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Interrelationships between selected blood markers of renal functions and milk components in dairy cows

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Summary

There are various methods for the assessment of internal metabolism and internal organ functions in the monitoring of highly productive dairy cows. The knowledge of various relationships, including significant correlations between different milk and blood parameters, based on the results of laboratory analyses is very important in veterinary diagnostics and useful in bovine practice. The aim of the present study was to analyze correlations between selected blood serum parameters included in the renal profile, such as concentrations of urea (U), creatinine (C), uric acid (UA), total protein (TP), albumin (A), sodium, and potassium, and the values of selected parameters of milk components, such as somatic cells count (SCC), bacteria colony forming units (CFU), milk fat (MF), milk protein (MP), lactose (ML), solids, solids non-fat (SNF), and milk yield (MY). Blood and milk samples were collected at the same time from 11 clinically healthy milking cows during the late lactation period. The animals were tested three times during 3 days at daily intervals, which yielded 33 sets of test results. This model of testing made it possible to obtain a highly homogeneous group of animals for testing and enabled us to determine statistical correlations between selected biochemical parameters of the renal blood profile and components of milk. Significant negative correlations were observed between U and MY, C and MY, UA and MY, as well as between C and TP. Significant positive correlations were observed between TP and SCC, TP and MP, TP and SNF, A and MP, A and SNF, sodium and SNF, as well as between TP and A. The results of blood and milk tests made it possible to precisely monitor the basic health status of cows in relation to selected blood parameters of the renal profile. The analysis of links between metabolic pathways in the relationship between blood and milk homeostasis in high-yielding dairy cows may have diagnostic significance in real cattle practice.

Keywords: cows, blood, urea, creatinine, uric acid, milk components, correlations

Biochemical analyses of blood and milk composition in cows are helpful in diagnosing health problems or monitoring diseases that occur in a subclinical form, but result in reduced production and reproduction parameters of these animals and therefore economic losses (6, 11, 23, 33). In veterinary medicine, especially in cattle practice, such analyses are particularly important in high-production dairy cows, which are at constant risk of metabolic disorders, deficiency, or functional overload of important organs resulting from pressure for higher milk yields and fertility (29, 31). The knowledge of hematological and biochemical blood parameters is necessary for the ongoing assessment of the health status of animals (19, 22, 26), the risk of subclinical metabolic and deficiency diseases (4, 18, 20, 25, 34), nutritional correctness (14-16), and the potential quality of raw materials obtained for food production (5, 13, 28). The assessment of the internal homeostasis of the body in terms of blood, milk, and urine parameters of cows is the basis of the veterinary laboratory diagnostics and assessment of animals during milk performance control, as well as the diagnostic monitoring of the herd in an occasional or seasonal system (7, 8, 12, 27, 34, 35).

Daily examination of the blood and milk parameters of cows makes it possible to precisely assess their inter-

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nal homeostasis and analyze correlations between these markers in a highly homogeneous group of animals under the same physiological, welfare, environmental, and nutritional conditions (23-25). This monitoring model, presented in three scientific papers (23-25), has proven useful for different metabolic blood profiles in cows depending on diagnostic needs or potential risks in the herd regarding liver function, macronutrient and electrolyte levels, or acid-base balance. Furthermore, this model is capable of monitoring animals and the herd in different blood profiles, as well as assessing correlations between blood and milk parameters, which translates into a better understanding of relationships between life processes and the functioning of organs related to milk production.

The renal diagnostic profile of blood serum, usually including such parameters as urea, uric acid, creatinine, total protein, albumin, and sodium and potassium electrolytes, can be analyzed statistically, both alone and in relation to the basic components of milk, to determine the state of internal homeostasis in high-producing cows monitored in the herd (8, 27). Urea is formed as a result of the breakdown of amino acids in the liver during the urea cycle, which makes it possible to remove excess nitrogen from these amino acids by the kidneys (10), while uric acid is formed during the breakdown of purines, but is also removed by the kidneys (9). Purines, such as adenine and guanine, are nitrogenous bases forming part of DNA and RNA. The decomposition of amino acids in the urea cycle results in the formation of ammonia, which is highly toxic to the body, but is converted into less toxic urea in the liver.

Creatinine is a nitrogenous organic compound, which, unlike the above markers, is formed as a result of metabolism, mainly in skeletal muscles. It occurs in blood as a product of creatine degradation and is then excreted in urine. According to Winnicka (35), in cattle, the total serum protein fraction with a concentration of 51-71 g/l includes 32-49 g/l of albumins, 7-23 g/l of α-globulins, 11-33 g/l β-globulins, and 10-32 g/l of γ-globulins. In addition, she provides reference ranges for electrolytes in blood serum: 134.8-156.5 mmol/l for sodium and 3.8-5.1 mmol/l for potassium. According to other authors (17), one liter of milk in healthy cows includes 30-35 g of total protein, of which 80% are casein proteins (αs1-casein 38%, αs2-casein 10%, β-casein 39%, κ-casein 13%), and the remaining 20% are whey proteins, consisting mainly of β-lactoglobulin (β-lg 56%) and α-lactoglobulin (α-la 21%) synthesized in the milk gland, as well as immunoglobulins (Ig – 14% lactoperoxidase and lysozyme), bovine serum albumin (BSA 7%), and lactoferrin (LF 2%).

Some authors point out the value of the analysis of mutual relationships between markers, such as urea and protein, in both blood serum and milk, especially in predicting milk yield in dairy cow herds (29). The complexity of the composition and properties of blood and milk is related to various complicated metabolic pathways, the understanding of which could be helpful in monitoring the health and milk production of cows. However, there are not many original research papers in the literature that analyze detailed mutual correlations between parameters of specialized blood metabolic profiles and parameters of the quantity and quality of milk obtained from cows. The aim of the present study was to analyze correlations between selected renal blood markers and the composition of milk from dairy cows in the late lactation period.

Material and methods

Animals and study design. The study protocol was approved by the 2nd Local Bioethics Committee in Wrocław (decision no. 24/2007). The research was conducted under an internal mission-related research grant from the Faculty of Veterinary Medicine, University of Environmental and Life Sciences in Wrocław, at the Agricultural Experimental Station Swojec with a dairy cattle farm. The material consisted of 11 randomly selected Holstein Friesian black and white clinically healthy cows aged 4-5 years. The cows had a body condition score (BCS) of 3.5 and were tested under similar conditions on the same dairy farm during the autumn season, in the same technological group, specifically in the second half of the lactation period. The cows were in their second or third lactation, and the average duration of the current lactation at the commencement of blood and milk analytical tests was 218 days. The herd of cows was under constant veterinary control, free from infectious diseases, and the average milk yield was approximately 8,500 kg per lactation. Each cow was examined three times over three days at 24-hour intervals, which resulted in a total of 33 separate sets of blood and milk samples for laboratory analyses.

The cows were kept under the same favorable environmental conditions. The animals were in a group of 90 dairy cows and had free access to feed on the feeding table and in automatic stations and to fresh water in automatic drinkers. The cows were fed the same total mixed ration (TMR) served on the feed table. The TMR was offered ad libitum twice a day in the morning and afternoon. The TMR was developed in accordance with the INRA system using the INRAtion 4.0 software, based on appropriate procedures for dietary components according to AOAC (1).

Detailed ingredients of the TMR nutrients for cows in the herd, as well as blood profiles assessing liver functions, acid-base and electrolyte balances, and bone-minerals, are shown in previous publications (23-25). This feeding was supplemented with a commercial concentrate fed to the cows in electronically controlled stations according to their current milk production. The animals were provided with a high level of comfort due to equipment, including insulating mattresses on the positions, an automatic manure removal system, and an automatic ventilation system.

The farm used an Alpro-Delaval milking system using a Parallel 2x4 milking machine with an automatic washing station and a 2,000-liter milk tank with an agitator and cooling. The milk yield during milking was recorded by the electronic system connected to the milking machine. Milk samples were collected during morning milking at around 5 am, and blood samples were taken immediately after milking.

Blood analysis. Blood samples were collected from the external jugular vein into 10 ml Sarstedt tubes, and the blood serum was analyzed biochemically. Blood tests were performed at a specialized laboratory of the Faculty of Veterinary Medicine (Department of Internal Medicine) at the University of Environmental and Life Sciences in Wrocław.

Blood biochemical parameters, such as urea, creatinine, uric acid, and total protein, were determined using a Pointe-180 (Pointe Scientific Poland) analyzer, while albumin values were determined with a Kodak – DTSC II Ektachem (Eastman Kodak Company USA) analyzer. Reference standard limits provided by Winnicka (35) were used for interpretation of individual diagnostic blood parameters.

Milk analysis. Milk samples were taken automatically during all milking phases and collected in a special tank, a device used in the trial milking. Subsequently, these samples were placed for transport in sterile polypropylene containers with a capacity of 50 ml (Prolab, Poland). They were analyzed for selected parameters, such as the somatic cell count (SCC), the number of colony forming units (CFU), milk fat (MF), milk protein (MP), milk lactose (ML), milk solids (S), solids non-fat in milk (SNF), and the milk yield obtained in the morning milking.

Analyses of milk samples were performed in a specialized laboratory for milk at our university. SCC tests were performed by the fluoro-opto-electronic method using an SOMACOUNT-150 Bentley (USA) analyzer. CFU values were determined by flow cytometry using a BACTO-COUNT-70 Bentley (USA) analyzer. Milk fractions, such as MF, MP, ML, S, and SNF, were measured with a Milko-Scan 133 B (Foss Electric, Denmark) analyzer.

Statistical analysis. Statistical analysis was carried out using the Statistica 10 StatSoft Inc. program. The samples were tested for normal distribution using the Shapiro-Wilk test. The r-Pearson correlation coefficient was used for correlation analysis because it can determine a linear relationship between two different variable blood and milk parameters taken in separate samples at different times. Each coefficient was also tested for statistical significance with Student's t-test for n-2 degrees of freedom. There were 33 samples, and the value of the correlation coefficient defining the threshold for a statistical significance level of 5% ($P < 0.05$) was $r = 0.344$. All values of Pearson's correlation coefficients between the variables, including those indicating their statistical significance, are presented in Tables 1 and 2.

Results and discussion

The values of correlations between selected blood markers of the renal profile and milk composition parameters are shown in Tables 1 and 2. Significant statistical correlations are specifically marked. Mean values and standard deviations of the blood and milk parameters are presented in Table 3. According to the results of the correlation analysis for the biochemical parameters of blood serum in the renal profile, the urea level was significantly positively correlated with the uric acid level ($r = 0.354$). The level of total protein in serum showed a significantly positive correlation with the serum albumin level ($r = 0.434$). The serum creatinine level was significantly negatively correlated with the serum total protein level ($r = –0.422$).

The obtained values of correlations between the blood and milk parameters show that the levels of urea, creatinine, and uric acid in blood serum showed significant negative correlations

Tab. 1. Coefficients of correlation between blood plasma markers

	Urea	Creatinine	Uric acid	Total protein	Albumin	Sodium	Potassium
Urea							
Creatinine	0.156						
Uric acid	0.354	0.275					
Total protein	–0.230	–0.422	–0.267				
Albumin	0.091	–0.240	–0.172	0.434			
Sodium	–0.010	–0.319	–0.186	–0.067	0.003		
Potassium	0.198	0.212	–0.315	0.027	–0.115	–0.115	

Explanations: * Significant statistical correlation at $r = 0.344$ ($P < 0.05$).

Tab. 2. Coefficients of correlation between selected blood plasma markers of the renal profile and milk components

	Urea	Creatinine	Uric acid	Total protein	Albumin	Sodium	Potassium
MY	–0.433	–0.367	–0.385	0.003	–0.185	0.320	–0.105
CFU	0.269	0.312	0.277	–0.323	–0.056	–0.183	0.285
SCC	–0.096	–0.242	–0.178	0.495	–0.033	0.155	0.065
MF	0.061	0.310	0.224	–0.282	–0.165	0.259	0.231
MP	0.207	–0.262	0.156	0.452	0.449	0.261	–0.018
ML	–0.188	–0.192	–0.211	–0.048	0.042	–0.097	–0.259
Solids	0.097	0.261	0.189	–0.217	–0.062	0.364	0.225
SNF	0.093	0.322	0.311	0.374	0.413	0.185	–0.140

Explanations: * Significant statistical correlation at $r = 0.344$ ($P < 0.05$); MY – milk yield; 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.

with milk yield (–0.433, –0.367, and –0.385, respectively). The level of total protein in blood serum showed a positive correlation with the values of milk parameters, such as SCC (r = 0.495), MP (r = 0.452), and SNF (r = 0.374). The concentration of albumin in serum correlated positively with the milk composition parameters MP (r = 0.449) and SNF (r = 0.413). The concentration of sodium in blood serum correlated positively with the level of solids in milk (r = 0.364).

The values of all markers of the renal profile, including urea, uric acid, and creatinine, in all samples did not exceed the reference limits for healthy cows, which demonstrates that the kidneys were functioning properly. In addition, the results indicate a significant positive correlation (r = 0.354) between the concentrations of urea and uric acid in blood serum, even though the biochemical transformation of urea into uric acid does not occur in the body. Both of these compounds are by-products of metabolism, but are formed in different biochemical processes. The positive correlation revealed by the present study may indicate the activation of parallel biochemical processes at the same time during lactation in cows. The results of the study also reveal significant negative correlations not only between the serum urea level and milk yield (r = –0.433) and between the serum uric acid level and milk yield (r = –0.385), but also between the serum creatinine level and milk yield (r = –0367). Blood serum concentrations of toxic or undesirable metabolites, such as urea, uric acid, and creatinine, which are successively excreted by various routes, may affect the cow's current milk yield, and the milk production process may also affect the concentrations of these metabolites in body fluids. This would be confirmed by observations in which dairy cows with a high milk urea (HMU) concentration have higher values of all blood serum parameters, such as urea, creatinine, and uric acid, compared to cows with a low milk urea concentration (LMU) (32). According to the cited authors, this may be related not only to the increased activation of the hepatic urea cycle or pathways synthesizing non-urea N metabolites, but also to higher levels of mitochondrial oxidative stress. In humans, elevated serum levels of uric acid may play an important role in many disease states, including gout and articular degenerative disorders, as well as vascular inflammation and atherosclerosis (21). It is well known that every negative external or internal factor affecting production animals, including dairy cows, usually results in a reduction in the quantity and quality of milk produced. For example, research results presented by other authors show that in healthy cows during lactation, the concentration of urea in blood serum 2.18-3.45 mmol/l was slightly lower than the concentration of urea in milk 2.87-5.53 mmol/l or the concentration of urea in urine 61.00-245.00 mmol/l (8). These findings can be used to approximately predict urea concentration levels on the basis of only one result obtained from serum, milk, or urine. Some authors observed a negative correlation between serum urea levels and milk production in HF cows (30), whereas others found no significant correlations between these parameters in Czech Pied (Simental) cows (3).

The results of the present study also reveal a significant negative correlation (r = –0.422) between serum creatinine and total protein levels. Similar relationships between serum creatinine and total protein levels were observed in cows in other studies at a significance level of $p < 0.01$ (29). The significant positive correlation between the concentration of total protein and albumin in blood serum results from the physiological fact that more than half of all serum proteins consist of albumin produced in hepatocytes, which matches reference standards (35) and has also been described in connection with bone (24) and liver (25) profiles in dairy cows. Similarly significant and positive correlations were found between TP and SCC levels. Comparable results were also observed by other authors, who concluded that this relationship may be caused by the positive effect of the increasing number of leucocytes in the udder on the level of globulins, which increases the concentration of total proteins in serum (2). In our analyses, a significant and positive correlation was also found between TP and MP values. This would

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

Parameters of blood and milk	Mean	SD	Unit	Limits acc. to Winnicka
Urea	3.39	1.13	mmol/l	1.66-7.47
Creatinine	97.63	10.73	µmol/l	88.4-183
Uric acid	1.97	0.24	mg/dl	0.0-2.7
Total protein	66.75	4.71	g/L	51-71
Albumin	29.78	2.36	g/L	32-49
Sodium	140.12	3.14	mmol/l	134.8-156.5
Potassium	4.19	0.33	mmol/l	3.8-5.1
MY	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: * The values of milk parameters (MY – milk yield, 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) and some blood parameters (total protein, albumin, sodium and potassium) shown in table have been published elsewhere (23-25). They are an essential comparative basis for assessment of correlations between other blood parameters and profiles characterizing the functions of other organs or other body systems, such as bones, electrolytes, and liver.

indicate a metabolic relationship between blood and milk proteins, which was also demonstrated by other authors in a different study design at a significance level of $p < 0.01$ (29). According to those authors, correlation values reveal that blood urea nitrogen (BUN), creatinine, milk protein, milk urea nitrogen (MUN), and some other markers, are useful indicators for animal breeding programs. Since daily fluctuations in urea, protein and lactose concentrations in milk from cows milked in the morning and afternoon may differ significantly, samples for comparative and analytical studies should be taken at the same time of the day (28). We followed this principle in our research. The results of other studies analyzing relationships between selected blood and milk metabolites in cows during different periods of lactation showed, among others, significant negative correlations between the level of total proteins in blood serum and the level of urea in milk ($r = -0.366$, $p < 0.05$), which proved helpful for monitoring the metabolic status of the herd of dairy cows and for the early diagnosis of subclinical metabolic disease (7).

The significant and positive correlations observed in the present study between TP and MP and between A and MP were clearly related to the positive significant correlations between TP and SNF, as well as between A and SNF, because solid non-fat components in milk contain many different types of these proteins (17). Therefore, some of our results are consistent with those of other authors. However many other results of this study are unique because they have not been published before, and because the method of monitoring blood and milk at daily intervals allowed us to obtain a highly homogeneous group of dairy cows for analysis.

The results of our tests revealed that the blood and milk parameters monitored at daily intervals were within the reference norms, which demonstrated the efficiency of kidney and mammary gland functions in randomly selected clinically healthy cows. The blood and milk tests made it possible to perform a precise correlation analysis in the highly homologous group of dairy cows. Serum levels of metabolites, such as urea, uric acid, and creatinine, were significantly negatively correlated with the current milk yield of dairy cows in late lactation. In the blood serum tests, it was found that the creatinine level significantly negatively correlated with the total protein level, and the urea level significantly positively correlated with the uric acid level. The total protein level tested in blood serum was significantly positively correlated with the milk protein level. A similar research methodology could be extended to other technological groups of cows in different stages of lactation, and the more ample research results obtained could be useful for comparison with the relationships revealed and signaled in this article.

The results obtained may be important for understanding biochemical processes related to milk production and its composition, as well as being helpful in the practical monitoring of dairy herds. In addition, these results may have a prognostic function in analyses of mutual relationships between individual blood markers and milk, and the relationships demonstrated may also be used in analytical computer programs or artificial intelligence databases.

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