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# Analysis of correlations between selected blood markers of acid-base balance, blood electrolytes, and milk components in dairy cows during late lactation

RYSZARD MORDAK[1], ZBIGNIEW DOBRZAŃSKI[2], JAROSŁAW POPIEL[1], ROBERT KUPCZYŃSKI[2]

[1]Department of Internal Medicine of Animals, Faculty of Veterinary Medicine, Wrocław University of Environmental and Life Sciences, Pl. Grunwaldzki 47, 50-366 Wrocław, Poland
[2]Department of Environment Hygiene and Animal Welfare, Faculty of Biology and Animal Science, Wrocław University of Environmental and Life Sciences, ul. Chełmońskiego 38c, 51-630 Wrocław, Poland

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Mordak R., Dobrzański Z., Popiel J., Kupczyński R.

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Summary

**The aim of the study was to assess the values of and correlations between selected markers of acid-base balance and electrolytes in the blood of late lactation cows, such as pH, $pCO_2$, $pO_2$, $HCO_3^-$, BE, BE(B), $Na^+$, $K^+$, $Cl^-$, as well as selected parameters of milk, such as the number of somatic cells (SCC), colony forming units (CFU), milk fat (MF), milk protein (MP), milk lactose (ML), milk solids, milk solids non-fat (SNF), and current milk production. Blood and milk samples were collected at the same time from 11 clinically healthy milking cows in the later lactation period. Those 11 selected cows were examined once a day for 3 consecutive days resulting in 33 sets of blood and milk samples used for laboratory and statistical analyses. Significant positive correlations were observed between pH and $HCO_3^-$, pH and BE, pH and BE(B), $pCO_2$ and $HCO_3^-$, $pCO_2$ and BE, $pCO_2$ and BE(B), $HCO_3^-$ and BE, $HCO_3^-$ and BE(B), BE and BE(B), $Na^+$ and $Cl^-$, $Na^+$ and milk production, $Cl^-$ and milk production, and $Cl^-$ and MP. Significant negative correlations were observed between pH and $K^+$, pH and $Cl^-$, $pCO_2$ and $Cl^-$, $HCO_3^-$ and $Cl^-$, BE and $Cl^-$, and BE(B) and $Cl^-$. The increase in blood pH was significantly negatively correlated with the current milk production, while the increase in $HCO_3^-$ or $pCO_2$ was associated with a significant decrease in the amount of protein in milk. These relations can indicate a significant negative influence of blood acid-base imbalance on the quantity and quality of cow's milk. The repeatable diagnostic model presented here proved to be a very useful tool for a detailed assessment of selected higly sensitive parameters of internal homeostasis in productive dairy cows.**

**Keywords: dairy cows, blood, acid-base balance, milk, correlations**

The acid-base and electrolyte balance of blood is very important for life processes, and its disturbances can have significant effects on the health and life of people and animals (8, 36, 39, 41). Disturbances in the acid-base and electrolyte balance of the blood usually affect the functioning of all body organs and can be particularly dangerous in high-yielding dairy cows, whose metabolism is additionally burdened with intense supra-physiological pressure on milk production and reproduction (22). These disturbances are largely due to the diet and the environment, but may not be easy to notice in herds (39). Therefore, modern dairy herds should be carefully monitored using a variety of clinical and laboratory diagnostic parameters to assess animal welfare and health and to detect cow health problems early (13, 26, 32, 36, 40, 43). Various metabolic disorders caused by inadequate quality and quantity of dietary ingredients or their incorrect proportions are particularly important because they can occur massively in dairy herds and can be very dangerous not only during the transitional period, but also in the later lactation period, which has a significant impact on health, reproduction, and the level of milk production in cows (28-30). The monitoring of the farm environment, hygiene, and nutrition, as well as early detection of disturbances in the internal homeostasis of animals by testing their blood and other samples has become the basis for prevention in many modern dairy farms.

Moreover, the assessment of milk quality and the functions of individual organs, including the liver and mammary gland, is usually very helpful in eliminating or minimizing health problems in the herd, and thus in reducing losses due to the treatment, culling, and mortality of dairy cows (2, 10, 16).

Blood and milk tests for dairy cows are usually designed to detect various infectious or non-infectious health problems, including metabolic ones. They are performed with appropriate frequency, depending on the disease risk determined by the milk production level, quality of nutrition, herd management, environment, technology, and number of animals (6, 11, 15, 17, 23, 38, 44). Herd health monitoring usually includes the analysis of parameters of milk production, reproduction, and clinical signs, as well as samples of blood, milk, urine, lattice fluid, and other material in a representative group of cows (13, 20, 37, 43). Laboratory diagnostics can include different biochemical blood parameters, depending on the type of health problem, and can be analyzed as specialized profiles, such as liver, kidney, bone and minerals, lipids, thyroid, heart, and acid-base or morphological balance profiles (2, 4, 12, 18, 19, 24, 25, 31). There are also published studies with an example of broader diagnostics assessing the impact of body condition (BCS) on the quantity and quality of milk and the acid-base balance of Holstein heifers (39). The results of these profiles can show the current internal homeostasis, functions of the most important organs, and the state of various metabolic processes, especially the levels of energy, proteins, minerals, water, electrolytes, and gases. Herd health monitoring can use milk samples not only to assess the mammary gland, but also to control the general health of dairy cows. This assessment is usually carried out by simple field methods during each milking on the farm or by systemic periodic monitoring of the morphological and biochemical parameters of milk samples tested under laboratory conditions or with new laser techniques (e.g. near infrared spectroscopy). In cattle practice, the assessment of the levels of milk production, fat, protein, lactose, SCC, and other parameters of milk makes it possible to analyze the quality of nutrition and to detect potential metabolic disorders or the occurrence of mastitis in cows throughout the lactation period (1, 7).

Unlike individual tests, which compare the results only with standard reference values, simultaneous and repeated biochemical or morphological tests of blood and milk can be used for comparative analysis to track changes in internal homeostasis and relationships between individual parameters and disease progression. The available scientific literature does not provide detailed data on the simultaneous testing of blood and milk, especially in late lactation in dairy cows when tests are repeated over a few days. In our previous studies, the usefulness of such a model was demonstrated in the assessment of the relationship between serum markers of liver function and milk composition (35), as well as the relationship between macro minerals, selected serum markers of bone profile, and components of milk from dairy cows in late lactation (34).

We know from our own practice that many dairy farms, for various reasons, are not interested in the systemic blood monitoring of high-yielding cows in the later stages of lactation, especially with regard to potential acid-base and electrolyte imbalances. On the other hand, we also know about the possible risk of clinical or subclinical metabolic problems and blood imbalances in late lactation. We hypothesize that the highly homogeneous, reproducible model of blood and milk samples presented here may reveal a precise relationship between the acid-base status of late lactating cows and the yield and composition of their milk, which can be used for accurate diagnosis in the monitoring of dairy herds. The aim of the present study was to test this hypothesis.

## Material and methods

**Animals and study design.** The research was conducted in October at the animal research center of the University of Environmental and Life Sciences. The study protocol was approved by the 2nd Local Bioethics Committee in Wrocław (decision no. 24/07). A total of 11 randomly selected black and white Holstein Friesian cows aged 4-5 years, with similar BCS values of about 3.5-3.75 and moderate milk production, were included in the study. The cows were from the same dairy farm and were representative of a technological group with a similar late lactation period – in the second half of the lactation – on average 218 days after calving. The herd of cows was free of common infectious diseases (monitored and controlled by the veterinary office) and the average milk yield was about 8500 kg per lactation. The 11 cows did not show signs of any disease during clinical examinations of the body, including the milk gland, but they were in a herd at a time of high risk for metabolic diseases and mastitis. Each of the selected cows was examined, and milk and blood samples were collected once a day for three consecutive days. Thus, within three days we obtained 33 sets of blood and milk samples for laboratory and statistical analyses. All samples were taken at the same time at around 5 am: milk samples during the morning milking and blood samples directly after the milking.

The cows were kept under the same, favorable environmental conditions in a hall designed for 90 milking cows with free access to beds and feed. The Alpro-Delaval milking system, parallel 2 × 4 was used on the farm. The animals had a high environmental comfort (mattresses, an automatic ventilation system, and a curtain system) and suitable nutrition.

The cows were fed a total mixed ration (TMR) supplemented with a commercial concentrate mixture (stations with an electronic system for dry ration) for lactating cows based on daily milk yield with free access to fresh water. TMR (*ad libitum*) was offered twice daily in the morning and afternoon. TMR was formulated according to the INRA system using the INRAtion 4.0 software, based on analyses of ingredients expressed as a percentage of dry matter (DM), such as corn silage 34.5%, alfalfa-grass silage 24.5%, high moisture corn 15.6%, sugar beet pulp 7.1%, rapeseed meal 1.9%, soybean meal 1,5%, alfalfa/grass hay 3.9%, barley straw 1.5%, complete mixture 5.20%, fodder chalk 0.15%, calcium bicarbonate 0.2%, vitamins and minerals 0.3%, as well as based on chemical composition expressed in NEL 6.12 MJ/kg and as a percentage for other components, such as crude protein 16.8%, crude fat 3.80%, NDF 36.2%, ADF 22.4%, calcium 0.78%, phosphorus 0.36%. Vitamin and mineral supplement included 11% Ca, 15% P, 12.5% Mg, 2% S, 2% K, 1.200 mg/kg of Fe, 100 mg/kg of J, 1.500 mg/kg of Cu, 3.300 mg/kg of Mn, 5.000 mg/kg of Zn, 36 mg/kg of Co, 22 mg/kg of Se, 550.000 IU/kg of vitamin A, 220.000 IU/kg of vitamin D, and 3.100 IU/kg of vitamin E. Samples of TMR were pooled twice during the experiment. The following parameters were determined: dry matter (method 934.01), crude protein (method 984.13; Kjeltec™ 2300 apparatus, FOSS Analytical AB, Sweden), crude fat (method 920.39), crude fiber (method 978.10; Fibertec™ 1020 (M6) apparatus, FOSS, Netherlands), according to AOAC (2005) procedures (5).

**Blood analysis.** Blood tests were performed at a specialized laboratory of our University. Blood samples were collected from the external jugular vein using 2 ml heparinized syringes for the acid-base profile parameters of full blood and 10 ml polypropylene tubes (Sarstedt, Germany) for the electrolyte profile of serum. The samples were transported to the laboratory in proper conditions in less than one hour. The blood parameters of the acid-base profile analyzed in this study were the potential of hydrogen ions (pH), partial pressure of carbon dioxide ($pCO_{2)}$, partial pressure of oxide ($pO_{2)}$, bicarbonate concentration ($HCO_3^-$), base excess standard (BE), and base excess in whole blood BE(B). The parameters were measured with an Osmetech OPTI CCA blood-gas analyzer (OPTI Medical Systems Onc. USA). Concentrations of electrolyte ions, such as sodium ($Na^+$), potassium ($K^-$), and chlorine ($Cl^-$), were measured with a Ciba-Corning 644 Na/K/Cl analyzer (CIBA-Corning, USA). Reference standards were used to interpret the values of individual diagnostic blood parameters.

**Milk analysis.** The study also used samples of milk from all phases of milking. These milk samples were collected in special reservoirs analogous to those used in the trial milking. The milk samples taken into sterile 50-ml polypropylene containers (Prolab, Poland) were analyzed for selected parameters of milk, such as somatic cell count (SCC), colony forming units (CFU), milk fat (MF), milk protein (MP), milk lactose (ML), milk solids, milk solids non-fat (SNF), and current milk production, during the morning milking, when the blood samples were also taken. Biochemical analyses of milk were carried out in a specialized milk laboratory of our University. An SCC assay was performed by the fluoro-opto-electronic method with a SOMACOUNT-150 (Bentley, USA) analyzer. CFU determination was performed by flow cytometry with a BACTOCOUNT-70 (Bentley, USA) analyzer. Determination of the fractions of milk fat, protein, lactose, DM, and FDM was done with a Milko-Scan 133 B (Foss Electric, Denmark) analyzer. Milk yield was recorded by the Alpro-Delaval electronic system.

**Statistical analysis.** Statistical analysis was carried using the Statistica 13.0 StatSoft Inc. program. The samples were tested for normal distribution with the Shapiro-Wilk test. Linear regression was used to assess the level of relationships between changes in the values of individual compared parameters and The Pearson Correlation Coefficient (PCC) (Pearson's r) was calculated. Each coefficient was then tested for statistical significance with Student's t-test for n-2 degrees of freedom. The number of samples was 33, and the value of the correlation coefficient determining the threshold for a statistical significance level of 5% ($p < 0.05$) was r = 0.344. Correlations were given using Pearson's correlation coefficient, and statistical significance was determined according to the adjusted p-value. They are presented in the tables.

## Results and discussion

Correlations between the selected blood markers of acid-base balance, blood electrolytes, and milk components, as well as their mutual relationships, are shown in Tables 1 and 2. The values of Pearson's correlation coefficients between variables exceeding the limit of statistically significant correlations are specially marked. The average values and standard deviations for blood and milk parameters are presented in Table 3.

Significant positive correlations ($p < 0.05$) were observed between pH and $HCO_3^-$ (r = 0.462), pH and BE (r = 0.624), pH and BE(B) (r = 0.651), $pCO_2$ and $HCO_3^-$ (r = 0.777), $pCO_2$ and BE (r = 0.798), $pCO_2$ and BE(B) (r = 0.786), $HCO_3^-$ and BE (r = 0.887), $HCO_3^-$ and BE(B) (r = 0.884), BE and BE(B) (r = 0.998), $Na^+$ and $Cl^-$ (r = 0.562).

Significant negative correlations were observed between pH and $K^+$ (r = –0.514), pH and $Cl^-$ (r = –0.455), $pCO_2$ and $Cl^-$ (–0.343), $HCO_3^-$ and $Cl^-$ (r = –0.474), BE and $Cl^-$ (r = –0.558), BE(B) and $Cl^-$ (r = –0.553).

Significant positive correlations were observed between $Na^+$ and milk solids (r = 0.364) and between $Cl^-$ and milk solids (r = 0.360). Significant negative correlations were observed between pH and milk production (r = –0.408), $pCO_2$ and MP (r = –0.361), $HCO_3^-$ and MP (r = –0.418).

Gasometry is the primary method for detecting metabolic or respiratory acidosis or alkalosis in humans and animals, and the basic acid-base relationship of blood parameters has been mathematically determined by the Henderson-Hasselbalch equation (42). Unfortunately, this method of diagnosis is usually not included in the typical monitoring of dairy herds. Moreover, in the literature, there are no detailed results of studies of the relationship between the acid-base and electrolyte status and the parameters of cow's milk in terms of its quantity and quality.

Our results indicate several significant relationships between the selected blood markers for the assessment of acid-base balance, blood electrolytes, and the param-

**Tab. 1. The Pearson Correlation Coefficients between selected blood markers of acid-base balance, and electrolytes in cows**

| | pH | $pCO_2$ | $pO_2$ | $HCO_3$ | BE | BE (B) | $Na^+$ | $K^+$ | $Cl^-$ |
|---|---|---|---|---|---|---|---|---|---|
| pH | – | | | | | | | | |
| $pCO_2$ | 0.220 | | | | | | | | |
| $pO_2$ | –0.304 | –0.175 | | | | | | | |
| $HCO_3$ | 0.462* | 0.777* | 0.074 | | | | | | |
| BE | 0.624* | 0.798* | –0.255 | 0.887* | | | | | |
| BE (B) | 0.651* | 0.786* | –0.266 | 0.884* | 0.998* | | | | |
| $Na^+$ | –0.036 | –0.261 | –0.274 | –0,311 | –0.278 | –0.276 | | | |
| $K^+$ | –0.514* | 0.052 | 0.006 | –0,185 | –0.234 | –0.245 | –0.115 | | |
| $Cl^-$ | –0.455* | –0.343* | –0.024 | –0.474* | –0.558* | –0.553* | 0.562* | 0.258 | – |

Explanation: * – statistically significant correlation at $p < 0.05$

**Tab. 2. The Pearson Correlation Coefficients between selected blood markers of acid-base balance, electrolytes and milk components in cows**

| | pH | $pCO_2$ | $pO_2$ | $HCO_3^-$ | BE | BE (B) | $Na^+$ | $K^+$ | $Cl^-$ |
|---|---|---|---|---|---|---|---|---|---|
| Production | –0.408* | –0.007 | 0.179 | –0.082 | –0.123 | –0.148 | 0.320 | –0.105 | 0.313 |
| CFU | –0.050 | 0.198 | 0.072 | 0.035 | 0.073 | 0.080 | –0.183 | 0.285 | 0.161 |
| SCC | –0.013 | –0.257 | –0.072 | –0.270 | –0.256 | –0.252 | 0.155 | 0.065 | 0.141 |
| MF | 0.027 | 0.048 | –0.150 | –0.040 | –0.011 | 0.001 | 0.259 | 0.231 | 0.257 |
| MP | 0.123 | –0.361* | –0.125 | –0.418* | –0.336 | –0.333 | 0.261 | –0.018 | 0.305 |
| ML | –0.173 | 0.048 | 0.174 | 0.085 | 0.010 | –0.006 | –0.097 | –0.259 | –0.172 |
| Solids | 0.043 | –0.033 | –0.191 | –0.146 | –0.101 | –0.087 | 0.364* | 0.225 | 0.360* |
| SNF | 0.030 | –0.290 | –0.029 | –0.325 | –0.288 | –0.293 | 0.185 | –0.140 | 0.185 |

Explanation: * – statistically significant correlation at $p < 0.05$

**Tab. 3. The mean values and standard deviations (SD) of selected parameters of blood and milk in cows**

| Parameters of blood and milk | Mean | SD | Unit |
|---|---|---|---|
| pH | 7.42 | 0.02 | |
| $pCO_2$ | 49.21 | 4.65 | mmHg |
| $pO_2$ | 28.56 | 2.41 | mmHg |
| $HCO_3$ | 31.83 | 2.60 | mmol/L |
| BE | 7.43 | 3.15 | mmol/L |
| BE (B) | 6.44 | 2.74 | mmol/L |
| $Na^+$ | 140.12 | 3.14 | mmol/L |
| $K^+$ | 4.19 | 0.33 | mmol/L |
| $Cl^-$ | 103.45 | 2.72 | mmol/L |
| Milk production (mean) | 16.53 | 2.63 | liters |
| CFU | 82.77 | 52.12 | × 1000/ml |
| SCC | 114.36 | 63.74 | × 1000/ml |
| MF | 4.30 | 1.81 | % |
| MP | 3.57 | 0.42 | % |
| ML | 4.64 | 0.22 | % |
| Solids | 13.14 | 1.56 | % |
| SNF | 8.82 | 0.48 | % |

Explanations: CFU – colony forming units; SCC – somatic cell count; MF – milk fat; MP – milk protein; ML – milk lactose; Solids – milk solids; SNF – milk solids non-fat

eters of milk production and composition in cows. In the group of randomly selected and clinically healthy cows, a shift towards alkalosis was observed in the blood picture, and the significantly negative correlation between blood pH and the milk yield showed that an increase in this imbalance results in a decrease in milk production. Blood pH change was significantly related to the current volume of milk production, and the increase in the $HCO_3^-$ or $pCO_2$ parameter was accompanied by a significant decrease in the amount of protein in milk. This indicates a significant negative effect of a blood acid-base imbalance on the quantity and quality of cow's milk obtained.

The pH value of blood depends on many nutritional and environmental factors, as well as the efficiency of the physiological functions of body systems, in particular the rumen, lungs, and kidneys. In clinically healthy cows, blood acid-base imbalances are constantly compensated by various regulatory mechanisms according to their performance and capacity (22). The dietary cation-anion difference (DCAD) has a significant impact on the acid-base balance of the cows' organism. Constable et al. (12) suggested that the optimal DCAD is approximately +275 to +400 mEq/kg of DM for cows in mid-lactation. The differences in DCAD values were achieved by manipulation of dietary Cl (21). It has been proved in other studies that rumen acidosis or rumen alkalosis not only affects the acid-base balance of blood in cows, but also has a significant impact on milk yield. Compared with high risk cows, low risk cows with subacute ruminal acidosis produced more fat-corrected milk (47.2 vs. 41.9 kg/d) and more milk fat (2.1 vs. 1.8 kg/d) (14). In our study, this was clearly demonstrated by our sample testing method, as the correlation coefficient between blood pH and milk yield was significant at –0.41. Other studies have revealed that even a slight reduction in rumen pH during subclinical subacute ruminal acidosis (SARA) was associated with a 0.22% decrease in milk fat, while a one unit increase in rumen fluid pH was associated with an increase in fat content in milk by 0.28% and an increase in dry matter in milk by 0.44% (27). It is also known that the maintenance of the osmotic pressure of body fluids in both blood and milk is caused by similar factors, especially water-electrolyte and protein factors (9). In addition, the decrease in blood chloride content after calving, convergent with $Na^+$ values, is due to the free flow of water in the body, which also accompanies milk production (39). In our study, we found significant positive correlations between serum $Na^+$ and solids in milk and between serum $Cl^-$ and solids in milk. Clear positive relations were noted between serum $Na^+$ and milk production, as well as between serum $Cl^-$ and milk production, although they did not reach minimal statistical significance. Studying the correlation between different blood markers of acid-base balance, electrolytes, and milk parameters in bovine practice may be important not only for better monitoring of internal homeostasis and health, but also for discovering important metabolic pathways and relationships, especially important in highly productive dairy cows (18, 39). The results of the cited studies showed that the body condition of 27 healthy Holstein primiparous heifers had a positive, but not statistically significant, effect on milk quantity and quality. However, the study of acid-base balance variables provides information on metabolic changes that may be difficult to observe and detect in the herd, especially in a single examination. Similar changes in blood and milk metabolite concentrations during the lactation period and the existence of milk-blood correlations, confirm that milk can be very useful in predicting the blood metabolites and metabolic status of cows (3). The authors of these studies indicate that many correlations showed the same trend across all lactation periods.

In our case, the repeated blood tests made it possible not only to interpret single, labile results in relation to the reference standard at a given moment, but also to determine very accurately the current health status, as well as the relationship between selected blood markers regarding the acid-base balance and blood electrolytes and selected parameters of milk in cows in the late lactation period. It has also helped us to better understand links between different biochemical processes, milk productivity, and milk quality in dairy cows. The knowledge of relationships between different blood markers of acid-base balance and milk parameters may be especially important in formulating a diagnosis and in preventing or correcting various blood acid-base imbalances and inadequate milk production in cows. In meta-regression analysis, milk fat and protein could be identified and confirmed as indicators of rumen pH parameters and thus, in a broader sense, also of subacute rumen acidosis (33). On the other hand, also the milk lactose content was selected by automatic selection of variables and showed the possibility of improving the predictability of rumen pH parameters. In bovine practice, an excess of crude protein is often observed in the ration during the lactation phase, and it can create a tendency to an alkaline state in the ruminal fluid and in the blood. Thus, the relations between selected blood and milk parameters analyzed here may also be important for assessing the potential health risks and losses in milk production, especially during the frequent occurrence of subclinical blood acidosis or alkalosis, which is often associated with nutritional and ruminal pH imbalances in high-yield dairy herds. Repeating the tests on the same, selected dairy cows within a few days made it possible to assess the degree and direction of changes measured by correlations between the parameters examined. Blood tests allowed us to formulate a precise diagnosis of subclinical alkalosis in cows. Our diagnostic model proved to be a good tool for the assessment of the very sensitive and dynamic blood acid-base balance in dairy cows. This may be important during the frequent occurrence of subclinical metabolic health problems.

The comparison of the parameters of the acid-base balance of blood and selected parameters of milk revealed a particularly significant correlation between the change in pH and the current volume of milk production. This relationship is of fundamental importance in cattle practice, both for herd health and the economy of dairy farms. Furthermore, the increase in $HCO_3^-$ or $pCO_2$ was accompanied by a significant decrease in the amount of protein in milk, which may indicate a negative effect on the qualitative composition of milk. These statistically significant correlations indicate a negative impact of a disturbed acid-base balance of the blood during mild metabolic alkalosis on the amount of milk obtained during milking and the qualitative composition of cow's milk. It is also worth noting that significant positive correlations were observed between $Na^+$ or $Cl^-$ in the blood and dry matter in milk.

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**Corresponding author: Ryszard Mordak, DVM, DSc, prof. univ., Department of Internal Medicine of Animals, Faculty of Veterinary Medicine, Wrocław University of Environmental and Life Sciences, Pl. Grunwaldzki 47, 50-366 Wrocław, Poland; e-mail: ryszard.mordak@upwr.edu.pl**