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Full Research Article

ACID-BASE, ELECTROLYTE AND OXIDATIVE STATUS IN DAIRY COWS AT DIFFERENT STAGES OF THE PRODUCTION CYCLE

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Abstract

Previous studies in the field of acid-base and oxidative status in cows have mainly focused on the transition period (three weeks before and three weeks after calving). The aim of this study was to determine the differences in the parameters of acid-base and oxidative status and electrolyte balance in cows not only during the transition period, but also at other stages of the production cycle. Holstein-Friesian cows were divided into four numerically equal groups (n = 6): early lactating cows (9±2 days in milk - DIM), peak lactating cows (50±5 DIM), late lactating cows (170±10 DIM) and dry cows (10±1 days before calving). Venous blood samples were taken from the cows to analyze acid-base status, electrolyte concentrations and oxidative stress parameters, and to compare group means. Significantly higher pH was observed in early lactating cows than in late lactating cows. Sodium (Na^+) concentration was significantly lower in early lactating and peak lactating cows compared to dry cows, while chloride (Cl^-) concentration was also lower in late lactating cows compared to dry cows. Plasma glutathione peroxidase (GSH-Px) activity was higher in early lactating cows compared to peak lactating cows. Thus, the highest blood pH, lowest Na^+ and Cl^- concentrations

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and highest serum GSH-Px activity were observed in early lactating cows compared to other groups, indicating the highest metabolic and oxidative stress during this period. In conclusion, it would be useful to consider the inclusion of these parameters in standard health assessment procedures in intensive dairy production.

Key words: acid-base, dairy cows, oxidative status

INTRODUCTION

In dairy science, metabolic profile testing and body condition scoring (BCS) are the commonly used approaches to assess the health, milk production, reproductive status and feed balance of dairy cattle herds (Roche et al., 2009; Horvat et al., 2014; Nozad et al., 2014). Recently, monitoring of acid-base status is also considered an important procedure in assessing the health of dairy cows (Gärtner et al., 2019).

Acid-base balance is essential for most physiological processes in dairy cows. Several mechanisms are involved in maintaining the acid-base balance: chemical buffer systems, and pulmonary and renal regulatory mechanisms (Wagner et al., 2019). The fixed ions (Na^+, K^+ and Cl^-) also determine acid-base status, with higher anion intake causing acidosis, while higher cation intake causes alkalosis (Afzaal et al., 2004). Alkalosis is known to decrease Ca^{++} concentration, predisposing cows to milk fever or subclinical hypocalcemia, especially during the first few days in milk (DIM) (Goff, 2008; Caixeta et al., 2017).

Also, intensive dairy production is accompanied by increased nutritive demands that are associated with increased oxygen requirement. This creates conditions for the generation of reactive oxygen species (ROS), which in the case of depletion of antioxidative capacities, can lead to oxidative damages of biomolecules and disruption of physiological integrity (Sharma et al., 2011; Halliwell and Gutteridege, 2015).

In the scientific literature, the value of standard parameters to determine the metabolic profile has been reported for each lactation phase and month (Nozad et al., 2014). However, previous research in the area of the acid-base status, electrolyte balance and oxidative status is focused on a narrow period around calving, the transition period (the period between 3 weeks before and 3 weeks after calving), while there is lack of data in the literature regarding the entire production cycle. Considering the role of acid-base homeostasis, electrolyte balance and antioxidant mechanisms in maintaining the physiological integrity of dairy cows throughout lactation, it would be important to study their status at different stages of the production cycle, as this will help to determine the critical phases. Based on this data, timely measures could be taken to prevent serious disturbances in acid-base balance, antioxidant protection and other associated pathological conditions.

The aim of this study was to determine differences in the parameters of acid-base status, blood electrolyte concentrations and antioxidant status in cows at different stages of the production cycle.

MATERIALS AND METHODS

The research was conducted during February 2020 on the Padinska Skela dairy farm, AlDahra Corporation, near Belgrade, Serbia.

Animals and Experimental Design. Twenty-four clinically healthy, multiparous Holstein-Friesian cows (aged 4-7 years) were chosen for the research and, according to stages of production cycle, were divided into four numerically equal groups (n=6). The first group (n=6) included early lactating cows (EL-cows; 9±2 DIM); the second group (n=6) included peak lactating cows (PL-cows; 50±5 DIM); the third group (n=6) included late lactating cows (LL-cows; 170±10 DIM), and; the fourth group (n=6) included dry cows (Dry-cows; 10±1 days before calving). The experimental cows belonged to the same farm, where they were housed in the same conditions (tie-stall barn) and fed twice daily (at 6.30 a.m. and 5.30 p.m.) with total mixed ration (TMR) that varied in quantity and quality according to the stage of the production cycle. In addition, the feed of EL- and PL-cows was supplemented with sodium bicarbonate (6 g/kg of DM) (Table 1). Water was available *ad libitum*. Cows were milked three times a day (at 7.00 a.m., 12.00 a.m. and 6.00 p.m.). Cows included in this study produced an average milk yield of 9,000±500 liters in 305 days in the previous lactation.

The animal-related component of the study was approved by the Ethical Committee of the Faculty of Veterinary Medicine, University of Belgrade (No. 323-07-11720/2020-05/1), Serbia, in accordance with the National Regulation on Animal Welfare.

Blood Sampling and Analyses of Acid-Base Status, Electrolyte Balance and Oxidative Status. Blood samples were taken from each animal on one day only. Two blood samples were collected from the jugular vein of each cow to study acid-base status and oxidative stress parameters. Blood samples for examination of acid-base status were collected anaerobically using 2.0-mL syringes containing electrolyte balanced heparin (PICO50^{TM}) with 18-G needles. All visible air bubbles were carefully expelled immediately after sampling and the tip of each syringe was capped after blood sampling. Within 3-5 minutes after collection, blood was analyzed using Wondfo Blood Gas Analyzer (China). In the analyses of acid-base status, the following parameters were measured: blood pH, partial pressure of carbon dioxide (pCO_2; mmHg), concentrations of L-lactate (Lac; mmol/L), ionized calcium (iCa^{++}; mmol/L), sodium (Na^+; mmol/L), potassium (K^+, mmol/L) and chloride (Cl^-; mmol/L). Concentrations of standard and actual bicarbonate (HCO_3-std and HCO_3-act; mmol/L), the base excess of extracellular fluid (BE-ecf; mmol/L), and the anion gap (AnGap; mmol/L) were calculated by the analyzer. Blood pH and pCO_2 were corrected according to the body temperature of the cow. In addition to the parameters of acid-base status, a Wondfo Blood Gas Analyzer also measured blood glucose concentration (mmol/L). Blood samples for the examination of one of the oxidative stress parameters (glutathione peroxidase – GSH-Px) were collected using 6.0-mL vacutainer tubes (BD Vacutainer®) with lithium heparin as the anticoagulant. Samples were subsequently centrifuged at 2000 g for 15 minutes and plasma was decanted and stored in Eppendorf tubes at

-20ºC until the determination of glutathione peroxidase activity. The determination of plasma GSH-Px activity was performed according to Günzler et al. (1974) using a spectrophotometer (Cecil 2000).

Table 1. Ingredients and chemical composition of the daily ration of cows at different stages of the production cycle

Item	Diet			
	EL- cows[1]	PL-cows[2]	LL-cows[3]	Dry-cows[4]
Ingredient (g/kg of DM)				
Maize silage	302	328	328	508
Alfalfa (Lucerne) silage		81	81	
Grass silage wet	175			79
Ryegrass silage		66	66	
Wheat straw		28	28	190
Alfalfa (Lucerne) hay		70	70	
Grass hay	124			
Brewers grains	47			
Ground maize	87	78	78	34
Triticale	50	78	78	34
Barley	53	78	78	34
Sunflower seed meal 33% CP		48	48	36
Soybean meal 44% CP	40	89	89	48
Soybean whole, extruded	45			
Dried beet pulp	51	19	19	18
Topsan®[5]		8	8	
Multisan Nektar®[6]		5	5	
DextroFat SC®[7]		18	18	
Sodium bicarbonate	6	6		
*Vitamin-mineral premix	19			14
Calcium	6.2	12.3	12.3	2.7
Phosphorus	3.1	3.8	3.8	2.7
Propylene glycol	12			
Chemical analysis				
Crude protein, g/kg of DM	161	164.5	164.5	138
Crude fat, g/kg of DM	21	27.1	27.1	28
NEL[8], MJ/kg of DM	7.3	7.1	7.1	6.7
ADF[9], g/kg of DM	221	196	196	241
NFC[10], % DM	31.8	24.3	24.3	30.6
Starch % DM	22.52			22.46
Ash % DM	6.91			6.89

[1]Early lactating cows (EL-cows; 9±2 days in milk (DIM)); [2]Peak lactating cows (PL-cows; 50±5 DIM);
[3]Late lactating cows (LL-cows; 170±10 DIM); [4]Dry cows (Dry-cows; 10±1 days before calving);
[5]Vitamin-mineral premix for dairy cows at very high performance levels;
[6]Cocktail sugar components for synchronization and optimization of rumen metabolism;
[7]Combination of rumen-protected sugars and fats for improving the energy balance of high performing cows;
[8]Net Energy Lactation; [9]Acid Detergent Fiber; [10]Non-fibre carbohydrates

Body condition score. Body condition score (BCS) was observed on the day of blood sampling by two trained evaluators based on the 1 to 5 scale, using increments of 0.25, described by Ferguson et al. (1994). The values they obtained separately coincided in most of the cases. However, when the values obtained by the evaluators differed, a third evaluator was involved and their scoring was accepted as final. Scores obtained by evaluators did not differ more than 0.25 points.

Statistical analysis. The statistical analysis of the obtained results was performed by calculation of the mean values (X) and standard deviations (SD) for each of the observed parameters. Determination of statistical significance among observed parameters was performed using Student's t test. The results in this research were statistically processed using the software STATISTICA 8 (StatSoft, SAD). The criterion for statistical significance was established as $p<0.05$.

RESULTS

Mean results for the observed parameters in groups of cows at different stages of the production cycle are presented in Table 2.

Table 2. Mean values of observed acid-base and oxidative status parameters in cows at different stages of the production cycle

Parameters[1]	EL-cows[2]	PL-cows[3]	LL-cows[4]	Dry-cows[5]
Blood pH	7.548±0.023^{a}	7.531±0.020^{ab}	7.517±0.024^{b}	7.524±0.041^{ab}
pCO_2 (mmHg)	40.10±2.28^{a}	43.23±3.37^{a}	42.97±3.81^{a}	40.38±3.99^{a}
L-lactate (mmol/L)	0.300±0.00^{a}	0.392±0.15^{a}	0.425±0.16^{a}	0.315±0.03^{a}
HCO_3-act (mmol/L)	34.95±3.22^{a}	36.05±3.78^{a}	34.65±3.16^{a}	33.00±1.84^{a}
HCO_3-std (mmol/L)	34.57±3.25^{a}	35.02±3.86^{a}	33.67±3.10^{a}	32.28±1.96^{a}
BE-ecf (mmol/L)	12.53±3.55^{a}	13.35±4.00^{a}	11.75±3.28^{a}	10.20±2.12^{a}
AnGap (mmol/L)	9.67±4.08^{a}	8.67±2.25^{a}	7.00±2.28^{a}	6.00±3.46^{a}
Na^+ (mmol/L)	138.50±1.38^{a}	139.00±1.79^{a}	139.17±2.71^{ab}	143.50±3.32^{b}
K^+ (mmol/L)	3.53±0.37^{a}	3.53±0.27^{a}	3.55±0.27^{a}	3.75±0.37^{a}
Cl^- (mmol/L)	97.67±4.08^{ab}	97.87±2.14^{a}	101.33±1.75^{b}	108.25±2.06^{c}
iCa^{++} (mmol/L)	1.19±0.11^{a}	1.22±0.04^{a}	1.10±0.23^{a}	1.25±0.11^{a}
GSH-Px (μKat)	253.67±30.03^{a}	200.01±12.83^{b}	209.30±21.99^{ab}	195.15±19.67^{ab}
Glucose (mmol/L)	3.47±0.43^{a}	3.40±0.79^{ab}	3.90±0.29^{ab}	4.23±0.28^{b}
BCS	3.29±0.33^{a}	2.71±0.19^{b}	3.00±0.37^{a}	3.45±0.20^{a}

[1]Concentrations of actual (HCO_3-std; mmol/L) and standard (HCO_3-std; mmol/L) bicarbonate; the base excess of extracellular fluid (BE-ecf; mmol/L); the anion gap (AnGap; mmol/L); glutathione peroxidase activity (GSH-Px; μKat); body condition score (BCS, unitless).
[2]Early lactating cows (EL-cows; 9±2 days in milk (DIM)); [3]Peak lactating cows (PL-cows; 50±5 DIM);
[4]Late lactating cows (LL-cows; 170±10 DIM); [5]Dry cows (Dry-cows; 10±1 days before calving);
[a,b,c] – values in the same rows with different superscripts are significantly different ($p<0.05$)

The blood pH was highest in EL-cows compared to cows at other stages of the production cycle, but a statistically significant difference for this parameter was found only between EL-cows and LL-cows. In contrast, no significant differences in pCO_2 or L-lactate concentration were found between cows in different stages of the production cycle. Our results show the concentrations of Na^+ and Cl^- increased as lactation progressed. Na^+ concentration was significantly lower in EL- and PL-cows compared to Dry-cows, while Cl^- concentration was significantly lower in EL-, PL- and LL-cows compared to Dry-cows. A similar trend was observed in K^+ concentration but without statistical significance. Concentrations of iCa^{++}, HCO_3-act and HCO_3-std and values of the BE-ecf and AnGap were not statistically significantly different in cows at different stages of the production cycle. Plasma GSH-Px activity was highest in EL-cows, and this difference was statistically significant compared to PL-cows. Blood glucose concentration was significantly lower in EL-cows compared to Dry-cows. BCS was lowest in PL-cows, and was significantly different compared to cows at other stages of the production cycle.

DISCUSSION

The mean blood pH in EL-cows (9±2 DIM) and PL-cows (50±5 DIM) was not in agreement with the results obtained in a study conducted by Gärtner et al. (2019), who studied cows that were from 1 to 15 and 43 to 76 DIM. In the aforementioned study, the average blood pH was in the range of 7.38-7.40, while in our study, the mean blood pH was higher than 7.50 (alkalemia) in all examined groups of dairy cows. Given that we used venous blood for acid-base analyses in our study, as did Gärtner et al (2019) in their study, the type of blood sample could be excluded as a factor that could potentially have caused differences between our study and theirs (in terms of blood pH). Also, the current study was conducted during winter, which excludes the possibility that increasing loss of CO_2 by pulmonary ventilation led to increase of blood pH. This loss of CO_2 is characteristic for periods with high daily temperatures and heat stress (West, 2003). However, the concentration of HCO_3-std (30.00-41.40 mmol/L, mean of 34.79 mmol/L) in the cows in our study was higher than those given by Gärtner et al. (2019) in cows at identical stages of the lactation cycle (27.00-28.80 mmol/L, mean of 27.90 mmol/L), which may explain the presented differences in blood pH. In addition, the results in our study show high values of BE-ecf, which in combination with high concentrations of HCO_3-std, indicates that the cows examined in our study were in metabolic alkalosis independently of production cycle stage. In support of this are the average values of the AnGap (8.00±3.10 mmol/L) obtained

in our study in cows at all stages of the production cycle, and the average value of the pCO_2 that was 41.79±3.35 mmHg and coincided with the pCO_2 values (42.68±8.34 mmHg) published by Fagnani et al. (2014) obtained from 67 clinically healthy cows. Interestingly, high concentrations of HCO_3-std were present in cows at all stages of the production cycle, especially when considering the fact that individual variations were minimal, as evidenced by the low standard deviation (SD) values. Considering the consistent values of standard bicarbonates, the influence of renal mechanisms for the production and reabsorption of filtered bicarbonates should be excluded. However, the composition of feed can have a significant effect on the concentration of bicarbonate and consequently on the blood pH. Sarwar et al. (2007) showed the addition of sodium bicarbonate to feed results in an increase of serum bicarbonate concentration (26.5 mmol/L) and blood pH (average 7.52). Similarly, the results of our study indicate the EL- and PL-cows, which were supplemented with sodium bicarbonate, had numerically higher blood pH and bicarbonate concentrations compared to other groups of cows (Table 2). The addition of this supplement is very attractive in dairy production, because increasing sodium bicarbonate intake leads to increased dry matter intake (DMI) and has a positive effect on milk production and conception rates in dairy cows. Accordingly, differences between our results and those published by Gärtner et al. (2019) could potentially be explained by the influence of environment and feed on the analyzed parameters of acid-base status. Unfortunately, Gärtner et al. (2019) did not show data related to feed of the cows in their study, and consequently, a detailed analysis cannot be performed.

The results of our study show mean Na^+ and Cl^- concentrations in the examined cows both statistically significantly differed at different stages of the production cycle, while significant differences in K^+ concentrations were not noted. These findings are in accordance with the reports of Dodomani et al. (2009) and Skrzypczak et al. (2014), but they are different from reports of Saleem et al. (2009), who did not find significant differences in Na^+ and Cl^- concentrations in cows at different stages of the production cycle. In contrast, Saleem et al. (2009) showed that Na^+ and Cl^+ concentrations were consistent during the lactation cycle. The study by Saleem et al. (2009) was performed on lactating Nili-Ravi buffaloes, which according to the available literature data, have an average milk yield of 2,000 liters in 305 days of lactation (Afzal et al., 2007). Therefore, differences in results between the current study and that of Afzal et al. (2007) could be related to differences in the amount of milk produced, which was significantly higher in the Holstein-Friesian cows (average milk yield of 9,000 liters in 305 days of lactation) included in our study. Given that Holstein-Friesian cows have high average milk yield, it can be hypothesized that intensive milk production contributes to the significant variations of concentrations of these elements (Na^+ and Cl^-) in the different stages of the production cycle. This relies on data that mineral requirements are highly dependent on the level of productivity, and that due to milk secretion, Na^+, K^+ and Cl^- output increases remarkably with the onset of lactation (Silanikove et al., 1997; McDowell, 2002). At the same time, the possible influence

of endocrinological status on these mineral elements cannot be neglected, especially in EL- and PL-cows, which had the lowest Na^+ and Cl^- concentrations in our study. Skrzypczak et al. (2014) reported that lower Na^+ concentration in the first week of lactation could be associated with decreased plasma rennin activity, resulting in Na^+ loss. Additionally, Asif et al. (1996) indicated that decrease in blood Na^+ concentration could be a consequence of high prostaglandin concentrations, which increase excretion of Na^+ by the kidneys. Although our study shows statistically significant differences in Na^+ and Cl^- concentrations, with consistent K^+ concentrations, in cows at different stages of the production cycle, it should be highlighted that they remained in the reference range (Cozzi et al., 2011).

No statistically significant difference was noted in lactate and iCa^{++} concentrations in cows at different production stages, indicating that the stages of the production (lactation) cycle did not have significantly affect these parameters. Another study (Seifi and Kia, 2018) indicated the presence of critical periods for homeostasis of these parameters, especially for Ca^{++}. Considering that our study did not include cows in any critical period around calving but only clinically healthy cows with clear anamnesis of previous calving, transition period and lactation, the possible impact of such periods on Ca^{++} homeostasis was excluded.

Serum glutathione peroxidase activity (GSH-Px) indicated the EL-cows were more exposed to oxidative stress than were cows in other stages of the production cycle. The results of our study are in agreement with the results of Pilarczyk et al. (2012); they observed the activity of GSH-Px and other parameters of oxidative stress in cows at different stages of lactation. Also, high GSH-Px activity in EL-cows was reported in other studies (Sharma et al., 2011; Konvičná et al., 2015), indicating the level of oxidative stress is higher in the early stage of lactation compared to the other stages of lactation.

Our results showed the blood glucose concentration was significantly lower in EL-cows than in Dry-cows. The onset of lactation in dairy cows is associated with negative energy balance, which leads to a decrease in glucose concentration, so the results obtained were expected and similar to the results of another study (Mohebbi-Fani et al., 2009). BCS changes depending on the stage of the production cycle detected in our study confirmed a previously established model of greater BCS loss in early lactation and at peak lactation and a gradual BCS increase towards the dry period (Roche et al., 2009; Puppel et al., 2016).

CONCLUSION

Our results show significant differences in blood pH, Na^+ and Cl^- concentrations and serum GSH-Px activity in cows at different stages of the production cycle. On average, the highest blood pH, the lowest Na^+ and Cl^- concentrations and the highest serum GSH-Px activity were measured in EL-cows, indicating greater metabolic and

oxidative stress in EL-cows than in the other examined cow groups. The originality of our study is reflected in the analysis of numerous parameters to assess acid-base status throughout the production cycle of cows, which should contribute to a better understanding of the differences between cows at different stages of the production cycle. Therefore, it would be useful to consider the inclusion of these parameters in standard procedures for health assessment in intensive dairy production, especially in the EL-cows.

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Authors' contributions

LJ, SN, RP, MS and DB conducted the field experiment and processed blood samples for acid-base status and electrolyte balance analyses. SM processed blood samples for oxidative status examination. DK, IV, LJ and DB were involved in study design, data analysis and draft manuscript preparation. All authors read and approved the final manuscript.

Competing interests

The author(s) declare that they have no competing interests.

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ACIDOBAZNI STATUS, BALANS ELEKTROLITA I OKSIDATIVNI STATUS KOD VISOKOMLEČNIH KRAVA U RAZLIČITIM FAZAMA PROIZVODNOG CIKLUSA

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Kratak sadržaj

Prethodna istraživanja na polju acidobaznog i oksidativnog statusa krava uglavnom su bila usredsređena na tranzicioni period (tri nedelje pre i tri nedelje nakon teljenja). Cilj ovog istraživanja bio je da se utvrde razlike u parametrima acidobaznog i oksidativnog statusa, kao i u balansu elektrolita, ne samo u tranzicionom periodu, nego i u ostalim fazama proizvodnog ciklusa krava. Krave holštajn-frizijske rase podeljene su u četiri jednake grupe (n=6): krave u ranoj laktaciji (9±2 dana laktacije – DL), krave u piku laktacije (50±5 DL), krave u kasnoj laktaciji (170±10 DL) i krave u zasušenju (10±1 dana pre očekivanog teljenja). Od ispitivanih krava prikupljeni su uzorci venske krvi za analizu acidobaznog i oksidativnog statusa i koncentraciju elektrolita. U ovom istraživanju zabeležena je značajno viša pH vrednost krvi kod krava u ranoj laktaciji nego kod krava u zasušenju. Koncentracija natrijuma (Na^+) bila je značajno niža kod krava u ranoj laktaciji i piku laktacije u poređenju sa kravama u zasušenju, dok je koncentracija hlora (Cl^-) bila značajno niža i kod krava u kasnoj laktaciji u poređenju sa kravama u zasušenju. Aktivnost glutation peroksidaze (GSH-Px) bila je značajno viša kod krava u ranoj laktaciji u poređenju sa kravama u piku laktacije. Najviše pH vrednosti krvi, najniža koncentracija Na^+ i Cl^- i najviša aktivnost GSH-Px zabeleženi su kod krava u ranoj laktaciji, što ukazuje na najveći metabolički i oksidativni stres tokom ovog perioda. Imajući u vidu dobijene razlike bilo bi značajno razmotriti uvođenje

parametara ispitivanih u ovom radu u standardne procedure za procenu zdravlja krava u intenzivnoj proizvodnji mleka.

Ključne reči: acidobazni status, mlečne krave, oksidativni status.

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