The prevalence, as well as the effect of subclinical ketosis on daily milk yield, was observed using 1,299,630 test-day records collected from January 2000 to December 2005 on 73,255 Slovenian Holstein cows. Subclinical ketosis was indicated by the fat to protein ratio (F/P ratio) higher than 1.5 in cows that yielded between 33 to 50 kg of milk per day (Eicher, 2004). The ketosis index was defined in relation to the timing of subclinical ketosis detection to the subsequent measures of test-day milk yields. The effect of subclinical ketosis on test day milk yields were studied separately for each parity using the mixed model analysis. The statistical model included the fixed effect of ketosis index, calving year-month, lactation stage and random effect of animals included in the study.

The prevalence peak of subclinical ketosis occurred in the first month of lactation. Subclinical ketosis had a significant negative effect on daily milk yield. Decrease in milk yield in the amount of 4.21 kg/day; 2.73 kg/day; 2.78 kg/day; 2.83 kg/day; and 3.72 kg/day in each parity were determined within 35 days after the detection of subclinical ketosis. The decrease continued in subsequent milk controls. The research results show that test-day records could be a useful tool for early detection of subclinical ketosis.

Key words: daily milk yield, detection, subclinical ketosis, test-day records

INTRODUCTION

Ketosis is a metabolic disorder that can occur both in clinical and subclinical forms where subclinical ketosis is defined as a preclinical stage of ketosis (Shaw, 1956). Clinical ketosis most frequently occurs in susceptible high-yielding dairy cows in the first days of lactation between the 2nd and 7th week after calving as a consequence of inadequate nutrition and management (Baird, 1982; Gillund et al., 2001). Prevalence of ketosis could be influenced by breed, parity, season and herd-related factors. Dahoo and Martin, 1984 reported that lactational incidence risk of ketosis was between 1.1 and 9.2%, while Rajala-Schultz et al. (1999) determined, depending to parity, the incidence risk to be in the interval between
2.5 to 4.2%. Baird (1982), Gustafsson and Emanuelson (1996), Rajala-Schultz and Gröhn (1999), as well as Østergaard and Gröhn (1999) quoted that clinical ketosis induces economic losses to the dairy farmer through treatment costs, decreased milk production, impaired reproduction efficiency, and increased involuntary culling. Andersson (1988) pointed out that subclinical ketosis causes delayed return of reproductive functions to normal after calving, increased intervals from calving to the first and the last service, and an increased frequency of ovarian cysts. Dohoo and Martin (1984) reported that subclinical ketosis has a detrimental effect on milk yield. The farmer's economic losses and the cow's malaise could be decreased or avoided by detection of the disease before the cow develops strong clinical symptoms. Subclinical ketosis can be revealed by determining levels of plasma glucose, plasma non-esterified fatty acids (NEFA), milk or urine ketone body concentrations (Andersson, 1988). Besides the named tests, records collected for official milk recording could be used (Duffield et al., 1997; Duffield, 2004; Eicher, 2004). These records include daily milk, fat and protein production, and the fat to protein ratio (F/P ratio). Since milk fat and milk protein percentages are altered in subclinical ketosis, these parameters have been investigated for their utility in defining subclinical ketosis. Beening (1993) and Gravert (1991) indicated that the ideal range for F/P ratio is 1 - 1.25, while Duffield et al. (1997) sets 1.33 as the upper margin. Haas and Hofirek (2004) reported that a F/P ratio higher than 1.4 indicates energy deficit and, if ketone bodies are present, subclinical ketosis. Duffield (2004) and Richardt (2004) defined a 1.5 value of F/P ratio as risk level for subclinical ketosis, while Eicher (2004) beside F/P ratio also took into account daily milk production.

The purpose of this study was to determine the prevalence of subclinical ketosis as well as the effect of subclinical ketosis on daily milk yield in Slovenian Holstein cows using monthly test day records.

MATERIAL AND METHODS

Data provided by the Agricultural institute of Slovenia consisted of 1 299 630 test-day yields of milk, fat, and protein from 73 255 Slovenian Holstein cows collected from January 2000 to December 2005. Cows were reared on 5 333 farms in Slovenia. Subclinical ketosis was indicated by the F/P ratio higher than 1.5 in cows that yielded between 33 to 50 kg milk per day (Eicher, 2004). Only the first occurrence of the above defined criteria was considered in this study. The basic statistical parameters for analysed traits according to parity are presented in Table 1.

Subclinical ketosis prevalence was defined as the lactational incidence risk was calculated as the frequency of cows indicated with subclinical ketosis in the total number of cows. For evaluation of subclinical ketosis daily milk yield, monthly test day milk yields taken at approximately 30-d intervals, were used.

The following mixed model was used for analysis:

\[
y_{ijkl}=\mu+MY_i+K_j+b_1(d_{ijkl}/305)+b_2(d_{ijkl}/305)^2+b_3\ln(305/d_{ijkl})+b_4\ln^2(305/d_{ijkl})+a_k+e_{ijkl}
\]
where:

\[ y_{ijk} = \text{estimated daily milk yield in kg}; \]
\[ \mu = \text{intercept}; \]
\[ \text{MY}_i = \text{fixed effect of calving year-month } i (i = 1999-7, ..., 2005-12); \]
\[ K_j = \text{fixed effect of ketosis index } j (j = K–0, AK–1, AK–2, AK–3); \]
\[ d_{ijk} = \text{stage of lactation, days (lactation curve by Ali and Schaeffer, 1987)}; \]
\[ a_k = \text{random effect of } k \text{ animal } (k = 1, ..., 1049); \]
\[ e_{ijkl} = \text{random effect of error}. \]

Table 1. Basic statistics for analysed traits according to parity

<table>
<thead>
<tr>
<th>Trait</th>
<th>Parity 1 ( (n = 392\ 354) )</th>
<th>Parity 2 ( (n = 327\ 747) )</th>
<th>Parity 3 ( (n = 257\ 958) )</th>
<th>Parity 4 ( (n = 188\ 993) )</th>
<th>Parity 5 ( (n = 132\ 578) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMY, kg</td>
<td>( \bar{x} = 14.55 ) SD = 4.78</td>
<td>( \bar{x} = 15.95 ) SD = 5.61</td>
<td>( \bar{x} = 16.77 ) SD = 5.95</td>
<td>( \bar{x} = 16.81 ) SD = 6.01</td>
<td>( \bar{x} = 16.63 ) SD = 5.89</td>
</tr>
<tr>
<td>DFC, %</td>
<td>4.3 SD = 0.70</td>
<td>4.32 SD = 0.73</td>
<td>4.28 SD = 0.73</td>
<td>4.25 SD = 0.73</td>
<td>4.21 SD = 0.72</td>
</tr>
<tr>
<td>DPC, %</td>
<td>3.40 SD = 0.36</td>
<td>3.47 SD = 0.38</td>
<td>3.43 SD = 0.37</td>
<td>3.42 SD = 0.37</td>
<td>3.40 SD = 0.37</td>
</tr>
<tr>
<td>F/P</td>
<td>1.27 SD = 0.22</td>
<td>1.25 SD = 0.22</td>
<td>1.25 SD = 0.22</td>
<td>1.25 SD = 0.23</td>
<td>1.25 SD = 0.23</td>
</tr>
</tbody>
</table>

Only cows with detected subclinical ketosis \( (n = 1,049) \) were included in the analyses. Milk yield measured on the control day when subclinical ketosis occurred was used as the reference level. The ketosis index was defined as follows: \( K – 0 = \) test-day milk yields collected when subclinical ketosis was detected, \( AK – 1 = \) test-day milk yields collected within 35 days after the diagnosis, \( AK – 2 = \) test-day milk yields collected between 35 and 70 days after the diagnosis, \( AK – 3 = \) test-day milk yields collected between 70 and 105 days after the diagnosis. The effect of subclinical ketosis on test day milk yields were studied separately for each parity (i.e., parities 1, 2, 3, 4 and 5). The significance of the differences between the levels of ketosis index was tested by Scheffe’s method of multiple comparisons using the MIXED procedure of SAS (SAS Institute Inc., 2000).

RESULTS AND DISCUSSION

During research 1,299,630 test-day yields of milk, fat, and protein collected on 73,255 Slovenian Holstein cows were analysed to observe subclinical ketosis effects on daily milk yield. An F/P ratio higher than 1.5 in cows that yielded between 33 to 50 kg per day (Eicher, 2004) was used as an indication for subclinical ketosis. From overall test-day records, subclinical ketosis had a total incidence of 0.08% (Table 2). The highest lactational incidence risks of subclinical ketosis were determined in the fourth lactation (0.15%), while the lowest frequency was observed in heifers (0.01%). A similar trend of lactational incidence risk was obtained by Rajala et al. (1999). The distribution of subclinical ketosis...
incidence risk during lactation according to each lactation number is given in Figure 1. For distribution analysis only records of cows with detected subclinical ketosis were used. Lactation was divided into 17 stages: milk records taken within 85 days after calving were grouped by 5-d intervals, while records from 85 days and later formed one stage. Only test day milk yields until 305 days after calving were considered. The prevalence peak occurred in the interval between 10th–35th day after calving in lactations after the first one (Figure 1). Low number of first parity cows with detected subclinical ketosis (n = 23, Table 2) induced a different prevalence trend in the first lactation. Dohoo and Martin (1984) determined a prevalence peak of positive ketosis test results in the 21–25 day period postpartum, and 56% of the positive results were in the interval between 11 and 35 days.

Table 2 Lactational incidence risk of subclinical ketosis according to parity

<table>
<thead>
<tr>
<th>Cows</th>
<th>Parity 1</th>
<th>Parity 2</th>
<th>Parity 3</th>
<th>Parity 4</th>
<th>Parity 5</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>%</td>
<td>0.01</td>
<td>0.07</td>
<td>0.14</td>
<td>0.15</td>
<td>0.12</td>
<td>0.08</td>
</tr>
<tr>
<td>no.</td>
<td>23</td>
<td>231</td>
<td>362</td>
<td>277</td>
<td>156</td>
<td>1,049</td>
</tr>
</tbody>
</table>

Figure 1 Subclinical ketosis incidence risk in regard to lactational stage per parity
For the analysis of subclinical ketosis effects on daily milk yield, only 8,916 monthly test day records of 1,049 cows with detected subclinical ketosis were used. All effects taken into account (fixed effect of calving year-month; fixed effect of ketosis index; fixed effect of lactation stage; as well as random effect of animal) in the mixed model showed to be highly significant (p<0.01). A significant negative effect of subclinical ketosis on daily milk yield per each parity was determined (Table 3). Decrease in milk yield in amount of 4.21 kg/day; 2.73 kg/day; 2.78 kg/day; 2.83 kg/day; and 3.72 kg/day in each parity (i.e., parities 1, 2, 3, 4 and 5) was determined within 35 days after the detection of subclinical ketosis. The milk reducing effect continued in subsequent milk controls. Dohoo and Martin (1984) determined losses of 1.0 and 1.4 kg milk/day associated with positive reactions (+1 and +2) on subclinical ketosis test. Decreased milk production as result of subclinical ketosis was also determined by Schwalm and Schultz (1976). The reduction of milk yield within 14 days before the diagnosis of clinical ketosis in the amount of 1.7 kg/day; 2.7 kg/day; 1.5 kg/day; and 4.9 kg/day in parity 1, 2, 3, and 4+ was found by Rajala et al. (1999). The same authors determined that milk yield started to decrease 2 to 4 weeks before the diagnosis of clinical ketosis and continued to decline for a varying period after it, with the greatest loss within the 2 week after diagnosis (from 3.0 to 5.3 kg/day, according to parity). Decline of milk yield in the interval from 2 to 4 weeks before diagnosis of clinical ketosis was also reported by Lucey et al. (1986).

Table 3. Effect of subclinical ketosis on daily milk yield (kg) according to parity

<table>
<thead>
<tr>
<th>Ketosis index</th>
<th>Parity 1 (n = 200)</th>
<th>Parity 2 (n = 2,007)</th>
<th>Parity 3 (n = 3,041)</th>
<th>Parity 4 (n = 2,357)</th>
<th>Parity 5 (n = 1,311)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate</td>
<td>SE</td>
<td>Estimate</td>
<td>SE</td>
<td>Estimate</td>
</tr>
<tr>
<td>K–0</td>
<td>31.303B</td>
<td>2.096</td>
<td>28.233B</td>
<td>0.665</td>
<td>29.137B</td>
</tr>
<tr>
<td>AK–1</td>
<td>26.804B</td>
<td>1.847</td>
<td>25.519B</td>
<td>0.518</td>
<td>26.353B</td>
</tr>
<tr>
<td>AK–2</td>
<td>25.699B</td>
<td>1.372</td>
<td>24.737B</td>
<td>0.422</td>
<td>25.294B</td>
</tr>
<tr>
<td>AK–3</td>
<td>24.042B</td>
<td>1.286</td>
<td>24.686B</td>
<td>0.394</td>
<td>25.216B</td>
</tr>
</tbody>
</table>

CONCLUSION

The prevalence peak of subclinical ketosis occurred in the interval between 10th – 35th day after calving. Subclinical ketosis had significant negative effect on daily milk yield. Decrease in milk yield began within 35 days after the detection of subclinical ketosis and continued in subsequent milk controls. The research results point out that test-day records collected in regular milk recording could be a useful tool for monitoring cows’ health status particularly for the detection of metabolic disorders. By early detection and treatment of subclinical ketosis farmer’s economic losses could be decreased or completely avoided.
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DETEKCIJA SUBKLINIČKE KETOZE UPOTREBOM ZAPISA NA KONTROLNI DAN

GANTNER VESNA, POTOCNIK K I JOVANOVAC SONJA

SADRŽAJ

Za analizu prevalencije i utjecaja subkliničke ketoze na dnevnu količinu mlijeka korišteno je 1 299 630 zapisa na kontrolni dan prikupljenih u razdoblju od januara 2000. do decembra 2005. godine na ukupno 73 255 grla holstein pasmine u Sloveniji. Subklinička ketoza je indicirana odnosom između mliječne masti i bijelančevina (F/P odnos) višim od 1,5 u krava koje su imale mliječnost u intervalu od 33 do 50 kg mlijeka/dan (Eicher, 2004). Ketoza indeks definiran je u odnosu na vrijeme proteklo od detekcije subkliničke ketoze do slijedećih mjerenja na kontrolni dan. Upotrebom mješanog statističkog modela utjecaj subkliničke ketoze na dnevnu količinu mlijeka analiziran je odvojeno za svaku laktaciju (1, 2, 3, 4. i 5.). Korištenim statističkim modelom uključeni su utjecaji ketoza indeksa, godine-mjeseca telenja i stadija laktacije kao fiksni, a utjecaj životinje kao slučajni.

Maksimum prevalencije subkliničke ketoze utvrđen je u prvom mjesecu laktacije. Signifikantan negativan utjecaj subkliničke ketoze i opadanje dnevne produkcije mlijeka u iznosu od 4,21 kg/dan; 2,73 kg/dan; 2,78 kg/dan; 2,83 kg/dan; i 3,72 kg/dan u pojedinoj laktaciji utvrđen je unutar 35 dana po detekciji subkliničke ketoze. Pad proizvodnosti zabilježen je i pri slijedećim kontrolama. Rezultati provedenog istraživanja ukazuju da se zapisi na kontrolni dan mogu koristiti u cilju rane detekcije subkliničke ketoze.