# Variance Estimation of Maternal Lineage Effect on Milk Traits in Croatian Holstein Cattle

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## Summary

The effect of maternal lineage (ML) on milk production traits: milk, fat and protein yield (MY, FY, and PY), fat and protein content (FC and PC) was evaluated in the Croatian Holstein cattle. Data included 102,961 records for 46,696 cows calved from January 2000 to July 2015 taken from the Central database of the Croatian Agricultural Agency. Pedigree file consisted of 77,398 animals. Variance components were estimated by REML method using VCE-6 program. Statistical model included parity, region, and calving season as fixed class effects, while age at first calving was fitted as quadratic regression. Random effects were: interaction herd-year, permanent environment, maternal lineage, and direct additive genetic effect. Identification of ML was based on the pedigree information. The contribution of ML to the phenotypic variance was 2% for FC and PC and 3% for MY, FY, and PY. The estimates of ML for milk traits were within the range of other studies.

## Key words

milk traits, Holstein cattle, variance components estimation, maternal lineage

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## Introduction

Most of economically important traits in livestock have a quantitative expression. These traits are influenced by many genes of predominantly small effect and environment which results in continuous variation of phenotypes (Falconer and Mackay, 1996). Genetic improvement of quantitative traits is commonly based on phenotypic and pedigree information that are used to estimate additive genetic effect of all the genes of an individual using the mixed model methodology formulated by Henderson (1984). The evaluation considers only the effect of nuclear genes. However, other sources of genetic variation such as mitochondrial genome could affect the economically important traits as well. The mitochondrial genome plays a central role in the metabolism of energy (Whittaker and Danks, 1978) and provides a mechanism of cytoplasmic inheritance being transmitted only through the maternal lineage (ML) (Hutchison et al., 1974; Giles et al., 1980). Several studies have been addressed the importance of cytoplasmic inheritance in dairy (Bell et al., 1985; Kennedy 1986; Boettcher et al., 1996a; Boettcher and Gibson, 1997) and beef cattle (Northcutt et al., 1991; Tess and MacNeil, 1994; Mannen et al., 1998; Mezzadra et al., 2005), either by estimation of the contribution of ML to total variance or by considering ML as a fixed effect. In these studies, cytoplasmic effects were analyzed under the assumption that ML derived from the pedigree reflect the mitogenome polymorphism. The models with cytoplasmic effect were evaluated in sheep (Hanford et al., 2003; Snowder et al., 2004), pigs (Fernandez et al., 2014), and poultry (Szwaczkowski et al., 1999) as well. The influence of ML either on milk or beef performance was significant in several studies (Schutz et al., 1994; Boettcher and Gibson, 1997; Mannen et al., 1998; Roughsedge et al., 1999) and accounted from 1% to 5% of the phenotypic variation (Mezzadra et al., 2005). On the other hand, several authors (Northcutt et al., 1991; Albuquerque et al., 1998; Rorato et al., 1999) stated that cytoplasmic genetic effect was not important sources of variation.

The importance of mitochondrial inheritance on cattle economically important traits have never been studied in Croatian dairy populations. Therefore, the objective of this study was to estimate the contribution of ML effect to the total variance for milk traits: milk, fat and protein yield (MY, FY, and PY) and fat and protein content (FC and PC).

## Material and methods

Data for the analysis were provided from the Central database of the Croatian Agricultural Agency for the Holstein cows calved from January 2000 to July 2015. Standard lactation records (305 days in milk) were utilized. Data were edited and records were deleted if: a) MY was less than 1,000 kg or more than 12,000 kg; b) FY and PY were less than 80 kg or more than 600 kg; c) FC and PC were less than 2% or more than 6%; d) cows with unknown birth or calving date; e) unknown parity, region or herd; and f) unexpected age at the first calving (younger than 18 months or older than 37 months). Calving season was defined as year-season interaction. Four seasons within the year were used as follows: spring (from March to May), summer (from June to August), autumn (from September to November), and winter (from December to February). After editing, 102,961 records for 46,696 cows were used in further analysis.

**Table 1.** Pedigree structure

| Item | Number |
|---|---:|
| Animals with records | 46,696 |
| Non–base animals | 73,201 |
| - both parents known | 60,193 |
| - only sire known | 8,997 |
| - only dam known | 4,011 |
| Base animals | 4,197 |
| Proportion of base animals (%) | 5.4 |
| Average number of progenies per sire | 14.7 |
| Average number of progenies per dam | 1.8 |
| Total number of animals | 77,398 |

All animals with records and their relatives tracing back for all known generations were included in the pedigree file (Table 1). The total number of animals in the pedigree was 77,398. The proportion of non–base animals was 94.6%. Among them, 82.2% had both parents known. Small proportion of animals (5.4%) was considered as the base population. The average number of progeny per sire was 14.7. Dams had on average 1.8 progenies. ML was identified by tracing female paths to the last known female ancestor in the herd. In the analysis, ML having at least three records per line were considered.

Lactation records for milk production traits ($y_{ijklmnop}$)were modelled using single-trait animal model. The following model present the best fit for analyzed traits and is shown in the scalar notation [1]:

$$y_{ijklmnop} = \mu + b_1\left(x_{ijklmnop} - \bar{x}\right) + b_2\left(x_{ijklmnop} - \bar{x}\right)^2 + P_i + S_j + R_k + h_{kl} + m_{mo} + p_{no} + a_o + e_{ijklmnop} \qquad [1]$$

Effects of parity ($P_i$) , calving season ($S_j$) , region ($R_k$) were considered as fixed class effects, while age at first calving ($x_{ijklmnop}$) was used as covariate and was modelled as quadratic regression. Common herd–year ($h_{kl}$), maternal lineage ($m_{mo}$), permanent environment ($p_{no}$), and direct additive genetic effect ($a_o$) were included in the model as random effects.

The GLM procedure in the statistical package SAS (SAS Inst. Inc., 2009) based on a least square method was used to define the fixed part of the model. The effects were included in the model based on significance level (p-value) and proportion of variation ($R^2$) explained by the effects and the model. MaGelLan 1.0 (Maternal Genealogy Lineage Analyzer) software, *mag sampl module*, was used to choose individuals for sampling based on assumptions that the number of samplings is defined by the available/active Holstein cows in Croatia and that the coverage of maternal pedigree lines was as diverse as possible within that number (Ristov et al., 2016). Covariance components were estimated by Residual Maximum Likelihood (REML) method using VCE-6 program package (Groeneveld et al., 2008).

## Results and discussion

Descriptive statistics for milk traits is given in Table 2. The average MY was around 7,000 kg with the maximum value of almost 12,000 kg. Similar averages (271.1 and 224.8 kg) and maximum values (600.0 and 582.8 kg) were obtained for FY and PY.

**Table 2.** Descriptive statistics for milk production traits

| Variable | Unit | N | Mean | SD | Min | Max |
|---|---|---|---|---|---|---|
| MY | kg | 101,887 | 6,780.6 | 1,890.5 | 1,053.3 | 11,999.9 |
| FY | kg | 102,553 | 271.1 | 83.9 | 80.1 | 600.0 |
| PY | kg | 101,911 | 224.8 | 64.2 | 80.0 | 582.8 |
| FC | % | 102,498 | 3.98 | 0.58 | 2.00 | 6.00 |
| PC | % | 102,305 | 3.28 | 0.25 | 2.00 | 5.26 |
| Age | months | 37,354 | 26.5 | 3.2 | 18.0 | 37.0 |

MY – milk yield, FY – fat yield, PY – protein yield, FC – fat content, PC – protein content

**Table 3.** Estimated variance components ratios (± standard error) for milk traits

| Trait | $h^2$ | $m^2$ | $c^2$ | $p^2$ |
|---|---|---|---|---|
| MY (kg) | 0.32±0.004 | 0.03±0.002 | 0.21±0.004 | 0.22±0.002 |
| FY (kg) | 0.29±0.004 | 0.03±0.002 | 0.23±0.004 | 0.22±0.002 |
| PY (kg) | 0.31±0.004 | 0.03±0.002 | 0.26±0.004 | 0.20±0.002 |
| FC (%) | 0.46±0.004 | 0.02±0.003 | 0.14±0.003 | 0.19±0.002 |
| PC (%) | 0.47±0.004 | 0.02±0.003 | 0.13±0.003 | 0.19±0.002 |

MY – milk yield, FY – fat yield, PY – protein yield, FC – fat content, PC – protein content, $h^2$ – heritability, $m^2$ – ratio for maternal lineage, $c^2$ – ratio for common herd-year, $p^2$ – ratio for permanent environmental effect

The average FC was slightly below 4.00%, while the determined range was between 2.00% and 6.00%. Holstein cows had the average protein content of 3.28%. Minimum PC was 2.00%, while maximum was near 5.30%. The proportion of variation ($R^2$) accounted for the fixed part of the model for milk traits ranged from 3.6% for FC up to 19.6% for PY. All fixed effects included in the model were significant ($P<0.05$).

Although the study was focused on the cytoplasmic effect on milk traits, heritability was also estimated (Table 3) in order to determine the genetic variability of the analyzed traits. The estimated heritabilities were 0.32 for MY, 0.29 for FY, and 0.31 for PY. These estimates agreed with results of several authors (De Jager and Kennedy, 1987; Chauhan and Hayes, 1991; Welper and Freeman, 1992; Albuquerque et al., 1995). However, estimates in this study were slightly lower compared to heritabilities estimated in the studies of Cue et al. (1987) and Van Vleck and Dong (1988). Heritability estimated for FC and PC (0.46 and 0.47) was similar to values reported by Meinert et al. (1989) and Welper and Freeman (1992), but lower than estimates reported in the study of Chauhan and Hayes (1991) and De Jager and Kennedy (1987). In general, estimated heritability was higher for FC and PC compared to yield traits which are in agreement with results reported in the literature (Chauhan and Hayes, 1991; De Jager and Kennedy, 1987). Herd-year interaction as common environment obtained a relatively large part of the phenotypic variance (from 21% to 26%) for yield traits. The same effect accounted 14% and 13% of FC and PC phenotypic variance. The variance ratio for permanent environmental effect covered between 19% and 22% of phenotypic variation for analyzed traits.

Altogether 8,583 ML were identified by the pedigree analysis with an average of 5.44 records per line. The number of records per ML ranged from three to 70. ML accounted 2% of the phenotypic variance for FC and PC. The proportion of explained phenotypic variance by ML was 3% for MY, FY, and PY. In several studies, the level of phenotypic variance attributable to ML was estimated using animal model (e.g. Gibson et al., 1997). In these analyses, ML was fitted as a random effect. Boettcher et al (1996b) reported the proportion of ML variance for MY, FY, and FC of 0.38%, 0.71%, and 2.90%, respectively. Furthermore, Schnitzenlehner and Essl (1999) estimated a component of phenotypic variance attributable to ML of 2% for the first lactation MY in the Austrian Simmental population. ML was also important component of phenotypic variance (4%) for the first lactation FY in UK Holstein cattle when data were restricted to five or more cows per ML. A significant component of ML variance (1.5%) was also estimated for the composite dairy type traits (Roughsedge et al., 2000a) as well as for persistency (4.4%) in the study of Schnitzenlehner and Essl (1999). Contrary, no significant component of the variance attributable to ML was found for 305-days milk yield, composition traits, and persistency (Roughsedge et al., 2000b). In this study, a contemporary record design was used to minimize pair-wise additive direct genetic relationships among cows within a ML and to remove both the effect of heterogeneous variance over time and the complications of permanent environment effects. Albuquerque et al. (1998) analyzed Holstein cattle data and concluded that the contributions of ML to the phenotypic variances of MY (1.1%), FY (0.8%), and FC (0.9%) were too small to be relevant for genetic evaluations. Rorato et al. (1999) investigated the same data set and came to the same conclusion suggesting that ML which accounted for 1.1% of the phenotypic variance for milk yield was not an important source of variation.

Lack of significance of ML effect reported in aforementioned studies could be attributed to the weaknesses within design of studies (Gibson et al., 1997). Mixed model methodology allows an adequate partition of variance and the unbiased estimation of ML effects. However, the methodology relies on pedigree records for the definition of ML. Pedigree recording errors and incomplete pedigree information could affect statistical power of designs as well as the underestimation of ML variance (Gibson et al., 1997). Roughsedge et al. (2001) using simulation studies also showed that pedigree errors could lead to the underestimation of true ML effect.

A positive mitochondrial effect is desirable for dams of cows, but not relevant for dams of sires, since they are not passed onto male progeny. Gibson et al. (1997) concluded that the predicted breeding values of progeny tested sires could be only slightly affected by the presence of ML. On the other hand, genetic merit is the sum of the additive and cytoplasmic genetic components in the case of selecting dams of commercial cows. The use of multiple ovulation and embryo transfer (MOET) make useful to determine the importance of cytoplasmic inheritance on production traits in dairy cattle. Therefore, female selection based on the MOET could benefit by including estimated ML in selection decisions for females.

## Conclusion

Maternal lineage showed significant effect on milk traits, 2-3% of phenotypic variability, in Croatian Holstein cattle. Results indicated strongest relationship of ML with MY, FY, and PY, compared to FC and PC. Accounting for that relationship may be of biological interest. Results obtained in this study will be upgraded after sequencing of mitochondrial whole genome in order to identify mutations associated with phenotypic variability of milk production traits.

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