The study included 4 herds of dairy cows (C, D, E, F) from Central-Eastern Poland. The objective of the study was to evaluate and compare selected haematological and biochemical indicators in the blood of dry and lactating cows from the studied region. Blood was sampled twice from the external jugular vein at two dates: 10-14 days prior to calving and after the first month of lactation. Determination of the RBC (red blood cell) count, as well as haemoglobin concentration (HGB) was made in the samples of whole blood by means of haematological MS 4-5 VET analyser. Glucose and total protein levels, as well as enzyme activities (AST, ALT, ALP and GGT) in the sera were determined with Cormay diagnostic set. Measurements of selected haematological and biochemical indicators indicated a reduced glucose level and increased activity of alkaline phosphatase in lactating cows. A particularly high activity of AST was found in the examined animals.

Key words: biochemical indicators, dairy cows, haematological indicators, serum

INTRODUCTION

The metabolic profile is a set of blood haematological, biochemical and enzymatic indicators which provide a more complete evaluation of health status of animals and, if necessary, makes it possible to undertake actions aiming at treating disorders (Kampl et al., 1991; Tornquist and Van Saun, 1999; Mordak and Nicpon, 2006; Šamanc et al., 2011).

Examination of physiological indicators is a diagnostic method for early recognition of a number of metabolic disorders of subclinical nature in dairy cows (Malecki, 2003; Stec et al., 2006). It is of great importance to diagnose as early as possible the latent diseases which may occur during the perinatal period spanning the range from the last 3 weeks of pregnancy to the first 3 weeks of lactation. Possible disorders occurring in this period influence further fertility and
health of cows and calves (Kupczynski and Chudoba-Drozdowska, 2002; Tothova et al., 2008).

Many authors have indicated the prognostic importance of determinations of, among others, glucose, total protein, haemoglobin in addition to the levels of blood morphological elements, as well as activities of liver enzymes (Roga-Franc et al., 1994, Freyer et al., 2006).

The purpose of this study was to evaluate and compare selected haematological and biochemical indicators in the blood of cows of varying physiological status raised in Central-Eastern Poland.

MATERIALS AND METHODS

Studies were conducted in cows from 4 dairy farms: C, D, E and F, located in Central-Eastern Poland. The cows were of Polish black and white Holstein-Frisian breed, aged between 3 and 6, and with average milk production of 4200 kg. The animals were clinically healthy and characterized by balanced breeding parameters. However, they differed with respect to physiological status. Over the winter period the animals were housed in barns where zootechnical parameters appropriate for the species were maintained. They received maize silage, meadow hay, barley straw and a concentrate mixture. In the summer feeding was based on pasture grazing supplemented with hay and barley straw. Feeds were offered so as to meet the animal feeding standards (INRA, 1993). Twenty two cows (11 lactating and 11 dry animals) were selected from each farm. Blood was sampled twice from the external jugular vein at two dates, that is 10-14 days prior to calving and after the first month of lactation. The RBC (red blood cell) counts and haemoglobin concentrations (HGB) were determined in whole blood samples by means of MS 4-5 VET analyser. After blood centrifugation, the following biochemical indicators were determined in the serum using the Cormay diagnostic set: glucose level, total protein level and activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma-glutamyltransferase (GGT). The results of the study were subjected to statistical analysis by means of Statistica 6.0 PL. Additionally, mean values and standard deviations were calculated. Significance of differences between mean values was checked using the Student's t-test, at significance level of p≤0.05 and p≤0.01.

RESULTS

In the present study the values of haemolytic indicators in cows stayed within the physiological limits (Winnicka, 2004). There was no statistically significant influence of physiological status on erythrocyte count and haemoglobin level in the blood of animals (Table 1).

Mean serum glucose concentration was low and fluctuated around the lower limit of normal glucose concentration in the serum of lactating cows in all the herds (2.13-2.55 mmol/L). By contrast, during the prenatal period the glucose
level in these animals was high (3.23-3.96 mmol/L). The differences observed were statistically highly significant (Table 1).

Table 1. Concentration of haematological parameters in the blood of cows during the peripartal period

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Farm</th>
<th>Time of testing</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before delivery</td>
<td>After delivery</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>Mean±SE</td>
<td>Mean±SE</td>
<td></td>
</tr>
<tr>
<td>RBC (10^{12}/L)</td>
<td>C</td>
<td>5.28±0.23</td>
<td>5.53±0.30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>5.33±0.22</td>
<td>5.33±0.17</td>
<td></td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>5.28±0.15</td>
<td>5.30±0.26</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>5.33±0.17</td>
<td>5.28±0.23</td>
<td></td>
</tr>
<tr>
<td>HGB (g/dL)</td>
<td>C</td>
<td>5.46±0.22</td>
<td>5.33±0.26</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>5.27±0.22</td>
<td>5.30±0.20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>5.26±0.22</td>
<td>5.36±0.14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>5.15±0.10</td>
<td>5.28±0.23</td>
<td></td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>C</td>
<td>3.23±0.28 B</td>
<td>2.23±0.38 A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>3.78±0.22 B</td>
<td>2.55±0.42 A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>3.65±0.50 B</td>
<td>2.13±0.10 A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>3.96±0.22 B</td>
<td>2.21±0.31 A</td>
<td></td>
</tr>
<tr>
<td>Total protein (g/L)</td>
<td>C</td>
<td>64.70±4.96 B</td>
<td>54.70±4.36 A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>62.33±5.24 b</td>
<td>53.66±4.32 a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>63.50±4.50 b</td>
<td>57.00±4.10 a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>66.83±2.92 B</td>
<td>52.50±1.87 A</td>
<td></td>
</tr>
</tbody>
</table>

AB – significant differences between groups at p ≤ 0.01
ab – significant differences between groups at p ≤ 0.05

The total protein level in dry cows, as well in cows in progressing lactation, was within the range of physiological values (Winnicka 2004) and ranged between 52.5 to 66.83 g/L (Table 1).

The level of alkaline phosphatase activity in the serum of lactating cows exceeded the upper normal limits (Winnicka, 2004). By contrast, in dry cows the activity of this enzyme assumed values within the physiological limits (61.16-81.00 U/L). An increase in serum ALP activity following calving was statistically highly significant (Table 2).

The level of both AST and ALT in the serum of the animals examined exceeded the physiological norms (Winnicka, 2004). Activity of alanine aminotransferase (298.83-400.16 U/L) was particularly high. The results showed that AST activity was higher in cows after calving (116.33-150.33 U/L). The
differences were statistically significant for herds D, E and F. Also ALT activity increased after calving on farms C and D. The reverse tendency was observed in herds E and F (Table 2).

Table 2. Enzyme activities in the blood sera of cows during the peripartal period

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Farm</th>
<th>Time of testing</th>
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<td></td>
<td></td>
<td>Before delivery</td>
<td>After delivery</td>
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<td></td>
<td></td>
<td>Mean±SE</td>
<td>Mean±SE</td>
<td></td>
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<tr>
<td>ALP (U/L)</td>
<td>C</td>
<td>71.16±10.62 A</td>
<td>119.16±7.41 B</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>81.00±11.59 A</td>
<td>122.16±5.52 B</td>
<td></td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>61.16±9.10 A</td>
<td>127.83±7.50 B</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>73.16±11.54 A</td>
<td>127.33±9.99 B</td>
<td></td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>C</td>
<td>108.83±10.61</td>
<td>116.33±14.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>107.00±8.07 A</td>
<td>150.33±16.41 B</td>
<td></td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>113.00±6.00 A</td>
<td>143.83±6.76 B</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>109.16±3.97 A</td>
<td>148.16±8.54 B</td>
<td></td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>C</td>
<td>298.83±62.66</td>
<td>305.66±27.60</td>
<td></td>
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<tr>
<td></td>
<td>D</td>
<td>346.83±45.09</td>
<td>369.5±37.52</td>
<td></td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>385.33±30.91</td>
<td>370.33±29.96</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>400.16±15.63 b</td>
<td>342.66±54.98 a</td>
<td></td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>C</td>
<td>55.16±5.60 B</td>
<td>42.33±5.68 A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>46.16±3.76</td>
<td>46.83±6.43</td>
<td></td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>45.83±7.08 b</td>
<td>37.66±3.61 a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>48.00±3.74</td>
<td>42.33±6.18</td>
<td></td>
</tr>
</tbody>
</table>

AB – significant differences between groups at $p \leq 0.01$
ab – significant differences between groups at $p \leq 0.05$

The values for GGT activity in the present study were within the normal limits (Winnicka, 2004). There was a depressed activity of this enzyme in lactating cows (37.66-46.83 U/L). The differences were statistically highly significant in herd C and statistically significant in herd E (Table 2).

DISCUSSION

Mean values of erythrocyte count and haemoglobin level are in accordance with those reported by Deptula and Dorynek (1993), as well as Saba et al. (1987). By contrast, other authors found an increased haemoglobin level in the blood of lactating cows compared with dry animals (Kupczynski and Chudoba-Drozdowska, 2002).
The physiological serum glucose level in cattle should range between 2.2 and 4.5 mmol/L (Winnicka, 2004). It is either increased or reduced in the case of many diseases (e.g. respiratory acidosis, ketosis) (Bombik et al., 2002). Blood glucose concentration is one of the biochemical indicators on the basis of which one may draw conclusions pertaining to body energy supply (Goff et al., 1991; Kupczynski and Chudoba-Drozdowska, 2002). Energy demand of the body is different in different production periods with the maximum observed in early-lactation (Kwiatkowski et al. 1989; Klebaniuk et al., 2009; Kasagi et al., 2011). In turn, energy supply should be smaller in the dry period so as to prevent an occurrence of postpartum disease symptoms (e.g. inflammation of uterine mucosa or ovarian cysts) (Kwiatkowski et al., 1987). Also other authors have recorded increased glucose levels in the blood of cows prior to calving which decreased following calving (Darul and Kruczynska, 2005; Kupczynski and Chudoba-Drozdowska, 2002). As glucose is necessary to produce lactose its lower concentration in the serum of cows following calving may result from a heavier demand of the mammary gland for this carbohydrate (Darul and Kruczynska, 2005).

Total protein content in the serum is one of the indicators of nitrogen metabolism in the body. The protein content in the serum changes depending on the amount of protein and energy supplied with the diet, as well as with the cow's age and feeding season (Dembinski et al., 1986; Kupczynski and Chudoba-Drozdowska, 2002; Kwiatkowski et al., 1989). There was no suitability of total protein level determination as one of the biochemical indicators in the detection of liver disorders (Nowakowski, 2008). According to Moskwa et al. (2001), an increased total protein concentration may result from losses in liquids, reduced hydration status of the body or impaired synthesis in the liver. The present results of determination of total protein amounts confirm the reports by other authors who indicated that the tendency towards reduction in the serum protein levels begins prior to calving and lasts up to the beginning of lactation (Kupczynski and Chudoba-Drozdowska, 2002; Mordak and Nicpon, 2006; Thothova et al., 2008). The reduction was statistically significant for farms D and E and statistically highly significant for farms C and F.

Determination of enzymatic activity (AST, ALT, ALP, GGT) in the serum is perceived by many authors as a useful tool to diagnose diseases of organs and tissues (Stec et al., 2006; Ramin et al., 2011). Attempts have been made to determine the activity of these enzymes, thus to evaluate if it is appropriate and safe to supply minerals (Hejlasz and Nicpon, 1989; Bronicki and Dembinski, 1994; Nicpon and Jonkisz, 1997; Moskwa et al. 2001; Darul and Kruczynska, 2005; Martyna et al., 2006).

According to Kuleta et al. (1988), increased activity of alkaline phosphatase may result from liver diseases. Other authors believe that it may be a result of disturbed Ca and P metabolism (e.g. calcium and phosphorus deficiencies) which is observed at the beginning of the postnatal period (Saba et al., 1999). As the results of the present study revealed that phosphorus supply to the animals was not sufficient (Górski et al. 2005), it can be inferred that the ALP level was a good diagnostic indicator in this case.
High activity of transaminases may be indicative of histopathological changes in internal organs, the liver in particular (Tyzenhauz-Malinowska and Twardowski, 1986; Bronicki and Dembinski, 1994; Saba et al., 1999; Moskwa et al. 2001; Darul and Kruczynska, 2005). According to Saba et al. (1987), high transaminase levels in cows after calving are connected with high physical effort and increased metabolism during progressing lactation. It is believed that transaminase activity during gestation which deviates from standards indicates the possibility of an occurrence of clinical symptoms of diseases of reproductive organs after calving (in particular inflammation of uterine mucosa) (Drewnowski et al., 1981). Increased AST and ALT activity may also be associated with disorders in the energy metabolism of the body, as well as stress (Krupczynski and Chudoba-Drozdowska, 2002; Darul and Kruczynska, 2005). An increased transaminase activity was also observed as a result of microelement deficiency (Martyna et al., 2006). Increased activity of liver enzymes after calving was observed in the studies by Kuleta et al. (1988), Kampl et al. (1991), Bronicki and Dembinski (1994), Kupczynski and Chudoba-Drozdowska (2002) and Kasagi et al. (2011).

Some authors believe that GGT is an enzyme which should be determined to aid detection of subclinical liver disorders. A substantial increase in the activity of this enzyme may indicate severe injury to the cell structure of hepatocytes (Bronicki and Dembinski, 1994).

Determination of metabolic profile indicators made it possible to confirm a significant influence of physiological status on glucose and total protein levels, as well as the activity of alkaline phosphatase, asparate aminotransferase, alanine aminotransferase and gamma-glutamyltransferase. Low glucose levels were found, also. Moreover, the activity of alkaline phosphatase exceeded the physiological standards for lactating cows. High activity of ALT and AST was especially prominent in the examined animals.

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PROMENE ODBRANIH HEMATOLOŠKIH I BIOHEMIJSKIH PARAMETARA U KRVI MLEČNIH KRAVA U ISTOPČNOM DELU CENTRALNE POLJSKE

GORSKI K I SABA L

SADRŽAJ

Ovo ispitivanje je obuhvatilo 4 stada mlečnih krava iz istočnog dela centralne Poljske. Osnovni cilj ispitivanja je bio procena i poređenje odabranih hematoloških i bioheminskih indikatora kod zasušenih krava i krava u laktaciji. Krv je uzorkovana iz jugularne vene i to dva puta: 10-14 dana pre teljenja i posle prvog meseca laktacije. Broj eritrocita i koncentracija hemoglobina su određivani u punoj krvi hematološkim analizatorom MS 4-5 VET. Koncentracija glukoze i ukupnih proteina, kao i aktivnost enzima (AST, ALT, ALP i GGT) su određivani Kormajevim dijagnostičkim setom. Dobijeni rezultati su ukazali na smanjen nivo glukoze i povećanu aktivnost alkalne fosfataze kod krava u laktaciji. Kod ispitivanih životinja je utvrđena visoka aktivnost enzima aspartat aminotransferaze.