

Hematological and biochemical blood parameters in dairy cows depending on the management system

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Monitoring the health of dairy herds is central to the assessment of animal health and welfare. The aim of this study was to analyse hematological and biochemical parameters in Holstein-Friesian dairy cows depending on the management system.

Thirty cows from the foundation stock with an average annual yield of 6500-7000 l of milk were selected for the experiment. The cows were characterized by similar yields and were in a similar lactation period (second and third lactation). The cows were divided into three groups of 10 cows each: confined to a barn (control group C), housed in a barn with free access to an outdoor run (O) and maintained on a pasture (P). Blood samples to determine hematological and biochemical parameters were collected from animals on an empty stomach before the onset of the experiment and towards the end of the pasture period. The present study showed a positive effect of pasturing dairy cows on their hematological parameters. In the group of pasture-fed cows, there were also significant ($P \leq 0.05$) differences between the results before and at the end of the trial. There was a significant ($P \leq 0.05$) increase in total white blood cell count and in basophil and neutrophil percentages. There were also significant ($P \leq 0.05$) increases in the concentration of hemoglobin (by 8%) and hematocrit (by 7%). Biochemical analysis showed slightly higher urea concentrations in the group of pastured cows when compared to the other groups. The high content of nitrogen compounds in the pasture forage could have a certain effect on hepatic nitrogen metabolism, which could increase serum concentrations of AST and ALT enzymes in cows from group P.

KEY WORDS: blood chemistry / dairy cows / housing systems / morphological parameters

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In dairy cattle farming it is critical to identify and monitor health status and disease incidence. In recent years there has been a growing concern for animal welfare, with consumers paying increasing attention to farm management conditions and procedures, especially those that may inflict pain and suffering on animals [Godyń *et al.* 2013]. The health status of animals maintained under specific conditions is one of the criteria for welfare assessment. It is important to monitor housing and feeding conditions, which translates directly into milk yield, reproductive parameters, absence of disease and mortality, mental comfort, absence of stress, good appetite, and determines the body's homeostasis or the maintenance of proper animal welfare levels. One of the most important challenges in modern herds is to maintain proper house climate, and the interrelations between air temperature and humidity are of prime importance for animal welfare and production profitability [Herbut and Angrecka 2012]. Changes taking place in the animal's body in response to external factors such as nutrition, housing system and microclimate can also affect the level of different blood parameters [Wójcik *et al.* 2004]. Testing of physiological parameters is essential to monitoring the health status of dairy herds. The most popular blood diagnostic test is the blood count determination, which includes erythrocyte, leukocyte and thrombocyte counts, as well as hemoglobin content, hematocrit value and red blood cell parameters. The content of blood components varies depending on factors that inhibit or stimulate the circulatory system. The main function of blood is to maintain the body's physiological balance, while the hematological blood indicators are the main determinant of the animals' environmental adaptation and thus their welfare [Anderson *et al.* 1999, Sattar and Mirza 2009]. Biochemical tests evaluate the body's internal condition, the function of different organs (including kidneys and the liver) and the course of metabolic changes in the body [Scamell 2006].

The aim of this study was to estimate the effect of the dairy cow housing system on basic hematological and biochemical parameters of blood.

Material and methods

The experiment was performed at the Experimental Station of the National Research Institute of Animal Production Chorzelów Ltd. on Polish Holstein-Friesian Black-and-White dairy cows (PHF-BW – 79.7%) during the grazing period between May and October. Thirty cows from the foundation stock with an average annual yield of 6500-7000 kg of milk were selected for the experiment. The cows characterized by similar yields and were in a similar lactation period (second and third lactation) and stage of lactation (120±10 days). The animals exhibited no signs of disease. The cows were randomly divided into three groups of 10 cows each.

The experiment involved three cow housing systems:

- without access to an outdoor run and pasture – the control (C);
- with access to an outdoor run – experimental group (O);
- maintained on the pasture – experimental group (P).

Cows from the control group (C) were kept on litter in a free-stall barn with separate lying areas. Cows from group O had unrestricted access to an outdoor yard, 4.15 m² per animal. Cows from group P were on pasture for 8-9 h/day between 8.00 and 16.00-17.00 h. The pasture was divided into six 0.4-ha run. Pastured cows had unlimited access to pasture waterers, to which water was delivered by water trucks. Prior to each grazing, was estimated pasture yield according to Różycki's method [Jamroz 2013]. During the evaluation, sward samples were collected for chemical analyses. Cows kept indoors (with and without outdoor access) were fed in accordance with IZ-PIB INRA standards [2009]. The diet of cows from groups C and O was based on TMR of the following nutritive value per kg d.m.: UFL – 0.80, PDIE – 91.0 g, PDIN – 99.0 g, FU – 0.72, CP – 149 g. TMR contained: maize silage – 23 kg, grass silage – 10 kg, rapeseed meal – 0.8 kg, extracted soybean meal – 1 kg, maize grain – 2 kg, concentrate mixture – 4 kg, wheat straw – 0.2 kg, fresh brewers' grains – 8 kg, limestone – 0.2 kg, and sodium bicarbonate – 0.25 kg. Pastured cows (group P) consumed approx. 50 kg·day⁻¹ of pasture sward and were supplemented with the same TMR as cows raised indoors (groups – C and O) at one-third the amount given to cows from groups C and O, which was administered twice daily. The dry matter content of pasture sward averaged 19.10%. In dry matter, crude protein accounted for an average of 16.5%, crude fibre for slightly over 20%, and sugars for approx. 22.9%. The nutritive value of pasture sward in 1 kg d.m. was as follows: UFL – 0.96, LFU – 0.97, PDIN – 104 g, PDIE – 95 g. TMR diets were formulated using INRAtion software (2006).

Blood for analysis was collected by a veterinary doctor twice: before the start of the pasture period (early May) and at the end of grazing (late September) from the jugular vein between 8.00 and 9.00 h, prior to feeding. Hematological determinations were made according to the recommendations for animal blood determination using an ADVIA 2120 automatic hematology analyser (Bayer Healthcare, Siemens) with software for blood determination in cattle. The following hematological parameters were analysed: red blood cell count (RBC), hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), white blood cell count (WBC) including eosinophils, basophils, neutrophils, lymphocytes and monocytes, and blood platelets (PLT). The results obtained were compared with the reference values for healthy cattle reported by Winnicka [2008]. Blood biochemical analysis included the determination of urea, triglycerides, total cholesterol, HDL cholesterol, glucose, total protein, ALT, AST and creatinine. These determinations were performed at the Central Laboratory of the National Research Institute of Animal Production in Aleksandrowice with the kinetic colorimetric method using kits by Pointe Scientific Polska, Ltd.

The differences among group means were examined by ANOVA and Duncan's test using Statistica 10.

Results and discussion

Hematological parameters in ruminants depend on many factors related to the animal's physiological status and management system, including housing hygiene and nutrition. Proper management conditions are essential for the organism to function normally. Hematological blood tests are primarily aimed to monitor the health status and to detect possible diseases [Brucka-Jastrzębska *et al.* 2007]. In addition, these parameters can be used to evaluate animal stress and welfare levels [Anderson *et al.* 1999].

Results of the morphological blood tests are shown in Table 1. The analyses showed that in all groups, mean results of the most important blood morphological parameters fell within the range of reference values for healthy dairy cows [Winnicka 2008].

Table 1. Hematological blood parameters in dairy cows depending on the management system

Parameter	Group without access to an outdoor run (C)		Group with access to an outdoor run (O)		Group grazing pasture (P)	
	I	II	I	II	I	II
WBC (G·l)	8.13 ^b	7.37 ^a	7.35 ^a	8.94 ^b	8.17 ^b	9.69 ^c
Eosinophil granulocytes (%)	8.5 ^b	10.8 ^c	10.4 ^c	7.6 ^a	8.6 ^b	7.6 ^a
Basophil granulocytem (%)	0.9 ^a	1.2 ^{cb}	0.8 ^a	0.9 ^b	0.7 ^a	1.0 ^b
Neutrophil granulocyte (%)	37.9 ^b	34.4 ^a	40.0 ^{bc}	41.9 ^c	34.2 ^a	39.4 ^{bc}
Lymphocyte (%)	46.0 ^b	47.6 ^b	43.2 ^a	43.8 ^a	49.8 ^c	46.9 ^b
Monocytes (%)	6.3 ^c	5.1 ^b	5.4 ^b	5.2 ^b	6.3 ^c	3.9 ^a
RBC (T·l ⁻¹)	6.62 ^a	6.29 ^a	6.69 ^{ab}	7.26 ^b	6.93 ^{ab}	7.23 ^b
HGB – hemoglobin concentration (g·dl ⁻¹)	10.22 ^a	10.71 ^{ab}	10.58 ^a	11.35 ^b	10.55 ^a	11.4 ^b
Hct – hematocrit value (l·l ⁻¹)	0.26 ^a	0.26 ^a	0.27 ^a	0.30 ^b	0.27 ^a	0.29 ^b
MCV – mean corpuscular volume (fl)	39.4 ^a	42.7 ^b	39.9 ^a	40.9 ^{ab}	39.5 ^a	40.3 ^{ab}
MCH – mean corpuscular hemoglobin (pg)	15.6 ^A	17.9 ^B	15.8 ^A	15.7 ^A	15.6 ^A	15.8 ^A
MCHC – mean corpuscular hemoglobin concentration (g·dl ⁻¹)	39.4 ^{ab}	39.3 ^{ab}	39.7 ^b	38.4 ^a	39.4 ^{ab}	39.0 ^a
PLT – platelets (G·l ⁻¹)	401 ^{ab}	472 ^b	397 ^{ab}	306 ^a	342 ^a	397 ^{ab}

^{aA...} Within rows means bearing different superscripts differ significantly at: small letters – P≤0.05; capitals – P≤0.01.

I – beginning of the experiment; II – end of the experiment.

In clinical diagnostics, information about changes in white blood cell count is of great importance. White cells are the basic cells of the immune system, which determine normal body function. In all the experimental groups, the mean white blood cell count fell within the reference range [Winnicka 2008].

At the end of the study in the group of barn-housed cows, a slight decrease in white and red cell counts and an increase in the concentration of hemoglobin were

observed in comparison to the initial determination. In the blood of cows from the group with outdoor access (O), significant ($P \leq 0.05$) increases in white cell count, hemoglobin concentration and hematocrit value were noted at the end of the study compared to the initial period. In the group of pasture-fed cows, there were also significant ($P \leq 0.05$) differences between the results before and at the end of the trial. There were significant ($P \leq 0.05$) increases in total white blood cell count and in basophil and neutrophil percentage. There were also significant ($P \leq 0.05$) increases in the concentration of hemoglobin (by 8%) and hematocrit (by 7%).

The present study showed that pasturing of cows had a beneficial effect on increasing most hematological parameters. White cell count was observed to increase significantly ($P \leq 0.05$) when compared to groups C (by 24%) and O (by 8%). The significantly higher number of white cells observed in the pastured group may suggest, among others, that the activity of the cows' immune system was enhanced, but the increase showed no evidence of pathology. Leukocyte count in cattle may also increase due to stress, including that related to blood collection [Brucka-Jastrzębska *et al.* 2007]. When compared to confined housing, pasturing and unrestricted access to outdoor runs also caused significant ($P \leq 0.05$) increases in red blood cell count and hematocrit value. Mean corpuscular hemoglobin (MCH) showed a highly significant ($P \leq 0.01$) inverse relationship, with the highest values recorded in group C.

The scientific literature contains studies concerning the effect of management system on blood parameters, but their findings are inconclusive. Research by Deptuła and Dorynek [1993] suggests that red blood cell parameters and hemoglobin concentration do not show large seasonal differences. Kumar and Pachaura [2000] found hemoglobin concentration, mean cell volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration to increase during summer, and hematocrit value to decrease during winter. Gutierrez-De La R *et al.* [1971] showed the mean corpuscular volume and the mean corpuscular hemoglobin concentration to decrease in response to high temperature, but found no changes in white and red blood cell counts, hematocrit value and hemoglobin concentration. Another study [El-Nouty *et al.* 1990] reported reductions in hemoglobin, hematocrit value, mean corpuscular volume and mean corpuscular hemoglobin concentration during the summer season, but no changes in red and white blood cell count and MCHC. In turn, Koubkova *et al.* [2002] proved that red blood cell count and hematocrit value increase in high temperature. In the present study, we observed increases in white and red blood cell counts, hemoglobin and hematocrit value in pastured cows and in cows with free-range access compared to cows housed indoors. In addition, at the end of the study, these groups (O, P) exhibited a significant increase in the value of the analysed parameters, also in relation to the period before the start of experiment. These trends are consistent with the findings of Deptuła and Dorynek [1993] and Wójcik *et al.* [2004]. A study with Charolais and Limousin beef cows [Wójcik *et al.* 2004] showed that the pasture period caused a slight increase in red blood cell count, white blood cell count, hemoglobin concentration and hematocrit value. Hematological parameters show

higher values during the stall period, when animals are confined indoors and have no access to pasture. This is probably related to feeding intensity and feed quality, which has a considerable effect on the level of hematological parameters [Pysera and Opalka 2000]. In the group with outdoor access and in the pastured group, mean corpuscular volume (MCV) was near the physiologically normal range. Red blood cell parameters are closely correlated and depend, among others, on the hemoglobin level, erythrocyte count and hematocrit value. Research shows that MCV can be used in hematological analysis of the response of cattle to thermal and humidity stress [Aengwanich *et al.* 2009]. The same study revealed that stress caused by high temperature and humidity conditions adversely affects hemoglobin synthesis, probably by reducing the available metabolites as a result of thyroid and body metabolism dysfunction. In the study under discussion, mean corpuscular hemoglobin concentration (MCHC) in all the experimental groups slightly exceeded the reference values both before and at the end of the trial. This may be due to the use of mineral mixtures containing the organic forms of elements stimulating hematogenesis. The slight increase in MCHC (by 2-3%) may also result from dehydration of the body or hemolysis of the analysed material [Brucka-Jastrzębska *et al.* 2007]. However, in the case of water-electrolyte imbalance (dehydration or overhydration), the hematocrit value is observed to increase or decrease, respectively, which was not observed in our study. The highest platelet count, still statistically insignificant, was observed in the group of barn-housed cows.

Biochemical tests are used to evaluate the body's internal condition, the function of organs (e.g. kidneys and liver), and metabolic processes in the body [Scamell 2006]. It is important to select proper biochemical parameters that determine the work of different organs or systems. In cattle the concentration of glucose, free fatty acids and beta-hydroxybutyric acid is considered an indicator of energy metabolism. The indicators of protein metabolism are urea, total protein and albumins. Liver condition is reflected in the activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyl transferase and total bilirubin concentration, while creatinine is the basic parameter reflecting kidney function [Stojević *et al.* 2005].

Most of the mean values of the biochemical parameters (Tab. 2) fell within the range of reference values [Winnicka 2008]. In our study no statistically significant differences in blood glucose and total protein contents were found in the cows, which is supported by Brzóška [2005]. After finishing the research, in the group of pastured cows there were slightly higher blood concentrations of triglycerides, HDL cholesterol, LDH and urea ($P \leq 0.05$). Urea concentration in blood and milk is one of the indicators of the nutritional status. The slightly higher blood urea concentration in the pastured compared to the other groups may be due to the fact that pasture vegetation, especially legumes, are rich in protein and poor in energy, which may increase the amount of nitrogen in the diet, and thus in the blood [Pelletier *et al.* 1985]. This is confirmed by the level of total protein in the blood of experimental animals, which was relatively high, but within normal limits. The urea content of milk indicates that feed protein is efficiently used by rumen microflora. Due to the pasture feeding in the summer period, cows ingest relatively

Table 2. Biochemical blood parameters of dairy cows depending on the management system

Parametr	Group without access to an outdoor run (C)		Group with access to an outdoor run (O)		Grazing pasture (P)	
	I	II	I	II	I	II
	Glucose (mg·dl ⁻¹)	68.15	70.86	67.41	69.28	67.98
Total protein (g·dl ⁻¹)	8.26	7.86	8.13	7.62	8.48	7.96
Triglycerides (mg·dl ⁻¹)	8.98 ^a	9.66 ^a	9.26 ^a	9.56 ^a	9.18 ^a	10.19 ^b
Total cholesterol (mg·dl ⁻¹)	184.6	179.0	189.1	180.8	181.6	174.6
HDL cholesterol (mg·dl ⁻¹)	95.1 ^a	95.7 ^a	93.8 ^a	94.3 ^a	91.1 ^a	103.9 ^b
AST – aspartate aminotransferase (IU·l ⁻¹)	62.5 ^a	59.1 ^a	63.8 ^a	69.4 ^b	61.1 ^a	73.8 ^b
ALT – alanine aminotransferase (IU·l ⁻¹)	22.9 ^a	21.4 ^a	23.3 ^a	25.4 ^b	23.5 ^a	27.1 ^b
LDH – lactate dehydrogenase (IU·l ⁻¹)	740.3 ^a	731.2 ^a	752.1 ^a	771.2 ^a	767.9 ^a	865.8 ^b
Creatinine (mg·dl ⁻¹)	0.90	0.80	0.95	1.01	0.85	0.89
Urea (mg·dl ⁻¹)	23.79 ^a	24.42 ^a	22.37 ^a	23.74 ^a	25.12 ^a	29.16 ^b

^{ab}–Within rows means bearing different superscripts differ significantly at P≤0.05.

I – beginning of the experiment; II – end of the experiment.

high amounts of dry matter with a high content of protein, which is rapidly degraded to ammonia in the rumen [Brzóska 2006]. The high content of nitrogen compounds in pasture forage may have a certain influence on hepatic nitrogen metabolism, which may be manifested by higher serum activity of AST and ALT enzymes in cows from group P. Similar reactions were observed by Iwańska *et al.* [1999]. The concentration of these enzymes can be also influenced by ambient temperature. Marai *et al.* [1995, 1997] found aminotransferase activity to increase during the summer in response to high temperature. The lower blood cholesterol content in cows from the pastured group is confirmed by El-Masry and Marai [1991] and Goswami *et al.* [2000], who demonstrated lower cholesterol concentrations in the plasma of cows during the summer. Biochemical parameters in groups C and O were similar, except for differences in the concentration of aminotransferases (AST and ALT) (P≤0.05).

The results of milk yield and cytological milk quality which come from this experiment were the subject of other publications [Radkowska 2012a, 2012b]. The research showed that in cows with free access to an outdoor run (O) or maintained on pasture (P) there is an insignificant decrease of milk yield, whereas their milk is characterized by a more beneficial chemical composition and cytological quality [Radkowska 2012a]. Milk from cows confined to a barn (C) had significantly higher (P≤0.05) somatic cell counts. In this group also a significantly higher (P≤0.05) frequency of *mastitis* or limb diseases could be observed [Radkowska 2012b]. Significantly lower somatic cell counts in milk of cows (P) and (O) may be evidence for the beneficial influence of these housing systems on dairy cow health [Radkowska 2012a].

The results of our study point to a beneficial effect of the pasture management system and outdoor access on the health of dairy cows. In the group of pastured cows, the most important hematological parameters increased in relation to the other experimental groups and compared to the parameters determined in the blood collected at the start of the trial, i.e. before the start of grazing. Biochemical tests showed slightly higher serum concentrations of urea and AST and ALT enzymes in the pastured compared to the other cows. The pastured group was characterized by the lowest plasma cholesterol concentration.

It can therefore be concluded that pasture management has a positive effect on the basic hematological parameters, and thus on the welfare of dairy cows. Where cows cannot use pastures, it is advisable to provide them with access to outdoor runs.

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