XXVI INTERNATIONAL
ECO-CONFERENCE® 2022
21–23th SEPTEMBER

XII SAFE FOOD

PROCEEDINGS

NOVI SAD, SERBIA

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SAFE FOOD

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2022

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Original Scientific paper

THE VARIABILITY OF BIOCHEMICAL PARAMETERS IN BLOOD SERUM AND MILK OF HOLSTEIN COWS REGARDING THE AGE AND HEALTH STATUS

Abstract

In order to determine the variability of biochemical parameters, blood and milk were sampled from 25 Holstein cows for a period of three months. The differences in the analyzed parameters due to the animal's health status (risk of acidosis or ketosis or normal health status; defined according to the fat/protein ratio) and animal's age (parity classes) were determined. The results of this research indicate the variability in biochemical parameters in both blood serum and milk as a result of variance in animals' health status and due to various age of animal implying that animals' response depends on animal age. Obtained results should be considered in the modelling for the prediction of metabolic disorders occurrence.

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

INTRODUCTION

One of the most challenging livestock productions is dairy cattle farming because the farmer must examine a big quantity of data every day and understand how to react in time to prevent potential problems in production (Gantner, 2020; Gantner et al., 2021). Furthermore, to optimize the management and realization of the genetic potential of dairy cows, the farmer must be conscious of the genetic and environmental aspects of different characteristics of interest for milk production. The term effective

dairy cattle farming means gravidity and calving on an annual basis, with the transitional period and the beginning of lactation being the most stressful period in the production cycle of dairy cows (Gantner, 2020). Various factors, such as changes of ration, negative energy balance, reduced food intake, weight loss and hypocalcemia can cause sets of different disorders in this period (Ametaj, 2017). After giving birth and during the first month of lactation, the most common metabolic disorders that appear in dairy cows 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 (Ametaj, 2017). Additionally, it is significant to point out that the occurrence of one metabolic disorder is highly associated with another one (Suthar et al., 2013; Ametaj, 2017). Accordingly, cows impacted by milk fever are more prone to mastitis, retained placenta, metritis, ketosis; at the same time cows impacted by acidosis are more tending to laminitis, milk fever, mastitis, and fatty liver (Suthar et al., 2013; Ametaj, 2017). Ruminal acidosis in subacute and acute forms frequently occurs in high-producing dairy herds in early lactation and cows with a high intake of dry matter (Ametaj, 2017), while ketosis usually occurs as a result of negative energy balance (usually due to increased daily milk production). Since subclinical disorders produce high economic losses to dairy farmers through therapy costs, decreased production, decreased reproduction characteristics and increased involuntary culling (Suthar et al., 2013), it is very important to detect and prevent disorders in the subclinical phase.

The purpose of this study was to determine the effect of cows age (parity) on the variability of biochemical parameters in blood serum and milk of Holsteins regarding the cow's health status (based on F/P ratio classes).

MATERIAL AND METHODS

The study was conducted in the east of Croatia on an indoor dairy cattle farm. The cows included in the research were healthy and did not have any disorders. The cows were fed with a total mixed ration with the addition of mixture accordingly to the daily milk production. During the three months of research, blood and milk was sampled, on monthly basis, from 25 Holstein cows (mean milk production near 40 kg/day, Table 1). Blood samples were taken from the coccygeal vein into tubes with lithium heparin anticoagulant (Becton Dickinson, Plymouth, England, UK) and centrifuged (1.500 g/10 min at 4°C) in order to separate the plasma. Milk samples were taken into clean tubes and centrifuged (12.000 g/30 min at 4°C) for plasma separation. The determination of the biochemical parameters in blood and milk plasma was performed operating an automatic clinical chemistry analyzer Beckman Coulter AU400 (Beckman Coulter, Germany). The concentration of β-hydroxybutyrate (BHB) was determined utilizing commercial kits (Randox Laboratories Ltd, Crumlin, UK) following the enzymatic colorimetric method. The test-day records of animals included in the research, obtained during the regular milk recording, were taken from the central database of the Croatian Agency for Agriculture and Food. Test-day records were corrected accordingly to the ICAR guidelines (2017). With reference to the F/P value, records

were divided into three classes: F/P ratio < 1.1 (acidosis risk); F/P ratio in [1.1, 1.5] (normal status); F/P ratio > 1.5 (ketosis risk). Table 1 presents basic statistical parameters of daily production (daily milk yield, daily fat and protein content along with F/P ratio) accordingly to the parity classes (cows in II. parity and cows in III. and higher parities).

Table 1. Basic statistical parameters of daily production traits of Holstein cows

Trait	N	Mean	SD	Minimum	Maximum
II. parity					
DMY, kg	28	38.79	8.33	18.60	55.70
DFC, %	27	3.50	0.94	2.12	6.94
DPC, %	27	3.34	0.25	2.70	3.81
F/P ratio	27	1.05	0.28	0.65	1.94
III.+ parity					
DMY, kg	47	39.61	9.48	19.90	59.80
DFC, %	47	4.02	1.26	1.99	8.61
DPC, %	47	3.41	0.36	2.66	4.26
F/P ratio	47	1.19	0.43	0.56	3.24

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

The variability of biochemical parameters due to F/P ratio classes separately for each parity class (II., and III. +) was tested using the least square means in the GLM procedure in SAS (SAS Institute Inc., 2019). The following statistical model was used:

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

Where:

y_{ijkl} = estimated biochemical parameters;
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 = 11 to 345 day);
M_j = fixed effect of experiment month k (k = May, June, July),
D_k = fixed effect of F/P ratio classes (acidosis risk, normal status, ketosis risk),
e_{ijkl} = residual.
Scheffe's multiple comparisons in PROC GLM (SAS) were used to test the significance
($p < 0.05$) of the differences in biochemical parameters due to F/P ratio classes.

RESULTS

The values of the biochemical parameters in the blood serum due to F/P ratio classes separately for each parity class are presented in Table 2. The highest values of γ-glutamil transferase (GGT), urea (UREA), β-hydroxybutyrate, Fe and Ca were determined in cows in the second lactation with an F/P ratio lower than 1.1 that is in cows at risk of acidosis. The lowest values of protein (PRO), albumin (ALB) and Fe were observed in cows in second parity in ketosis risk (F/P > 1.5). The concentration of triglyceride in blood plasma did not show variability due to the F/P ratio regardless of the parity class. The concentration of all biochemical in blood serum, with exception of glucose, was higher in older cows at risk of acidosis prevalence. Furthermore, almost all biochemical parameters (except urea, β-hydroxybutyrate, and Ca) were lower in older cows at risk of ketosis occurrence.

Table 2. LSmeans of the biochemical parameters in blood serum in regard to F/P ratio classes separately for each parity class

Trait	**F/P ratio < 1.1**		**F/P ratio in [1.1, 1.5]**		**F/P ratio > 1.5**	
	Acidosis risk		**Normal status**		**Ketosis risk**	
Parity	**II.**	**III.+**	**II.**	**III.+**	**II.**	**III.+**
Aspartate amino transferase (U/L, AST)	143.51	152.16[a]	176.36	122.61[a]	148.31	63.20[b]
γ-glutamil transferase (U/L, GGT)	33.18	39.59	31.62	31.05	32.42	25.96
Glucose (mmol/L, GUK)	3.00	2.96	3.10	3.16	3.21	2.85
Urea (mmol/L, UREA)	4.41	4.58	3.88	4.79	3.07	4.30
Protein (g/L, PRO)	84.59	84.67	84.82	83.95	82.35	87.78
Albumin (g/L, ALB)	31.70	32.23	32.13	32.61	30.88	29.41
Triglyceride (mmol/L, TGC)	0.12	0.11	0.11	0.12	0.11	0.13
β-hydroxybutyrate (mmol/L, BHB)	0.47	0.47	0.31	0.59	0.36	0.41
Fe (μmol/L)	24.48	25.04	25.08	23.53	20.33	12.90
Ca (mmol/L)	2.13	2.20	2.11	2.17	2.10	2.15

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

The variability of the biochemical parameters in milk regarding F/P ratio classes separately for each parity class is presented in Table 3. The highest value of GGT and the lowest values of glucose, urea, albumin, Fe and Ca in milk were determined in cows in the second lactation that was at risk of ketosis prevalence. Also, the highest

values of aspartate aminotransferase (AST), glucose and Ca in milk were observed in younger cows in normal status. Furthermore, older cows with acidosis risk had a higher concentration of GGT, glucose, urea, protein, albumin, and Fe than the younger ones. On the other hand, older cows at ketosis risk had a lower concentration of GGT, glucose, protein, and Fe than the younger animals implying that animal's age, besides the individual health status, affects the concentration of biochemical parameters both in blood serum and milk.

Table 3. LSmeans of the biochemical parameters in milk in regard to F/P ratio classes separately for each parity class

Trait	**F/P ratio < 1.1**		**F/P ratio in [1.1, 1.5]**		**F/P ratio > 1.5**	
	Acidosis risk		**Normal status**		**Ketosis risk**	
Parity	**II.**	**III.+**	**II.**	**III.+**	**II.**	**III.+**
Aspartate amino transferase (U/L, AST)	13.99	13.12[a]	17.66	16.56[a]	16.39	33.17[b]
γ-glutamil transferase (U/L, GGT)	312.46	322.87	374.49	377.08	419.42	354.25
Glucose (mmol/L, GUK)	0.57	0.69[a]	0.61	0.43[a]	0.53	0.27[b]
Urea (mmol/L, UREA)	5.33	5.58	4.84	5.61	3.74	4.85
Protein (g/L, PRO)	35.48	36.22	34.81	37.07	34.86	34.48
Albumin (g/L, ALB)	22.28	22.68	21.85	23.21	21.04	21.17
Fe (μmol/L)	22.80	27.50	21.24	26.43	17.30	14.53
Ca (mmol/L)	3.16	3.10	3.37	3.39	3.15	3.41

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

DISCUSSION

The inadequate feeding management and imbalanced ration (regarding the forage to concentrate ratio) accompanying the reduced absorption capacity of short-chain fatty acids through the rumen due to underdeveloped rumen papillae after parturition significantly increases the probability of metabolic disorders prevalence (Dieho et al., 2016). The occurrence of some metabolic disorders frequently results in variations in the concentration of biochemical parameters both in the blood and milk of dairy cows. Understanding the essentials underlying the development of metabolic disorders by using biochemical parameters of blood and milk to assess animal health could improve disease prevention.

The aspartate aminotransferase (AST) and γ-glutamyl transferase (GGT) as important catabolic enzymes play a significant role in proper animal liver function. Similarly like in this research, Liu et al. (2012) determined that GGT enzyme concentrations in milk were higher than in blood plasma, while in the case of AST

concentrations were the opposite (higher in blood plasma). The highest concentration of urea in the blood serum and milk of animals at risk of acidosis could indicate inefficient utilization of nitrogen from food. Another useful parameter is milk urea nitrogen (MUN) because it shows nitrogen metabolism during the whole 24 hours. Stefanska et al. (2020) pointed out that in cows with low rumen pH, MUN rises significantly.

The results of this research indicate the differences in biochemical parameters in both blood serum and milk due to animals' health status and due to animal age implying that animals' response depends on animal age. Obtained should be considered in the modelling for the occurrence prediction of metabolic diseases.

CONCLUSIONS

The obtained results indicate the variability in biochemical parameters in both blood serum and milk due to animals' health status (classes of F/P ratio that is acidosis or ketosis risk) and due to animal age (parity classes) implying that animals' metabolism and response to feeding management and production needs depends on animal age. Obtained should be considered in the modelling for the occurrence prediction of metabolic diseases.

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Оригинални научни рад

ВАРИЈАБИЛНОСТ БИОХЕМИЈСКИХ ПАРАМЕТАРА У КРВНОМ СЕРУМУ И МЛЕКУ ХОЛСТЕИН КРАВА У ОДНОСУ НА СТАРОСТ И ЗДРАВСТВЕНО СТАЊЕ

Сажетак

У циљу утврђивања варијабилности биохемијских параметара узорковани су крв и млеко од 25 холштајн крава у трајању од три месеца. Утврђене су разлике у анализираним параметрима у зависности од здравственог статуса животиње (ризик од ацидозе или кетозе или нормалног здравственог стања; дефинисано према односу масти/протеина) и старости животиње (паритетне класе). Резултати овог истраживања указују на варијабилност биохемијских параметара у крвном серуму и млеку као резултат варијансе здравственог стања животиња и због различите старости животиња, што имплицира да одговор животиња зависи од старости животиње. Добијене резултате треба узети у обзир при моделирању за предвиђање појаве метаболичких поремећаја.

Кључне речи: *Холштајн краве, биохемијски параметри, млеко, крв, метаболички поремећаји*

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