

## Estimation of chosen blood diagnostic indices in imported cows and their progeny

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### ABSTRACT

The investigation was carried out on 13 pregnant Holstein-Friesian heifers imported from Germany in the second trimester of pregnancy and on 10 local cows of the same breed. Blood diagnostic indices: total leukocyte counts, erythrocyte counts, haematocrit, haemoglobin, total protein, albumin, cholesterol content, aspartate aminotransferase and alkaline phosphatase activity, albumin:globulin ratio, and lysozyme activity, were measured in the cows in the perinatal period, and in the calves at the end of the first month of life. The results of the determinations indicate that immunosuppression can be a response to the stress of many hours' transport and adaptation to new maintenance conditions. The low level of total protein in the plasma of the cows' progeny (5.13 vs 6.90 g/dl in control calves) is proof of a low concentration of immunoglobulins adsorbed from colostrum. This low level is insufficient for full protection of the calves' health and is interpreted as a consequence of the long-lasting stress in cows.

**KEY WORDS:** pregnant heifers, calves, blood indices, transport, adaptation, stress

### INTRODUCTION

The Holstein-Friesian variety is used to improve many breeds of Black-and-White cattle. Bull semen and heifers in calf are imported most often. The procedure of animal import is connected with transport lasting for many hours, followed by the animal's adaptation to new maintenance conditions. In the case of pregnant heifers, stress of a physiological nature is an additional factor. Such conditions may cause a response referred to as polyetiological stress, which is regarded as very

dangerous, especially for breeds of high milking capacity, particularly sensitive to environmental changes. Biochemical signals (the so-called stress hormones) sent by the pituitary-adrenocortical axis enable an adequate response to stress-generating factors. Excess stress acting over a long time unnaturally burdens the organism, causing a loss of physiological balance and weakening of the immune system.

Ninety pregnant heifers, which were imported from Germany to a farm located in the Żuławy region, where the object of the investigation. Compared with local cows of the same age, abortion and endometrial (47%) and mammary gland inflammation (49%) after deliveries occurred significantly more often in these animals. It was assumed that this condition might be a consequence of the stress caused by transportation and adaptation. This hypothesis was verified by a comparison of blood diagnostic indices determined in imported and local animals of the same breed. Cows and their progeny, not showing disease symptoms, were studied. This way the effects caused by the transport and adaptation stress were exposed and the influence of diseases and treatment eliminated.

#### MATERIAL AND METHODS

The investigation was carried out on 13 Holstein-Friesian pregnant heifers, which were imported from Germany to Poland in the second trimester of pregnancy and on 10 local cows of the same breed, the same age and physiological state, and on their progeny. The animals were transported from Germany in trucks for 16 h, in compliance with the conditions contained in Directive EU 91/628. The animals were kept in the conventional system both in Germany and in Poland. The nutrition of the animals before and after the transport was similar. Before the import, conserved bulky feeds (silage and hay) and an addition of concentrates were used according to DLG standards (1997). In Poland, maize silage (27% DM), hay-crop silage made from a mixture of lucerne and grasses (40% DM), meadow hay and concentrate mixture B according to INRA nutrition standards (1989) were fed. Blood was sampled from the cows twice, in the last month of pregnancy (period I) and about 1 month after calving (period II), while in calves - once, at the end of the first month of life.

Total leukocyte counts (WBC) and erythrocyte counts (RBC) were determined in whole blood using a PICOSCALE apparatus, haematocrit (Ht), using a haematocrit centrifuge, haemoglobin (Hb) concentration was measured by a standard method employing cyanmethaemoglobin. In addition, total protein and albumin concentrations, cholesterol content, as well as aspartate aminotransferase (AST) and alkaline phosphatase (AP) activity, using a biochemical analyzer (COBAS MIRA), were determined in blood plasma. The value of the albumin:globulin ratio (A/G) and lysozyme bacteriological activity, defined by the turbidimetric method

with the use of *Micrococcus lysodeikticus* as the substrate (Metzger, 1970), were determined, too.

The results of the determinations of blood indices in cows were analyzed statistically by analysis of variance according to the following model:

$$Y_{ijk} = \mu + a_i + b_{ij} + e_{ijk}$$

where:

- $\mu$  – mean
- $a_i$  – effect of the group
- $b_{ij}$  – effect of the period of investigation
- $e_{ijk}$  – error

The results of the determinations in calves were analyzed by variance analysis according to the following model:

$$Y_{ij} = \mu + a_i + e_{ij}$$

where:

- $\mu$  – mean
- $a_i$  – the effect of origin
- $e_{ij}$  – error

## RESULTS

The effect of variation of the level of blood diagnostic indices caused by the different physiological condition of the cows at subsequent times of examination and membership in the group of imported and local animals is given in Table 1. With regard to several traits, a significant influence of the period of investigation and the group was found. On the other hand, no statistically significant interaction between the action of both analyzed factors was observed, which indicates that the variations in the values of blood indices caused by pregnancy and lactation had a similar tendency of changes in both the local and imported cows.

The physiological condition of the animals was not the main subject of analysis. However, the influence of factors varying the values of blood indices in cows in the last month of pregnancy (period I) and at the beginning of lactation (period II) was taken into consideration (Table 1). The results of haematological investigations were similar in cows in periods I and II. Significant variations were noted in the level of blood plasma components. After calving (period II), there was an increase in total protein ( $P < 0.05$ ) with a simultaneous decrease in the concentration of albumin and the albumin:globulin ratio ( $P < 0.01$ ). A lower alkaline phosphatase activity (AP) and an increase in aspartate aminotransferase activity (AST) ( $P < 0.01$ ) were also found.

A complex effect of stress-generating factors is illustrated by the results presented in Table 1. In the imported cows, exposed to stress, there was a higher concentration of haemoglobin and a higher haematocrit ( $P < 0.01$ ), while the total leukocyte counts were significantly lower compared with the control group. Significant differences between the analyzed groups of cows also concerned total plas-

TABLE 1

The influence of the period of investigation and the group on the level of blood diagnostic indices in cows

Blood indices	Period of investigation		Cows	
	I	II	imported	local
Leukocytes, $10^9/l$	*7.59 ± 0.56	7.32 ± 0.50	6.16 <sup>C</sup> ± 0.41	8.88 <sup>D</sup> ± 0.44
Erythrocytes, $10^{12}/l$	5.67 ± 0.40	5.14 ± 0.25	5.62 ± 0.32	5.13 ± 0.20
Haematocrit, l/l	0.31 ± 0.009	0.30 ± 0.006	0.32 <sup>C</sup> ± 0.005	0.28 <sup>D</sup> ± 0.004
Haemoglobin, mmol/l	5.73 ± 0.20	5.62 ± 0.13	6.10 <sup>C</sup> ± 0.12	5.21 <sup>D</sup> ± 0.10
Total protein, g/dl	7.25 <sup>a</sup> ± 0.15	7.61 <sup>b</sup> ± 0.16	7.06 <sup>C</sup> ± 0.11	8.03 <sup>D</sup> ± 0.15
Albumin, g/dl	3.77 <sup>A</sup> ± 0.08	3.50 <sup>B</sup> ± 0.06	3.74 <sup>C</sup> ± 0.10	3.47 <sup>D</sup> ± 0.06
A/G	1.15 <sup>A</sup> ± 0.07	0.89 <sup>B</sup> ± 0.05	1.16 <sup>C</sup> ± 0.06	0.79 <sup>D</sup> ± 0.05
Lysozyme, % of lysis	9.74 ± 0.55	12.42 ± 1.58	11.75 ± 1.31	9.99 ± 0.68
AST, U/l	56.80 <sup>A</sup> ± 2.61	85.88 <sup>B</sup> ± 4.33	73.00 ± 4.50	68.65 ± 5.10
AP, U/l	65.14 <sup>a</sup> ± 5.43	49.76 <sup>b</sup> ± 3.92	61.88 ± 3.34	50.25 ± 7.28
Cholesterol, mmol/l	2.14 ± 0.14	2.25 ± 0.17	2.36 ± 0.10	1.92 ± 0.18

\* mean ± standard error

<sup>AB</sup> -  $P \leq 0.01$ , <sup>ab</sup> -  $P \leq 0.05$  for period of investigation

<sup>CD</sup> -  $P \leq 0.01$ , <sup>cd</sup> -  $P \leq 0.05$  for groups

TABLE 2

Means and standard errors for blood indices in calves from imported cows and local cows

Blood indices	Calves from cows	
	imported	local
Leukocytes, $10^9/l$	8.35 ± 0.67	9.60 ± 0.91
Erythrocytes, $10^{12}/l$	5.08 ± 0.17	5.01 ± 0.30
Haematocrit, l/l	0.35 ± 0.01	0.34 ± 0.01
Haemoglobin, mmol/l	5.86 ± 0.23	5.68 ± 0.18
Total protein, g/dl	5.13 <sup>A</sup> ± 0.20	6.90 <sup>B</sup> ± 0.20
Albumin, g/dl	3.21 ± 0.16	3.49 ± 0.13
A/G	1.70 <sup>A</sup> ± 0.10	1.07 <sup>B</sup> ± 0.09
Lysozyme, % of lysis	7.33 ± 1.25	10.12 ± 0.76
AST, U/l	48.60 ± 4.36	58.05 ± 5.09
AP, U/l	184.23 ± 20.3	172.50 ± 17.3

<sup>AB</sup> -  $P \leq 0.01$

ma protein, the albumin concentration and the A/G ratio. The cows exposed to stress were characterized by a lower content of total protein ( $P<0.01$ ), a higher level of albumin ( $P<0.05$ ) and a higher A/G ratio ( $P<0.01$ ).

To check whether the stress in heifers in calf might influence the physiological condition of their progeny, a comparative analysis of the values of diagnostic blood indices of the calves, in which the control group was made up of the progeny of the local cows, was carried out. The mean values of haematological and biochemical determinations of the blood of calves are presented in Table 2. Statistically significant differences were found for the total protein concentration and albumin:globulin ratio. The calves from the imported cows were characterized by a lower level of total protein in plasma ( $P<0.01$ ) and a higher value of the A/G ratio ( $P<0.01$ ).

## DISCUSSION

Blood analyses performed twice in the cows showed that the values of some plasma components change during the perinatal period. The values given in Table 1, reflecting the effect of advanced pregnancy and the initial period of lactation, correspond to other authors' observations. During pregnancy the volume of circulating blood increases, which causes increased synthesis of albumins that play a major role in maintaining fluids in blood vessels. Therefore, the total protein content decreases and the A/G ratio changes. In the last period of pregnancy, an increase in the activity of alkaline phosphatase is observed (Minakowski, 1981; Miecznikowski, 1983; Deptuła et al., 1993). The investigations concerning the effect of milk secretion, conducted in dairy cattle and beef cattle (Peterson and Waldern, 1981; Doornbal et al., 1988) indicate an increase in the total protein concentration, a decrease in the level of albumins, and an increase in the AST activity in blood serum.

A comparative analysis of diagnostic blood indices in the imported and local cows showed significant differences in the level of haematological and biochemical determinations (Table 1). A significantly lower leukocyte count was found in the cows exposed to transport and adaptation stress. Secretion of glucocorticosteroids is a physiological response to stress, which leads to an ejection of neutrophils from the bone marrow reserve and a decrease in lymphocyte counts in peripheral blood. In adult cattle, the reserve pool in the bone marrow is relatively low, therefore stress-related neutrophilia causes no increase in total leukocyte counts. Leucopenia usually occurs under such conditions, because lymphocytes constitute the largest percentage in the total leukocyte counts in cattle (Andreson, 1970; Lan et al., 1995; Cole et al., 1997; Hopster et al., 1998). A higher haematocrit and haemoglobin concentration, found in the imported cows, may also be a symptom of their response to stress. This is indicated by a highly significant positive correlation between the cortisol and Ht content found in ruminants (Lee et al., 1976; Guerrini and

Bertchinger, 1982). A lack of detailed data concerning the influence of prolonged stress on the erythropoietic system makes it difficult to interpret the results explicitly. In addition, a significant variation in the level of blood plasma components was found in the presented investigations. The cows exposed to stress were characterized by a lower concentration of total protein ( $P < 0.01$ ), a higher level of albumin ( $P < 0.05$ ), and a higher A/G ratio. A lower level of total protein in plasma, resulting from a lowered percentage of globulin fractions in imported cows (a high value of the A/G ratio) may be a consequence of a low immunoglobulin content. These proteins have the largest influence on the globulin content in blood plasma (Szutowicz, 1996). Excess corticosteroids may have lowered the immunoglobulin level. Cortisol's immunosuppressive action increases catabolism as well as inhibits immunoglobulin synthesis, especially IgG and IgA (Roth, 1985; Lasek et al., 1995; Kegley et al., 1997). Chronic overproduction of cortisol caused by stress acting over a long time considerably weakens the immune system, leading to immunosuppression and thus increasing susceptibility to diseases (Paape et al., 1981; Rubello et al., 1992; Anderson et al., 1999). Despite the lack of glyco-corticosteroid level measurements in this investigation, the findings signalize that the procedure of importing heifers near to calving can expose them to the immunosuppressive consequences of stress.

The values given in Table 2 illustrate the differences between the progeny of the imported cows and the local cows. The results of the haematological investigation carried out in the calves are in line with the range of normal values for 1-month-old dairy breed calves - Jersey and Hf (Tennant et al., 1974). The values of plasma components, such as albumin, the AP and AST activity are also close to the values reported by other authors (Roussel et al., 1982; Doornenbal et al., 1988). However, detailed comparisons are impossible due to the fact that the published data concern calves of different breeds, including beef cattle studied in different age ranges. The differences between calf groups concerned mainly the level of total protein in plasma and the albumin:globulin ratio. Calves from the imported cows were characterized by a significantly lower concentration of total protein ( $P < 0.01$ ) and a higher A/G ratio ( $P < 0.01$ ). The level of total protein is the lowest in the newborns' plasma, amounting to 4.72 g/dl on average in Hf cattle. Absorption of colostrum increases this value by about 2 g/dl and this level is stable for the next two months (Naylor et al., 1977). Total protein in the blood plasma of calves over 24 h of age is clinically useful as an indicator passive transfer of humoral immunity. The usefulness of this index in the evaluation of the calves' susceptibility to diseases in the first weeks of life is comparable with the radial immunodiffusion test (Naylor et al., 1977; Selim et al., 1995). The correlation between the measurement of the total protein content and the concentration of immunoglobulins determined by the radial immunodiffusion method was defined at the level of  $r = 0.84$  (Selim et al., 1995). Some authors (Naylor et al., 1977; Selim et al., 1995; Wittum and Perino, 1995) have indicated

that a protein concentration in blood plasma below 6g/dl is associated with an increased incidence of disease in calves in the period before weaning. In the presented investigations, a lower level of total protein was found in the plasma of the calves from the imported mothers (5.1 g/dl), while in the local calves this value was much higher and amounted to 6.9 g/dl. The low protein level and the high A/G ratio in the calves born by imported cows are a sign of a low concentration of immunoglobulins absorbed from colostrum, not sufficient for full health protection. The results obtained confirm the immunosuppressive action of stress on cow-mothers; they also point to its unfavourable consequences in the progeny.

## CONCLUSIONS

The results of haematological and biochemical investigations of blood in heifers imported in the second trimester of pregnancy indicate lower leukocyte counts, a lower concentration of total protein and a higher A/G ratio compared with local cows of the same age. The observed regularities may be the effect of immunosuppression caused by the transport and adaptation stress. The unfavourable action of stress is confirmed by a low concentration of total protein in the blood plasma of the progeny, ranging at the level indicating increased susceptibility of calves to pathogenic factors.

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## STRESZCZENIE

**Ocena wybranych wskaźników diagnostycznych krwi u krów importowanych i ich potomstwa**

Badania przeprowadzono na 13 pierwiastkach holsztyno-fryzach, które w drugim trymestrze ciąży sprowadzono z Niemiec, oraz na 10 krowach miejscowych, tej samej rasy i podobnym wieku, a także na ich potomstwie. U krów w okresie okołoporodowym, a u cieląt pod koniec pierwszego miesiąca życia, oznaczono wskaźniki diagnostyczne krwi: ogólną liczbę leukocytów, liczbę erytrocytów, hematokryt, hemoglobinę, białko całkowite, albuminy, zawartość cholesterolu, aktywność aminotransferazy asparaginianowej i fosfatazy alkalicznej, stosunek albumin do globulin oraz aktywność lizozymu. Na podstawie otrzymanych wyników stwierdzono, że odpowiedzią na stres wielogodzinnego transportu i adaptacji do nowych warunków utrzymania może być immunosupresja. Jako konsekwencję długotrwałego stresu krów przyjęto niski poziom białka całkowitego w osoczu ich potomstwa (5,13 vs 6,90 g/dl u kontrolnych), świadczący o niewielkiej ilości zaadsorbowanych z siary immunoglobulin, niewystarczających dla pełnej ochrony zdrowia cieląt.