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Evaluation of score for subclinical ketosis risk in Czech Holstein cows

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This study aimed to evaluate the score for subclinical ketosis risk, which is routinely monitored in Czech Holstein cows. The score is based on milk recording indicator traits which include fat-to-protein ratio, fat-to-lactose ratio, citric acid, β-hydroxybutyrate, and acetone concentrations. The score was significantly (P <0.001) affected by the age of cow at calving, days in milk (DIM) and season of test-day recording. Variance components were estimated with a univariate linear animal model for the score on the first test-day and with a multivariate linear animal model for the score in 3 successive test-days (6-40, 30-70, 60-100 DIM). The heritability estimate was lower at the beginning of lactation (0.08) and increased gradually to 0.11 at the end of the recorded period. Genetic correlations between the score at the first and the other two test-days were lower than 1 indicating that they are genetically different traits. Estimated breeding values were normally distributed with mean 0.20 and reliabilities up to 0.66 in females and 0.98 in males. Breeding values were negatively correlated with most of other routinely evaluated traits, with the strongest correlations with milk fat percentage (0.39), body condition score (-0.26) and fertility of cows (-0.25). The score for subclinical ketosis risk showed sufficient genetic variability and had the potential to be used in genetic improvement of resistance to (sub)clinical ketosis of Czech Holstein cows.

Keywords: metabolic status, indicator trait, ketone body

1 Introduction

Ketosis is one of the most prevalent metabolic disorders in dairy cows with incidence up to 17.2% for its clinical and 36.6% for its subclinical form (Pryce et al., 2016). Ketosis in clinical form is manifested as reduced feed intake, weight loss and production decline. Both clinical and subclinical ketosis are characterized by the accumulation of ketone bodies (acetone, acetoacetate, β-hydroxybutyrate) in body fluids, including milk (Pryce et al., 2016). The genetic improvement of the resistance to ketosis is possible, but it is hampered by its low heritability, the significant impact of environment, difficult recording of accurate phenotypic data and rather subjective diagnostics, which might influence the estimates of genetic parameters (van der Drift et al., 2012).

Czech breeders record diseases/disorders of dairy cows through the web application "Diary of Diseases and Medication" launched in 2017 (Šlosárková et al., 2016). Records of metabolic disorders are, however, infrequent (Kašná et al., 2019). Ketone bodies content can be used as an indicator trait (Koeck et al., 2014, Vosman et al., 2015, Belay et al., 2017), as it is regularly measured under the test-day milk recording programme, and it reflects the physiological status of the cow (Bastin et al., 2016). Risk of subclinical ketosis within the first 100 days in milk (DIM) is routinely evaluated using five indicators based on milk components predicted with mid-infrared analysis of test-day milk samples in the Czech Republic. These indicators include fat-to-protein ratio (FP), fat-to-lactose ratio (FL), citric acid (CA), milk β-hydroxybutyrate (BHB), and milk acetone (ACE) content. The total score for subclinical ketosis risk is calculated for each cow as a sum of cases when the predicted indicator was higher than the previously determined threshold (Hanuš et al., 2013).

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Our objective was to evaluate the score for subclinical ketosis risk and its variability in early lactation of first-parity Holstein cows.

2 Material and methods

Data from milk production recording were provided by the Czech Moravian Breeders' Corporation, Inc.. The dataset included 641,980 test-day records taken between 6 and 100 DIM from 244,115 first-lactation Holstein cows in 903 herds in the years 2016 to 2019. The percentages of cows with 1, 2, 3, and 4 test-days were 10, 24, 60, and 4%, respectively. An individual score for subclinical ketosis was calculated for each cow as a sum of cases when the values of partial indicators crossed the thresholds used in routine evaluation (Table 1). The values of the score ranged from 0 to 5, with 0 = healthy cow, 1 = the possible onset of subclinical ketosis, 2 = subclinical ketosis, 3 = severe subclinical ketosis, 4 and 5 = possible onset of clinical ketosis.

Table 1 Threshold values routinely used for the evaluation of the score for subclinical ketosis risk

Indicator	Threshold value	% of samples crossing the threshold value
Fat-to-protein ratio	≥ 1.25	37
Fat-to-lactose ratio	≥ 0.80	33
Citric acid (%)	≤ 0.16	41
Acetone (mmol/l)	≥ 0.11	22
BHB (mmol/l)	≥ 0.07	18

Variance and covariance components were estimated for (1) the score of subclinical ketosis on the first test-day (DIM 6-40) and (2) the score on the first three test-days (DIM 6-40, DIM 30-70, DIM 60-100; records from the fourth test-day were discarded). The data were edited for genetic parameter estimation. The first dataset included 202,924 cows by 1,324 sires kept in 2,411 herd-years. The pedigree file consisted of 629,575 animals and included cows with phenotypic records and their ancestors to the fourth generation. The second dataset included only cows with three subsequent test-day records and contained 153,274 cows by 1,192 sires kept in 2,256 herd-years. Pedigree file (4 generations) consisted of 524,507 animals.

Univariate and multivariate linear animal models were applied for the estimation of variance components. Model equation in matrix notation was:

$$\mathbf{y} = \mathbf{X\beta} + \mathbf{Z}_{hy}\mathbf{hy} + \mathbf{Z}_{a}\mathbf{a} + \mathbf{e},$$

where **y** is a vector of score observations; **β** is a vector of fixed effects including the age of cow at calving (8 levels: < 23 months, 23, 24, 25, 26, 27-28, 29-31 and >31 months), year-season (8 levels: a combination of 2 seasons, summer (June - September) and winter (October - May) with 4 levels of the year (2016-2019)), and DIM (each day represented a single class with 35 levels for the first test-day and 41 levels for the second and third test-day); **hy** is a vector of random herd-year effects of test-day recording; **a** is a vector of random animal additive genetic effects; **e** is a vector of random residuals; and **X**, $\mathbf{Z}_{hy}$, and $\mathbf{Z}_{a}$ are the corresponding incidence matrices.

Random effects were assumed to be normally distributed with means equal to zero and (co)variance structure equal to

$$Var\begin{bmatrix}\mathbf{hy}\\ \mathbf{a}\\ \mathbf{e}\end{bmatrix} = \begin{bmatrix}\mathbf{H}_0 \otimes \mathbf{I} & 0 & 0\\ 0 & \mathbf{G}_0 \otimes \mathbf{A} & 0\\ 0 & 0 & \mathbf{R}_0 \otimes \mathbf{I}\end{bmatrix},$$

where $\mathbf{H}_0$ is the (co)variance matrix for herd-year of test-day recording effect; $\mathbf{G}_0$ is the additive genetic (co)variance matrix; $\mathbf{R}_0$ is the residual (co)variance matrix; **I** is an identity matrix of appropriate order; **A** is the additive relationship matrix, and $\otimes$ is the Kronecker product.

Variance components were used to estimate heritability as

$$h^2 = \frac{var(a)}{var(a) + var(hy) + var(e)},$$

where *var(a)* + *var(hy)* + *var(e)* is the total phenotypic variance.

Estimated breeding values (EBV) of bulls for the score on the first test-day (6-40 DIM) with reliability ≥0.70 were correlated with EBV for routinely evaluated traits to approximate the genetic associations between them. Pearson correlations were computed with EBV for 23 type traits (20 single traits and three composite indexes for body, udder, and feet and legs), six milk production traits (milk, fat and protein yield, fat and protein percentage, somatic cell count), one longevity trait (length of production life) and six fertility traits (fertility of heifers, cows and combined; calving ease – direct, maternal, direct in primiparous).

Data editing and basic statistics were carried out in the SAS software v. 9.4 (SAS Institute Inc., 2017), and (co)variance components estimation was carried out using the average information-restricted maximum likelihood (AI-REML) procedure in the DMU package (Madsen & Jensen, 2013).b

3 Results and discussion

3.1 Evaluation of fixed effects

Means of the score according to test-days (Table 2) and least squares means of the score according to DIM (Figure 1) showed the highest values at the beginning of the lactation which reflects a more severe negative energy balance experienced by the cow after calving (Martens, 2020).

Table 2 Summary statistics of the scores for the subclinical ketosis risk in evaluated Czech Holstein cows

Trait	N	DIM	Mean	SD
$Score_1$	153,274	6–40	2.11	1.46
$Score_2$	153,274	30–70	1.41	1.17
$Score_3$	153,274	60–100	1.25	1.07

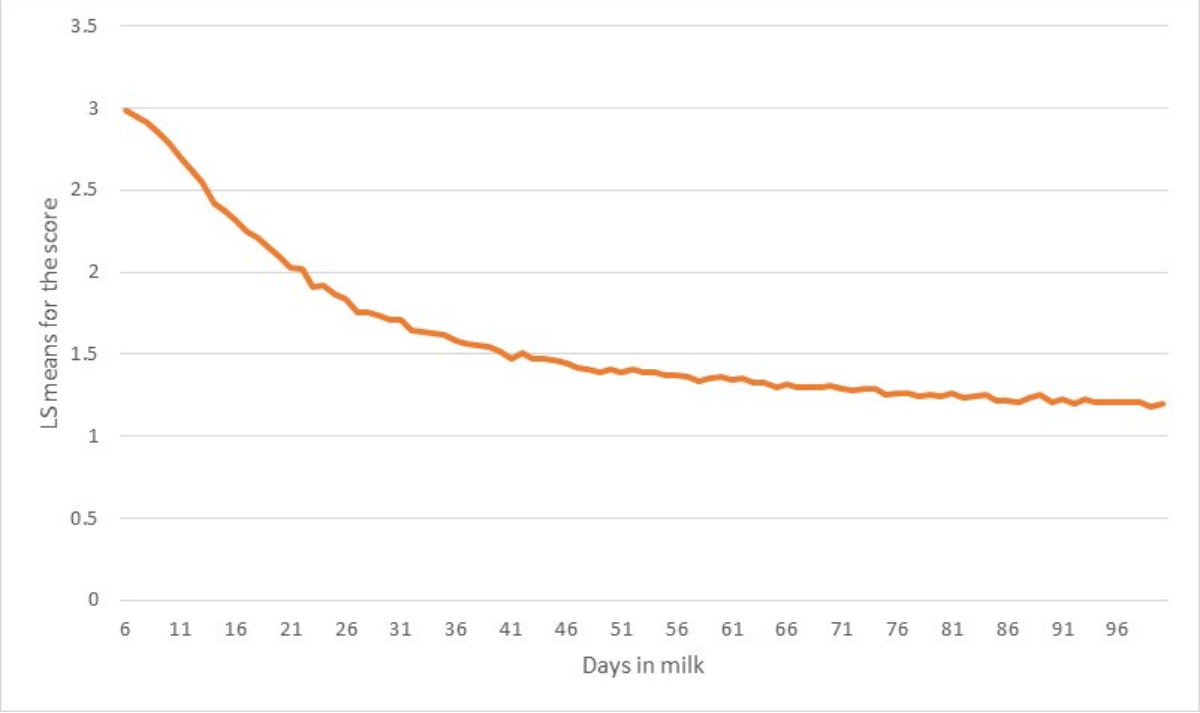

Figure 1 Least squares means for the score of subclinical ketosis risk according to the days in milk

The effect of DIM was statistically significant (P <0.001), but its importance decreased in later stages of lactation with a lower variability of the score. The same trend with the highest milk and plasma BHB content in the first month of lactation and significant decline later was reported by Koeck et al. (2014) and Belay et al. (2017). Additionally, Belay et al. (2017) reported the decrease of BHB concentration up to 20 DIM and its second increase between 20 and 30 DIM, which might be due to ketosis type I that occurs because cows approached the peak of lactation.

The score was affected by the month of test-day recording (P <0.001) with lower values from June to September. Contrary to our result, Vosman et al. (2015) found a more than doubled incidence of ketosis in spring and summer compared to autumn and winter, which they explained with the possible impact of heat stress. Hanuš et al. (2017) found lower ACE content, FP and FL in the summer season compared to the present study. However, only the difference in FL was significant in the first third of lactation.

The score also increased with the age of cows at first calving (P <0.001), which might be related to a higher body condition score and excessive mobilization of fat in older animals.

3.2 Evaluation of random effects

The largest proportion (80-81%) of the total score variance was residual, due to the effects not described by the applied model. Random herd-year effect explained 7 to 11% of the total variability, and 8 to 11% of the variability was explained by the additive genetic effect. Estimated heritability (Table 3) was higher than most of the linear estimates for clinical ketosis reported in the review of Pryce et al. (2016). In this review, heritabilities ranged from 0.01 to 0.16; usually, they are <0.05 (Koeck et al., 2014, Jamrozik et al., 2016). Genetic but also phenotypic variances were the highest in the first stage of lactation (DIM 6-40), which led to the lowest heritability estimate. A similar trend with gradually increasing heritability of BHB throughout the first third of lactation was observed by Koeck et al. (2014) and Belay et al. (2017). Variance attributable to the herd-year showed a reversed pattern, i.e. its effect was the strongest in DIM 6-40 (0.11) and then its proportion gradually decreased to be lower than the additive genetic variance. A similar trend was observed by Belay et al. (2017), who suggested that events on test-day such as feeding and management have less influence on the aetiology of ketosis than genetic differences between cows.

Table 3 Variance components of the scores for subclinical ketosis risk in different lactation stages (standard errors in brackets) estimated with univariate model

	var(a)	var(hy)	var(e)	h^2_a	h^2_{hy}	h^2_e
$Score_1$	0.159 (0.009)	0.209 (0.007)	1.515 (0.009)	0.08 (0.004)	0.11 (0.007)	0.80 (0.028)
$Score_2$	0.139 (0.007)	0.112 (0.004)	1.092 (0.006)	0.10 (0.005)	0.08 (0.003)	0.81 (0.031)
$Score_3$	0.129 (0.007)	0.084 (0.003)	0.911 (0.006)	0.11 (0.006)	0.07 (0.003)	0.81 (0.035)

var(a) – additive genetic variance, var(hy) – herd-year variance, var(e) – residual variance, and ratios with respect to phenotypic variance for additive genetic (h^2_a), herd-year (h^2_{hy}), and residual (h^2_e) effects

Nevertheless, a large proportion of variability remained unexplained by the fitted model. As summed up by van der Drift et al. (2012), variation between cows exists in individual feed intake, fat and protein mobilization, and metabolic gene expression in the liver. Additionally, Gebreyesus et al. (2020) found out that rumen microbial composition explained a larger proportion of ACE and BHB variability than host genetic. Higher estimated heritabilities (0.13 to 0.18) were presented by Vosman et al. (2015) for a similar ketosis indicator used in the Netherlands based on Fourier transform infrared spectroscopy measurements for milk FP, ACE and BHB concentrations in first 60 DIM. Higher estimates might be partly because Dutch evaluation included all parities of dairy cows, and ketosis incidence together with variability is usually higher in older compared to primiparous cows. Heritability estimates of partial score components are usually also higher. For example, van der Drift et al. (2012) reported heritabilities of 0.16 and 0.10 for milk BHB and ACE, respectively, recorded in the first 60 DIM in cows of various parities. Koeck et al. (2014) estimated heritabilities for BHB in different lactation stages from 0.14 to 0.28 in DIM 5 to 100 in first-lactation Holsteins. Jamrozik et al. (2016) reported heritability of FP which was 0.16 in first lactation and 0.10 in later lactations, and heritability of BHB which was 0.13 in

first lactation and 0.07 in later lactations. Jamrozik et al. (2016) also suggested that FP was a relatively good indicator of metabolic disorders in first lactation, but in later parities, the genetic relationship between those traits was weak and nonsignificant. Belay et al. (2017) reported heritabilities from 0.25 to 0.27 for milk BHB predicted from milk spectra in the Norwegian Red cows from 6 to 65 DIM, and Costa et al. (2019) estimated a moderate heritability (0.45) of milk lactose-to-fat ratio in the first 150 DIM in Austrian Fleckvieh cows.

Variance components from the multivariate linear animal model (Table 4) were comparable to the parameters from the univariate model. Genetic correlations between the score in first and other test-days suggest that score for subclinical ketosis risk on the first test-day is genetically different from the score for subclinical ketosis risk in later stages of lactation.

Table 4 Heritabilities (on the diagonal) and genetic correlations (above diagonal) for scores of subclinical ketosis risk based on first three test-day records from multivariate analysis (standard errors in brackets)

	$Score_1$ (DIM 6-40)	$Score_2$ (DIM 30-70)	$Score_3$ (DIM 60-100)
$Score_1$ (DIM 6-40)	0.08 (0.01)	0.86 (0.02)	0.72 (0.03)
$Score_2$ (DIM 30-70)		0.10 (0.01)	0.96 (0.01)
$Score_3$ (DIM 60-100)			0.12 (0.01)

Generally, the strongest genetic correlations were estimated between subsequent stages of lactation. Koeck et al. (2014) and Belay et al. (2017) detected strong genetic correlations of BHB content between DIM 5-20 and DIM 21-40 (0.95) and between DIM 11-30 and DIM 31-60 (0.92). Most studies focused on the evaluation of indicator traits on the first test-day (up to 40-60 DIM), where the genetic correlation with clinical ketosis and possibly other diseases is the strongest and decreases as the lactation progress. For example, Koeck et al. (2014) reported genetic correlations of 0.48 between BHB and clinical ketosis, 0.56 between FP and ketosis, 0.07 between BHB and displaced abomasum, and 0.25 between FP and displaced abomasum, and Jamrozik et al. (2016) reported genetic relationships between indicator traits (BHB, FP) and clinical diseases (ketosis, displaced abomasum) that were weaker in multiparous than primiparous cows. The study of the relationship between the score and clinical diseases has not been performed in the Czech population yet. However, the preliminary analysis showed that genetic correlations of FP with clinical ketosis and displaced abomasum were 0.38 and 0.23, respectively (Kašná et al., 2020).

3.3 Estimated breeding values

Estimated breeding values for the score were normally distributed with mean 0.04, minimum -0.97 and maximum 0.95. Mean reliability of EBV was 0.20 with a maximum of 0.66 for females and 0.98 for males. Breeding values estimated with reliability ≥0.25 were expressed as relative breeding values (REBV) standardized to mean 100 and standard deviation 12. Mean REBV according to sex and birth year are plotted in Figure 2 to assess the genetic trend in the population. While female means were stable over time, the means of bulls tended to decrease, indicating lower susceptibility to ketosis. That might be a favourable result of selection on other genetically correlated traits, mainly daughters' fertility and functional longevity, which both were included in the Czech Holstein selection index in 2008 with weights of 12% and 7%, respectively. However, this result should be interpreted with caution, as the number of bulls with reliability ≥0.25 was low (from 38 to 246, with the maximum in 2010), which may suggest an insufficient amount of information, rather than a real genetic trend to better resistance to ketosis.

The genetic correlations with other routinely evaluated traits were approximated by correlations between EBV for bulls with score reliability ≥0.70. All statistically significant correlations are shown in Figure 3. Their values indicate that animals with higher EBV for score (higher score means higher risk of ketosis) would have a genetic predisposition for higher milk protein and fat content (%), higher fat yield, and lower milk and milk protein yield in 305 days of lactation. Contrary to our result, the positive genetic correlation between milk yield and ketosis indicator is expected, as the genetic selection for high milk production would result in larger negative energy balance and higher risk of ketosis (Koeck et al., 2014, Vosman et al., 2015, Belay et al., 2016). However, Belay et al. (2017) reported a negative genetic correlation between BHB and protein yield on the second, third and fourth test-day, together with a negative association between BHB and fat and lactose content (%).

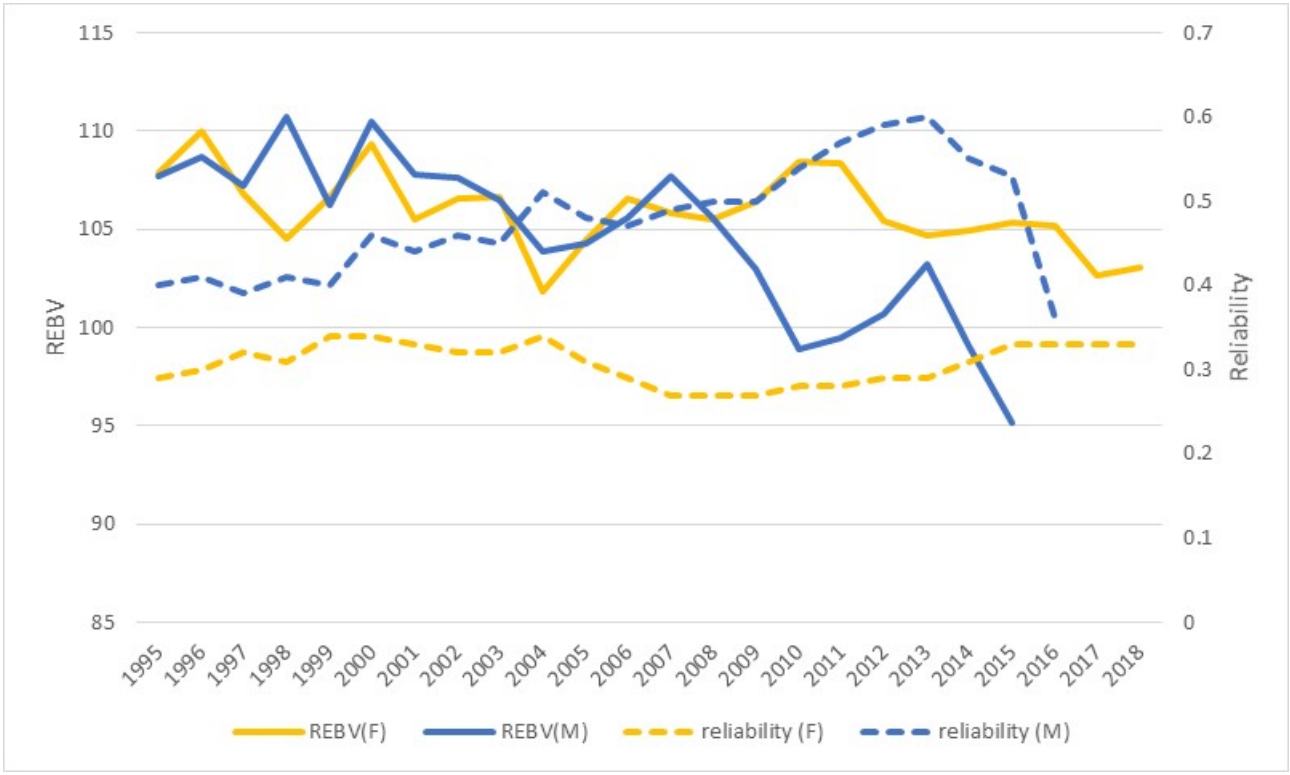

Figure 2 Mean relative breeding values (REBV) for subclinical ketosis risk with reliability ≥0.25 according to the sex and birth year of animals (lower values are favourable as they indicate lower ketosis risk)

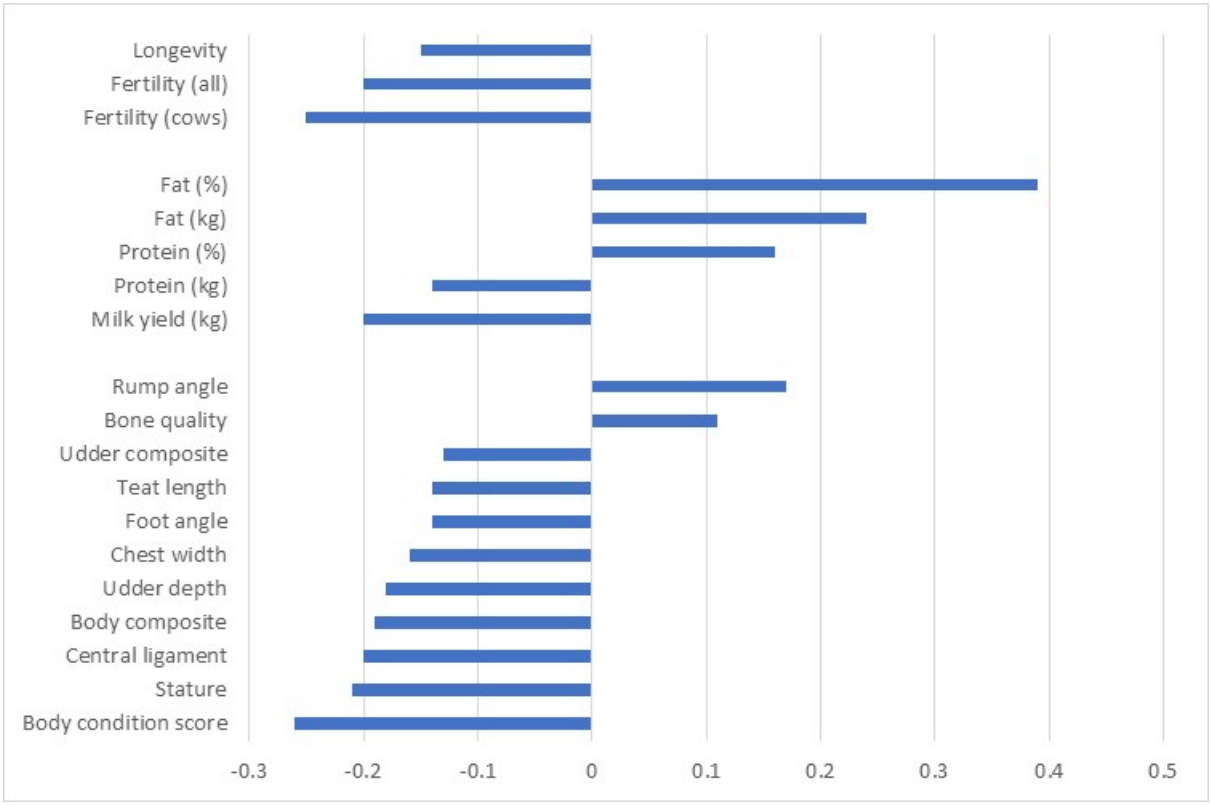

Figure 3 Pearson correlations between EBV for score of subclinical ketosis risk and EBV of routinely evaluated traits in sires (n = 361) with reliability ≥0.70. All correlations were statistically significant (P <0.05)

The correlations between EBV indicated a predisposition for poorer fertility and shorter production life in animals with higher score EBV. Unfavourable association of mid-infrared predicted phenotypes and fertility was also reported by Vosman et al. (2015) and Bastin et al. (2016), while negative Pearson correlation of BHB with direct herd life was described by Koeck et al. (2014).

The correlations suggested a genetic association with type traits, mainly with body and udder traits. Animals with a genetic predisposition for a higher score would also have a genetic predisposition to shorter stature, narrow chest, sloping rump, worse body condition, finer and flatter leg bones, lower foot angle, deeper udder with weaker cleft, and short teats. A negative genetic correlation of body condition score with ketosis indicators was assessed by Vosman et al. (2015) and Koeck et al. (2014), who also described significant Pearson correlations between EBV for BHB and EBV for overall conformation and overall feet and legs.

4 Conclusions

The score for subclinical ketosis risk has the potential to be used for genetic improvement of resistance to (sub)clinical ketosis of Czech Holstein cows. The score is routinely recorded at the population level, and it also showed enough genetic variability to be exploited for selection purposes. It does not, however, fully reflect the biological variability of its partial components, or the severity of hyperketonaemia in cows. The relationship of score with clinical ketosis, which is currently limited by a lack of data on ketosis incidence, should be further investigated.

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