THE EFFECT OF METABOLIC DISORDER RISK ON THE VARIABILITY OF BIOCHEMICAL PARAMETERS IN COWS' BLOOD AND MILK REGARDING THE SAMPLING MONTH*

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Summary

Aiming the determination the variability of biochemical parameters in blood plasma and milk of Holstein cows regarding the metabolic disorder risk (based on F/P ratio classes) separately for each sampling month (May, June, July), blood and milk were sampled from 75 high-yielding Holsteins reared on a dairy cattle farm in East Croatia. The metabolic disorder risk was defined concerning the daily fat/protein ratio (F/P) obtained from test-day records (F/P < 1.1 indicating acidosis risk; F/P in [1.1, 1.5], the normal status of an animal; F/P > 1.5, indicating ketosis risk).
The conducted analysis showed variability in the values of the biochemical parameters in both blood plasma and milk due to metabolic disorder risk and month of sampling. Accordingly, when different metabolites are used as an indicator of the metabolic disorder risk, correction for the systematic effects (stage of lactation and parity, sampling month) should be applied.

Key words: *biochemical parameters, blood, milk, metabolic disorders, Holstein*

INTRODUCTION

Reduced profitability of dairy cattle farms is frequently caused by the occurrence of animal disorders/diseases. The best way to realize the economically and ecologically sustainable functioning of a dairy farm is to prevent the occurrence of certain disorders/diseases and solve problems in a subclinical form. The above implies constant monitoring of the state of animals in production and the use of various indicators to assess the state of the animal from the health, production, reproductive and welfare aspects. Since farmers have to monitor and analyse a big quantity of data on daily basis as well as react in time to prevent potential problems in production, dairy cattle farming represents one of the most demanding animal productions (Gantner, 2020; Gantner

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et al., 2021). Furthermore, to optimize all segments of dairy cattle farm functioning and to enable the expression of the genetic potential of an animal, the farmer must be aware of animals' needs and the characteristics of the production environment. The intensive selection for high milk production reduced the resistance of the animal to negative external influences and increased the stress level of the organism, which results in an increased frequency of occurrence of various disorders/diseases in dairy cows. Puppel and Kuczyńska (2016) stated that the disproportion between the genetic production potential and the limitations in the digestive system could cause the occurrence of metabolic disorders. The disproportion of the level of production and the digestive system, especially at the beginning of lactation, significantly burdens the functioning of the liver and consequently creates prerequisites for the occurrence of abomasal displacement, mastitis, parturient paresis, placenta retention and endometritis, acidosis and ketosis. Ametaj (2017) emphasised that different factors, such as changes in the ration, reduced food intake, weight loss, negative energy balance and hypocalcemia can cause sets of different disorders during this period postpartum period. The same autor continued that in the period after calving and in early lactation, the most common metabolic disorders are sub-acute and acute ruminal acidosis, laminitis, ketosis, fatty liver, displaced abomasum, milk fever, downer cow, retained placenta, liver abscesses, metritis, mastitis and bloat. furthermore, the occurrence of one metabolic disorder is highly correlated with the occurrence of another one (Suthar *et al*., 2013; Ametaj, 2017). Ametaj (2017) noticed that ruminal acidosis in subacute and acute forms often occurs in high-producing dairy cows in the first month of lactation and cows with a high intake of dry matter, while ketosis is generally a result of negative energy balance (usually due to increased daily milk production). Since the occurrence of subclinical disorders frequently results in high economic losses caused by decreased production, decreased reproduction and increased involuntary culling as well as therapy costs (Suthar *et al*., 2013), it is essential to notice and prevent disorders in the subclinical phase before the development of clinical signs and possible occurrence of some other disorder. Hu *et al.* (2021) stated that the various metabolites can indicate the status of living cells. For instance, metabolites in the blood can indicate the physiological and biochemical state of the organism of a dairy cow, so it is often used to detect the occurrence of some disorders/diseases in the animal. Furthermore, different metabolites in milk can also indicate the metabolic and healthy status of an animal. Therefore, these metabolites could be used for the prevention of disorders/diseases occurrence.

This research aimed to determine the variability of biochemical parameters in blood plasma and milk of Holstein cows regarding the risk of metabolic disorder occurrence (based on F/P ratio classes) separately for each sampling month (May, June, July).

MATERIALS AND METHODS

During the research, blood and milk were sampled from 75 high-yielding Holsteins reared on a dairy cattle farm in East Croatia. Blood was taken from the animals' coccygeal veins into tubes with lithium heparin anticoagulant (Becton Dickinson, Plymouth, England, UK). In the laboratory, samples were centrifuged (1.500 g/10 min at 4°C) and plasma was separated and frozen at -80°C until analyses. Furthermore, milk was taken into clean tubes, centrifuged (12.000 g/30 min at 4°C) and milk plasma was separated and stored at -80°C until analyses. Using the automatic clinical chemistry analyser Beckman Coulter AU400 (Beckman Coulter, Germany) biochemical parameters were determined in prepared samples. The concentration of β-hydroxybutyrate (BHB) was determined by the enzymatic colourimetric method (using commercial kits, Randox Laboratories Ltd, Crumlin, UK).

Furthermore, test-day records (obtained in regular milk recording that performs accordingly to the AT4 method) of selected Holsteins were taken from the central database of HAPIH (Croatian Agency for Agriculture and Food). During the preparation of data, test-day records were logically controlled accordingly to the ICAR guidelines (ICAR, 2017). Concerning fat/protein ratio (F/P), cows were divided into three classes: F/P < 1.1, acidosis risk; F/P in [1.1, 1.5], normal; F/P > 1.5, ketosis risk. Tab. 1 presents the basic statics of daily milk yield and content of selected Holstein cows regarding the sampling month.

Tab. 1. Basic statistics of daily milk yield and content of selected Holstein cows regarding the sampling month

Trait	N	Mean	SD	CV	Minimum	Maximum
May						
DMY, kg	30	39.36	9.48	24.10	18.60	59.80
DFC, %	30	3.60	1.09	29.68	1.99	6.94
DPC, %	30	3.34	0.34	10.28	2.70	4.11
F/P ratio	30	1.10	0.31	27.89	0.56	1.94
June						
DMY, kg	26	38.36	8.94	23.31	24.20	54.80
DFC, %	26	3.92	1.10	27.96	2.12	7.56
DPC, %	26	3.42	0.25	7.38	3.02	4.07
F/P ratio	26	1.15	0.34	29.45	0.65	2.48
July						
DMY, kg	19	40.50	8.70	21.48	19.90	55.70
DFC, %	19	3.93	1.42	36.26	2.15	8.61
DPC, %	19	3.41	0.37	10.91	2.66	4.26
F/P ratio	19	1.18	0.55	46.64	0.58	3.24

*DMY – daily milk yield; DFC – daily fat content; DPC – daily protein content; F/P – fat/protein

The variability of biochemical parameters in blood plasma and milk due to fat/protein classes were tested using least square means in GLM procedure in SAS (SAS Institute Inc., 2019) separately for each sampling month (May, June, July). Following statistical model was used:

$$y_{ijkl} = \mu + b_1(d_i/305) + b_2(d_i/305)^2 + b_3\, ln(305/d_i) + b_4\, ln^2(305/d_i) + P_j + D_k + e_{ijkl}$$

Where:
y_{ijkl} = estimated biochemical parameter in blood serum or milk;
μ = intercept;
b_1, b_2, b_3, b_4 = regression coefficients (lactation curve by Ali and Schaeffer, 1987);
d_i = days in milk i (i = 11 to 537 day);
P_j = fixed effect of parity i (i = II., III+),
D_k = fixed effect of fat/protein ratio classes k (k = acidosis risk / normal / ketosis risk),
e_{ijkl} = residual.
Tukey-Kramer's studentized range test in GLM procedure in SAS (SAS Institute Inc., 2019) were applied in order to test the significance ($p < 0.05$) of the differences in biochemical parameters in blood plasma and milk due to fat/protein ratio classes separately for each sampling month (May, June, July).

RESULTS AND DISCUSSION

The effects included in the used statistical model (lactation stage, parity, and F/P ratio class statistically significantly ($P < 0.01$) affected values of biochemical parameters in blood plasma and milk of selected Holsteins. The variability of the biochemical parameters in blood plasma regarding the metabolic disorder risk (F/P ratio classes) separately for each sampling month (May, June, July) is presented in Table 2. Differences in the values of the biochemical parameters in blood plasma regarding the risk of metabolic disorder separately for each month of sampling (May, June, July) were found, although they were not statistically significant ($p > 0.05$).
The value of aspartate aminotransferase (AST) in blood plasma was highest in cows with acidosis risk. Also, the decreasing trend of AST was observed in cows in normal status and in acidosis risk regarding the sampling month. Similarly, the highest value of γ-glutamyl transferase (GGT) in blood plasma was determined in animals at risk of acidosis with an increasing trend regarding the month of sampling. The concentration of glucose in blood plasma also varied regarding the sampling month with the lowest values observed in June while the highest concentration was measured in the blood plasma of cows at ketosis risk in May. Furthermore, the highest concentration of urea in May and June was in healthy cows, while in July the highest urea concentration was determined in the blood plasma of cows at acidosis risk. The highest concentration of protein was observed in the blood plasma of cows at risk of ketosis in May and June, while in July highest protein concentration was in normal cows. Regarding the albumin concentration, the lowest value was observed in June in cows at ketosis risk. The lowest concentration of triglyceride was observed in acidosis-risk animals, while the lowest β-hydroxybutyrate was in June in ketosis-risk cows. The highest concentration of Fe in blood plasma was determined in acidosis-risk animals, while the highest value of Ca was observed in ketosis-risk cows in May.

Tab. 2. LSmeans of the biochemical parameters in blood plasma regarding fat/protein ratio classes separately by sample month (May, June, July)

Trait	**Month**	**Fat/protein ratio**		
		< 1.1 Acidosis risk	**1.1 – 1.5 Normal status**	**> 1.5 Ketosis risk**
Aspartate amino transferase (u/l)	May	144.195 ^{A}	132.912 ^{A}	88.996 ^{A}
	June	174.313 ^{A}	157.299 ^{A}	80.525 ^{A}
	July	129.657 ^{A}	107.023 $^{\acute{A}}$	137.727 ^{A}
γ-glutamil transferase (u/l)	May	32.489 ^{A}	24.715 ^{A}	26.628 ^{A}
	June	36.573 ^{A}	34.665 ^{A}	24.934 ^{A}
	July	41.789 ^{A}	34.335 ^{A}	37.226 ^{A}
Glucose (mmol/l)	May	3.195 ^{A}	3.381 ^{A}	3.621 ^{A}
	June	2.533 ^{A}	2.734 ^{A}	2.447 ^{A}
	July	3.292 ^{A}	3.206 ^{A}	3.208 ^{A}
Urea (mmol/l)	May	4.354 ^{A}	4.689 ^{A}	3.478 ^{A}
	June	4.614 ^{A}	4.767 ^{A}	4.297 ^{A}
	July	4.599 ^{A}	4.340 ^{A}	2.902 ^{A}
Protein (g/l)	May	85.717 ^{A}	83.626 ^{A}	86.235 ^{A}
	June	82.799 ^{A}	80.230 ^{A}	83.341 ^{A}
	July	86.700 ^{A}	88.751 ^{A}	79.283 ^{A}
Albumin (g/l)	May	32.170 ^{A}	32.499 ^{A}	32.593 ^{A}
	June	31.889 ^{A}	33.129 ^{A}	29.385 ^{A}
	July	32.229 ^{A}	32.409 ^{A}	25.964 ^{A}
Triglyceride (mmol/l)	May	0.112 ^{A}	0.122 ^{A}	0.130 ^{A}
	June	0.113 ^{A}	0.115 ^{A}	0.131 ^{A}
	July	0.107 ^{A}	0.117 ^{A}	0.107 ^{A}
β-hydroxybutyrate (mmol/l)	May	0.584 ^{A}	0.574 ^{A}	0.464 ^{A}
	June	0.451 ^{A}	0.445 ^{A}	0.338 ^{A}
	July	0.366 ^{A}	0.515 ^{A}	0.455 ^{A}
Fe (µmol/l)	May	26.892 ^{A}	24.105 ^{A}	17.551 ^{A}
	June	24.390 ^{A}	23.976 ^{A}	11.911 ^{A}
	July	23.647 ^{A}	23.224 ^{A}	21.325 ^{A}
Ca (mmol/l)	May	2.303 ^{A}	2.240 ^{A}	2.332 ^{A}
	June	1.983 ^{A}	2.055 ^{A}	1.949 ^{A}
	July	2.174 ^{A}	2.174 ^{A}	2.172 ^{A}

** Values within the same row marked with different letter differ statistically significant (P<0.05)*

The values of the biochemical parameters in milk regarding the risk of metabolic disorder separately for each month of sampling (May, June, July) are presented in Table 2. Differences in the values of the biochemical parameters in milk regarding the metabolic disorder risk were found, and were statistically significant ($p < 0.05$) only for AST and albumin concentration. The highest concentration of AST was determined in the milk of ketotic-risk cows in June, while the highest value of GGT was observed in ketotic-risk cows in July. The concentration of glucose was highest in the milk of acidosis-risk and lowest in ketosis-risk cows. A similar trend regarding the risk of metabolic disorders was also observed for the value of urea in milk with the highest values determined in June. The lowest determined concentration of protein and albumin was in the milk of ketotic-risk cows in May. Furthermore, in the same animals, the lowest concentration of Fe and Ca in milk were observed.

Tab. 3. LSmeans of the biochemical parameters in blood plasma regarding fat/protein ratio classes separately by sample month (May, June, July)

Trait	**Month**	**Fat/protein ratio**		
		< 1.1 Acidosis risk	**1.1 – 1.5 Normal status**	**> 1.5 Ketosis risk**
Aspartate amino transferase (u/l)	May	7.024 ^{A}	9.486 ^{A}	10.829 ^{A}
	June	19.153 ^{A}	20.786 ^{A}	43.134 ^{A}
	July	15.399 ^{A}	19.771 ^{A}	15.376 ^{A}
γ-glutamil transferase (u/l)	May	279.661 ^{A}	315.916 ^{A}	332.696 ^{A}
	June	308.205 ^{A}	394.518 ^{B}	375.193 ^{AB}
	July	342.663 ^{A}	442.823 ^{A}	468.324 ^{A}
Glucose (mmol/l)	May	0.562 ^{A}	0.457 ^{A}	0.411 ^{A}
	June	0.560 ^{A}	0.537 ^{A}	0.288 ^{A}
	July	0.621 ^{A}	0.434 ^{A}	0.407 ^{A}
Urea (mmol/l)	May	5.168 ^{A}	5.730 ^{A}	3.934 ^{A}
	June	6.402 ^{A}	5.989 ^{A}	5.556 ^{A}
	July	4.752 ^{A}	4.546 ^{A}	3.467 ^{A}
Protein (g/l)	May	30.676 ^{A}	34.662 ^{A}	28.382 ^{A}
	June	40.354 ^{A}	39.059 ^{A}	38.418 ^{A}
	July	35.507 ^{A}	35.315 ^{A}	40.450 ^{A}
Albumin (g/l)	May	20.686 ^{A}	23.129 ^{A}	18.494 ^{B}
	June	23.129 ^{A}	23.242 ^{A}	22.255 ^{A}
	July	23.020 ^{A}	22.182 ^{A}	23.818 ^{A}
Fe (μmol/l)	May	11.895 ^{A}	17.751 ^{A}	7.518 ^{A}
	June	30.939 ^{A}	27.002 ^{A}	16.412 ^{A}
	July	28.036 ^{A}	24.139 ^{A}	45.251 ^{A}
Ca (mmol/l)	May	2.768 ^{A}	3.067 ^{A}	2.658 ^{A}
	June	3.086 ^{A}	3.699 ^{A}	3.851 ^{A}
	July	3.460 ^{A}	3.546 ^{A}	3.425 ^{A}

** Values within the same row marked with different letter differ statistically significant (P<0.05)*

Dieho *et al*. (2016) pointed out that inadequate feeding management and imbalanced ration significantly raise the possibility of metabolic disorders occurrence. Furthermore, the occurrence of some metabolic disorders could result in variability of the concentration of biochemical parameters in cows' blood and milk. Determining the differences in the biochemical parameters in blood and milk concerning animal health could enable the detection and prevention of disorders/diseases development.

The results of the conducted analysis showed that the differences in the values of the biochemical parameters in blood plasma and milk regarding the metabolic disorder risk were present, but were statistically significant ($p < 0.05$) only for Aspartate amino transferase (AST) and albumin concentration in milk. Also, there were notable differences in all analysed parameters due to sampling months (May, June, and July). The determined effect of sampling month could be the consequence of variations in feeding management and microclimatic conditions on the farm. Similarly, fluctuations of catabolic liver enzymes in blood plasma vs milk as in this research were determined by Liu *et al*. (2012). The same authors reported higher GGT and lower AST concentrations in milk than in blood plasma. Furthermore, the higher urea concentration in the blood serum, determined mainly in acidosis-risk cows, could indicate inefficient nitrogen utilization. For instance, Stefanska *et al*. (2020) reported that in cows with low rumen pH, milk urea nitrogen rises significantly. Although β-hydroxybutyrate (BHB) did not vary significantly in this research (variability was determined regarding the indication of metabolic disorder and the month of sampling, and the highest values did not undoubtedly indicate only subclinical ketosis), Guan *et al*. (2020) emphasised that the elevated BHB levels are mainly related to ketosis in dairy cows. The obtained results pointed out the lowest concentrations of protein, albumin, Fe and Ca in the milk, as well as the lowest concentrations of Fe in the blood plasma of ketotic-risk cows. Tsukano and Suzuki (2020) noted that the differences in the blood Fe concentration could be an indicator of the same inflammatory disease.

CONCLUSIONS

This work aimed to determine the variability of biochemical parameters in blood and milk samples due to the classes of fat/protein ratio (which indicates the risk of metabolic disorders) regarding the sampling month (May, June, and July). The effects included in the used statistical model (lactation stage, parity, and F/P ratio class statistically significantly ($P < 0.05$) affected values of biochemical parameters in blood plasma and milk of selected Holsteins. Also, the conducted analysis showed variability in the values of the biochemical parameters in both blood plasma and milk due to metabolic disorder risk and month of sampling. Accordingly, when different metabolites are used as an indicator of the metabolic disorder risk, correction for the systematic effects (stage of lactation and parity, sampling month) should be performed.

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UTJECAJ RIZIKA OD METABOLIČKIH POREMEĆAJA NA VARIJABILNOST BIOKEMIJSKIH PARAMETARA U KRVI I MLIJEKU KRAVA S OBZIROM NA MJESEC UZORKOVANJA

Rezime

U cilju utvrđivanja varijabilnosti biokemijskih parametara u krvnoj plazmi i mlijeku Holstein krava s obzirom na rizik od pojave metaboličkih poremećaja (na temelju razreda omjera F/P) zasebno za svaki mjesec uzorkovanja (svibanj, lipanj, srpanj), krv i mlijeko uzorkovani su iz 75 visokoproizvodnih krava Holstein pasmine uzgojanih na farmi mliječnih krava u istočnoj Hrvatskoj. Rizik od metaboličkog poremećaja definiran je u temeljem dnevnog omjer masti/protein (F/P) dobiven iz zapisa na kontrolni dan – test-day records (F/P < 1,1 što ukazuje na rizik od acidoze; F/P u [1.1, 1.5], normalan status životinje ; F/P > 1,5, što ukazuje na rizik od ketoze).
Provedena analiza pokazala je varijabilnost vrijednosti biokemijskih parametara u krvnoj plazmi i mlijeku uslijed razreda rizika od metaboličkih poremećaja i mjeseca uzorkovanja. U skladu s tim, kada se različiti metaboliti koriste kao pokazatelj rizika od metaboličkog poremećaja, potrebno je primijeniti korekciju za fiksne utjecaje (stadij laktacije i pariteta, mjesec uzorkovanja).

Ključne riječi: *biokemijski parametri, krv, mlijeko, metabolički poremećaji, Holstein*