

## Effect of fat – protein ratio on somatic cell count in milk

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International genetic trend study in Brown Swiss bulls has revealed the largest changes in genetic trend direction for somatic cell count (SCC) (Gorjanc *et al.*, 2011). Somatic cells in milk are represented by epithelial cells and leukocytes (immune cells), where the latter predominate and in case of udder inflammation (mastitis) increase to extreme extent. The relation between increase of SCC and immune response is confirmed by several studies (e.g. Concha, 1986; Burvenich *et al.*, 1994). Due to the low economic value of milk fat in the last decades, high selection pressure on the protein content (PC) has been applied. At the same time no selection pressure, or in some populations even negative one, has been applied on fat content (FC). Taking into account also the fact that precursors of milk components enter the mammary system from the blood, the hypothesis is that milk composition changes expressed as narrow fat - protein ratio (FPR) affect cow's immune response and result in higher SCC. Hypothesis was tested on Slovenian Brown Swiss dairy cattle population included in national milk recording scheme. Test day records (TD) from years 2004 to 2017 were used. Data set included 862,780 TD of 44,821 cows. Distribution of raw SCC values was right skewed hence the data were transformed using binary logarithm and the resulting values were almost normally distributed afterwards. To estimate variance and covariance parameters animal TD models were used. Two-trait model (model 1) included SCC and FPR while three-trait model (model 2) included SCC, FC and PC. Statistical model was the same as the model in the routine national genetic evaluation. Results of described models were compared with results of variance component estimates from routine single-trait evaluation for SCC, FC and PC. Heritability estimates for SCC were almost the same for all three evaluations (0.36). Heritability for FC increased for 0.01 in model 2 in comparison to national evaluation whereas heritability for PC decreased for 0.01. Estimated heritability was lower for FPR than for the other traits (0.17). Estimated phenotypic correlation between SCC and FPR in model 1 was negative and very low (-0.001) while genetic correlation among these traits was higher though still negative (-0.100). In model 2, phenotypic correlations were low for all three trait combinations (0.062 - 0.065) but genetic correlations showed to be quite different. Genetic correlation between FC and PC was moderate and positive (0.541), between SCC and PC was low and positive (0.064) whereas genetic correlation between SCC and FC was low and negative (-0.043). Moderate and positive genetic correlation between FC and PC indicates opposite orientation compared to the estimated genetic correlations between SCC and each of these two traits. This difference is supported

### Summary

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by negative genetic correlation between SCC and FPR. The results show the complexity of the relations between considered traits. Especially traits with low and negative genetic correlations could on long term result in unexpected negative (unwanted) consequences. These results will also be tested on the other two Slovenian dairy cattle breeds. More conservative approach will have to be taken when making selection pressure changes on individual traits in breeding programs if the hypothesis is confirmed.

*Keywords: Brown Swiss cattle, somatic cell count, fat-protein ratio, immune response.*

## Introduction

Study on Brown Swiss cattle made by Gorjanc *et al.* (2011) shows a positive genetic trend for milk's protein yield for a last couple of decades, and also for somatic cell count (SCC) since 1995. Interestingly, while protein yield seems to be raising continuously, trend for SCC has reverted from negative to a raising positive one in a span of a decade (between years 1990 and 2000). Moderate positive correlation was estimated between fat content (FC) and protein content (PC) ( $r = 0.53$ ), whereas weaker but also positive correlation was estimated for SCC to PC and FC ( $r = 0.24$  and  $0.13$ , respectively) (Rajcevic *et al.*, 2003). Cinar *et al.* (2015) also presented positive correlation estimates between SCC and PC ( $r = 0.291$ ) and between SCC and FC ( $0.103$ ), confirming the results of Rajcevic *et al.* (2003). Moreover, fat - protein ratio (FPR), which is a trait used for determining cow's energy balance and health status, has shown to be a heritable trait correlated to SCC and clinical mastitis (Negussie *et al.*, 2013).

Genetic trends that reflect selection pressure in dairy cattle can be explained by human dietary guidelines' and diet changes in the past decades. Nutritional recommendations issued by health organisations have been changing, following the progress in discoveries of studies researching connections among macronutrient intake and health. For instance, a strong positive correlation between dietary, saturated fat intake and increased risk factors associated with the occurrence of coronary heart disease was estimated (e.g. Knox, 1977; Menotti *et al.*, 1999). Findings such as these further influenced diet recommendations (e.g. Department of Agriculture, 1980) toward reduced dietary fat intake (with emphasis on cholesterol and saturated fat) and simultaneously toward protein promotion. Rising awareness of health importance has led to substituting the origin of fat; animal for vegetable, and thus lessened proportion of fat consumed in form of milk fat (Weir, 1974; Munro, 1974). Increased demand for lower-fat milk and dairy products and declined milk consumption resulted in a decrease of milk fat's economic value. Since milk price is mainly determined by demand and supply, but also by the value of the products made from it, milk pricing started to take into account also more highly regarded part of milk, PC, which is important for cheese production (Graf, 1974; Sims, 1998). As a response to the milk market changes, high selection pressure on PC and no or negative selection pressure on FC started to be applied (e.g. Welper, 1991).

SCC is another economically important trait. It is considered a marker of milk quality control and milk hygiene in ruminants, but also an indicator of mammary health (subclinical mastitis). Somatic cells in the udder and milk are predominantly represented by epithelial cells and leucocytes (polymorphonuclear neutrophils, macrophages, and lymphocytes). The latter cell type is associated with the immune response of the mammary gland, thus reflecting the health of the udder (Concha, 1986; Burvenich *et al.*, 1994). As leucocytes provide the first defence response in case of udder inflammation, their elevated number is associated with occurrence of mastitis; an inflammatory reaction within the mammary gland. The primary threshold for udder

inflammation in cows is  $SCC \geq 200,000$  cells/ml milk, and the threshold for significant udder infection is  $SCC \geq 300,000$  cells/ml milk (Burvenich *et al.*, 1994; Li *et al.*, 2014; AHDB Dairy, 2018). Raw cow's milk in the European Union is considered to be fit for human consumption and further processing if the criteria  $SCC \leq 400,000$  cells/ml milk (three month average) is met (Regulation (EC) No 853/2004).

Milk is composed for optimal nutrition of calves. Aspiring to moderate positively correlated milk component proportions such as FC and PC in the opposite direction seems not only difficult to attain but also not completely free of negative consequences on the animal's health. Animals have a biologically limited capability to physiologically adapt to the selection pressure on production traits. If animal's production is being emphasized genetically, one or several other biological traits are going to be genetically restricted (Oltenucu and Broom, 2010). Since elements of milk components stem from blood (Strucken *et al.*, 2015), genetic milk component alteration could mean genetic blood element alteration, which could have an effect on immune response and reflect as changed (elevated) SCC.

Based on the described premises, it is hypothesized that milk composition changes, expressed as narrow FPR affect cow's immune response and result in higher SCC. This study gives an insight of genetic consequences caused by low negative genetic correlation in Slovenian Brown Swiss cattle; a result of short-sighted selection decisions. It estimates the heritabilities for SCC, FPR, FC and PC, and genetic as well as phenotypic correlations between them in two- and three-trait model, comparing them to the estimates from the routine national single-trait variance component evaluation.

The data records of Slovenian Brown Swiss dairy cattle population included in national milk recording scheme were used. The data set consisted of 862,780 test day records (TD) stemmed from 44,821 cows, gathered in years 2004 to 2017. The data were obtained from the National dairy milk recording database of Agricultural Institute of Slovenia.

Traits considered in the analysis were FC (%), PC (%), SCC (x 1,000 cells/ml), and FPR. Distribution of raw SCC values was right skewed hence the data were transformed using binary logarithm ( $\log_2$ ). The resulting SCC values were almost normally distributed.

To estimate variance and covariance components by software package VCE-6 (version 6.0.3-dev; 95% Bayesian credibility region) animal TD models were used. Two-trait model (model 1) included SCC and FPR while three-trait model (model 2) included SCC, FC and PC. Statistical models were the same as for the routine national genetic evaluation:

$$y_{ijklm} = \mu + C_i + b_{ij} + b_{ijj} (t/305)^2 + b_{ijj} \ln(t/305) + b_{ivj} \ln^2(t/305) + P_j + hy_k + pe_{ji} + a_{ijkl} + e_{ijklm} \quad (1)$$

In the given model (1)  $y_{ijklm}$  represented trait of interest (FC, PC, SCC and/or FPR; standard lactation), and  $\mu$  population mean. Fixed effects were  $C_i$  as a calving season (calving year x month interaction; from 2003 to 2017);  $b_{ij} (t/305) + b_{ijj} (t/305)^2 + b_{ijj} \ln(t/305) + b_{ivj} \ln^2(t/305)$  as state of lactation with lactation curve shaped by

## Material and methods

Ali-Schaffer, nested within parity; and  $P_j$  as parity ( $j = 1, 2, \dots, 5$ ). Random effects in the model were  $hy_k$  as herd-year;  $pe_{ij}$  as permanent environmental effect; and  $a_{ijk}$  as additive genetic effect;  $e_{ijklm}$  represented residual in the model.

## Results and discussion

Estimates of variance components from routine national single-trait evaluation for SCC, FC and PC are given in Table 1, and results of models 1 and 2 are shown in Table 2. Heritability estimates for SCC were very similar for all three evaluations (0.36). Heritability for FC from national evaluation (0.22) was lower but similar to heritability from model 2 (0.23). Heritability for PC was on the contrary little lower in model 2 (0.39) compared to the one from the national evaluation (0.40). Although converse, the change of heritability for FC and PC comparing the routine evaluation and evaluation made for this study was the same ( $\pm 0.01$ ). Estimated heritability for FPR (0.17; Table 2) was lower than for any other trait. SCC heritability estimates in this study were almost 4 times higher than the highest estimate of Negussie *et al.* (2013), who reported heritability for SCC in Nordic Red cattle from 0.05 to 0.10. But the estimate of heritability for FPR seems to be in agreement with theirs which was between 0.13 and 0.25. Welper (1991) also reported lower but similar heritability for SCC (0.16) in Holstein breed comparing by our estimate.

Missanjo *et al.* (2013) estimated heritabilities of 0.08, 0.42 and 0.44, genetic variances of 0.0252, 0.0587 and 0.0105, and error variances of 0.25, 0.071 and 0.0117 for SCC, FC and PC, respectively in Jersey breed, while Bouver *et al.* estimated similarly low heritability (0.07) for SCC in South African Dairy Swiss population. Their heritabilities for SCC were quite lower than ours, but their heritability for FC was almost double compared to ours. Heritabilities for PC were similar, though ours were lower.

In Canadian Holstein population, Jamrozik *et al.* (2011) also estimated low heritability for SCC (0.17) but a higher one (0.71) for FPR. Compared to ours, the latter is substantially higher.

Estimated phenotypic correlation between SCC and FPR in model 1 was negative and very low (-0.001) and genetic correlation among these traits was also negative although higher (-0.100). Phenotypic correlations in model 2 were low and similar for all trait combinations (0.062 – 0.065) (Table 3).

As shown in Table 3, genetic correlation between SCC and PC (0.064) and also between FC and PC (0.541) were low to moderate and positive. Genetic correlation between SCC and FC was low and negative (-0.043) which is consistent with estimates reported by Negussie *et al.* (2013) that ranged from -0.01 to 0.20. They also estimated genetic correlation between FPR and clinical mastitis, which was positive and ranged from 0.12 to 0.21, confirming the FPR being an 'indicative trait' for udder health status.

Negative weak genetic (-0.01) and phenotypic (-0.01) correlations between SCC and PC for Jersey breed estimated by Missanjo *et al.* (2013) were lower and oppositely oriented than ours. The only data that we found to compare SCC-FPR genetic correlation with was from Jamrozik *et al.* (2011) for Holstein cows. Their estimation of genetic correlation was weak and positive (0.04) and thereby not in agreement with ours.

Table 1. Variance component estimates from routine single-trait evaluation.

Trait	$^a h^2$	$^b \delta_a^2$	$^c \delta_e^2$
SCC	0.36	1.35	1.53
FC	0.22	0.10	0.33
PC	0.40	0.04	0.05

$^a h^2$ : heritability

$^b \delta_a^2$ : genetic (animal) variance

$^c \delta_e^2$ : error variance

Table 2. Variance component estimates from model 1 and 2.

Model	Trait	$^a h^2$	$^b \delta_a^2$	$^c \delta_e^2$
1	SCC	0.36	1.35	1.53
	FPR	0.17	0.01	0.03
2	SCC	0.36	1.35	1.53
	FC	0.23	0.11	0.33
	PC	0.39	0.04	0.05

$^a h^2$ : heritability

$^b \delta_a^2$ : genetic (animal) variance

$^c \delta_e^2$ : error variance

Table 3. Genetic and phenotypic correlations of SCC, FPR, FC and PC.

Model	Trait	$r_g^1$	$r_p^2$
1	SCC-FPR	-0.100	-0.001
	SCC-FC	-0.043	0.065
2	SCC-PC	0.064	0.062
	FC-PC	0.541	0.065

$r_g^1$ : genetic correlation;

$r_p^2$ : phenotypic correlation

Higher positive genetic correlation between FC and PC, when compared to the estimated genetic correlations between SCC and each of these two traits, indicates pronounced orientation in the opposite direction. Negative genetic correlation between SCC and FPR furthers the contrast. Similar phenotypic correlation estimates between SCC and FC or PC lead to conclusion that regardless the differences in genetic correlation estimates, alteration of milk components in form of selection on FC or PC increases the SCC with similar intensity. However, estimates of (co)variance components for these traits, especially for FPR are scarce and the results are hard to compare.

Nevertheless, relations between considered traits are more complicated than it seems at first sight. Results show the importance of considering the impact of traits with current low negative genetic correlations. These traits could, on long term, result in unexpected negative (unwanted) outcome.

## Conclusion

Somatic cell count (SCC) is a highly heritable udder health trait; its elevation usually marks an inflammatory reaction such as mastitis. Besides being used as a health indicator, it is also a milk quality indicator upon which the dairy can reduce the payment or reject the milk. Fat – protein ratio (FPR) is another heritable trait used to determine cow's health status, and shows to be positively correlated to clinical mastitis.

Due to the high selection pressure on protein content (PC), and concurrent non-existent or even negative selection pressure on fat content (FC), FPR has doubled in favour of the PC. As previously reported, PC and FC are positively correlated, whereas results of this study show the negative genetic correlation between FPR and SCC pointing out the possibility of cow's health alteration by unbalancing milk's composition.

These results are also going to be tested on the other two Slovenian dairy cattle breeds. Current indications stress the importance of a conservative but comprehensive approach in decision making when the selection pressure changes on individual traits are in question. Considering the results, we recommend a cautious approach even with traits that show weak genetic correlation. Changes in these traits, as a consequence of the indirect impact of high selection pressure on other traits, can only be seen in the long term, when they may be difficult to reverse. Further confirmation of the hypothesis will back up the significance of a cautious approach.

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