

1 **Genetic parameters for endocrine and traditional fertility traits, hyperketonemia**  
2 **and milk yield in dairy cattle**

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11 Short title: Fertility and hyperketonemia in dairy cattle

12

13 **Abstract**

14 High-yielding cows may suffer from negative energy balance during early lactation,  
15 which can lead to ketosis and delayed ability of returning to cyclicity after calving. Fast  
16 recovery after calving is essential when breeding for improved fertility. Traditionally  
17 used fertility traits, such as the interval from calving to first insemination (CFI), have  
18 low heritabilities and are highly influenced by management decisions. Herd  
19 Navigator™ management program samples and analyses milk progesterone and  $\beta$ -  
20 hydroxybutyrate (BHB) automatically during milking. In this study, the genetic  
21 parameters of endocrine fertility traits (measured from milk progesterone) and  
22 hyperketonemia (measured from milk BHB) in early lactation were evaluated and

23 compared with traditional fertility traits (CFI, interval from calving to the last  
24 insemination and interval from first to last insemination) and the milk yield in red dairy  
25 cattle herds in Finland. Data included observations from 14 farms from 2014 to 2017.  
26 Data were analysed with linear animal models using DMU software and analyses were  
27 done for first parity cows. Heritability estimates for traditional fertility traits were low and  
28 varied between 0.03 and 0.07. Estimated heritabilities for endocrine fertility traits  
29 (interval from calving to the first heat (CFH) and commencement of luteal activity (C-  
30 LA)) were higher than for traditional fertility traits (0.19-0.33). Five slightly different  
31 hyperketonemia traits divided into two or three classes were studied. Linear model  
32 heritability estimates for hyperketonemia traits were low, however when the threshold  
33 model was used for binary traits the estimates became slightly higher (0.07-0.15).  
34 Genetic correlation between CFH and C-LA for first parity cows was high (0.97) as  
35 expected since traits are quite similar. Moderate genetic correlations (0.47-0.52) were  
36 found between the endocrine fertility traits and early lactation milk yield. Results  
37 suggest that the data on endocrine fertility traits measured by automatic systems is a  
38 promising tool for improving fertility, specifically when more data is available. For  
39 hyperketonemia traits, dividing values into three classes instead of two seemed to work  
40 better. Based on the current study and previous studies, where higher heritabilities  
41 have been found for milk  $\beta$ -hydroxybutyrate traits than for clinical ketosis, milk  $\beta$ -  
42 hydroxybutyrate traits are a promising indicator trait for resistance to ketosis and  
43 should be studied more. It is important that this kind of data from automatic devices is  
44 made available to recording and breeding organizations in the future.

45

46 **Keywords:** progesterone, ketosis,  $\beta$ -hydroxybutyrate, heat detection, nordic red

47

## 48 **Implications**

49 The genetic parameters of endocrine fertility traits (measured from milk progesterone)  
50 and hyperketonemia (measured from milk  $\beta$ -hydroxybutyrate) in early lactation were  
51 evaluated and compared with traditional fertility traits and the milk yield in dairy cattle.  
52 Traditional fertility traits are commonly used in breeding schemes even though they  
53 are highly influenced by management decisions and have low heritabilities. Heritability  
54 estimates for endocrine fertility traits were higher than for traditional fertility traits,  
55 consequently data on fertility traits measured by automatic systems is a promising tool  
56 for improving fertility, specifically when more data is available. For hyperketonemia  
57 traits, heritability estimates were quite low.

58

## 59 **Introduction**

60 Good fertility is an important determinant of dairy production efficiency. Nordic total  
61 merit (NTM) aims for healthy, fertile, well producing and long-lasting cows (NAV, 2018).  
62 For red dairy cattle (RDC), the relative weight for yield represents currently about 33%  
63 of the total merit. The correlation between yield index and NTM is 0.63, while the  
64 correlation between fertility index and NTM is 0.22. Hence considerably greater genetic  
65 response can be expected on production traits. Many previous studies have shown an  
66 unfavorable genetic correlation between milk production and fertility traits (e.g. Berry  
67 *et al.*, 2014). Detecting the heats and inseminating the cows at the correct time is  
68 crucial for achieving high conception rates. In addition, high yielding cows are  
69 susceptible to ketosis, a metabolic disorder, which has been associated to descended  
70 health, fertility and milk production (e.g. Duffield, 2009). Insemination measurements,  
71 such as the interval from calving to the first insemination, are generally used to  
72 evaluate cow's ability of returning to cyclicity after calving. However, insemination traits

73 are highly influenced by management decisions and tend to have low heritabilities (e.g.  
74 Berry *et al.*, 2014). Instead, endocrine fertility traits are more accurate and have been  
75 found to have higher heritabilities than traditional fertility traits (Royal *et al.*, 2002;  
76 Petersson *et al.*, 2007).

77

78 DeLaval Herd Navigator™ (HN, DeLaval International, Tumba, Sweden) management  
79 program samples and analyses four biological parameters (progesterone,  $\beta$ -  
80 hydroxybutyrate, lactate dehydrogenase and urea) in milk automatically during milking.  
81 HN uses frequent progesterone (P4) measurements to determine the reproduction  
82 status of the cow and detect the heat. If frequent P4 measurements are used, heat can  
83 be identified accurately even if the cow is not showing any visible signs of heat.  
84 Progesterone is sampled at varying intervals depending on the cow's estrus cycle  
85 (Friggens *et al.*, 2008).

86

87 Hyperketonemia or ketosis is primarily found in early lactation and is caused by a  
88 severe negative energy balance. In subclinical ketosis, no clinical signs can be seen  
89 but ketone body concentrations, such as  $\beta$ -hydroxybutyrate (BHB), increase in the  
90 blood, urine and milk (e.g. Koeck *et al.*, 2014). Concentrations of BHB in the blood  
91 have been used to diagnose hyperketonemia (Duffield, 2009) but this procedure is not  
92 practical and suitable for routine analysis. Previous studies have shown a high  
93 correlation between blood and milk BHB (Denis-Robichaud *et al.*, 2014) and that milk  
94 BHB is a useful tool for diagnosing hyperketonemia (e.g. Koeck *et al.*, 2014). In  
95 previous studies, lower conception rates have been found for cows with larger BHB  
96 concentrations than for healthy cows (e.g. Blom *et al.*, 2015). The incidence of clinical  
97 ketosis is low in Finland, in a study by Rajala-Schultz *et al.* (1999) the incidence for

98 Finnish Ayrshire was 3.3% and in the 2016 national dairy cattle health recording results  
99 (treatments by veterinarians) even lower being 1.04% (Faba, 2017). However,  
100 subclinical ketosis is more common, is not recorded, and can be costly to farmers as  
101 it has a negative effect on cows overall performance (Duffield *et al.*, 2009).

102

103 Traditional fertility traits and clinical ketosis are currently included in the Nordic  
104 breeding goal. For fertility traits, interval from calving to the first insemination (CFI),  
105 interval from first to last insemination (IFL), number of inseminations, non-return rate,  
106 conception rate and heat strength (data only from Sweden) are included, the heritability  
107 estimates for these traits are very low varying from 0.015 to 0.04 for first parity RDC  
108 cows. Heritability for clinical ketosis is 0.01 for first parity RDC cows.

109

110 The objective of this study was to estimate the genetic parameters of endocrine (P4)  
111 fertility traits and hyperketonemia (measured from milk BHB concentrations) in early  
112 lactation measured by the HN system and compare these traits with the traditional  
113 fertility traits (CFI, interval from calving to the last insemination (CLI) and IFL) and milk  
114 yield in Nordic RDC herds in Finland.

115

## 116 **Materials and Methods**

117 In this study HN data from P4 and BHB concentrations from 14 Finnish dairy farms  
118 were provided by Lattec I/S (Hillerød, Denmark). Data were available from 2014 to  
119 2017 although for 6 herds data was available from 2015 onwards and for 3 herds only  
120 from 2016. Milk P4 data (17-365 days postpartum) included 2531 observations from  
121 1622 RDC cows from parities 1-3. Some of the cows had records from multiple parities  
122 and the average number of observations per herd was 181 (range 15-795). For

123 endocrine fertility traits, early lactation observations within 100 days postpartum were  
124 included in the analysis. In this dataset, many cows which had observation for the  
125 second and / or third parity were missing the first parity record due to recent investment  
126 to HN. Including animals with only later parity records can lead to biased estimates of  
127 genetic parameters, since information on selection process is missing. Therefore,  
128 genetic analyses were performed only for the first parity cows in this study.

129

130 HN starts to record and analyze P4 concentrations from ca. 20 days after calving and  
131 uses immunoassay based dry-stick technique (Samsonova *et al.*, 2015). An extended  
132 Kalman filter is used to smooth the raw P4 values and a biological model is applied to  
133 predict the cows' reproduction status (Friggens *et al.*, 2008). Cows are classified to  
134 three categories based on their reproduction status (0 = postpartum anestrus, 1 =  
135 oestrus cycling and 2 = potentially pregnant), additionally time (days) to the next  
136 sample (DNS) is defined based on the reproduction status (Friggens *et al.*, 2008). Two  
137 P4 traits were studied, days from calving to the first heat (CFH, restricted to  $\leq 100$  days)  
138 identified by HN system and days from calving to luteal activity (C-LA, restricted to  
139  $\leq 100$  days). C-LA was calculated as a reproduction status change from 0 to 1 or 2 and  
140 it is assumed to describe accurately the first real heat since it is measured within a few  
141 days after the first heat has occurred. The CFH is difficult to detect from the P4 curve,  
142 since the P4 concentrations tend to be constantly low after calving (Figure 1). Hence,  
143 the model identifies P4 concentration change from high to low, determines passed heat  
144 and will be searching for new heat from around 17 - 18 days later. Therefore, CFH is  
145 recorded when the progesterone concentration changes from high to low and it actually  
146 describes the second real heat after calving.

147

148 Insemination and pedigree data for the 14 HN farms studied were provided by Faba  
149 Coop (Vantaa, Finland). Three traditional fertility traits were analyzed, the interval  
150 (days) from calving to the first insemination (CFI, restricted to  $\leq 230$  days), interval  
151 (days) from calving to the last insemination (CLI, restricted to  $\leq 365$  days) and interval  
152 (days) from first to last insemination (IFL). Two different datasets with different trait  
153 groups were created, first dataset included CFH and traditional fertility traits (CFI, CLI,  
154 IFL) and second dataset had observations for CFH, C-LA, milk yield (5 to 70 days  
155 postpartum) and two ketosis traits derived from smoothed BHB values (5 to 60 days  
156 postpartum).

157

158 For first parity cows, smoothed BHB values, based on prior measurements, between  
159 5 and 60 days postpartum were used in the analysis. Cows (16 animals) with less than  
160 4 BHB records were removed after which total of 16 851 observations from 794 first  
161 parity cows remained. The mean number of observations were 21.2 (range 4-55). Two  
162 BHB traits were defined which divided observations into three levels (0/1/2) with slightly  
163 different BHB thresholds (ket\_s1 and ket\_s2) to describe the cows' hyperketonemia  
164 status. In ket\_s1 the value "negative" implied that milk BHB  $< 0.10$  mmol/l; animals with  
165  $0.10 \leq$  milk BHB  $< 0.20$  mmol/l were "suspect" and animals with milk BHB  $\geq 0.20$  mmol/l  
166 were "positive". Thresholds used for ket\_s2 were: negative (milk BHB  $< 0.15$  mmol/l),  
167 suspect ( $0.15 \leq$  milk BHB  $< 0.20$  mmol/l) or positive (milk BHB  $\geq 0.20$  mmol/l). These  
168 thresholds were chosen based on the previous studies where BHB values between  
169 0.10 and 0.20 mmol/l have been used (e.g. Denis-Robichaud *et al.*, 2014; Koeck *et al.*,  
170 2014). Leblanc (2010) suggested that if individual cows are inspected instead of group-  
171 level monitoring 0.20 mmol/l threshold should be used, therefore also binary trait (0/1  
172 trait: ketosis\_b) with a threshold of 0.20 mmol/l was studied. Only one, highest,

173 observation per cow was used in the analysis. Because BHB baseline can vary among  
174 cows due to the diet (Nielsen *et al.*, 2010) also two binary (0/1) traits (ket\_r1 and ket\_r2)  
175 based on the ketosis risk values calculated by HN were studied. HN calculates the risk  
176 of ketosis (value between 0 = no risk and 1 = clinical ketosis) with biological models  
177 and uses the cow level baseline of BHB (based on first few samples) in the  
178 calculations. Based on the literature, the thresholds selected for ketosis risk traits were  
179 55 % for ket\_r1 and 60 % for ket\_r2 (e.g. Nielsen *et al.*, 2005).

180

181 HN data included the information about milk yields from all milkings. The sum of milk  
182 yields from 5 to 70 days postpartum was used to study the early lactation milk yield.  
183 Cows with less than 60 milking records available were removed from the analysis  
184 (because of the fixed time frame of data collection, some cows had either missing  
185 beginning of the recording period or end of the period). Most of the cows had milking  
186 records for all days in the recording period but in the case of missing records the  
187 previous or following record was used in order to get 66 records for all cows. For  
188 example, if the recording period ended when cow was on 68 day postpartum the 68  
189 day milk yield was used also for days 69 and 70.

190

### 191 *Statistical analysis*

192 Data were analysed with linear animal models using DMU software (Madsen and  
193 Jensen, 2013). Fixed effects were chosen based on the models used in Nordic cattle  
194 genetic evaluations and the structure of the data. Interactions (e.g. herd x year) were  
195 not included because of the small data size. Pedigree was traced 4 generations back.  
196 Univariate and multivariate models were used in genetic analyses. Univariate models  
197 were used for all traits in this study. Bivariate model was used to calculate the



198 correlation between endocrine fertility trait CFH and two traditional fertility traits which  
199 are included in the Nordic total merit (CFI, IFL). Multivariate models, with four traits at  
200 the time, were used to calculate the correlations between CFH, C-LA, milk yield and  
201 two ketosis traits derived from smoothed BHB values.

202 In matrix notation, the model was defined as follows:

$$203 \quad \mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}_a\mathbf{a} + \mathbf{e},$$

204 where  $\mathbf{y}$  is a vector of observations;  $\boldsymbol{\beta}$  is a vector of the fixed effects of herd, calving  
205 year, calving month (not for CLI and IFL), first insemination month (for CLI and IFL)  
206 and calving age;  $\mathbf{a}$  is a vector of random animal effects. For single trait analysis  $\mathbf{a} \sim N$   
207  $(0, \mathbf{A}\sigma_a^2)$  where  $\mathbf{A}$  is the additive genetic relationship matrix among animals and  $\sigma_a^2$  is  
208 the additive genetic variance and  $\mathbf{e}$  is a vector of random residuals [ $\mathbf{e} \sim N(0, \mathbf{I}\sigma_e^2)$  where  
209  $\mathbf{I}$  is the identity matrix and  $\sigma_e^2$  is the residual variance].  $\mathbf{X}$  and  $\mathbf{Z}_a$  are the corresponding  
210 incidence matrices. Calving age was divided into 7 classes <24, 24, 25, 26, 27, 28,  
211 >28 months.

212

213 In multivariate analysis, the variance-covariance structure for animal effects were  
214 defined as  $\mathbf{G} = \mathbf{A} \otimes \mathbf{G}_0$ , where  $\mathbf{G}_0$  denotes the additive genetic variances and  
215 covariance's between animal effects,  $\otimes$  the Kronecker product, and  $\mathbf{A}$  is the additive  
216 genetic relationship matrix. Because not all animals had all records available the  
217 variance-covariance structures for residual effects were defined as a block-diagonal  
218 matrix  $\mathbf{R} = \text{diag}(\mathbf{R}_i)$ , where  $\mathbf{R}_i$  is the animal specific submatrix depending on the number  
219 of available records.

220

221 To remove the dependency with the incidence levels, the linear model heritability  
222 estimates from binary hyperketonemia traits were converted to underlying scale using

223 Dempster and Lerner (1950) approximation. In hyperketonemia analysis, also a  
224 threshold model, using same fixed and random factors as in the linear model, was  
225 tested to model binary traits using logit and probit link functions. The heritability  
226 estimates for the logit model were calculated using the correction of the residual  
227 variance by  $\pi^2/3$ , which is the variance of standard logistic distribution.

228

## 229 **Results**

230 The means for traditional fertility traits and CFH increased together with the parity  
231 (Table 1). For C-LA the mean number of days were 39.8 (n=766), 37.7 (n=694) and  
232 40.6 (n=473) for parities 1, 2 and 3, respectively. Most of the cows were inseminated  
233 first time to the second heat identified by the HN system (First 17.3%, Second 31.2%  
234 and Third 18.5%). The descriptive statistics for endocrine fertility traits and early  
235 lactation milk yield used in multivariate analysis with hyperketonemia traits for first  
236 parity cows are shown in Table 2. Distribution of the BHB values in hyperketonemia  
237 trait classes are shown in Table 3.

238

239 Heritability estimates, analysed with univariate models, for traditional fertility traits were  
240 low and varied between 0.03 and 0.07 in first parity cows (Table 4). The heritability  
241 estimates for endocrine fertility traits were higher (Table 5). For first parity cows, the  
242 heritability estimates for CFH were 0.19 and 0.33 in univariate and multivariate models,  
243 respectively (Table 5). For C-LA, the univariate model heritability estimate for first  
244 parity cows (n=766) was 0.24 (Table 5). The heritability estimate for 70 days  
245 postpartum milk yield was as expected being 0.29 and 0.30 in the univariate and  
246 multivariate analysis, respectively.

247

248 Linear model heritability estimates for ket\_s1 and ket\_s2 varied between 0.07 and 0.10  
249 and were quite similar in univariate and multivariate analysis (Table 5). The heritability  
250 estimates for other hyperketonemia traits were also low (Table 6). Spearman's rank  
251 correlation between ket\_s1 and ket\_s2, when animals were ordered based on their  
252 BLUP predictions, was very high (0.995). Spearman's rank correlation between ket\_r1  
253 and ket\_r2 was 0.988 and among the ketosis (ket\_s1 and ket\_s2) and risk level (ket\_r1  
254 and ket\_r2) traits varied from 0.950 to 0.961.

255

256 Correlations between the endocrine and traditional fertility traits were positive and  
257 favorable. Genetic correlation between CFH and CFI was  $0.12 \pm 0.55$  and between CFH  
258 and CLI  $0.33 \pm 0.54$  for first parity cows. Correlation between CFH and C-LA for first  
259 parity cows was very high and favorable, genetic correlation being  $0.97 \pm 0.02$  and  
260 phenotypic correlation being 0.92 (Table 7). The unfavorable moderate genetic  
261 correlations were found between 5 to 70 days postpartum milk yield and endocrine  
262 fertility traits (Table 7). Genetic correlations between ket\_s1 and endocrine fertility  
263 traits (CFH, C-LA) were small, negative and had high standard errors (Table 7).  
264 Unfavorable and quite high genetic correlation was found between ket\_s1 and  
265 postpartum milk yield, however the estimate had a high standard error ( $0.59 \pm 0.39$ ).  
266 Correlations for ket\_s2 with endocrine fertility traits and milk yield were similar than for  
267 ket\_s1.

268

## 269 **Discussion**

270 The overall mean for C-LA in first parity cows was 39.9 days which is close to one  
271 Tenghe *et al.* (2015) found for Holstein-Friesian cows in the Netherlands. (38.1 days).  
272 Bullman and Lamming (1978) indicated that C-LA occurs ca. 5 days after ovulation and

273 can be used to study the interval from calving to first ovulation. Based on previous  
274 results, the first ovulation occurred ca. 35 days postpartum in our study. In addition,  
275 shorter mean C-LA figures of 24.7-33.8 days have been reported in previous studies  
276 (e.g. Bullman and Lamming, 1978; Petersson *et al.*, 2007). Differences between the  
277 figures may be due to different breeds, feeding practices, data editing and recording  
278 practices. For example, in HN herds the recording of P4 starts 20 days postpartum,  
279 whereas in some studies recording can start as early as 10 days postpartum. In  
280 addition, previous studies have indicated that declined dairy fertility and population  
281 differences can affect the length of this period (e.g. Pollott and Coffey, 2008).

282

283 In general, traditional fertility traits such as CFI has been used to evaluate cow's ability  
284 of returning to cyclicity and these traits tend to have low heritabilities. In the current  
285 study, heritability estimates for traditional fertility traits were low and in similar  
286 magnitude than in previous studies. For example, Muuttoranta *et al.* (2015) found  
287 slightly lower heritability estimates for traditional fertility traits in RDC (0.03 for CFI and  
288 0.02 for IFL) and Tenghe *et al.* (2015) reported slightly higher heritability for CFI  
289 (0.11±0.06) in Holstein-Friesian cows. The length of the voluntary waiting period and  
290 the visual checks of heats can differ widely among herds which can have an effect on  
291 the heritability estimates and lower the accuracy of the traditional fertility traits in dairy  
292 genetic evaluations.

293

294 In this study, estimated heritabilities for endocrine fertility traits (CFH and C-LA) were  
295 higher than for traditional fertility traits (0.19-0.33). Because of the small number of  
296 animals in the data, some of the estimates had high standard errors indicating limited  
297 accuracy of the estimates. The difference between the heritability estimates for CFH

298 between the univariate and multivariate model (0.19 and 0.33) were large most likely  
299 due to small data size, which is reflected by the standard errors of the estimates. It is  
300 also likely that with production information included the multi-trait models were better  
301 able to model the herd-year effects.

302 However, the magnitudes of the estimates are in line with previous studies where  
303 heritability estimates from 0.12 to 0.30 have been reported for endocrine fertility traits  
304 (e.g. Royal *et al.*, 2002; Petersson *et al.*, 2007; Tenghe *et al.*, 2015). In addition,  
305 heritability estimates between 0.12 and 0.18 have been reported for traits based on  
306 cow activity, such as, calving to the first sign of high activity (CFHA) (Løvendahl and  
307 Chagunda, 2009; Ismael *et al.*, 2015). Endocrine fertility traits reflect better cows´  
308 reproductive physiology and tend to have higher heritabilities than traditional fertility  
309 traits and could therefore perform better in dairy breeding schemes. However, P4 are  
310 currently available only for the small number of cows and although activity  
311 measurements are widely used in management they are seldom collected to central  
312 data base. Before they can be used in the national genetic evaluations the recording  
313 of these traits should be done more commonly.

314

315 For hyperketonemia traits, linear model heritability estimates were low, however when  
316 the threshold model was used for binary traits or when the linear model estimates were  
317 transformed to the underlying scale the estimates became slightly higher (0.07-0.15).

318 In a literature review, Pryce *et al.* (2016) reported low heritability estimates for clinical  
319 ketosis ranging from 0.01 to 0.16. In previous studies, most of the estimates for milk  
320 and serum BHB traits are low or moderate but higher than estimates for clinical ketosis  
321 (van der Drift *et al.*, 2012; Koeck *et al.*, 2014; Koeck *et al.*, 2016; Miglior *et al.*, 2014;  
322 Lee *et al.*, 2016). For example, in a study of Lee *et al.* (2016) the heritability for milk

323 BHB 30 days postpartum was 0.10 (the average heritability for the first parity was 0.08).  
324 In a study of Koeck *et al.* (2014) the heritability of milk BHB between 5 to 40 days  
325 postpartum was 0.14 and in a study of van der Drift *et al.* (2012) the heritability of milk  
326 BHB between 5 to 60 days postpartum was 0.16. In a study of Miglior *et al.* (2014)  
327 higher heritability estimates for milk BHB were found varying between 0.13 and 0.29  
328 and increasing with days in milk. The differences in the estimates between studies can  
329 partly depend on the different traits used, for example maximum BHB concentration  
330 (e.g. Koeck *et al.*, 2016), BHB concentration measured at first or some specific test  
331 day (e.g. van der Drift *et al.*, 2012; Koeck *et al.*, 2014; Lee *et al.*, 2016) and  
332 classification based on different BHB thresholds (e.g. Miglior *et al.*, 2014) have been  
333 used in previous studies. Most of the estimates found in literature are based on single  
334 or few BHB samples in the postpartum period. Although high sensitivity and specificity  
335 have been found in studies comparing milk and blood BHB measurements the use of  
336 single BHB values can be misleading if used to divide cows into ketotic or nonketotic  
337 since BHB levels can change considerably between days (Oetzi, 2007). In the current  
338 study, the frequent measurements of BHB were used which is deemed to be more  
339 accurate. There were only slight differences in the estimates, and Spearman's rank  
340 correlations between predicted breeding values for different traits were high. The BHB  
341 baseline can vary between cows due to their dietary composition (Nielsen *et al.*, 2010).  
342 Risk level traits (ket\_r1 and ket\_r2) had lower heritability estimates than traits based  
343 on smoothed BHB concentrations and divided into three classes (ket\_s1 and ket\_s2),  
344 which was expected since continuous values contain more information. Based on the  
345 current study and previous studies, where higher heritabilities have been found for milk  
346 BHB traits than for clinical ketosis, it seems that including milk BHB traits as indicator  
347 traits to the national genetic evaluation for resistance to ketosis could be useful.

348 Identification of animals more susceptible to subclinical ketosis may provide additional  
349 ways to producers to reduce costs (e.g., subsequent decrease in health, fertility and  
350 production) that would not be identified if only using clinical ketosis.

351  
352 Genetic correlation between CFH and C-LA for first parity cows was very high (0.97)  
353 as expected since both traits are measured from milk P4 concentration and describe  
354 the cow's ability of returning to cyclicity after calving. No previous studies were found  
355 where these two traits were compared. Based on these results, both of the studied P4  
356 traits can be used in genetic analysis when analyzing cow's ability of returning to  
357 cyclicity after calving. In this study, CFH was chosen to be used in the multivariate  
358 analysis with traditional fertility traits and milk yield. Correlation between CFH and CFI  
359 (genetic  $0.12 \pm 0.55$ , phenotypic 0.21) for first parity cows was lower than in many  
360 previous studies, however the standard error for this estimate was very high. In  
361 previous studies genetic correlations between 0.35 and 0.37 have been found among  
362 C-LA and CFI, however many of these estimates had high standard errors as well (e.g.  
363 Nyman *et al.*, 2014; Tenghe *et al.*, 2015). Positive genetic correlations indicate that  
364 selection for shorter C-LA will also reduce the interval for CFI. On the contrary, Royal  
365 *et al.* (2002) found a genetic correlation of  $-0.03 \pm 0.27$  between the natural logarithm of  
366 milk C-LA (lnC-LA) and CFI and Ismael *et al.* (2015) found very high positive genetic  
367 correlation (0.91) between CFHA (based on activity) and CFI.

368  
369 Moderate genetic correlations (0.47-0.52) were found between the endocrine fertility  
370 traits and 5 to 70 days postpartum milk yield, which is in line with previous studies  
371 where undesirable genetic correlations have been found between milk yield and  
372 different fertility traits. For example, the genetic correlations of 0.14-0.44 have been

373 reported between CFI and the milk yield (e.g. Hoekstra *et al.*, 1994; Tenghe *et al.*,  
374 2015). In addition, also unfavorable genetic correlations (0.18-0.45) between milk InC-  
375 LA and milk yield have been reported (Royal *et al.*, 2002; Nyman *et al.*, 2014; Tenghe  
376 *et al.*, 2015). For example, Nyman *et al.* (2014) found a genetic correlation of 0.45  
377 between InC-LA and 60 days postpartum energy corrected milk yield.

378

379 In this study, hyperketonemia traits divided into three classes (ket\_s1 and ket\_s2)  
380 seemed to perform better than bivariate traits in the univariate analysis and were  
381 included in the multivariate analysis with endocrine traits and 70 days milk yield. In the  
382 current study, genetic correlations between hyperketonemia traits (ket\_s1 and ket\_s2)  
383 and endocrine fertility traits were small, negative and had high standard errors. Instead,  
384 genetic correlation between hyperketonemia traits (ket\_s1 and ket\_s2) and 5 to 70  
385 days milk yield were high, however the standard errors were high as well. In a study  
386 by Koeck *et al.* (2014) moderate genetic correlations (0.13-0.22) were found between  
387 EBV of milk BHB and milk yield. Genetic associations found between the milk yield and  
388 traits measured from milk BHB concentrations are not surprising as selection for high  
389 milk production has negative impacts on cows energy balance and demand a larger  
390 fat mobilization in early lactation (e.g. Veerkamp *et al.*, 2003), consequently increasing  
391 the risk of hyperketonemia.

392

### 393 **Conclusions**

394 Endocrine fertility traits measured from milk progesterone concentration (CFH, C-LA)  
395 had higher heritability estimates than traditional fertility traits and the correlation  
396 between these traits was very high. Some of the estimates had high standard errors  
397 because of the small data size. However, these results suggest that the data on



398 endocrine fertility traits measured by automatic systems is a promising tool for  
399 improving fertility, specifically when more data is available. For hyperketonemia traits,  
400 heritability estimates were quite low and there were only slight differences between the  
401 different traits studied, however dividing values into three classes instead of two  
402 seemed to work somewhat better in the current study. Based on the current study and  
403 previous studies, where higher heritabilities have been found for traits based on milk  
404 BHB than for clinical ketosis, milk BHB is a promising indicator trait for resistance to  
405 ketosis and should be studied more. It is important that this kind of data from automatic  
406 devices is made available to recording and breeding organizations in the future.

407

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#### 417 **Declaration of interest**

418 The authors declare that they have no conflict of interest.

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#### 420 **Ethics statement**

421 Herd Navigator™ management program samples and analyses milk progesterone and  
422 β-hydroxybutyrate (BHB) automatically during routine milking. No ethics statement is  
423 thus required.

424

#### 425 **Software and data repository resources**

426 No data repository resources are available for this publication.

427

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553 **Table 1** *Descriptive statistics for CFH and traditional fertility traits for dairy cows*

Trait (days)	Parity	n	Mean	SD	Min	Max
CFI	1	676	78.9	15.8	46.0	184.0
CFI	2	625	79.9	17.6	40.0	213.0
CFI	3	391	83.5	20.8	51.0	228.0
CLI	1	676	109.4	43.0	52.0	296.0
CLI	2	625	112.8	40.3	48.0	299.0
CLI	3	391	117.0	46.3	51.0	279.0
IFL	1	676	30.5	40.4	0.0	234.0
IFL	2	625	32.9	43.9	0.0	220.0
IFL	3	391	33.5	41.6	0.0	213.0
CFH	1	676	49.9	17.7	22.0	100.0
CFH	2	625	51.2	17.1	21.0	100.0
CFH	3	391	53.0	17.2	22.0	100.0

554 CFI = calving to first insemination restricted to  $\leq 230$  days; CLI = calving to last insemination restricted  
555 to  $\leq 365$  days; IFL = interval from first to last insemination; CFH = first heat identified by Herd Navigator  
556 restricted to  $\leq 100$  days

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566 **Table 2** *Descriptive statistics for CFH, C-LA and milk yield used in multivariate*  
 567 *analysis with hyperketonemia traits for first parity cows*

Trait	n	Mean	SD	Min	Max
CFH, d	763	49.3	17.5	22.0	100.0
C-LA, d	766	39.8	18.0	21.0	100.0
milk, kg	670	1774.3	328.3	485.7	2894.4

568 CFH = first heat identified by Herd Navigator restricted to  $\leq 100$  days; C-LA = commencement of luteal  
 569 activity restricted to  $\leq 100$  days; milk = milk yield 5-70 days postpartum

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589 **Table 3** *Distribution of hyperketonemia values (%) 5 to 60 days postpartum in*  
590 *different classifications of  $\beta$ -hydroxybutyrate values (BHB) and ketosis risk values for*  
591 *first parity cows*

Trait	negative	suspect	positive
ket_s1 <sup>1</sup>	640(80.6)	86(10.8)	68(8.6)
ket_s2 <sup>2</sup>	696(87.7)	30(3.8)	68(8.6)
ket_bin <sup>3</sup>	726(91.4)		68(8.6)
ket_r1 <sup>4</sup>	701(88.3)		93(11.7)
ket_r2 <sup>5</sup>	704(88.7)		90(11.3)

592 <sup>1</sup> Smoothed BHB values (<0.10, ≥0.10 & <0.20, ≥0.20).

593 <sup>2</sup> Smoothed BHB values (<0.15, ≥0.15 & < 0.20, ≥0.20).

594 <sup>3</sup> Smoothed BHB values (<0.20, ≥0.20).

595 <sup>4</sup> Ketosis risk values (<0.55, ≥0.55).

596 <sup>5</sup> Ketosis risk values (<0.60, ≥0.60).

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611 **Table 4** Heritability estimates ( $h^2 \pm$  standard error) from univariate model for  
 612 traditional fertility traits for first parity cows

Trait (days)	$h^2$
CFI	0.07±0.07
CLI	0.06±0.07
IFL	0.03±0.06

613 CFI = calving to first insemination restricted to  $\leq 230$  days; CLI = calving to last insemination restricted  
 614 to  $\leq 365$  days; IFL = interval from first to last insemination

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633 **Table 5** Heritability estimates ( $h^2 \pm$  standard error) for CFH, C-LA, milk yield and  
 634 hyperketonemia traits for first parity cows

Trait	$h^2$ , multivariate model	$h^2$ , univariate model
CFH, d	0.33±0.13	0.19±0.11
C-LA, d	0.32±0.12	0.24±0.12
milk, kg	0.30±0.13	0.29±0.13
ket_s1 <sup>1</sup>	0.10±0.07	0.09±0.07
ket_s2 <sup>1</sup>	0.08±0.07	0.07±0.07

635 CFH = first heat identified by Herd Navigator restricted to  $\leq 100$  days; C-LA = commencement of luteal  
 636 activity restricted to  $\leq 100$  days; milk = milk yield 5-70 days postpartum; ket\_s1 = smoothed BHB  
 637 values ( $<0.10$ ,  $\geq 0.10$  &  $<0.20$ ,  $\geq 0.20$ , restricted 5 to 60 days); ket\_s2 = smoothed BHB values ( $<0.15$ ,  
 638  $\geq 0.15$  &  $<0.20$ ,  $\geq 0.20$ , restricted 5 to 60 days)

639 <sup>1</sup> Either ket\_s1 or ket\_s2 were included in the multivariate model

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654 **Table 6** Heritability estimates ( $h^2 \pm$  standard error) for hyperketonemia traits recorded  
 655 from 5 to 70 days postpartum for first parity cows from univariate analysis

Trait	$h^2$ linear	$h^2$ underlying	$h^2$ logit	$h^2$ probit
ket_s1 <sup>1</sup>	0.09±0.07			
ket_s2 <sup>2</sup>	0.07±0.07			
ket_bin <sup>3</sup>	0.03±0.06	0.12	0.15	0.07
ket_r1 <sup>4</sup>	0.05±0.06	0.15	0.14	0.10
ket_r2 <sup>5</sup>	0.04±0.06	0.12	0.13	0.09

656 <sup>1</sup> Smoothed BHB values (<0.10, ≥0.10 & <0.20, ≥0.20).

657 <sup>2</sup> Smoothed BHB values (<0.15, ≥0.15 & <0.20, ≥0.20).

658 <sup>3</sup> Smoothed BHB values (<0.20, ≥0.20).

659 <sup>4</sup> Ketosis risk values (<0.55, ≥0.55).

660 <sup>5</sup> Ketosis risk values (<0.60, ≥0.60).

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675 **Table 7** Genetic (above diagonal) and phenotypic (below diagonal) correlations  
 676 among CFH, C-LA, milk yield and ket\_s1 for first parity cows - Standard errors of  
 677 estimate in parenthesis

Trait	CFH	C-LA	milk	ket_s1
CFH		0.97 (0.02)	0.52 (0.27)	-0.17 (0.42)
C-LA	0.92		0.47 (0.28)	-0.08 (0.42)
milk	0.01	0.001		0.59 (0.39)
ket_s1	0.07	0.06	0.10	

678 CFH = first heat identified by Herd Navigator restricted to  $\leq 100$  days; C-LA = commencement of luteal  
 679 activity restricted to  $\leq 100$  days; milk = milk yield 5-70 days postpartum; ket\_s1 = smoothed BHB  
 680 values ( $<0.10$ ,  $\geq 0.10$  &  $<0.20$ ,  $\geq 0.20$ , restricted 5 to 60 days)

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694 **Figure captions**

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696 **Figure 1** Milk progesterone curve of a hypothetical dairy cow, used to define  
697 endocrine fertility traits. Commencement of luteal activity (C-LA) occurs few days  
698 after the first ovulation and the first heat identified by Herd Navigator (CFH) is  
699 recorded when the concentration changes from high to low

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