldsymbol{y}$ was the vector of phenotypic records; β was the vector of fixed effects that included farm (1,2,…,8), lactation number (1,2,…,7), interaction farm × lactation number, and the linear effects of days in milk and $e^{-0.05}$ × days in milk as covariates; u was the vector of random genetic additive effects [~$N(0, A\sigma^2_A)$], where A is the additive genetic relationship matrix among animals and σ^2_A is the direct additive genetic variance; and $\boldsymbol{e}$ was the vector of residual random effects [~$N(0, I_N\sigma^2_e)$], where I_N is an identity matrix of order the

number of observations, and σ^2_e is the residual variance. X and Z were incidence matrices relating phenotypic records to the corresponding vectors. The *Asociación Mexicana de Criadores de Ganado Suizo de Registro* supplied pedigree information of 2616 animals. Heritability (h^2) was defined as:

$$h^2 = \frac{\sigma^2_A}{\sigma^2_A + \sigma^2_e}$$

where σ^2_A and σ^2_e are as previously defined.

Genetic correlations (r_g) were estimated using a bi-variate mixed model:

$$\begin{bmatrix} y_1 \\ y_2 \end{bmatrix} = \begin{bmatrix} X_1 & 0 \\ 0 & X_2 \end{bmatrix} \begin{bmatrix} b_1 \\ b_2 \end{bmatrix} + \begin{bmatrix} Z_1 & 0 \\ 0 & Z_2 \end{bmatrix} \begin{bmatrix} u_1 \\ u_2 \end{bmatrix} + \begin{bmatrix} e_1 \\ e_2 \end{bmatrix}$$

with the subscripts 1 and 2 identifying traits 1 and 2, with the same fixed and random effects and assumptions as those for single-mixed model. X_1, X_2, Z_1, and Z_2 were incidence matrices relating phenotypic records with the corresponding vectors.

Genetic correlation was defined as:

$$r_g = \frac{\sigma_{A1,A2}}{\sqrt{\sigma^2_{A1} \text{ x } \sigma^2_{A2}}}$$

where $\sigma_{A1,A2}$ was the additive genetic covariance between traits 1 and 2, σ^2_{A1} and σ^2_{A2} were additive genetic variances for traits 1 and 2, provided by results of the corresponding bi-variate analysis.

Results

Descriptive general statistics for the studied variables are shown in **Table 2**. Among FA, the C16, C18, and C18:1cis9 were found with the highest concentrations. FA composed approximately 88% of total fat. In general, the results showed considerable phenotypic variation. Except for lactose (%), total solids (%), C6, C8, and C18:2cis9cis12 (g FA/100 g milk) that had CV minor than 15%, the rest of traits evaluated had a CV close or superior to 20%.

Changes in milk components and FA trough lactation are shown in **Table 3**. Traits with large variation through lactation were observed in FA with 16 or more carbons. Estimates of h^2, r_g, phenotypic correlation (r_f) and standard errors (SE) are shown in **Table 4**. Heritability estimates for milk components were low to high (0.05 to 0.64), while h^2 estimates for FA were moderate within a short range of values

(0.20 to 0.37). It was observed that h^2 estimates for FA shorter than 16 carbons were slightly smaller than h^2 for longer carbon chain FA. The magnitude of the correlation coefficients for FA varied according to the specific combination of FA. Genetic correlations were positive covering a wide range (0.07 to 1.00), while r_f had a wider range, almost covering the range -1.00 to 1.00. Correlation estimates between FA with a similar length of carbon chain were high; the correlation decreased as the carbon chains differed more in size, even becoming negative. Standard errors ranged from 0.11 to 0.26 for h^2 estimates, and from 0.00 to 0.95 for correlation estimates. Some SE were larger than or almost as large as the estimate of the parameter, probably as a consequence of the small sample size.

Table 2 Descriptive statistics for milk components and fatty acid (FA) content in milk of Mexican Brown Swiss cattle

Trait	Mean	SD[1]	CV[2] (%)	Min	Max
Casein (%)	2.68	1.66	61.94	1.91	5.32
Fat (%)	2.04	1.04	50.98	0.37	8.06
Lactose (%)	4.49	0.62	13.81	0.21	5.33
Protein (%)	3.41	0.62	18.18	2.39	7.00
Total solids (%)	10.92	1.20	10.99	7.18	17.00
C4 (g FA/100 g milk)	0.09	0.03	33.33	0.04	0.29
C6 (g FA/100 g milk)	0.05	0.006	12.00	0.02	0.06
C8 (g FA/100 g milk)	0.03	0.003	10.00	0.02	0.05
C10 (g FA/100 g milk)	0.07	0.02	28.57	0.05	0.16
C14 (g FA/100 g milk)	0.23	0.10	23.48	0.07	0.82
C16 (g FA/100 g milk)	0.55	0.26	47.27	0.14	2.04
C18 (g FA/100 g milk)	0.24	0.13	54.17	0.04	0.97
C18:*1cis9* (g FA/100 g milk)	0.47	0.27	57.45	0.05	2.01
C18:*2cis9cis12* (g FA/100 g milk)	0.07	0.01	14.29	0.05	0.15

[1] SD = standard deviation; [2] CV = coefficient of variation

Discussion

For milk components, all the phenotypic values estimated in the present study were lower than those reported by Tullo et al. (2014), Pegolo et al. (2016), and El-Tarabany et al. (2018) for BS. Lactose and

protein percent showed differences lower than 10%, casein and TS percent around 20%, while fat (%) was the trait with the most significant difference observed, higher than 80%, and sometimes almost 100%. In BS, the individual FA has been reported as g FA/g 100 g total FA (Pegolo et al. 2016), g FA/100 g fat (El-Tarabany et al. 2018), and as a percentage to express FA grouped by their degree of saturation (Tullo et al. 2014). Results obtained here had slight similarities with those reported, as g FA/dL, by Bastin et al. (2011; 2012; 2013) for the Holstein breed; even by those reported by Soyeurt et al. (2008) for a mixed breed. The largest differences were observed in C14, C16, C18, and C18:1cis9, results from the literature were almost twice as high as those obtained in the present study.

Additional to differences inherent to animals, phenotypic variability could be explained by the heterogeneity (lactation number and stage, feeding strategies, animal health, among others factors) among farms considered in the study. Several factors could explain differences in phenotypic records. However, the food factor plays a primary role in the differences observed. The nutritional control to modify the FA profile has received particular attention in recent years aiming at improving the concentration of FA, especially those with beneficial effects for human health. According to Schönfeldt et al. (2012), modifications to grain feeding systems, as well as the administration of bio-hydrogenated fats, among others, have been used in the industry to make these changes. Results published (Khan et al. 2012; Hristov et al. 2013; Neveu et al. 2014) showed a variation in the effect of the inclusion of ingredients on the FA profile in dairy cattle. It has been pointed out in different literature reviews (Palmquist et al. 1993, Jensen, 2002, Elgersma et al. 2006) that feeding strategies could modify the FA profile. Although not all studies indicate the feeding regime of the animals included, in the present study, the animals considered were taken from commercial herds with some differences in food management. Also, differences in milk and fat content are sources of variability in FA.

The CV published by other authors for milk composition and fat composition in BS, cover up to 75% (Pegolo et al. 2016) so that those values obtained here are below this maximum. However, when comparing specifically each trait, the values reported by Pegolo et al. (2016) are lower than those obtained in this study.

Table 3 Milk components and fatty acid (FA) content during lactation for Mexican Brown Swiss cattle

Trait	Lactation stage, days										
	30	45	75	105	135	165	195	225	255	285	301
Casein (%)	2.82	2.51	2.69	2.53	2.55	2.60	2.64	2.72	2.77	3.11	2.68
Fat (%)	1.89	1.94	2.06	1.62	1.93	2.01	2.12	2.08	2.23	2.11	2.48
Lactose (%)	4.47	4.46	4.63	4.55	4.66	4.59	4.45	4.37	4.41	4.37	4.4
Protein (%)	3.64	3.29	3.4	3.29	3.29	3.35	3.45	3.51	3.52	3.55	3.4
Total solids (%)	10.97	10.67	11.09	10.51	10.81	10.95	11.00	10.96	11.09	10.98	11.27
C4 (g FA/100 g milk)	0.09	0.09	0.09	0.08	0.09	0.09	0.09	0.09	0.10	0.09	0.11
C6 (g FA/100 g milk)	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
C8 (g FA/100 g milk)	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
C10 (g FA/100 g milk)	0.07	0.07	0.07	0.07	0.07	0.07	0.08	0.07	0.08	0.08	0.08
C14 (g FA/100 g milk)	0.22	0.23	0.24	0.19	0.23	0.23	0.24	0.24	0.25	0.24	0.28
C16 (g FA/100 g milk)	0.51	0.53	0.56	0.45	0.52	0.54	0.57	0.56	0.60	0.57	0.66
C18 (g FA/100 g milk)	0.22	0.23	0.24	0.19	0.23	0.24	0.25	0.25	0.27	0.25	0.29
C18:1cis9 (g FA/100 g milk)	0.44	0.45	0.48	0.37	0.45	0.47	0.49	0.48	0.52	0.49	0.59
C18:2cis9cis12 (g FA/100 g milk)	0.07	0.07	0.07	0.06	0.07	0.07	0.07	0.07	0.07	0.07	0.08

Table 4 Phenotypic (above) and genetic (below diagonal) correlations ± standard error, and heritability ± standard error (on diagonal) for milk components (%) and milk fatty acids (g FA/100 g milk) for Mexican Browns Swiss cattle

Trait	Casein	Fat	Lactose	Protein	TS[Z]	C4	C6	C8	C10	C14	C16	C18	C18:1cis9	C18:2*cis9cis12*
Casein	**0.50**$^{\pm 0.12}$	0.07$^{\pm 0.06}$	-0.24$^{\pm 0.06}$	0.89$^{\pm 0.03}$	0.41$^{\pm 0.06}$	0.13$^{\pm 0.70}$	-0.01$^{\pm 0.20}$	0.06$^{\pm 0.45}$	0.02$^{\pm 0.19}$	-0.08$^{\pm 0.18}$	-0.09$^{\pm 0.18}$	-0.04$^{\pm 0.18}$	-0.05$^{\pm 0.18}$	-0.13$^{\pm 0.30}$
Fat	0.53$^{\pm 0.86}$	**0.49**$^{\pm 0.13}$	-0.03$^{\pm 0.06}$	0.07$^{\pm 0.06}$	0.88$^{\pm 0.03}$	0.57$^{\pm 0.47}$	0.12$^{\pm 0.18}$	0.12$^{\pm 0.35}$	0.16$^{\pm 0.17}$	0.31$^{\pm 0.16}$	0.28$^{\pm 0.16}$	0.34$^{\pm 0.16}$	0.35$^{\pm 0.16}$	0.19$^{\pm 0.25}$
Lactose	0.10$^{\pm 0.20}$	0.14$^{\pm 0.42}$	**0.15**$^{\pm 0.21}$	-0.52$^{\pm 0.06}$	0.10$^{\pm 0.06}$	-0.53$^{\pm 0.49}$	0.07$^{\pm 0.18}$	0.21$^{\pm 0.35}$	0.01$^{\pm 0.18}$	0.00$^{\pm 0.16}$	0.00$^{\pm 0.17}$	-0.01$^{\pm 0.16}$	-0.03$^{\pm 0.17}$	-0.29$^{\pm 0.25}$
Protein	0.92$^{\pm 0.04}$	0.56$^{\pm 0.06}$	-0.40$^{\pm 0.20}$	**0.64**$^{\pm 0.26}$	0.35$^{\pm 0.05}$	-0.10$^{\pm 0.52}$	0.10$^{\pm 0.18}$	0.10$^{\pm 0.35}$	0.08$^{\pm 0.18}$	0.02$^{\pm 0.17}$	0.01$^{\pm 0.17}$	0.01$^{\pm 0.16}$	0.01$^{\pm 0.17}$	0.15$^{\pm 0.25}$
TS	0.86$^{\pm 0.99}$	0.95$^{\pm 0.33}$	0.09$^{\pm 0.30}$	0.84$^{\pm 0.38}$	**0.40**$^{\pm 0.19}$	0.14$^{\pm 0.57}$	0.09$^{\pm 0.18}$	0.38$^{\pm 0.33}$	0.22$^{\pm 0.17}$	0.18$^{\pm 0.16}$	0.22$^{\pm 0.16}$	0.20$^{\pm 0.16}$	0.20$^{\pm 0.16}$	0.08$^{\pm 0.26}$
C4	0.75$^{\pm 0.22}$	0.98$^{\pm 0.71}$	0.13$^{\pm 0.46}$	0.32$^{\pm 0.40}$	0.96$^{\pm 0.04}$	**0.31**$^{\pm 0.23}$	1.00$^{\pm 0.00}$	1.00$^{\pm 0.00}$	0.93$^{\pm 0.24}$	0.79$^{\pm 0.35}$	0.78$^{\pm 0.36}$	-0.74$^{\pm 0.38}$	-0.74$^{\pm 0.39}$	-0.67$^{\pm 0.52}$
C6	0.55$^{\pm 0.06}$	0.86$^{\pm 0.35}$	0.25$^{\pm 0.06}$	0.45$^{\pm 0.83}$	0.85$^{\pm 0.89}$	0.95$^{\pm 0.07}$	**0.37**$^{\pm 0.18}$	0.95$^{\pm 0.04}$	0.91$^{\pm 0.08}$	0.81$^{\pm 0.11}$	0.82$^{\pm 0.10}$	-0.73$^{\pm 0.12}$	-0.74$^{\pm 0.12}$	-0.89$^{\pm 0.18}$
C8	0.16$^{\pm 0.09}$	0.93$^{\pm 0.95}$	0.20$^{\pm 0.35}$	0.32$^{\pm 0.12}$	0.99$^{\pm 0.03}$	0.72$^{\pm 0.24}$	0.96$^{\pm 0.56}$	**0.37**$^{\pm 0.20}$	0.99$^{\pm 0.01}$	0.88$^{\pm 0.06}$	0.68$^{\pm 0.06}$	-0.31$^{\pm 0.14}$	-0.40$^{\pm 0.16}$	-0.45$^{\pm 0.18}$
C10	0.10$^{\pm 0.16}$	0.71$^{\pm 0.18}$	0.20$^{\pm 0.08}$	0.11$^{\pm 0.10}$	0.92$^{\pm 0.54}$	0.65$^{\pm 0.47}$	0.82$^{\pm 0.14}$	1.00$^{\pm 0.29}$	**0.34**$^{\pm 0.32}$	0.84$^{\pm 0.06}$	0.65$^{\pm 0.05}$	-0.26$^{\pm 0.09}$	-0.65$^{\pm 0.09}$	-0.86$^{\pm 0.13}$
C14	0.38$^{\pm 0.32}$	0.90$^{\pm 0.03}$	0.32$^{\pm 0.50}$	0.56$^{\pm 0.15}$	0.96$^{\pm 0.07}$	0.75$^{\pm 0.10}$	0.80$^{\pm 0.10}$	0.98$^{\pm 0.12}$	0.99$^{\pm 0.10}$	**0.28**$^{\pm 0.11}$	0.98$^{\pm 0.03}$	-0.27$^{\pm 0.04}$	-0.57$^{\pm 0.04}$	-0.57$^{\pm 0.06}$
C16	0.62$^{\pm 0.48}$	0.90$^{\pm 0.35}$	0.23$^{\pm 0.36}$	0.26$^{\pm 0.30}$	0.93$^{\pm 0.61}$	0.80$^{\pm 0.15}$	0.80$^{\pm 0.22}$	0.78$^{\pm 0.23}$	0.74$^{\pm 0.20}$	0.89$^{\pm 0.15}$	**0.30**$^{\pm 0.13}$	0.94$^{\pm 0.06}$	0.93$^{\pm 0.06}$	0.95$^{\pm 0.08}$
C18	0.33$^{\pm 0.24}$	1.00$^{\pm 0.12}$	0.15$^{\pm 0.70}$	0.20$^{\pm 0.17}$	0.96$^{\pm 0.09}$	0.57$^{\pm 0.47}$	0.53$^{\pm 0.18}$	0.40$^{\pm 0.01}$	0.39$^{\pm 0.27}$	0.59$^{\pm 0.15}$	0.70$^{\pm 0.12}$	**0.29**$^{\pm 0.11}$	0.99$^{\pm 0.01}$	0.97$^{\pm 0.06}$
C18:1*cis9*	0.56$^{\pm 0.28}$	0.69$^{\pm 0.42}$	0.13$^{\pm 0.30}$	0.34$^{\pm 0.04}$	0.93$^{\pm 0.61}$	0.49$^{\pm 0.04}$	0.38$^{\pm 0.07}$	0.30$^{\pm 0.18}$	0.29$^{\pm 0.13}$	0.35$^{\pm 0.08}$	0.51$^{\pm 0.15}$	0.63$^{\pm 0.44}$	**0.20**$^{\pm 0.13}$	0.97$^{\pm 0.06}$
C18:2*cis9cis12*	0.07$^{\pm 0.11}$	0.99$^{\pm 0.23}$	0.25$^{\pm 0.16}$	0.13$^{\pm 0.11}$	0.91$^{\pm 0.59}$	0.20$^{\pm 0.46}$	0.06$^{\pm 0.15}$	0.20$^{\pm 0.18}$	0.25$^{\pm 0.19}$	0.30$^{\pm 0.12}$	0.35$^{\pm 0.15}$	0.30$^{\pm 0.05}$	1.00$^{\pm 0.15}$	**0.22**$^{\pm 0.20}$

[Z] TS = total solids

The effect of lactation stage has been studied and recognized as an important source of variation for FA profile (Jensen 2002, Soyeurt et al. 2008, Narayana et al. 2017) due to changes in feeding strategies. In this study, changes in FA content through lactation were not identified. Nevertheless, these changes could be hidden due to the short range of DIM that the animals covered during the sampling period. It is possible to observe that at early-lactation most of the traits studied had lower values than at those registered in late-lactation.

Based on the feeding strategy, changes in FA due to the stage of lactation follow different patterns. For example, Bastin et al. (2011) observed, during early-lactation, a more significant variation in long-chain FA compared to those of medium- or short-chain; while Garnsworthy et al. (2006) did not identify differences in medium- or late-lactation. In general, it is recognized that with the advance of lactation the proportion of most of the FA-derived from synthesis *de novo* (FA synthesized in the mammary gland from 4 to 12 carbons) increase, while FA from the diet decrease (Palmquist et al. 1993), attributed to the physiological capacity of animals to satisfy their nutritional requirements (Jensen 2002).

With the exception of h^2 estimated for lactose, that was within the published range for these trait (0.17 to 0.25), the rest of h^2 estimated for milk components were higher than those previously reported estimates (Samoré et al. 2007; 2012; Tullo et al. 2014) for the same traits in BS; around 0.30, 0.13, and 0.28, for casein, fat, and protein percent, respectively. Estimates for TS were not identified in the literature. The size of the data and the edition of it could explain the observed differences.

In general, a decrease in the magnitude of the h^2 was observed as the FA carbon chain length increases. The differences between h^2 estimates for FA could be associated with their biosynthesis pathway (Garnsworthy et al. 2010; Bouwman et al. 2011; Gion et al. 2011). Bovine FA originate from two primary sources: *de novo synthesis* and dietary uptake of preformed FA. Almost all of the C4:0 to C14:0 and approximately half of the C16:0 (Bauman and Griinari 2003) are synthesized *de novo*, whereas the remaining C16:0, and higher carbon chain FA are mainly derived from the diet (Bauman and Griinari 2003), which could vary depending on fat composition (Garnsworthy et al. 2010). According to Garnsworthy et al. (2010) and Bastin et al. (2011), *de novo* synthesized FA have a stronger genetic control (expected to have a higher h^2) than milk FA-derived from the diet and body fat mobilization. However, lower h^2 estimates for long-chain FA indicates that processes involved in the inclusion of these FA, such as biohydrogenation in

the rumen, absorption in the intestine, or mobilization of FA from adipose tissue, may be partially influenced by genetic control (Bastin et al. 2011).

For BS, r_f including lactose had been reported as negative, or even close to zero (from -0.18 to 0.04; Samoré et al. 2007; 2012). The rest of the correlations among milk components obtained here were lower than what was reported for BS (Samoré et al. 2007; 2012). As r_f, in other studies for BS (Samoré et al. 2007; 2012), the r_g including lactose had been reported negative (from -0.08 to -0.03), except for r_g between casein and lactose (from 0.04 to 0.06). The rest of r_g among other milk components (casein, fat, protein, TS) agree with the meaning of the published estimates (Samoré et al. 2007; 2012). The highest similarity was observed in r_g between protein and casein, and it was generally higher than 0.90. As previously mentioned, studies that report TS content were not identified for BS. Studies reporting r_g between individual FA and milk components for BS were not identified in the literature. The only one identified for BS (Tullo et al. 2014) was focused on FA grouped by their degree of saturation. Results of r_g, in addition to r_f, among milk components and individual FA, expressed as a concentration in milk (g FA/dL milk) have been reported mainly for Holstein cattle (Bastin et al. 2011; 2012; Petrini et al. 2016). However, even for Holstein, no studies were identified that report relationships between individual FA and casein or TS.

Correlation estimates between lactose and individual FA (%) have limited published reports (Petrini et al. 2016). In this case, the r_g obtained here was superior to those reported by Petrini et al. (2016); however, the r_f, in both cases, were close to zero. When FA was reported as a concentration in milk (g FA/dL of milk; Bastin 2011; 2013), r_g among fat (%) and individual FA in the present were similar, higher (20%) for C4, C8, and C18, than those reported in the literature for Holstein. However, r_f were not reported in the studies previously discussed. Similar to the trend observed between fat (%) and FA, r_f between protein (%) and individual FA have limited reports. However, r_g among protein (%) and FA, obtained in the present study, had magnitudes comparable with those reported by Bastin et al. (2011; 2013), with the exception of the correlation obtained between C8, C10, and C16 with protein (%), which were lower (around 50%) to what was reported by authors above mentioned.

In general, r_g estimates among individual FA could be comparable in magnitude and direction with those reported by Bastin et al. (2011; 2013), with slight differences for that r_g including C4 or C6, where the estimates obtained in the present study were lower; and the specific correlation between C14 and C18, whose

magnitude in this study was higher than that reported in the literature by the previously mentioned authors. It is possible to observe that the r_g among FA with similar chain length showed positive correlations of large magnitude (>0.8 and even close to 1.0), the magnitudes of estimates decrease as FA of different sizes are related. Genetic correlations among FA illustrates similarities and differences in their origin, and it is based on the length of the carbon chain, which is explained by the metabolic pathway of FA synthesis. Although there are differences in the definition of FA and the model used for data analysis, results of this study support the direction of the association between FA previously reported (Bastin et al. 2011; 2013).

In addition to other traits unexplored (for example, production of methane gas) in dairy cattle, FA has an excellent opportunity to be part into future breeding programs. However, before being added to these programs and due to the lack of published reports, it is necessary more research focused on correlation, genetic and phenotypic, among FA and milk components in order to have a deeper understanding of these relationships and thus be able to apply them to meet the different demands of society for functional dairy products.

There are some proposals (Hayes and Khosla 1992; Pascal et al. 1996) of the ideal composition of FA that would have positive effects on human health, and this proportion should be one-third of saturated, one-tenth of poly-unsaturated and the rest of mono-unsaturated FA. However, due to the different functions that FA play in human health, the properties that they transfer to dairy products and their relationship with other productive traits, there are still some questions to be resolved before FA traits are included in a selection program. Among the factors that need to be studied or to have a higher number of studies, for example, is the economic weighting of these traits in breeding programs or the acceptance of dairy products with different FA profiles by consumers. When there are answers to the different scenarios of these questions, genetic improvement of FA will take a direction more in line with the needs of consumers, so more studies of these traits should be carried out.

According to the results of this study, there is enough genetic variability for FA in the studied population, suggesting that milk fat composition could be changed by directional selection. Due to the relationship among FA, simultaneous evaluations should be considered when these traits are evaluated genetically. Also, the present work evidences the need for this type of studies in Brown Swiss cattle.

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Compliance with ethical standards

Conflicts of interest. The authors declare they have no conflicts of interest concerning the work presented in this report.

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This chapter is an article accepted to be published in *Tropical Health Animal and Production*

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5. Genetic parameters for nitrogen fractions content in Mexican Brown Swiss cattle milk

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Abstract

Nitrogen plays an important role in the metabolism of living organisms due to the variety of physiological functions involving molecules that contain it. This study aimed to estimate genetic parameters for milk nitrogen fractions and the casein:protein ratio in a Mexican Brown Swiss population. Milk samples from 317 cows were used to determine total (TN), non-protein (NPN), and non-casein (NCN) nitrogen. Then, crude (CP), true (TP) and whey (WP) proteins, and casein (Cas) percentages were obtained. In addition, the ratios (%) Cas:CP (CCP) and Cas:TP (CTP) were calculated. (Co)variance components and genetic parameters were estimated using an animal model. The pedigree included 2616 animals. Heritabilities were obtained from single-trait and genetic correlations from bi-variate analyses. The variation among cows was large for the studied traits. Heritability estimates could be regarded as high (>0.7) for NCN, WP, CCP, and CTP, while for the rest of traits, the estimates were from moderate to low magnitude. Genetic correlations estimates differed in magnitude, ranging from -0.9 to 0.9. There is enough additive genetic variability to achieve genetic improvement for the traits studied; therefore, they could be considered in a breeding program for the studied population.

Keywords: Casein, Casein:Protein ratio, Total nitrogen, Whey protein

Introduction

Human population is increasing and this would increase the need for animal products of high nutritional quality, such as bovine milk. Among milk components, proteins are important for the human population, and although they are not considered as an energy source, their energy contribution promotes the reduction of fat in dairy products, which is one of the reasons why market niches have emerged. There are opportunities to feed both very young and older adults with products fortified with protein. Likewise, athletes need proteins for regeneration and muscle recovery. Hence, milk composition must be an important part of any genetic improvement program in dairy cattle.

According to Rodrigues et al. (2010), Ruska and Jonkus (2014), and Rafiq et al. (2016), protein (%) in milk could be estimated from the total amount of nitrogen times 6.38. Milk crude protein (CP) is composed of casein (77.2%; Cas), whey protein (17.5%; WP), and non-protein nitrogen (5.2%; NPN) (Guo et al. 2007). Caseins play an important role in cheese production (Emmons et al. 2003). Whey protein is

dispersed in the serum, but do not contribute to cheese (Samoré et al. 2012). It has all essential amino acids, showing immune-enhancing properties, ability as antioxidant, and prevention of cardiovascular diseases and osteoporosis (Marshall 2004); it is also an ingredient in infant formula, performance beverage, and supplements (Lagrange et al. 2015). The NPN consists of amino acids, peptides, creatinine, and other constituents (DePeters and Ferguson 1992). Casein and WP, synthesized in the mammary gland and pre-formed in the blood (DePeters and Fergusson, 1992), compose the true protein (TP); a higher concentration of these protein fractions is desirable in the dairy industry (Emmons et al. 2003).

Nowadays, breeding programs should consider supplying high-quality animal products at a reasonable price (Berry 2013). There is a wide field of study in milk components to cope with the current demands of the human population. For this reason, studies on milk composition are focusing on protein fractions from different perspectives (Bonfatti et al. 2011; Rafiq et al. 2016). Studies with Brown Swiss (BS) cattle, on milk proteins, indicate the existence of genetic variability among cows for protein traits (Ghiroldi et al. 2004; Samoré et al. 2007; 2012). These studies report estimates of heritability (h^2) from 0.23 to 0.31, and from 0.22 to 0.40 for protein and Cas (%), and genetic correlations (r_g) between these traits close to 1.0. This breed is of economic importance in Mexico, but genetic evaluation is limited to milk production (Núñez et al. 2018). There is an interest by some BS Mexican breeders to produce special milk for market niches; however, there is no information about the additive genetic variability of other traits that include other measurement processes (as nitrogen fraction), preventing their inclusion as a possible alternative into breeding programs. Therefore, the objective of this study was to estimate genetic parameters for milk nitrogen fractions and casein:protein ratio in a Mexican Brown Swiss population.

Materials and methods

Collection and preparation of milk samples

Individual milk samples (240 mL) of 317 BS cows reared in eight commercial herds of Mexico (with averages of lactation number 3.3 ± 2.0 and days in milk 154.8 ± 99.8; **Table 1**) were taken from June to August 2016. Milk sampling occurred once per animal during the morning milking. These farms had slight differences in management. For example, 70% of the herds had one milking and the rest had two. Only one herd had manual milking. Around 25% were confined herds with feeding based on corn silage,

another 25% were grazing herds using mainly native grass, and the remaining herds fed the cows based on grazing and supplementation with commercial food during the milking routine.

Table 1 Number of cows (n), means (± standard deviation) of lactation number and days in milk (DIM) by herds involved in the study

Herd	n	Lactation	DIM
1	26	3.2 ± 1.5	181.9 ± 102.8
2	47	3.4 ± 1.9	161.8 ± 90.7
3	19	2.8 ± 2.1	147.5 ± 89.7
4	39	4.0 ± 2.5	144.8 ± 85.4
5	63	4.1 ± 2.1	150.3 ± 95.8
6	18	2.9 ± 1.7	142.2 ± 126.5
7	69	2.8 ± 1.6	153.5 ± 113.0
8	36	2.3 ± 1.4	156.7 ± 99.9

Milk samples were labeled and formaldehyde was added (2 mL L^{-1}) as a preservative. After collection, milk samples were stored at 4 °C until their analysis. Milk samples were prepared according to the Norm ISO 8968-1:2001 (ISO-IDF 2001).

Total nitrogen (TN). Ten mL of milk were introduced in a 100 mL volumetric flask. Distilled water was added until complete the volume. Then, the flasks were shaken by 30 s and 2 mL aliquots were taken to determine TN.

Non-protein nitrogen (NPN). In a 50 mL volumetric flask, 20 mL of milk and 20 mL of 24% TCA were introduced. The flasks were shaken by 30 s. The final volume was adjusted with 12% TCA; the flasks were shaken and filtered with Whatman® filter paper number 542. Finally, 2 mL aliquots were taken to determine NPN.

Non-casein nitrogen (NCN). Twenty mL of milk were introduced in a 50 mL volumetric flask, 20 mL of distilled water and 2 mL of 10% AcOH were added. The flasks were shaken by 30 s and let stand for 5 min. Then, 2 mL of 1 M NaOAc were introduced into the flask. The final volume was adjusted with distilled

water. The flask was shaken and filtered with Whatman® filter paper number 542. Two mL aliquots were taken to determine NCN.

Laboratory determination and studied traits

Determination of nitrogen content. The TN, NPN and NCN contents (g 100 g^{-1}), were determined by Kjeldahl method, according to standard protocols.

Studied traits. After determination of nitrogen content, they were converted to crude (CP), true (TP) and whey (WP) proteins, and casein (Cas) using:

CP (%) = TN × 6.38

TP (%) = (TN - NPN) × 6.38

Cas (%) = (TN - NCN) × 6.38

WP (%) = (NCN - NPN) × 6.38

The ratios Cas:CP (CCP) and Cas:TP (CTP) were calculated as proposed by Lacroix et al. (1996):

CCP (%) = [(TN - NCN) / TN] x 100

CTP (%) = [(TN - NCN) / (TN - NPN)] x 100

Statistical analyses

Genetic parameters and (co)variance components were estimated for each trait with an animal model, using ASReml software (Gilmour et al. 2002). Heritability was estimated using a single-trait mixed model:

$$\boldsymbol{y} = \boldsymbol{X\beta} + \boldsymbol{Zu} + \boldsymbol{e}$$

where **y** was the vector of phenotypic records; $\boldsymbol{\beta}$ was the vector of fixed effects that included farm (1,2,…,8), lactation number (1,2,…,7), the interaction farm × lactation number, and the linear effects of days in milk and $e^{-0.05}$ × days in milk as covariates; $\boldsymbol{u}$ was the vector of random genetic additive effects [~N(0, $A\sigma^2_A$)], where A is the additive genetic relationship matrix among animals and σ^2_A is the direct additive genetic variance; and $\boldsymbol{e}$ was the vector of residual random effects [~N(0, $I_N\sigma^2_e$)], where I_N is an identity matrix of order the number of observations, and σ^2_e is the residual variance. *X* and *Z* were incidence matrices relating

phenotypic records to the corresponding vectors. The *Asociación Mexicana de Criadores de Ganado Suizo de Registro* supplied pedigree information of 2616 animals. Heritability was defined as:

$$h^2 = \frac{\sigma_A^2}{\sigma_A^2 + \sigma_e^2}$$

where σ_A^2 and σ_e^2 were previously defined.

Genetic correlation (r_g) was estimated using a bi-variate mixed model:

$$\begin{bmatrix} \boldsymbol{y_1} \\ \boldsymbol{y_2} \end{bmatrix} = \begin{bmatrix} X_1 & 0 \\ 0 & X_2 \end{bmatrix} \begin{bmatrix} b_1 \\ b_2 \end{bmatrix} + \begin{bmatrix} Z_1 & 0 \\ 0 & Z_2 \end{bmatrix} \begin{bmatrix} u_1 \\ u_2 \end{bmatrix} + \begin{bmatrix} e_1 \\ e_2 \end{bmatrix}$$

with the subscripts 1 and 2 identifying traits 1 and 2, with the same fixed and random effects and assumptions than for a single-mixed model. X_1, X_2, Z_1, and Z_2 were incidence matrices relating phenotypic records with the corresponding vectors.

Genetic correlation was defined as:

$$r_g = \frac{\sigma_{A1,A2}}{\sqrt{\sigma_{A1}^2 \; x \; \sigma_{A2}^2}}$$

where $\sigma_{A1,A2}$ was the additive genetic covariance between traits 1 and 2, σ_{A1}^2 and σ_{A2}^2 were additive genetic variances for traits 1 and 2, provided by results of the corresponding bi-variate analysis.

Results

Descriptive general statistics for studied variables are shown in **Table 2** and changes through days in milk in **Table 3**. Phenotypic variability was detected for all traits, especially in WP (%) and NCN (g 100 g^{-1}) (coefficient of variation, CV, > 70%). These two traits also showed high variability throughout days in milk but a specific trend was not observed, and NPN (g 100 g^{-1}) showed values almost constantly throughout days in milk. The traits with the lowest variability were CCP and CTP (%) ratios (CV = 10%).

Estimates of h^2 and standard errors (SE) are shown in **Table 4**. Estimates of h^2 could be considered high (>0.40) for NCN, NPN (g 100 g^{-1}), WP, CCP, and CTP (%); meanwhile, for TN (g 100 g^{-1}), CP, TP and Cas (%) could be considered as medium (0.20 to 0.40). Standard errors for h^2 estimates were high, between 0.13 and 0.28; for CP, their SE was almost as large as the estimate of the parameter. It should be noted that the h^2 estimates for NCN (g 100 g^{-1}), TP, WP, CCP, CTP (%) were different from zero.

Table 2 Descriptive statistics of nitrogen fractions and protein content, and the casein to protein ratio in milk of Mexican Brown Swiss cattle

Trait	Mean	SD[1]	CV[2]	Min	Max
Total Nitrogen (g 100 g^{-1})	0.51	0.06	12	0.34	0.63
Non-protein nitrogen (g 100 g^{-1})	0.02	0.01	25	0.01	0.05
Non-casein nitrogen (g 100 g^{-1})	0.07	0.05	71	0.04	0.36
Crude protein (%)	3.24	0.39	12	2.19	4.01
True protein (%)	3.11	0.39	13	1.99	3.89
Casein (%)	2.77	0.43	16	1.26	3.65
Whey protein (%)	0.34	0.29	85	0.12	2.20
Casein:Crude protein (%)	85.36	8.60	10	35.04	93.02
Casein:True protein (%)	89.11	8.79	10	36.34	96.10

[1] SD: standard deviation; [2] CV: coefficient of variation

Phenotypic ($r_f \pm$ SE) and genetic ($r_g \pm$ SE) correlations (**Table 4**) had a wide variation, covering almost the entire range -1 to 1. Standard errors for r_f were equal or lower than 0.06 for all correlations, while SE for r_g estimates covered a wider range, and even some correlations had SE > 1. For some r_g estimates convergence was not attained.

Discussion

Total nitrogen content (g 100 g^{-1}) obtained in the present study was lower than that reported by Cerbulis and Farrell (1975; 0.63 g N 100 mL^{-1} milk) and Carroll et al. (2006; 0.57 g N kg^{-1} milk) for BS cattle. Non-protein nitrogen in milk could be a quality parameter and indicator of nutritional practices. It is composed around 50% of urea (Samoré et al. 2007). For BS, NPN represents an average of 5.4% from TN, which could mean losses of up to 0.18% of CP (%) (Cerbulis and Farrel 1975). From the present study, NPN was 4.13% of TN, representing a loss of 0.14% CP. This value was lower than those reported by Freitas et al. (2010; 0.19%), Ruska and Jonkus (2014; 0.20%), and Rafiq et al. (2016; 0.33%) for other

breeds. Non-casein nitrogen has been reported as a response to substitution of ingredients in the diet. Different sources of ionophores (Rodrigues et al. 2010), nitrogen (Aquino et al. 2008) or fat (Freitas et al. 2010) have not had an influence on NCN. Results obtained by Freitas et al. (2010; 0.60%), Rodrigues et al. (2010; 0.77%) and Rafiq et al. (2016, 0.77%) were higher than those obtained in the present study.

Crude protein (%) in milk of BS cattle has been reported around 3.6% (Samoré et al. 2007; Cecchinato et al. 2011; Samoré et al. 2012), which is 11% higher than results from this study (**Table 2**). However, minimum (2.2%) and maximum (4.0%) estimates obtained here are within the range for BS, 1.7 to 5.9% (Malossini et al. 1996; Ghiroldi et al. 2004; Cecchinato et al. 2011). Casein comprises between 76.0 and 86.0% of CP (DePeters and Cant 1992) or 82.0% of TP (Cerbulis and Farrell 1975). In this study, Cas represented 85.4 and 89.1% of CP and TP, respectively. Cas content was around 2.8%, estimate that agrees with the range (from 0.8 to 4.8%) and average published for BS (Ghiroldi et al. 2004; Samoré et al. 2007; Cecchinato et al. 2011). According to DePeters and Fergusson (1992), 16.5% of TN is associated with WP, value that was 36% larger than the average obtained in the present study.

Casein:protein ratio could be an indicator of the suitability of milk for the industry (Carroll et al. 2006). For dairy cattle, CCP ranged between 58.0 and 90.0% (Ghiroldi et al. 2004; Cecchinato et al. 2011), and between 76.0 and 85.0% for CTP (Lacroix et al. 1996; Aquino et al. 2008; Reid et al. 2015). Casein:crude protein ratio was >8% than published values for BS (around 78%; Malossini et al. 1996; Samoré et al. 2012).

In general, similarity among findings in the present study and those from literature reflects the common origin of BS. Differences among results could be a consequence of environmental conditions and experimental design in each study; it could include lactation number and lactation stage, nutritional management, animal health, season, number of herds, sampling conditions, quantification methodology, among others. These factors could generate fluctuation in the components studied, so more studies covering a wider variety of conditions should be carried out.

Studies using similar laboratory techniques have reported low CV (≤ 10%) for CP, TP, and Cas (%). For example, Aquino et al. (2008) reported CV similar to the estimates of the present study; they determined that WP had the highest (22%) and CTP the lowest (4%) CV. Conversely, NCN had a higher CV in the present study, but not in estimates of Aquino et al. (2008), where their CV was intermediate. Both

studies demonstrating heterogeneity of NCN. Research using infrared technology (Ikonen et al. 2004; Samoré et al. 2007; Cecchinato et al. 2011) reported similar or even lower CV for CP and Cas (%) than those obtained using the Kjeldahl method.

Similar to findings published by Ghiroldi et al. (2004), Bonfatti et al. (2011), and Reid et al. (2015), phenotypic variability of traits studied by herd, lactation and days in milk (**Tables 2** and **3**) for this BS population confirmed the need to include those effects in the estimation of genetic parameters of the studied traits.

The h^2 estimate for CP (%) in this study (**Table 4**) was below the range, 0.28 to 0.31, found in the literature for BS (Samoré et al. 2007; Cecchinato et al. 2011; Samoré et al. 2012). Conversely, h^2 estimate for Cas (%) in our study was above the range, 0.22 to 0.31, published for BS (Ghiroldi et al. 2004; Samoré et al. 2007; 2012). In the present study, h^2 for CCP (%) was higher than estimates obtained by Samoré et al. (2012), who reported a low h^2 estimate (0.11). Differences in estimates could be due to the method used to fully capture variability in these traits. According to estimates of the present study, the additive genetic variability for TN, NPN, NCN and WP is enough to achieve a response to selection. However, these traits have not been explored within the dairy industry, and although their inclusion in future breeding programs still faces limitations, as a faster method of measurement, and there is a vast opportunity to study them and demonstrate the advantages of their inclusion into breeding programs.

The use of TP, Cas, CCP or CTP, instead CP in breeding programs could represent advantages, since the use of TP, for example, could reduce fluctuations due to NPN; if the objective were Cas, it would allow larger response to the selection due to the larger h^2 estimates, and even would support nutritional programs if CTP or CCP were used. Selection of these traits instead CP could improve the technological and nutritional properties of milk products, besides it would reflect more accurately the economic value of milk.

Table 3 Nitrogen fraction (mean ± SD) milk composition during lactation for Mexican Brown Swiss cattle

	Lactation stage, days										
Trait[1]	30	45	75	105	135	165	195	225	255	285	301
TN (g 100 g^{-1})	$0.51^{\pm0.06}$	$0.50^{\pm0.06}$	$0.50^{\pm0.06}$	$0.49^{\pm0.04}$	$0.51^{\pm0.06}$	$0.49^{\pm0.06}$	$0.52^{\pm0.07}$	$0.53^{\pm0.08}$	$0.51^{\pm0.07}$	$0.52^{\pm0.05}$	$0.53\pm^{0.06}$
NPN (g 100 g^{-1})	$0.02^{\pm0.00}$	$0.02^{\pm0.00}$	$0.02^{\pm0.01}$	$0.02^{\pm0.01}$	$0.02^{\pm0.00}$	$0.02^{\pm0.01}$	$0.02^{\pm0.00}$	$0.02^{\pm0.01}$	$0.02^{\pm0.00}$	$0.02^{\pm0.01}$	$0.02^{\pm0.00}$
NCN (g 100 g^{-1})	$0.07^{\pm0.04}$	$0.08^{\pm0.05}$	$0.08^{\pm0.07}$	$0.07^{\pm0.04}$	$0.06^{\pm0.04}$	$0.07^{\pm0.05}$	$0.07^{\pm0.03}$	$0.09^{\pm0.06}$	$0.07^{\pm0.03}$	$0.07^{\pm0.04}$	$0.07^{\pm0.04}$
CP (%)	$3.24^{\pm0.41}$	$3.17^{\pm0.36}$	$3.20^{\pm0.37}$	$3.11^{\pm0.28}$	$3.27^{\pm0.38}$	$3.13^{\pm0.39}$	$3.33^{\pm0.42}$	$3.35^{\pm0.49}$	$3.27^{\pm0.47}$	$3.30^{\pm0.31}$	$3.35^{\pm0.38}$
TP (%)	$3.10^{\pm0.42}$	$3.03^{\pm0.37}$	$3.06^{\pm0.37}$	$2.97^{\pm0.28}$	$3.14^{\pm0.38}$	$3.00^{\pm0.41}$	$3.20^{\pm0.41}$	$3.21^{\pm0.50}$	$3.14^{\pm0.46}$	$3.18^{\pm0.31}$	$3.22^{\pm0.38}$
Cas (%)	$2.78^{\pm0.40}$	$2.66^{\pm0.43}$	$2.67^{\pm0.51}$	$2.63^{\pm0.38}$	$2.86^{\pm0.39}$	$2.69^{\pm0.36}$	$2.88^{\pm0.39}$	$2.76^{\pm0.56}$	$2.83^{\pm0.46}$	$2.87^{\pm0.36}$	$2.88^{\pm0.40}$
WP (%)	$0.33^{\pm0.27}$	$0.36^{\pm0.31}$	$0.39^{\pm0.43}$	$0.34^{\pm0.27}$	$0.28^{\pm0.25}$	$0.31^{\pm0.30}$	$0.32^{\pm0.20}$	$0.45^{\pm0.35}$	$0.31^{\pm0.22}$	$0.30^{\pm0.23}$	$0.34^{\pm0.26}$
CCP (%)	$85.85^{\pm7.34}$	$84.13^{\pm9.58}$	$83.63^{\pm12.51}$	$84.52^{\pm8.66}$	$87.39^{\pm7.16}$	$86.05^{\pm7.94}$	$86.61^{\pm5.74}$	$82.22^{\pm10.29}$	$86.35^{\pm6.50}$	$87.09^{\pm7.00}$	$85.87^{\pm7.37}$
CTP (%)	$89.63^{\pm7.62}$	$88.04^{\pm9.87}$	$87.56^{\pm12.92}$	$88.43^{\pm8.85}$	$91.04^{\pm7.19}$	$90.11^{\pm8.10}$	$90.14^{\pm5.74}$	$86.08^{\pm10.63}$	$89.98^{\pm6.74}$	$90.44^{\pm7.03}$	$89.49^{\pm7.49}$

[1] TN: total nitrogen; NPN: non-protein nitrogen; NCN: non-casein nitrogen; CP: crude protein; TP: true protein; Cas: caseins; WP: whey protein; CCP: casein:crude protein ratio; CTP: casein:true protein ratio

Table 4 Phenotypic (above diagonal) and genetic (below diagonal) correlations ± standard error, and heritability ± standard error (on diagonal) for nitrogen fractions and protein contents, and the casein to protein ratio for Mexican Brown Swiss cattle

Trait[1]	TN	NPN	NCN	CP	TP	Cas	WP	CCP	CTP	Fat	TS
TN	**0.30±0.19**	-0.04±0.06	0.23±0.06	1.00±3E-08	1.00±0.01	0.73±0.04	0.25±0.06	-0.04±0.06	-0.11±0.06	0.20±0.06	0.26±0.06
NPN	-0.13±0.39	**0.41±0.22**	0.30±0.05	-0.05±0.06	-0.13±0.06	-0.25±0.06	0.19±0.06	-0.32±0.05	-0.20±0.06	-0.06±0.06	-0.01±0.06
NCN	0.40±0.24	0.54±0.18	**0.81±0.25**	0.24±0.06	0.21±0.06	-0.49±0.05	0.99±0.01	-0.98±0.01	-0.98±0.01	0.11±0.06	0.12±0.06
CP	0.98±0.24*	-0.14±0.49	0.81±1.77	**0.23±0.24**	1.00±0.01	0.74±0.04	0.25±0.06	-0.04±0.06	-0.12±0.06	0.20±0.06	0.26±0.06
TP	0.98±0.24*	-0.57±0.69	0.72±1.97	1.00±0.50*	**0.30±0.13**	0.75±0.04	0.23±0.06	-0.01±0.06	-0.09±0.06	0.20±0.06	0.26±0.06
Cas	0.87±0.05	-0.05±1.80*	-0.89±0.20	0.87±0.05	0.88±0.05	**0.30±0.16**	-0.47±0.05	0.64±0.04	0.59±0.05	0.10±0.06	0.15±0.06
WP	0.76±1.39*	0.36±0.16	1.00±0.00	1.00±0.33*	0.57±0.84	-0.99±0.25*	**0.80±0.25**	-0.97±0.01	-0.99±0.01	0.12±0.06	0.12±0.06
CCP	0.20±0.33	-0.59±0.19	-0.99±0.00	0.23±0.48	0.39±0.75	0.98±0.20*	-0.99±0.04	**0.73±0.25**	0.99±0.01	-0.08±0.06	-0.07±0.06
CTP	-0.10±0.42	-0.40±0.18	-0.99±0.00	-0.16±0.80	-0.02±0.77*	0.92±0.12	-1.00±0.00	1.00±0.20	**0.71±0.25**	-0.10±0.06	-0.08±0.06
Fat	0.66±0.42	-0.36±1.03	0.36±0.45	0.30±0.57	0.35±0.60	0.99±0.42*	0.59±0.56	1.00±1.29*	0.99±1.36*	**0.11±0.28**	0.68±0.05
TS	0.72±1.54*	0.36±1.29	0.26±0.53	0.60±0.85	0.61±1.05	0.61±1.23	0.26±0.54	-0.31±1.24	-0.30±1.21	0.94±0.27	**0.18±0.24**

[1] TN: total nitrogen; NPN: non-protein nitrogen; NCN: non-casein nitrogen; CP: crude protein; TP: true protein; Cas: caseins; WP: whey protein; CCP: casein:crude protein ratio; CTP: casein:true protein ratio; Fat (%), TS: total solids (%)

* Convergence was not attained

For a trait to be considered as an objective in selection programs is that it must be important, preferably, economically. However, it could have social value. A greater obstacle for including these traits, without apparently economic value, is the inability to identify genetic differences at a reasonable cost (Berry 2013). Many researchers believe that it is too much work to measure milk components by chemical methods, which could be a limitation to generate large datasets for breeding programs, which need relatively large and accurate phenotypic datasets for estimation of (co)variance components (Berry 2013). For these reasons, the trend is to replace chemical methods with instruments that allow faster measurements (O'Sullivan et al. 1999), such as infrared analyzers. Comparisons between reference methods and infrared analyzers point out the great similarity, with correlation around 0.9 for fat and protein (Šustová et al. 2007). Whether or not phenotypic data are available, measures on genetically correlated traits, indirect selection or merely collecting the necessary data could be an alternative (Berry 2013). Nowadays, another option is to use genotype information, with the additional advantage of obtaining a smaller SE (Lassen et al. 2016).

Although the Kjeldahl method could be regarded as old, and despite negative factors of this (hazardous, labor intense and expensive; O'Sullivan et al. 1999; Šustová et al. 2007), results of this method could be used as additional phenotypic data for the genetic evaluation of Mexican BS, with the advantage of the precision that it has for estimating components. However, it is recognized the need to join results from Kjeldahl method and infrared technology to increase the amount of data. Using both datasets would allow periodic monitoring of the calibration curves for infrared measurement (O'Sullivan et al. 1999) so that it reflects the true conditions of the Mexican production systems.

The relationship between CP and Cas (**Table 4**) has been probably the most studied in dairy cattle; even in BS, the $r_g > 0.9$ (Ghiroldi et al. 2004; Samoré et al. 2007; 2012). These results are higher to those observed in our study, while correlation estimates between CP and TP (%) in the present study were similar to those reported by Samoré et al. (2012). Increases of TN in milk would likely increase other nitrogen fractions, as suggested by a positive r_g estimated (<0.4) in this study between TN and NCN (g 100 g^{-1}), and NCN and NPN (g 100 g^{-1}). The r_g between TN and NPN (g 100 g^{-1}) was negative but low. The trend of estimates of phenotypic correlations (r_f) was similar, although of lower magnitude. Increases in Cas (%) mean decrease in WP (%) ($r_g \approx -0.90$, and $r_f \approx -0.40$), and in NPN (g 100 g^{-1}) ($r_g \approx -0.04$, and, r_f -0.25). Casein (%) had positive r_g and r_f (>0.60) with TN (g 100 g^{-1}), CP, TP, CCP, and CTP (%). In this way,

negative r_g (≈0.80) and r_f (≈0.50) were expected between Cas (%) and NCN (g 100 g^{-1}). The r_g estimates for CCP or CTP with CP or TP (%) were low. On the other hand, WP (%) had a negative r_g and r_f close to one with CCP and CTP, which could suggest that both ratios depend more on Cas than on protein content in milk.

Rafiq et al. (2016) studied nitrogen fractions similar to the present study. They reported positive correlation estimates (0.10 to 0.86) among NCN, CP, TP, Cas, and WP; however, they used information of Sahiwal cattle. Besides, the sample size used by Rafiq et al. (2016) might not be representative. The most common r_g or r_f studied among nitrogen fraction and dairy traits include fat (%) with CP (%). Correlations estimated here (**Table 4**) were lower than those reported in the literature (Ikonen et al. 2004; Samoré et al. 2007; 2012), and ranged from 0.63 to 0.71, and from 0.28 to 0.40, for r_g or r_f, respectively.

Modifying the environmental component to improve the traits studied could be the fastest option; however, these changes could be reduced or even lost if the environmental conditions are not kept permanent. On the other hand, selection programs offer permanent results, at a lower speed. Given the objective of offering products for a niche market, it is required positive changes over time, so genetic improvement represents a good option to face the new challenges. Considering the request of Mexican BS breeders to study alternative traits, and although there is a lack of information on genetic parameters, the inclusion of alternative traits that influence economic, environmental, social or farm management profit could be fruitful.

Based on heritability estimates obtained in the present study, there is significant additive genetic variability for total, non-protein and non-casein nitrogen; crude, true and whey proteins; casein, and for casein:crude protein and casein:true protein ratios. These traits could be considered for genetic improvement in the studied Mexican Brown Swiss population. It is recommended to continue studying the considered traits, but including a higher number of farms and animals, and identifying the perspective of cost-benefit of including this type of traits in a selection program.

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Statement of Animal Rights

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Conflict of interest Statement

The authors declare that they have not conflict of interest.

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6. Phantom parents and their effect on the genetic evaluation of growth traits in Mexican Braunvieh cattle

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Abstract

In livestock pedigrees, unknown parents could be assigned to phantom parent groups (PPG). This paper aimed to compare the effect of grouping strategies for unknown parents on genetic evaluation of growth traits in Mexican Braunvieh cattle. Phenotypic data included records for birth (BW, n = 31,654), weaning (WW, n = 21,333) and yearling (YW, n = 14,439) weights. Pedigree was traced back to 1970, for animals with phenotype for any of the traits analysed. Two grouping strategies were studied. The first strategy involved 12 PPG based on birth year of their progeny and the sex of unknown parent. The second involved 24 PPG based on the birth year of their progeny and the selection pathway. The statistical models included the fixed effects of contemporary group, sex, and management (WW and YW), linear and quadratic effect of age of dam (BW and WW) and percentage of breed purity, and the random effects of direct additive genetic, maternal genetic, and maternal permanent environment (WW). Criteria for comparison were product-moment and rank correlation between BLUP with and without PPG, genetic trend, root-mean-square deviation, and changes in solutions for fixed effects. Product-moment and rank correlation ranged from 0.78 to 0.99, 0.41 to 0.91 and 0.45 to 0.85, for BW, WW and YW, respectively. Inclusion of PPG modified the genetic trend depending on the variable studied. Considering PPG in the model decreased the bias in the prediction of breeding values up to 11%, and changed solutions for fixed effects. Different grouping strategies could be applied depending on the evaluated trait.

Keywords: Beef cattle, Breeding values, Genetic groups, Rank correlation, Unknown parents

Introduction

There are unknown parents in any population which might be related to the first generation of animals in the pedigree, or spread over several generations. Unknown parents affect genetic progress in several ways: they reduce selection intensity for animals with unknown parents, parentage uncertainty decreases the accuracy of evaluations, and parent miss-identification yields bias in heritability estimation and estimated breeding values (EBV) (Van Vleck 1970). Best linear unbiased prediction (BLUP) regresses genetic merit predictions of animals to unknown parents of mean zero. Depending on the genetic background and the generation to which unknown parents belong to, their expected genetic merit could be different from zero. One strategy to deal with missing parent information (Quass 1988) is assigning

unknown parents to phantom parent groups or genetic groups (PPG). These parents are assumed to be unrelated, non-inbred and to have a single descendant. Although PPG are not of interest themselves, they are considered to facilitate modelling and computation (Westell et al. 1988). Furthermore, along statistical correction for non-random missing pedigree information, PPG enables direct estimation of quantitative genetic parameters (Wolak and Reid 2017). Assigning unknown parents to PPG with a possibly non-zero average of genetic merit, would increase or reduce the genetic merits of their descendants.

Since there are no specific rules to determine PPG, its definition is based on the researcher's criteria, but it usually includes a time component (Fikse 2009). Other factors commonly considered into the grouping strategies are sex of the parent or selection intensity (Westell et al. 1988; Theron et al. 2002; Petrini et al. 2015). Defining PPG to balance the number of groups *versus* the number of unknown parents in each PPG is not likely to affect significantly an animal model's ability to estimate PPG effects with acceptable precision (Wolak and Reid 2017). However, any strategy for assigning unknown parents to PPG should reflect the average genetic level of unknown parents (Pollak and Quaas 1983).

Inclusion of PPG in the model reduced the bias in the genetic trend of milk yield for South African Holsteins (Theron at al. 2002). Similarly, in Nelore cattle, Oliveira Junior et al. (2013) observed a reduction in EBV bias by including PPG in the genetic analyses for weaning and yearling weight, postweaning weight, scrotal circumference and muscling score. PPG caused a significant change in milk yield genetic trend of South African Holsteins (Theron et al. 2002). The purpose of this research was to compare two grouping strategies for unknown parents on genetic evaluation of growth traits in Mexican Braunvieh cattle.

Materials and Methods

Data

Pedigree and phenotype databases were obtained from *Asociación Mexicana de Criadores de Ganado Suizo de Registro*. Phenotypic records included animals born between 1985 and 2017, from 229 farms located all over Mexico. The evaluated traits were birth (BW), weaning (WW), and yearling weight (YW). Weaning and yearling weights were adjusted to 240 and 365 days of age, according to procedures proposed by Beef Improvement Federation (BIF 2010). Records exceeding ± 3 standard deviations were not included in the analyses. Similarly, WW and YW records outside 240 ± 45 and 365 ± 45 d, respectively,

were excluded from the analyses. Pedigree considered animals with phenotypic records born from 1970 and included 57,341, 2,746 and 27,015 individuals, sires and dams, respectively.

Contemporary groups were formed considering herd, year, and season of birth (rainy or dry season). If a herd-year-season had less than four animals, it was excluded from the analyses. **Table 1** shows the final number of records used for EBV and descriptive statistics for each trait. Connectedness between contemporary groups was tested using the AMC program (Roso and Schenkel 2006).

Table 1 Descriptive statistics for growth traits in the Mexican Braunvieh population

Trait	n	Min	Mean ± SD	Max
Birth weight	31,654	23.00	38.11 ± 4.84	53.00
Weaning weight	21,333	100.59	235.07 ± 42.85	372.62
Yearling weight	14,439	146.66	324.07 ± 56.07	504.84

SD = Standard deviation

Genetic analyses

The genetic analyses comprised estimation of genetic parameters, and BLUP (Henderson 1975) for the Mexican Braunvieh population, using the following single-trait models:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Z_1u} + \mathbf{e}, \qquad (1)$$

for BW and YW, and

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Z_1u} + \mathbf{Z_2m} + \mathbf{Wmpe} + \mathbf{e}, \qquad (2)$$

for WW,

where **y**, **b**, **u**, **m**, **mpe,** and **e** are vectors of phenotypic records, and fixed, direct additive genetic, maternal genetic, maternal permanent environmental, and residual effects, respectively. **X**, $\mathbf{Z_1}$, $\mathbf{Z_2}$, and **W,** are incidence matrices relating records to **b**, **u**, **m**, and **mpe**, respectively. Fixed effects were sex and the percentage of breed purity for BW, WW, and YW; linear and quadratic effects of age of dam at calving for BW and WW; birth contemporary group, birth to weaning contemporary group and management, and weaning to yearling contemporary group and management for BW, WW, and YW, respectively. There were

1,778, 1,450, and 1,038 contemporary groups for BW, WW, and YW, respectively. The (co)variance structures were defined as:

$$Var\begin{pmatrix}\mathbf{u}\\ \mathbf{e}\end{pmatrix} = \begin{pmatrix}\mathbf{A}\sigma_u^2 & 0\\ 0 & \mathbf{I}_N\sigma_e^2\end{pmatrix} \text{ (for BW and YW), and}$$

$$Var\begin{pmatrix}\mathbf{u}\\ \mathbf{m}\\ \mathbf{pe}\\ \mathbf{e}\end{pmatrix} = \begin{pmatrix}\mathbf{A}\sigma_u^2 & 0 & 0 & 0\\ & \mathbf{A}\sigma_m^2 & 0 & 0\\ & & \mathbf{I}_{Nd}\sigma_{mpe}^2 & 0\\ Sym. & & & \mathbf{I}_N\sigma_e^2\end{pmatrix} \text{ (for WW),}$$

where **A** is the pedigree-based additive genetic relationship matrix among animals, $\mathbf{I}_{Nd}$ and $\mathbf{I}_N$ are identity matrices of order equal to the number of dams and number of observations, respectively; $\sigma_u^2, \sigma_m^2, \sigma_{mpe}^2$, and σ_e^2 are the direct additive genetic, maternal genetic, maternal permanent environment, and residual variances, respectively. Variance components were obtained with derivative-free REML algorithm, using the MTDFREML software (Boldman et al. 1995), **Table 2**. The same variance component estimates were used in analyses with and without PPG.

Table 2 Estimates of variance components for birth, weaning, and yearling weights

Trait	$\boldsymbol{\sigma_u^2}$	$\boldsymbol{\sigma_e^2}$	$\boldsymbol{\sigma_m^2}$	$\boldsymbol{\sigma_{mpe}^2}$
Birth weight	2.688	8.540		
Weaning weight	87.758	435.853	8.798	23.125
Yearling weight	86.274	692.965		

σ_u^2 = direct additive genetic variance; σ_e^2 = residual variance; σ_m^2= maternal genetic variance; σ_{mpe}^2 = maternal permanent environment variance

Genetic groups

The evaluation of genetic grouping strategies was done through comparison of EBV predicted using a complete relationship matrix and those obtained from the inclusion of PPG. Criteria used to group missing parents were:

1. Year of birth: Year of birth of the missing parent was considered to be five years before the year of birth of its progeny. Missing parent birth years were grouped into six classes: 1965-69, 1970-74, 1975-79, 1980-84, 1985-89, and 1990-96.

2. Sex of missing parent.
3. Pathway of selection (sire of son, sire of daughter, dam of son, and dam of daughter).

Two strategies were studied:

1. **G12:** Class of year of birth (6 levels)×sex of missing parent (2 levels).
2. **G24:** Class year of birth (6 levels)×pathway of selection (4 levels).

Table 3 shows the frequencies of missing parents in each PPG, for each strategy.

Table 3 Criteria and frequency of unknown parents in phantom parent groups

Strategy[1]	Unknown parent	Year group[2]					
		1965-69	1970-74	1975-79	1980-84	1985-89	1990-96
G12	Sire	540	513	820	941	678	433
	Dam	647	457	664	891	564	35
G24	Sire of son	119	58	72	90	87	143
	Sire of daughter	421	455	748	851	591	290
	Dam of son	145	57	51	84	73	9
	Dam of daughter	502	400	613	807	491	26

[1] Phantom parent group with 12 (G12) and 24 (G24) levels; [2] Progeny's birth year – five years

In order to apply PPG, the term $\mathbf{Z}_1\mathbf{Qg}$ was added to equations [1] and [2], where **g** is the vector of PPG, and **Q** is the incidence matrix-relating animals to PPG. The mixed model equations for BW and YW without PPG were:

$$\begin{bmatrix} \mathbf{X}'\mathbf{X} & \mathbf{X}'\mathbf{Z} \\ \mathbf{Z}'\mathbf{X} & \mathbf{Z}'\mathbf{Z} + \lambda\mathbf{A}^{-1} \end{bmatrix} \begin{bmatrix} \hat{\mathbf{b}} \\ \hat{\mathbf{u}} \end{bmatrix} = \begin{bmatrix} \mathbf{X}'\mathbf{y} \\ \mathbf{Z}'\mathbf{y} \end{bmatrix}, \qquad (3)$$

where λ is the ratio σ_e^2/σ_u^2. The mixed model equations with PPG added were (Quaas 1988):

$$\begin{bmatrix} \mathbf{X}'\mathbf{X} & \mathbf{X}'\mathbf{Z} & \mathbf{X}'\mathbf{Z}\mathbf{Q} \\ \mathbf{Z}'\mathbf{X} & \mathbf{Z}'\mathbf{Z} + \mathbf{A}^{-1}\lambda & \mathbf{Z}'\mathbf{Z}\mathbf{Q} \\ \mathbf{Q}'\mathbf{Z}'\mathbf{X} & \mathbf{Q}'\mathbf{Z}'\mathbf{Z} & \mathbf{Q}'\mathbf{Z}'\mathbf{Z}\mathbf{Q} \end{bmatrix} \begin{bmatrix} \hat{\mathbf{b}} \\ \hat{\mathbf{u}} \\ \hat{\mathbf{g}} \end{bmatrix} = \begin{bmatrix} \mathbf{X}'\mathbf{y} \\ \mathbf{Z}'\mathbf{y} \\ \mathbf{Q}'\mathbf{Z}'\mathbf{y} \end{bmatrix} \qquad (4)$$

After incorporating PPG effects, the predicted genetic value of animals becomes **EBV** = **û** + **Qĝ**. This prediction could be made directly in the mixed model equations, using Quaas and Pollak (1981) transformation that involves absorption of PPG equations, which gives (Quaas 1988):

$$\begin{bmatrix} \mathbf{X'X} & \mathbf{X'Z} & \mathbf{0} \\ \mathbf{Z'X} & \mathbf{Z'Z} + \mathbf{A}^{-1}\lambda & -\mathbf{A}^{-1}\mathbf{Q}\lambda \\ \mathbf{0} & -\mathbf{Q'A}^{-1}\lambda & \mathbf{Q'A}^{-1}\mathbf{Q}\lambda \end{bmatrix} \begin{bmatrix} \hat{\mathbf{b}} \\ \hat{\mathbf{u}} + \mathbf{Q}\hat{\mathbf{g}} \\ \hat{\mathbf{g}} \end{bmatrix} = \begin{bmatrix} \mathbf{X'y} \\ \mathbf{Z'y} \\ \mathbf{0} \end{bmatrix} \quad (5)$$

The use of this procedure avoids the extra step of calculating **û** + **Qĝ**, after Equation [4], and the need for creating and exporting the matrix **Q**, which is computationally heavy.

Equation 5 could not be solved with MTDFREML software. Therefore, we applied Equations [3] and [4] in this study, for BLUP without and with PPG. Predicted breeding values accounting for PPG (**û** + **Qĝ**) were obtained using R package "ggroups" (Nilforooshan 2018). This package calculates the matrix of PPG contributions to individuals in a pedigree (**Q**) and adds PPG contributions (**Qĝ**) to the genetic merit of animals, obtained from MTDFREML (**û**).

Comparison and validation strategies

The evaluation of grouping strategies were:

1. Product-moment (Pearson) and rank (Spearman) correlations between BV obtained with and without PPG were estimated for each trait and PPG strategy.
2. Genetic trends were obtained for each analysis, by averaging BV by birth year.
3. In order to compare how well BV from each analysis fit the data, root mean square deviation (RMSD) between BV and phenotypes, and between BV and corrected phenotypes were calculated as:

$$RMSD_{(EBV,y)} = \sqrt{\frac{\sum_{i=1}^{n}(EBV_i - y_i)^2}{n}}, \text{ and } RMSD_{(EBV,(\boldsymbol{y}-\boldsymbol{Xb}))} = \sqrt{\frac{\sum_{i=1}^{n}(EBV_i - (\boldsymbol{y}-\boldsymbol{Xb})_i)^2}{n}},$$

where EBV_i is the predicted breeding value for individual *i*, y_i is the phenotypic record for individual *i;* $(\boldsymbol{y} - \boldsymbol{Xb})_i$ is the corrected phenotype for individual *i*, with **Xb** from BLUP without PPG; and, *n* is the number of phenotypic records.

4. Changes in the solutions for fixed effect were estimated as deviation from solution without PPG for each trait

Results

Analyses without PPG considered 3,925 and 3,258 unknown sires and dams, which were reduced to 12 (G12) and 24 (G24) phantom parent groups. G12 ranged from 35 to 941, and from nine to 851 unknown parents in G24 (**Table 3**). The product-moment (and rank correlation) coefficient estimates for EBV with and without PPG are shown in **Table 4**. Correlation coefficient estimates between EBV without PPG and EBV for G24 ($r_{EBV,G24}$) were lower than those between EBV without PPG and EBV for G12 ($r_{EBV,G12}$) for all traits and groups considered. The greatest effects of grouping were observed for WW in the group of females without phenotypic record, followed by males in the same condition. These estimates, product-moment (and rank correlation), were from moderate to high, ranging from 0.799 to 0.988 (0.781 to 0.982), 0.407 to 0.914 (0.491 to 0.886), and 0.445 to 0.853 (0.515 to 0.846), for BW, WW, and YW, respectively.

Figure 1 illustrates the effect of whether including or not PPG on genetic trend estimation. In general, inclusion of PPG did not modify the slope. Inclusion of G24 yielded larger differences in genetic trends than inclusion of G12 respect to when PPG were not considered. The genetic trends by sex were similar with the inclusion of G12 or G24 (**Figure 1**). In the first years of the pedigree considered, it seems that there is not a clear trend, so variations in the average of EBV could be seen at the beginning of the period studied. However, this period coincides with the largest amount of missing genealogical information (**Figure 2**) and with the lack of phenotypic records.

The results of empirical deviation, RMSD, are reported in **Table 5**. Grouping strategies studied yielded different responses depending on the trait analyzed. Nonetheless, the inclusion of PPG in genetic evaluation could reduce RMSD, either when it is estimated between EBV and phenotype, or EBV and corrected phenotype. Inclusion of PPG in BW analyses, under any scenario studied, decreased RMSD values compared with analyses without PPG. The most significant reduction (5.7%) were observed when G24 was considered. Inclusion of G12 decreased $RMSD_{(EBV,y)}$ and $RMSD_{(EBV,\ y\text{-}Xb)}$ estimates (5.8 and 7.5%, respectively) for WW compared with EBV predicted excluding PPG. Conversely for YW, G12 increased estimates of $RMSD_{(EBV,y)}$ and $RMSD_{(EBV,\ y\text{-}Xb)}$, but G24 reduced deviation, up to 11.7%.

Solutions for fixed effects, as well as deviations, are presented in Table 6. Solutions for sex and age of dam (linear or quadratic), did not change by the inclusion of PPG in any trait analyzed, but solutions

for contemporary groups and percentage of breed purity experimented changes of different magnitude and meaning in the traits and scenarios studied**.**

Table 4 Pearson (and Spearman) correlation coefficients between predicted breeding values without phantom parent groups (EBV), and EBV with 12 phantom parent groups (EBV_G12), and EBV with 24 phantom parent groups (EBV_G24), in the Mexican Braunvieh population

	Trait		
	Birth weight	Weaning weight	Yearling weight
Males with phenotype	n = 15,810	n = 10,748	n = 7,384
$r_{(EBV, EBV\_G12)}$	0.988 (0.982)	0.914 (0.886)	0.853 (0.846)
$r_{(EBV, EBV\_G24)}$	0.975 (0.964)	0.786 (0.763)	0.743 (0.737)
Males without phenotype	n = 2,879	n = 7,941	n = 11,305
$r_{(EBV, EBV\_G12)}$	0.941 (0.923)	0.796 (0.797)	0.719 (0.760)
$r_{(EBV, EBV\_G24)}$	0.861 (0.830)	0.606 (0.627)	0.587 (0.636)
Females with phenotype	n = 15,844	n = 10,585	n = 7,055
$r_{(EBV, EBV\_G12)}$	0.986 (0.979)	0.901 (0.879)	0.844 (0.840)
$r_{(EBV, EBV\_G24)}$	0.972 (0.960)	0.752 (0.749)	0.710 (0.743)
Females without phenotype	n = 22,808	n = 28,067	n = 31597
$r_{(EBV, EBV\_G12)}$	0.895 (0.877)	0.635 (0.659)	0.596 (0.634)
$r_{(EBV, EBV\_G24)}$	0.799 (0.781)	0.407 (0.491)	0.445 (0.515)

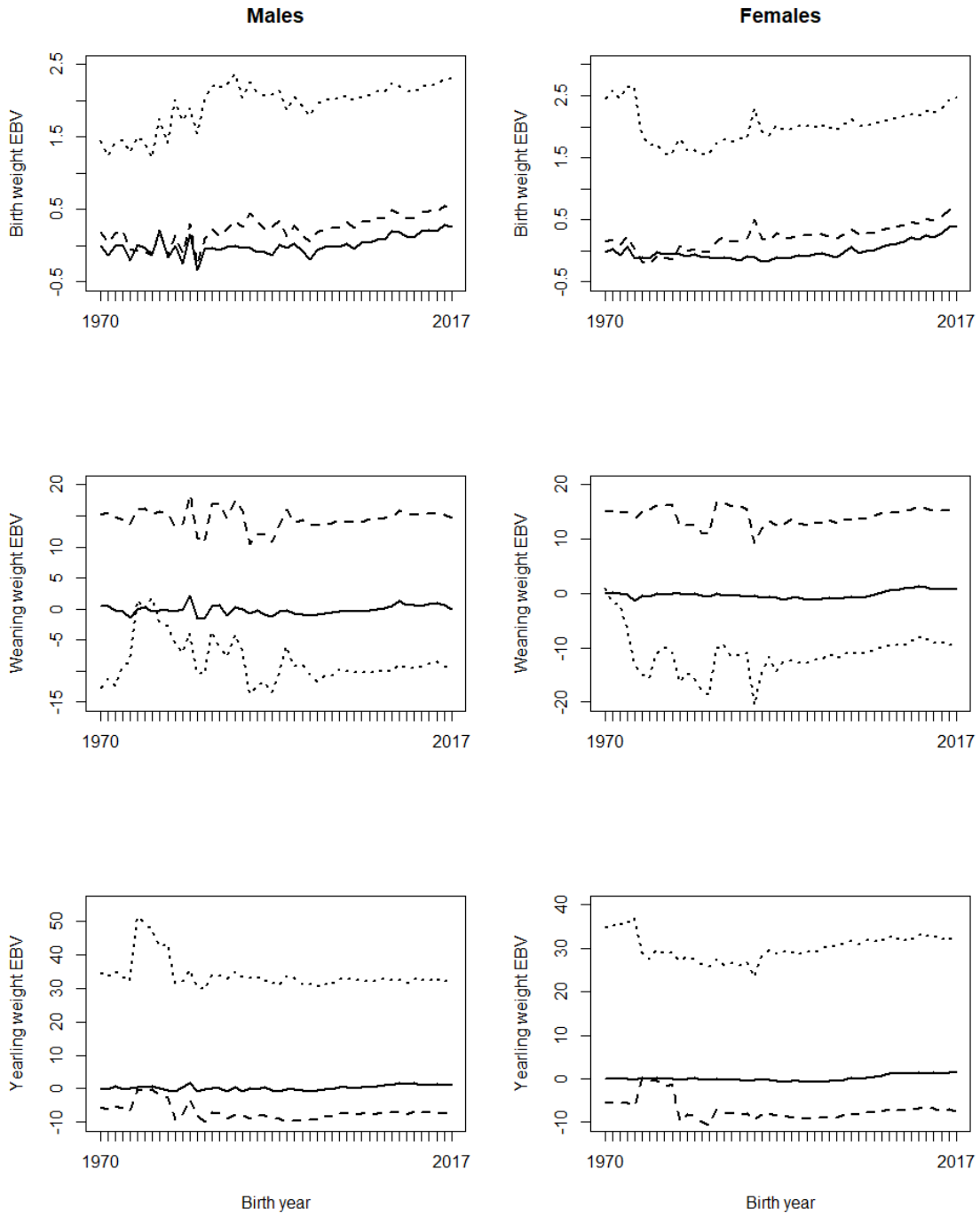

Figure 1 Genetic trends of growth traits for BLUP (EBV (solid line)), BLUP with 12 phantom parent groups (EBV_G12 (dashed line)), and BLUP with 24 phantom parent groups (EBV_G24 (dotted line))

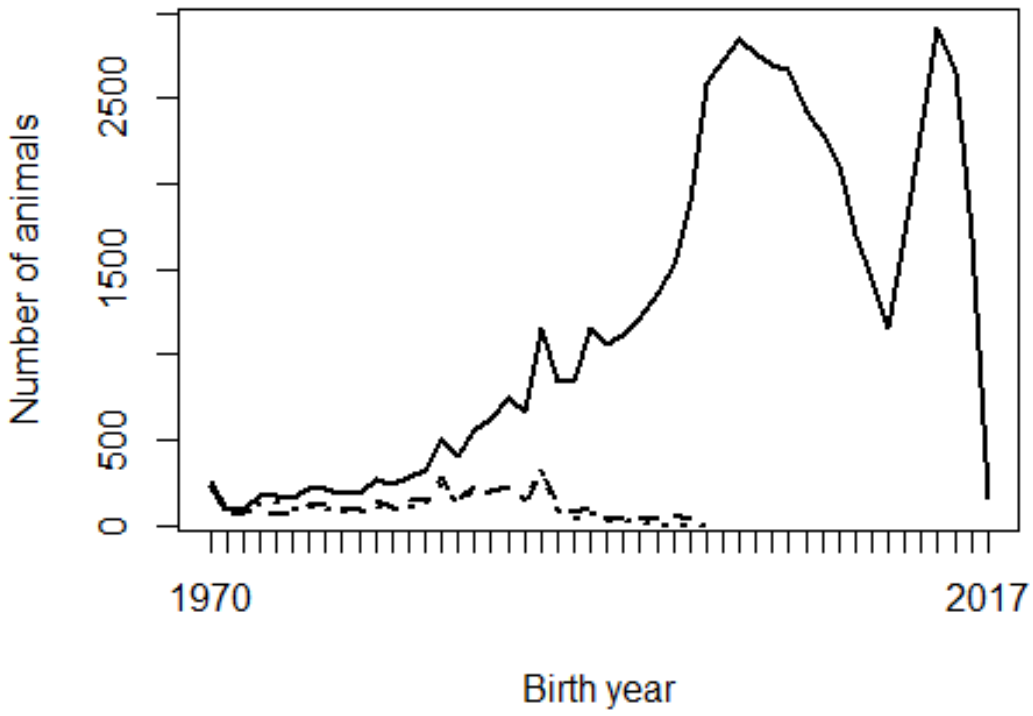

Figure 2 Number of animals (solid line), unknown sires (dashed line) and unknown dams (dotted line) per year of birth

Discussion

Formation of homogeneous size PPG is not an easy topic and sometimes could represent some problems. According to INTERBULL (2001), PPG should have a minimum size of 10 to 20 animals, and different groups should be merged to obtain a reasonable size. Thus, groups with few animals restrain the linkage between them, impairing the estimation of PPG effect (Petrini et al. 2015). In the present study, not all the groups had at least 10 animals (**Table 3**). However, merging of PPG was not necessary because the priority was given to the establishment of the time trend (**Figure 2**).

Genetic analyses of livestock data have shown that considering PPG have a different effect on ranking of animals, and it has been proposed that correlation coefficients between EBV lower than 0.70 could indicate changes in the ranking of animals for genetic evaluation (Crews and Franke 1998). Therefore, findings obtained in the present study suggest possible changes in the EBV ranking for WW and YW, depending on the sample of animals. Similar to the results in this study, Petrini et al. (2015) concluded that

changes were expected, due to the inclusion of PPG, in the rank of EBV for WW, supported by their Pearson and Spearman correlation coefficient estimates that ranged from 0.50 to 0.69; and from 0.61 to 0.70, respectively. On the contrary, in this study minimum or null changes in animal ranking for BW, non-depending of grouping strategy applied were observed. These results for BW agree with those reported by Petrini et al. (2015) for scrotal circumference and muscling score, Shiotsuki et al. (2013) for yearling weight and post-weaning weight gain, and Theron et al. (2002) for milk production. These authors concluded that grouping did not change noticeably the results across models with or without inclusion of PPG. These results could indicate that different grouping strategies could be applied to different traits, but based on the evidence showed here and in the study by Petrini et al. (2015), grouping strategies with more PPG, or more complex, could result in lower correlations.

Table 5 Root-mean-square deviation (RMSD) of estimated breeding values[1] and raw phenotypes (y), and between estimated breeding values and corrected phenotypes (y - Xb), for growth traits, in the Mexican Braunvieh population

RMSD	Birth weight	Weaning weight	Yearling weight
EBV, y	37.75	193.31	218.67
EBV_G12, y	37.48	**181.99**	224.05
EBV_G24, y	**35.76**	201.39	**199.35**
EBV, y – Xb	30.91	193.01	270.55
EBV_G12, y – Xb	30.63	**178.64**	278.55
EBV_G24, y – Xb	**28.88**	202.98	**239.00**

[1]EBV = predicted breeding values from BLUP, EBV_G12 = EBV with 12 phantom parent groups, EBV_G24 = EBV with 24 phantom parent groups

The inclusion of PPG in the genetic evaluation could have variable and substantial effects on the estimated genetic trend. In our study, including PPG did not change the slope of the trend. In other study (Petrini et al 2015), the inclusion of PPG in genetic analyses for WW, scrotal circumference and muscling score showed a lower genetic trend compared to when PPG were not included. However, those authors did not observe differences between grouping strategies, indicating small differences in the averages of the EBV. In contrast, Shiotsuki et al. (2013) observed greater genetic trends, for post-weaning weight gain and

YW, when the model included PPG, with small differences between averages of EBV with and without PPG, especially at the beginning of the period studied. Likewise, Theron et al. (2002) showed that inclusion of PPG in genetic evaluation had a drastic effect in milk production traits, having a higher response (almost double) when PPG were included in the analysis. Additionally, Theron et al. (2002) concluded that inclusion of PPG in genetic evaluation reduced of bias in genetic trend.

The effectiveness of including PPG on genetic evaluation depends primarily on the genetic structure of the population and trait studied (Petrini et al. 2015); also, it depends on criteria adopted to define PPG. Petrini et al. (2015) proposed that definition of PPG should be associated with the balance between complexity of criteria and the appropriate representation of genetic differences; on the other hand, formation of PPG should consider the selection criteria adopted by breeders, as the average generational interval, to define with greater precision the temporal component of the grouping strategy. Missing pedigree information from unknown parents could bias animal model because the resulting pedigree underestimates relatedness and inbreeding, and this bias is even more severe when parents' identifications are missing concerning to the phenotypic values (Wolak and Reid 2017). In the present study, there was no overlap among phenotypes and animals with missing parents' information. The evidence obtained in the present study indicates that inclusion of PPG could reduce RMSD values, for some trait×strategy studied, and therefore, models that included PPG could estimate with less bias or more accurately EBV. These results are supported by observations made by Theron et al. (2002) and Oliveira Junior et al. (2013). Those authors concluded that substitution of missing parents with PPG in traits such as WW, YW, scrotal circumference, milk production, among others, predicted with less bias EBV of animals with absent paternity.

Nowadays, there are different tools to improve the knowledge of genealogical information, such as DNA methods, which could be expensive for breeders. Therefore, using PPG could be considered a viable alternative in the absence of complete pedigree data. It is clear that PPG should be included in the model as an approach to improve the accuracy of EBV of animals with some degree of unknown paternity.

No scientific evidence was found of how inclusion of PPG in genetic evaluation affects the solutions for the fixed effects. Here, the addition of PPG caused changes in the solutions for contemporary groups and percentage of breed purity; the changes detected were function of the grouping strategy and trait analyzed. The covariate and the "sex" factor were not affected by the inclusion of PPG.

Table 6 Solutions for fixed effects (sex, contemporary group (mean ± standard deviation), age of dam and, degree of breed purity) obtained with and without phantom parents groups and differences between solutions in the Mexican Braunvieh population

Trait	Effect	Solution[1]			Δ^2	Δ^3
		EBV	EBV_G12	EBV_G24		
Birth weight	Sex_male	0.00	1.85	0.00	-1.85	0.00
	Sex_female	-1.85	0.00	-1.85	-1.85	0.00
	CG[4]	0.99 ± 3.55	-1.01 ± 3.55	-0.02 ± 3.55	2.00	1.01
	Age of dam[5]	0.16	0.16	0.16	0.00	0.00
	Age of dam[6]	-0.03	-0.03	-0.03	0.00	0.00
	Breed purity	6.94	8.48	8.67	-1.54	-1.73
Weaning weight	Sex_male	14.68	14.68	14.64	0.00	0.04
	Sex_female	0.00	0.00	0.00	0.00	0.00
	CG	-2.03 ± 37.50	-9.33 ± 37.46	2.58 ± 37.36	7.30	-4.61
	Age of dam	0.86	0.85	0.86	0.01	0.00
	Age of dam	-0.19	-0.19	-0.19	0.00	0.00
	Breed purity	40.71	24.18	23.19	16.53	17.52
Yearling weight	Sex_male	0.00	0.00	0.00	0.00	0.00
	Sex_female	-29.64	-27.13	-27.09	-2.51	-2.55
	CG	23.51 ± 49.37	43.52 ± 48.00	-9.65 ± 47.96	-20.01	33.16
	Breed purity	55.16	34.62	-3.54	20.54	58.7

[1]EBV = solution for fixed effects without phantom parent groups, EBV_G12 = solution for fixed effect with 12 phantom parent groups, EBV_G24 = solution for fixed effect with 24 phantom parent; [2] Difference between EBV and EBV_G12; [3] Difference between EBV and EBV_G24; [4] solution for contemporary groups; [5] solution for lineal age of dam; [6] solution for quadratic age of dam

Replacing missing parents with PPG in the genetic evaluation for birth, weaning, and yearling weights for Mexican Braunvieh cattle changed the ranking of EBVs, modified the magnitude of genetic

trend, reduced the bias in the estimation of BV, and changed solutions for fixed effects, in function of the grouping strategy and the trait studied. It is recommended to apply different grouping strategies based on the trait analysed. Additional studies are still needed to evaluate other group classification criteria, including their effects on EBV reliability, even simulating absence of pedigree data.

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Compliance with ethical standards

Statement of animal rights

Authors declare that their research was solely on the available pedigree and on-farm performance data, not conflicting with animal ethics and rights.

Conflict of interest

Authors declare they do not have any conflict of interest.

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