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Relationship between environmental conditions and physiological indicators of horses' welfare

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Summary

The aim of this study was to assess the relationship between living conditions (microclimate, aerial contaminations, box dimension, access to the paddock/pasture) of horses kept in 3 stables and physiological indicators (protein and its fractions in serum, haptoglobin).

The study was carried out for 4 months during autumn and winter in 3 stables. Blood samples were collected from 10 clinically healthy adult horses from each stable (30 in total) in 4 terms – every 4 weeks. Environmental conditions were evaluated according to zoohygiene methodology and current law regulation. In the blood serum, the analyzed parameters were: total protein (g/L) – with the biuret test, the contribution of individual serum protein fractions (%) (albumin and α1-, α2-, β- and γ-globulin) – set to serum protein electrophoresis on agarose gels in an SAS-MX electrophoresis chamber; haptoglobin concentration (g/L) – immunoturbidimetric test.

Living conditions meeting the requirements of welfare were provided in stable 3 in terms of both microclimatic parameters as well as the dimensions of the boxes and access to paddock and pasture. Slightly worse conditions were recorded in stable 2, while stable 1 was characterized by not only the worst microclimatic parameters, but also the surface of the boxes was too small. The different living conditions of horses in different stables were reflected in the diverse values of the blood indicators. Although the obtained ratios of protein indicators corresponded to the values of normal adult healthy horses, it was demonstrated that in the serum of horses in stables 1 and 2, as compared to the stable 3, a significantly lower fraction of albumin and higher α2-globulin and a higher concentration of Hp was found – suggesting the effect of different living conditions on organism homeostasis indicators. It may be stated that living conditions significantly influence the physiological condition of the horse.

Keywords: horse welfare, microclimate, aerial contamination, protein

Ensuring the welfare of animals is the responsibility of livestock owners. Of the many interpretations of the term welfare proposed in the literature, the definition presented by Wiepkema and Koolhaas (27) seems to accurately reflect the problem, while also indicating its complexity. According to these authors, welfare occurs when an animal, from both ethological and physiological point of view, is in harmony with the surrounding environment and is able to adapt appropriately to changes in the environment. The welfare concept thus involves maintaining a wide range of homeostasis of the organism. The need to take into account a great number of factors in the assessment of animal welfare has led to a long-lasting debate on what is the best way to assess the 'good status' of the organism (14).

The current animal welfare assessment methods are based on parameters concerning both the living environment (i.e. the living conditions of the animals) and the animal itself (i.e. the behavioral and physiological reaction of the organism to the living conditions) (14). The mentioned authors emphasize the importance of taking into account the living conditions of the animals. For example, inappropriate microclimatic conditions and defective functional solutions in buildings are common causes of poor welfare, which is manifested, among others, by changes in behavioral and physiological indicators. Among the many available physiological indicators, the suitability of the parameters of protein metabolism in acute phase proteins has been suggested (15, 19). The selection of methods for the assessment of animal welfare depends on many circumstances, such as the purpose of the assessment, its feasibility, and cost. It is important, however, that regardless of whether the selected parameters are

related to the living conditions of animals, or to the animals themselves, it should be possible to draw the same conclusions about the level of welfare. The aim of this study was to assess the relationship between living conditions (microclimate, aerial contaminations, box dimension, access to the paddock/pasture) of horses kept in 3 stables, and physiological indicators (protein and its fractions in serum, haptoglobin), which reflect the physiological response of horses to these conditions.

Material and methods

This study involving animals was approved by the Local Ethics Committee for Animal Experimentation at the University of Warmia and Mazury in Olsztyn (December 21, 2005, No 60/N)

Buildings. The study was carried out for 4 months in autumn and winter (November to February) in 3 stables located in the central part of Greater Poland. These facilities varied in size, number and surface of the boxes.

Animals. The investigation included horses which were all comparable in terms of breed (Wielkoposka and Polish Warmblood Horse), age and gender. All of the horses were used for non-competitive recreational riding. Horses from each stable spent approximately 2 hours outside the stables for their daily training; however, horses from stable 3 also had access to paddocks where they additionally spent approximately 6 h/day.

Stable 1. Stable 1 was a building with an attic, without access to a paddock. The usable area was 616.8 m^2 with a cubic capacity of 2,035 m^3. Forty-seven boxes for horses, each with an area of 7.8 m^2 were located on both sides of the corridor. Fifty single-glazed windows were placed in the side walls of the building at a height of 1.85 m. The windows were permanently closed, without the possibility of opening them. In each wall of a box, at a height of 2.0 m, air supply inlets with dimensions of 14.14 cm (in cold seasons plugged with straw) were placed, but there were no exhaust air outlets.

Stable 2. Stable 2 was also a building with an attic, without access to paddocks. The usable area was 413.2 m^2 and the capacity was 1,467 m^3. There was a central aisle with 26 boxes placed on both sides, each with an area of 11.0 m^2. Twenty-six single-glazed windows were placed in the side walls of the building at a height of 2.0 m. Twenty-two air inlets measuring 25.25 cm were placed in the side walls at a height of 3.0 m, but there were no exhaust air outlets.

Stable 3. Stable 3 was a building without an attic, with access to 7 grass-sand paddocks, with a total area of 4.5 ha. The usable area was 223 m^2 and the capacity was 1,228 m^3. Twelve boxes, each with an area of 12.6 m^2, were placed on both sides of the central aisle. Twelve double-glazed windows were placed in the side walls of the stable at a height of 1.9 m. Air exchange was provided by an efficient natural ventilation system assisted by Chanard air outlets.

Microclimate and Aerial Contaminants. Throughout the entire period of study, continuous measurements of air temperature and humidity in the stables were conducted using electronic thermo-hygrometers LB-520 (LAB-EL, Poland), which registered specified indicators at 2-hour intervals. Recorders were placed inside each stable in fixed points.

The daylight supply was evaluated based on the window – floor (W : F) ratio (the ratio of the glazed surface of the windows to the floor surface) and was calculated based on measurements by a lux meter (SONOPAN, Poland), daylight coefficient (DC). Artificial illumination was assessed based on the number of bulbs and recalculating its power per 1 m^2 of a stable.

Once a week, instantaneous measurements of airflow rate, cooling rate, microbiological contamination, dust pollution and ammonia and carbon dioxide concentration were measured three times during a day in the stables. Measurements and air sampling collections were performed at several fixed points at a height of 1.5 m from the floor. Cooling rate and air flow rate were measured by a Hill dry kata-thermometer (TOGO, Poland). Microbiological air samples were collected by the collision method using the air IDEAL sampler (bioMérieux Corp., France) at a flow rate of 10 l/min. Mesophilic bacteria were cultured on commercial tryptic soy agar with casein-peptone and soy meal-peptone (TSA, Merck Corp., Germany). Airborne fungi were collected on commercial Sabouraud medium (Merck Corp., Germany) and incubated at 25°C for about one week. The cultures were incubated at 35°C for 24 hours. The number of colonies on Petri dishes was determined using an automated colony counter (Schuett colonyQuant, Schuett-Biotec Corp., Germany). The results were corrected using Feller's conversion formula and microorganism counts were expressed in terms of colony-forming units per m^3 of air (cfu/m^3). The total dust concentration in the air was determined by DustScan Model 3020 gravimetric apparatus (Rupprecht&Patashnick Co., USA). NH_3 and CO_2 concentration measurements were performed with indicator tubes (Dräger Tubes Measurement System, Germany).

Blood Indicators. Blood samples for the study were collected from the external jugular vein from 10 clinically healthy adult horses from each stable (30 in total) in 4 terms: every 4 weeks during the period from November to February. The samples were collected in the morning before the feeding.

In the blood serum, the analyzed parameters were: total protein (g/L) – with the biuret test using the Cobas Integra 800 analyzer (Roche Diagnostics, Switzerland), the contribution of individual serum protein fractions (%) (albumin and α_1-, α_2-, β- and γ-globulin) – set to serum protein electrophoresis on agarose gels in an SAS-MX electrophoresis chamber; haptoglobin concentration (g/L) – immunoturbidimetric test (Roche Diagnostics, Switzerland) using the Cobas Integra 800 analyzer.

Statistical Analysis. Statistical analysis (Statistica 8.0, StatSoft Inc., OK) included characteristics of the studied traits (mean ± SD) and determination of the significance level of differences between mean values from experimental groups using a single-factor analysis of variance in an orthogonal design and the new multiple range test. The normal distribution of the data was checked by the Kolmogorov-Smirnov test.

Results and discussion

Illumination Conditions. The data presented in Tab. 1 shows that the illumination parameters in stables 2 and 3 were similar. The daylight coefficient (DC) in these buildings differed by 0.23%, the ratio of the window area-to-floor area (W : F) ranged from 1 : 12 to 1 : 11 and artificial lighting was 3.56 W/m^2 and 5.98 W/m^2, respectively. However, in stable 1 the obtained DC (0.35%) and artificial lighting (0.95 W/m^2) were the lowest and the windows-to-floor area ratio (1 : 26) was unfavorable.

Tab. 1. Illumination conditions in stables

Specification	Stable		
	1	2	3
Daylight coefficient DC (%)	0.35	0.89	1.12
Ratio of window area-to-floor area (W : F)	1 : 26	1 : 12	1 : 11
Artificial lighting (W/m^2)	0.95	3.56	5.98

Microclimatic Parameters. The values of microclimatic parameters shown in Tab. 2 differed between the stables, especially stable 3 from objects 1 and 2. This applied to both the entire period and the subsequent months of research. The mean air temperature through-

Tab. 2. Microclimatic parameters in stables in the following months and throughout the entire study period (mean ± SD)

Indicators	Stable	Month*				Entire period**
		November	December	January	February	
Temperature [°C]	1	^{A}8.74 ± 1.08	^{A}8.59 ± 1.89	^{A}7.01 ± 1.12	^{A}6.75 ± 1.22	^{X}7.79 ± 2.15
	2	^{B}7.49 ± 1.67	^{A}8.14 ± 1.67	^{A}7.25 ± 0.78	^{A}7.09 ± 1.29	^{X}7.51 ± 1.92
	3	^{B}6.97 ± 1.23	^{B}5.26 ± 2.21	^{B}4.84 ± 1.52	^{B}3.14 ± 1.24	^{Y}5.09 ± 2.11
Relative humidity [%]	1	^{a}89.31 ± 4.42	^{Aa}93.61 ± 2.81	^{A}92.20 ± 2.53	^{A}94.04 ± 2.78	^{X}92.26 ± 3.41
	2	^{b}86.39 ± 5.64	^{Ab}90.71 ± 4.42	^{Aa}89.36 ± 2.89	^{B}87.91 ± 5.52	^{Y}88.59 ± 4.57
	3	87.01 ± 7.03	^{B}87.13 ± 8.08	^{Bb}87.81 ± 4.12	^{C}82.10 ± 7.53	^{Z}85.14 ± 7.83
Air flow rate [m/s]	1	0.24 ± 0.36	^{B}0.25 ± 0.21	^{B}0.15 ± 0.10	^{A}0.21 ± 0.14	^{Y}0.21 ± 0.11
	2	^{a}0.28 ± 0.21	^{A}0.40 ± 0.34	^{A}0.22 ± 0.12	^{A}0.22 ± 0.26	^{X}0.28 ± 0.21
	3	^{b}0.14 ± 0.10	^{B}0.15 ± 0.08	^{B}0.14 ± 0.12	^{B}0.09 ± 0.03	^{Z}0.13 ± 0.06
Cooling power [mW/cm^2]	1	45.69 ± 11.67	^{B}47.53 ± 8.22	45.59 ± 5.28	46.55 ± 9.04	^{X}46.42 ± 8.52
	2	^{a}48.04 ± 9.78	^{A}51.61 ± 10.63	46.25 ± 8.38	44.74 ± 7.48	^{X}47.68 ± 10.64
	3	^{b}42.44 ± 7.81	^{B}46.07 ± 7.20	45.29 ± 7.41	44.22 ± 6.41	^{Y}44.52 ± 6.72

Explanations: *Letters A, B and C indicate P values < 0.01 in columns (stables in the following months); *Letters a and b indicate P values < 0.05 in columns (stables in the following months); **Letters X, Y and Z indicate P values < 0.01 in column (stables, the entire period)

Tab. 3. Aerial contamination indicators in stables in the following months and throughout the entire study period (mean ± SD)

Indicators	Stable	Month*				Entire period**
		November	December	January	February	
Total bacteria [$\log_{10}$ cfu/m^3]	1	^{A}6.16 ± 1.46	^{A}6.02 ± 1.24	^{A}6.07 ± 1.46	^{A}6.01 ± 1.40	^{X}6.07 ± 1.43
	2	^{B}6.03 ± 1.65	^{B}5.85 ± 1.35	6.01 ± 1.68	^{B}5.88 ± 1.47	^{Y}5.95 ± 1.53
	3	^{C}5.91 ± 1.58	^{C}5.31 ± 1.11	^{B}5.64 ± 1.60	^{C}5.62 ± 1.50	^{Z}5.65 ± 1.50
Total fungi [$\log_{10}$ cfu/m^3]	1	^{A}5.59 ± 1.24	^{A}5.57 ± 1.34	^{A}5.68 ± 1.38	^{A}5.69 ± 1.41	^{X}5.64 ± 1.43
	2	5.49 ± 1.24	^{Ba}5.51 ± 1.00	^{B}5.53 ± 1.18	^{B}5.44 ± 1.21	^{Y}5.50 ± 1.39
	3	^{B}5.42 ± 1.23	^{Bb}5.38 ± 1.10	^{C}5.32 ± 1.04	^{C}5.21 ± 0.91	^{Z}5.35 ± 1.26
Total dust [mg/m^3]	1	^{A}7.69 ± 5.67	^{A}6.36 ± 5.61	^{Ba}3.64 ± 1.92	^{Ab}4.74 ± 2.91	^{Xx}5.71 ± 4.22
	2	^{B}2.75 ± 1.41	^{B}2.75 ± 1.38	^{A}5.72 ± 3.89	^{a}6.69 ± 4.36	^{Xy}4.48 ± 3.52
	3	^{B}1.75 ± 0.59	^{B}1.71 ± 0.67	^{Bb}2.19 ± 1.58	^{B}2.69 ± 2.11	^{Y}2.05 ± 1.42
NH_3 [ppm]	1	^{A}19.33 ± 9.47	^{A}18.03 ± 8.89	^{A}19.56 ± 8.81	^{A}22.56 ± 8.22	^{X}19.88 ± 8.89
	2	^{B}13.35 ± 5.91	^{B}12.82 ± 5.62	^{B}12.49 ± 5.67	^{B}11.79 ± 5.68	^{Y}12.62 ± 5.64
	3	^{C}3.01 ± 0.83	^{C}3.00 ± 0.78	^{C}3.08 ± 0.78	^{C}2.93 ± 0.92	^{Z}3.01 ± 0.76
CO_2 [ppm]	1	^{Aa}3389.1 ± 1020.4	^{A}2444.3 ± 1280.3	^{A}3055.6 ± 1540.2	^{A}3323.3 ± 1890.2	^{X}3051.1 ± 1200.4
	2	^{Ab}2819.2 ± 1420.3	^{A}2722.2 ± 1620	^{A}2638.9 ± 1980.1	^{B}2736.1 ± 1850.3	^{Y}2728.7 ± 1970.3
	3	^{B}1806.3 ± 1310.4	^{B}1847.2 ± 1350.2	^{B}1805.6 ± 1021.5	^{C}1625.0 ± 980.1	^{Z}1770.8 ± 1240.4

Explanations: *Letters A, B and C indicate P values < 0.01 in columns (stables in the following months); *Letters a and b indicate P values < 0.05 in columns (stables in the following months); **Letters X, Y and Z indicate P values < 0.01 in column (stables, the entire period); **Letters x and y indicate P values < 0.05 in column (stables, the entire period)

out the study period in stable 3 was lower ($P \leq 0.01$) than in stables 1 and 2 (respectively: 5.09°C, 7.79°C and 7.51°C). The highest average relative air humidity was found in stable 1 (92.26%), the lowest was in stable 3 (85.14%), while stable 2 was between these values (88.59%). These values differed significantly ($P \leq 0.01$). Significant differences ($P \leq 0.01$) were also demonstrated in the case of air flow rate. The lowest value of this parameter was found in stable 3 (0.13 m/s), higher in stable 1 (0.21 m/s) and the highest in stable 2 (0.28 m/s). The cooling values in stable 1 and 2 (46.42 mW/cm^2 and 47.68 mW/cm^2, respectively) were significantly higher ($P \leq 0.01$) compared to the cooling in stable 3 (44.52 mW/cm^2).

Aerial Contaminations. The aerial contamination in the stables is presented in Tab. 3. The average total bacterial concentrations of each stable during the study were significantly different ($P \leq 0.01$). The highest bacterial count (exceeding 6 $\log_{10}$ cfu/m^3) was observed in stable 1, the lowest was in stable 3 and the difference was about 0.4 $\log_{10}$ cfu/m^3. A similar trend was observed for the total fungi counts. In stable 1, the level of fungi concentration was the highest (5.64 $\log_{10}$ cfu/m^3), whereas in stable 3, the fungi count was about 0.3 $\log_{10}$ cfu/m^3 lower than in stable 1. The differences between each stable were statistically confirmed at $P \leq 0.01$. The aerial bacteria and fungi counts in particular months of the study revealed the same trend.

The concentration of dust in the air throughout the study period was significantly higher ($P \leq 0.01$) in stables 1 and 2 (5.71 mg/m^3 and 4.48 mg/m^3, respectively) than in stable 3 (2.05 mg/m^3). A similar tendency was observed in particular months of the study.

The ammonia and carbon dioxide concentrations also differed statistically ($P \leq 0.01$) between the stables. The lowest concentration of gases was found in the air in stable 3 (NH_3 – 3.01 ppm, CO_2 – 1770.8 ppm), while in stable 1 (NH_3 – 19.88 ppm, CO_2 – 3051.1ppm) and in stable 2 (NH_3 – 12.62 ppm, CO_2 – 2728.7 ppm) the values were significantly higher.

Protein Indicators. The data in Tab. 4 show that, for the entire period, the mean values of the protein metabolism in the blood serum of horses differed between the stables, especially stable 3 from stables 1 and 2. Horses from stable 3 had a higher ($P \leq 0.01$) total protein concentration (69.2 g/L) compared to the values in stables 1 and 2 (respectively 65.8 g/L and 65.0 g/L). The fraction of albumin in stable 3 (53.48%) was also significantly higher than in stable 1 and 2 (50.58% and 50.53%). The fraction of α_1- and α_2-globulin was higher ($P \leq 0.05$) in stable 1 (respectively: 5.06% and 8.45%) than in stable 3 (4.49% and 7.87%), with intermediate

Tab. 4. Protein indicators in the following blood sampling terms and throughout the entire period (mean ± SD)

Blood indicators	Stable	The blood collection term*				Entire period**
		November	December	January	February	
Total protein [g/L]	1	67.50 ± 4.90	^{B}59.50 ± 6.90	68.50 ± 4.90	68.00 ± 3.20	^{Y}65.80 ± 6.20
	2	^{b}65.80 ± 5.10	^{b}64.40 ± 6.70	65.20 ± 6.30	^{b}64.70 ± 5.60	^{Y}65.00 ± 5.50
	3	^{a}69.10 ± 3.80	^{Aa}70.50 ± 5.70	68.10 ± 4.90	^{a}69.10 ± 2.90	^{X}69.20 ± 4.80
Albumin [%]	1	^{b}53.37 ± 3.57	^{b}48.82 ± 4.20	51.29 ± 4.36	^{b}48.83 ± 4.20	^{Y}50.58 ± 4.60
	2	^{b}53.78 ± 3.86	^{b}48.87 ± 3.54	50.48 ± 5.10	^{b}48.87 ± 3.54	^{Y}50.53 ± 4.43
	3	^{a}56.31 ± 4.50	^{a}53.26 ± 2.92	51.86 ± 4.78	^{a}52.91 ± 2.92	^{X}53.48 ± 4.64
α1-globulin [%]	1	^{Aa}4.93 ± 0.57	4.83 ± 1.13	5.22 ± 0.62	^{a}5.28± 0.39	^{x}5.06 ± 0.71
	2	^{b}4.37 ± 0.69	5.13 ± 0.72	4.77 ± 0.62	5.13± 0.72	4.85 ± 0.80
	3	^{Bc}3.71 ± 0.91	4.85 ± 0.95	4.72 ± 0.58	^{b}4.67± 0.60	^{y}4.49 ± 1.24
α2-globulin [%]	1	8.01 ± 1.06	8.79 ± 1.10	8.21 ± 1.08	^{a}8.79 ± 1.10	^{x}8.45 ± 1.38
	2	7.42 ± 1.27	8.54 ± 1.04	8.25 ± 0.65	8.36 ± 0.83	8.14 ± 1.26
	3	7.48 ± 0.89	8.43 ± 1.71	7.85 ± 1.21	^{b}7.73 ± 1.09	^{y}7.87 ± 1.32
β-globulin [%]	1	^{b}22.63 ± 2.91	23.23 ± 3.52	23.84 ± 3.31	23.23 ± 3.52	^{X}23.23 ± 3.15
	2	^{a}24.14 ± 2.39	22.14 ± 2.36	22.94 ± 2.70	24.62 ± 2.53	^{X}23.46 ± 2.68
	3	^{b}22.74 ± 2.66	22.10 ± 2.28	21.70 ± 3.30	22.10 ± 2.82	^{Y}22.16 ± 3.13
γ-globulin [%]	1	12.72 ± 1.13	^{b}11.83 ± 2.34	^{B}10.79 ± 1.82	^{B}10.20 ± 1.75	^{y}11.39 ± 2.17
	2	^{a}13.57 ± 2.30	12.14 ± 1.36	^{B}10.95 ± 1.33	^{b}10.88 ± 1.48	^{y}11.88 ± 2.65
	3	^{b}12.07 ± 2.52	^{a}12.43 ± 1.67	^{A}14.41 ± 2.29	^{Aa}12.42 ± 2.89	^{x}12.83 ± 2.35
Haptoglobin [g/L]	1	0.12 ± 0.05	0.14 ± 0.05	^{a}0.21 ± 0.09	0.20 ± 0.08	^{X}0.17 ± 0.07
	2	0.10 ± 0.05	0.16 ± 0.05	^{b}0.13 ± 0.08	0.13 ± 0.05	^{Y}0.13 ± 0.06
	3	0.09 ± 0.04	0.14 ± 0.05	^{b}0.13 ± 0.05	0.12 ± 0.02	^{Z}0.12 ± 0.03

Explanations: *Letters A and B indicate P values < 0.01 in columns (stables in the following months); *Letters a, b and c indicate P values < 0.05 in columns (stables in the following months); **Letters X, Y and Z indicate P values < 0.01 in column (stables, the entire period); **Letters x and y indicate P values < 0.05 in column (stables, the entire period)

values of these fractions in stable 2 (4.85% and 8.14%). Values of the β-globulin fraction in stable 1 and 2 were similar (23.23% and 23.46%) and significantly higher ($P \leq 0.01$) compared to stable 3 (22.16%). The values of the γ-globulin fraction found in stables 1 and 2 were similar (11.39% and 11.88%) and were significantly lower ($P \leq 0.05$) than in the serum of horses in stable 3 (12.83%). Horses from stable 1 had the highest concentration of haptoglobin (0.17 g/L) than the horses from stable 2 (0.13 g/L) and the lowest concentration was noted from horses in stable 3 (0.12 g/L). The significance of differences between the obtained values was statistically high. The data presented in Tab. 4 also shows that the differences in the parameters between the stables in each of the following periods of blood collection were generally similar to the results reported for the entire study period.

The obtained data characterizing illumination conditions (Tab. 1) showed that in stables 2 and 3, the parameters W: F and DC and the power of artificial light were consistent with the recommendations of Fiedorowicz (7) and Bombik et al. (1). However, the illumination conditions in stable 1 did not meet the required standards. Although an appropriate level of visible light in the stables is essential for normal growth and development and proper physical and mental condition of the horses, the results of the authors own research, as well as other authors (1, 18), have shown that the stables did not always meet the requirements in this regard.

In analyzing the obtained results of temperature, relative humidity and airflow rate, the parameters determining the thermal comfort necessary for the good welfare, it should be noted that the mean temperature in stable 3 over the whole period of study (5.09°C) was lower than in stables 1 and 2 and the relative humidity and air flow rate were also lower in stable 3. Considering that horses tolerate lower ambient temperatures if they are not exposed to excessive humidity and air flow rate (8), it can be stated that the thermal conditions in the stable 3 were more favorable than in stables 1 and 2. This was confirmed by the cooling power values, which in a stable 3 were significantly lower ($P \leq 0.01$) and were within the recommended range of 29-45 mW/cm^2 (8). The temperature and air flow rate data in Tab. 2 did not differ significantly from the obtained results by other authors (1, 8, 12, 17). However, in the present study, a higher relative air humidity (especially in stable 1) was demonstrated and the obtained values in this stable were similar to those reported by Kwiatkowska-Stenzel et al. (18).

High dust, microorganisms and gas pollution in the stable air is a common cause of respiratory diseases of horses, especially in the absence of the use of a paddock or pasture. Respiratory diseases of horses directly affect their sport efficiency and, in the long term, affect their welfare (26).

Data concerning the concentration of bacteria, fungi, dust and ammonia in the air (Tab. 3) indicate the different levels of these pollutants in different stables: the highest in stable 1, lower in stable 2 and the lowest in stable 3. The results of several studies demonstrate the differences in the concentrations of airborne microorganisms in stables during the winter season (5, 6, 12, 29). The lowest average microbiological air contamination occurred in a study conducted by Houben (12) in stables with natural ventilation and straw bedding. In that study, the concentration of bacteria ranged from 2 $\log_{10}$ cfu/m^3 to 4 $\log_{10}$ cfu/m^3 and the average value was 3.46 $\log_{10}$ cfu/m^3. The count of fungi ranged between 3 $\log_{10}$ cfu/m^3 and 4 $\log_{10}$ cfu/m^3 and the mean was 3.53 $\log_{10}$ cfu/m^3. Witkowska et al. (29) found a level of aerial bacteria (4 $\log_{10}$ cfu/m^3) and fungi (3-4 $\log_{10}$ cfu/m^3) in a stable, which was lower than in the current study. Higher concentrations of airborne bacteria and fungi were noted by Elfman et al. (6) in January in the Swedish climate. In their study, the levels of bacteria colony units exceeded 6 $\log_{10}$ cfu/m^3 (the range was 6.3-6.7 $\log_{10}$ cfu/m^3) and fungi colonies exceeded 1 log10 cfu/m^3. The results of our study conducted in the winter season demonstrate similar values, although compared to the above study greater aerial contamination was observed in stable 1 and lower in stables 2 and 3. There are no regulations which determine the maximum permissible level of microbial air contamination in livestock buildings (5, 29) but, according to Dutkiewicz et al. (5), a concentration of airborne microorganisms between 3-8 $\log_{10}$ cfu/m^3 may increase the risk of respiratory diseases.

The results obtained in our study of the total concentration of airborne dust in all of the stables were higher compared to the values presented by other authors (6, 12), who found airborne dust concentrations of 0.16 mg/m^3 to 1.20 mg/m^3. Taking into consideration that the allowable concentration of dust in the air of livestock buildings is 3 mg/m^3 (7), it is clear that the level of dust pollution in stables 1 and 2 was too high.

The mean concentration of ammonia in none of the stables exceeded the allowable 20 ppm value (7) in stables, although Seedorf et al. (22) believe that due to the welfare requirements, the concentration of ammonia in a stable's air should not exceed 10 ppm. Studies conducted by other authors have shown diverse levels of ammonia in the stable air. The concentration of this gas in the range of 2-11 ppm was found by Fiedorowicz and Łochowski (8) and Houben (12), whereas Curtis et al. (3) and Elfman, et al. (6) found ranges of 20-27 ppm. The mean concentration of CO_2 in stable 1 exceeded the permissible value of 3,000 ppm (7, 13), while in the other stables it was under the recommended level. However, similar to stable 1, in stable 2 the maximum concentration of carbon dioxide was higher than suggested by Fiedorowicz (7) and Jodkowska (13). This was probably caused by irregular air exchange. Fiedorowicz and Łochowski (8) reported

a lower concentration of CO_2 in stables during winter season (mean 1,507.4 ppm, range 490.0-3,370.0 ppm).

From the data presented in Tab. 1-3 it can be stated that living conditions meeting the welfare requirements were provided only in stable 3, both in terms of microclimatic parameters, as well as the dimensions of the boxes and access to paddock and pasture. Slightly worse conditions were provided in stable 2, and stable 1 was characterized by not only the worst microclimatic conditions, but also by the smallest surface of boxes, which did not meet the requirements of the Regulation of Ministry of Agriculture and Rural Development (Official Journal of Laws no 116 item 778) (20). In addition, the level of welfare of horses in stables 1 and 2 was affected by the lack of access to open air.

The diverse conditions provided for horses in individual stables was reflected in the values of the studied physiological indicators (Tab. 4). Differences in total protein content and its fractions in the horse serum, mostly confirmed statistically, were found between the stable 3 (the best conditions) and stables 1 (the least favorable conditions) and 2 (slightly better conditions). It should be noted that the total protein concentration in all investigated horse blood serum samples were in the range of reference values (28). The lower concentration of total protein and a lower proportion of albumin and γ-globulin, a higher proportion of α- and β-globulin in the horses' serum from stables 1 and 2, compared with the data obtained in stable 3, all suggest the impact of housing conditions on the formation of these indicators. A similar trend in the formation of noticeable differences between the stables also appeared between stables 1 and 2, but this was not confirmed statistically. However, significant differences ($P \leq 0.01$) in Hp concentration between all stables may confirm the impact of the provided conditions on the development of the value of this acute phase protein.

Discussion and comparison of these results with those obtained by other authors is difficult because the results of studies concerning the evolution of similar blood parameters in horses kept under different conditions are not known. However, studies concerning the influence of various factors on the concentration of total protein and its fractions in horse serum have been conducted by many authors, but were primarily related to horse health status (2, 4), the type of work (15), gender, breed and seasonal fluctuation (9). However, the adverse environmental impact on reducing the concentration of total protein, albumin and the fraction of γ-globulin has been shown in cattle (23), chicken (31) and pigs (25). Decreased albumin levels were accompanied by increased concentrations of other acute phase proteins (15). In the electrophoresis of proteins, it is demonstrated by increased participation *inter alia* of fractions of α_2-globulin, which shows the activity of haptoglobin. The growth of Hp concentration (in the absence of an infectious agent), both under aggravated conditions and under different stressors, indicates the possibility of using this protein to assess animal welfare (15, 24). Although there are no study results concerning the applicability of using Hp concentrations to assess the welfare of horses, there are some results of research conducted on pigs (10, 11), cattle (21) and sheep (16).

The protein ratios obtained in our study are similar to those reported by other authors considered as valid for adults, healthy horses (4). However, the significantly lower level of the albumin fraction, the higher α_2-globulin (in stable 2) and the higher Hp concentrations shown in the serum of horses in stables 1 and 2, compared to stable 3, may suggest the effect of different living conditions on the homeostasis indicators.

It should be noted that both environmental and physiological factors display similar conclusions concerning the level of horse welfare. The results demonstrate that only horses in stable 3 had very good welfare. The conditions in stables 1 and 2 did not meet the environmental needs of the horses and did not provide them with good welfare. This fact was confirmed by physiological indicators pointing to some disruption of homeostasis and increased activation of the immune system of horses (30) from stables 1 and 2. To summarize it may be stated that living conditions significantly influence the physiological condition of the horse.

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