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BLOOD ACID-BASE BALANCE OF HEAVILY PREGNANT AND LACTATING EWES SUPPLEMENTED WITH SELENIUM

ACIDO BAZNA RAVNOTEŽA VISOKO GRAVIDNIH OVACA I OVACA U LAKTACIJI S DODATKOM SELENA U OBROCIMA

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SUMMARY

This research aimed to determine the influence of selenium supplements (organic, inorganic) in the rations of heavily pregnant and lactation ewes on indicators of acid-base balance. The research was conducted on 30 pregnant ewes Merinolandschaf breeds. The ewes were divided into three groups of 10 animals. The research started in autumn, by selecting the heavily (90th days of pregnancy) pregnant ewes with a single lamb pregnancy, continued during the spring of next year with the same ewes after lambing in lactation. The control group of ewes (group I) was fed without the selenium supplement, the second group of ewes (group II) was supplemented with 0.3 mg/kg of organic selenium, and the third group of ewes (group III) was supplemented with the 0.3 mg/kg inorganic selenium. In the blood of heavily pregnant and lactating ewes pH, gases (pCO₂, pO₂), electrolytes (Ca, Na⁺, K⁺, Cl⁻ and HCO₃⁻), were determined and calculated z - value, BE_{fw}, BE_{Cl}, BE_{alb}, anion gap (AG) and the strong ion difference (SID). Most of the indicators of acid base balance in all three groups were in reference range and supplementation with selenium especially inorganic helps move their values to reference and avoid acid base imbalance and disorders connected, which can cause reduced productivity and economic results. Determined acid base indicators are a sign of proper, timely, and good feeding adjustments of ewes in demanding reproduction statuses.

Keywords: acid base balance, selenium supplementation, ewes, heavy pregnancy, lactation

INTRODUCTION

Acid-base balance (ABB) is a complex concept that requires a detailed understanding of metabolic pathways used to eliminate H⁺ ions from the body. Blood, cells, and the respiratory system play key roles in maintaining acid-base balance within the physiological values of the animal. Extracellular H⁺ concentration is one of the most accurate blood

variables (Wojtas et al., 2013). Disorders that can occur in this regulatory system can endanger the life of the individual since the structure of proteins and enzymes depends on the pH value in the body. Anions and cations from feed, i.e., sodium (Na⁺), potassium (K⁺), and chlorides (Cl⁻) have the most significant influence on the acid-base balance. Some other minerals also participate in this process. When determining the acid-base balance, the degree of

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oxygen and carbon dioxide blood saturation is also an important indicator. The concentration of bicarbonate ions (HCO_3^-) in plasma depends on the level of oxygen saturation of hemoglobin. The decrease in hemoglobin concentration in blood is accompanied by an increase in the concentration of HCO_3^- in erythrocytes, which consequently passes through cell membranes into plasma (Adams et al., 1991). After that, the erythrocytes within the blood infiltrate the lungs, where the reverse process takes place. Hemoglobin undergoes an oxygen enrichment process that increases its acidity, and as a result, releases hydrogen ion which is then neutralized by HCO_3^- anion producing CO_2 and H_2O which are secreted by alveolar air due to the increase in partial pressure. Nutrition may strongly influence the acid-base balance in domestic animals. Pregnancy and lactation in small ruminants are phases that modify the metabolism of dams (Piccione et al., 2012). Lactation increases the need for milk synthesis nutrients, so animals' physical condition and nutrition have to be adequate (Antunović et al., 2017). An important practical application in nutrition is the manipulation of the diet to prevent hypocalcemic postparturient paresis. This disease is responsible for major economic losses and a sound strategy for its prevention is of top importance (Riond, 2001). A substantial research effort has been made to understand the pathophysiology of this disorder and how dietary prevention functions. Changes in acid-base balance have been found to have an impact on production indicators in animals (Relman, 1972; Chan, 1974). Micronutrient selenium (Se) and antioxidants vitamins E, A, and C have been evaluated as nutrients to potentially reduce the negative effects of oxidative stress in sheep. Supranutritional doses of dietary Se can reverse these effects (Chauhan, 2015). Selenium deficiency influences fertility reduction, abortion, retained placenta (Sunde, 2012), health problems in young animals, such as increased neonatal mortality, lower vitality of newborns, reduction of suckling reflex, and frequent occurrence of lamb diseases (Natasha et al., 2018). There are various methods of overcoming selenium deficiency (Novoselec et al., 2018), but, generally, sources of selenium supplement in feed can be classified into two categories: organic and inorganic. Current regulations allow the amount of 0.3 mg of Se/kg of ewe diet as organic or inorganic form (FDA, 2018). According to research (Weiss, 2005), in sheep, the true digestibility of selenium

from diets containing selenite (inorganic form) has shown to be about 50% while one from selenium yeast (organic form) would be about 66% due to a different metabolism. In many parts of the world, the concentration of selenium in the soil, and therefore in plants, is low (Schiavon et al., 2020). The area of the Pannonian Basin, where Croatia belongs, is included in low-selenium terrains (Antunović et al., 2010; Manojlović and Lončarić 2017). Therefore, this research aimed to determine the influence of selenium supplements (organic and inorganic) on indicators of acid-base balance in ewes demanding production phases (heavy pregnancy and early lactation).

MATERIAL AND METHODS

The research was conducted on 30 pregnant Merinolandschaf ewes average age of 4 years in good condition. Ewes, used for this research were selected from a flock of 200 animals. The research started in autumn, by selecting the heavily (90th days of pregnancy) pregnant ewes with a single lamb pregnancy, continued during the spring of next year with the same ewes after lambing and in lactation. The number of lambs was determined and the length of pregnancy was estimated trans-abdominal by using ultrasound Tringa 50S (PIE Medical, Netherlands). In total, the research lasted four months, two months with ewes in heavy pregnancy and two months with the same lactating ewes. The ewes were divided into three groups of 10 animals. The control group (group I) was fed a meal that consisted of 300 g/day/animal feed mixtures without selenium enrichment and 150 g/day/animal of barley. The second group of animals (group II) were fed a meal that consisted of 300 g/day/animal feed mixtures further enriched with 0.3 mg/kg of organic selenium (Selplex®, Alltech), and 150 g/day/animal of barley. The third group of animals (group III) were fed a meal, which consisted of 300 g/day/animal feed mixtures further supplemented with the 0.3 mg/kg inorganic selenium (sodium selenite) and 150 g/day/animal of barley. Basal feed mixture was composed of corn 511 g/kg dry matter, wheat flour 150 g/kg dry matter, molasses 40 g/kg dry matter, yeast 30 g/kg dry matter, dehydrated alfalfa meal 20 g/kg dry matter, sunflower meal 90 g/kg dry matter, soybean meal 90 g/kg dry matter, animal salt 4 g/kg dry matter,

limestone 20 g/kg dry matter monocalcium phosphate 10 g/kg dry matter, complete milk replacer 25 g/kg dry matter and premix 10 g /kg dry matter. All three groups of ewes fed Alfalfa hay *ad libitum*. Animals had access to fresh water *ad libitum*. The chemical composition of the basal feed mixture is shown in Table 1.

Table 1 Chemical composition of basal feed mixture, barley, and alfalfa hay

Tablica 1. Kemijski sastav osnovne krmne smjese, ječma i sijena lucerne

Indicator – Sastojak, g/kg dry matter	Feed mixture Krmna smjese	Barley Ječam	Alfalfa hay Sijeno lucerne
Dry matter / Suha tvar	880	880	860
Crude proteins / Sirove bjelančevine	177	117	146.5
Crude fiber / Sirova vlakna	64	65	260
Ash / Pepeo	70	22	13
Crude fat / Sirova mast	30	24	53

The laboratory analysis of the basal feed mixture revealed the following quantities selenium DM: 0.092 ± 0.01 mg/kg in the control group, 0.30 ± 0.05 mg/kg in the second, and 0.299 ± 0.07 mg/kg in the third group. Blood sampling was carried out in heavily pregnant ewes and ewes in lactation (on days - 14 and 23 relative to parturition). Blood samples were taken in the morning before feeding from the jugular vein into a sterile vacuum tube Venoject® (Leuven, Belgium). The blood was then placed at + 4 °C and analyzed after an average of two hours. From the blood samples, pH, partial pressure of carbon dioxide (pCO_2), partial pressure of oxygen (pO_2), and electrolytes (Ca, Na^+ , K^+ , Cl^- and HCO_3^- - bicarbonate) were determined by an automatic analyzer, Rapid Lab 348, that works based on ion-selective electrodes. Strong ions difference (SID) was calculated according to the formula: $[(Na^+ + K^+) - Cl^-]$ according to Stewart et al. (1983), z - value according to the formula: SID / reference value according to Whitehair et al. (1995), and the anion gap (AG) according to the formula: $[(Na^+ + K^+) - (Cl^- + HCO_3^-)]$ according to Kaneko et al. (2008). Also, indicators were calculated: Base

excess free water - $BE_{fw} = Z$ for ewes $\times (Na^+_{measured} - Na^+_{reference \text{ for ewes}})$, Base excess chloride - $BE_{Cl^-} = Cl^-_{reference \text{ ewes}} - Cl^-_{corrected}$, Base excess albumin - $BE_{alb} = 3.7 (albumin_{reference} - albumin_{measured})$, $Cl^-_{corrected} = Cl^-_{measured} (Na^+_{reference} / Na^+_{measured})$ according to Whitehair et al (1995). Normality distribution of data was checked by the Shapiro-Wilk W test. The mean values of the obtained results were calculated by the MEANS procedure in the computer program TIBCO Statistica® 13.3.0. Differences between mean values of analyzed parameters were examined by ANOVA at a significance level $P < 0.05$ or less.

RESULTS AND DISCUSSION

The addition of inorganic selenium in lactating sheep significantly reduced ($P < 0.05$) the partial pressure of carbon dioxide (pCO_2) compared to the control group of ewes without added selenium in the feed mixture (Table 2).

Table 2 shows the effect of selenium supplementation in sheep feed on acid-base balance indicators.

A similar partial pressure of CO_2 in sheep was found by Sobiech et al. (2005) and Chauhan et al. (2015). Contrary, in lactating sheep, significantly higher partial pressure of oxygen (pO_2) was found in a group of ewes supplemented with sodium selenite in relation to a control group of ewes without selenium supplement. The lowest partial pressure of oxygen (pO_2) was found in a group of ewes supplemented with selenized yeast and it was significantly ($P < 0.01$) lower compared to group III. with the addition of inorganic selenium in the feed mixture. Selenium supplementation in ewes did not significantly affect ($P > 0.05$) SID and z-values.

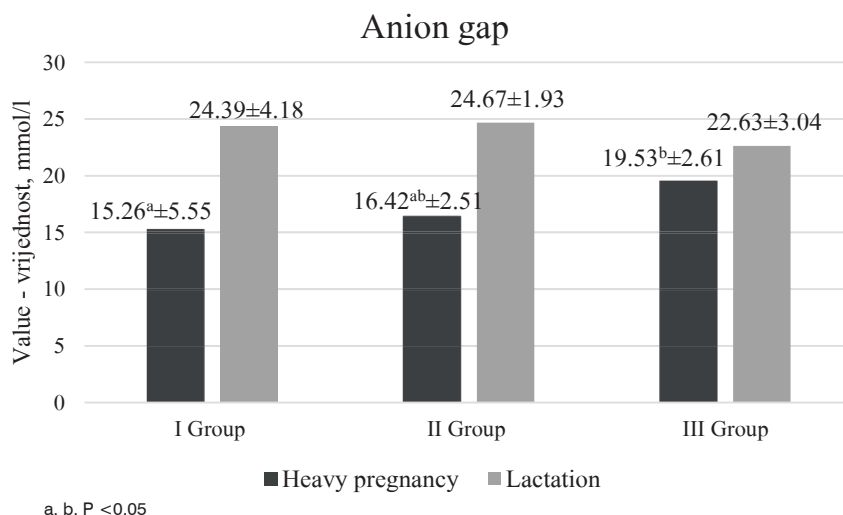
However, the addition of inorganic selenium to the feed mixture of heavily pregnant sheep led to a significant increase in the anion gap (AG value) (Graph 1). The Anion gap value is an indicator used to determine the presence of unmeasured anions. The mentioned indicator may also significantly differ with changes of pCO_2 and HCO_3^- (Castillo et al., 1998). Antunović et al. (2017) determined a similar value in the blood of lactation goats for HCO_3^- (25.94 mmol/l), pCO_2 (6.45 kPa), and content of AG (23.81 mmol/l) in comparison with present research especially in the group of ewes supplemented with sodium selenite.

Table 2 Indicators of acid-base balance in the blood of heavily pregnant and lactating ewes

Tablica 2. Pokazatelji acidobazne ravnoteže u krvi visokogavidnih ovaca i ovaca u laktaciji

Indicator Pokazatelj	R. S.	Group / Skupina			SE
		I.	II.	III.	
		Mean ± sd	Mean ± sd	Mean ± sd	
pH	L.P. V.G.	7.41 ± 0.04	7.44 ± 0.05	7.41 ± 0.02	0.01
	L. L.	7.37 ± 0.05	7.37 ± 0.04	7.41 ± 0.07	0.01
pCO ₂ , kPa	L.P. V.G.	6.74 ^{Aa} ± 1.77	5.64 ^b ± 1.24	5.27 ^B ± 0.47	2.09
	L.	6.86 ^a ± 0.91	6.52 ^{ab} ± 0.87	6.07 ^b ± 0.95	0.16
pO ₂ , kPa	L.P. V.G.	5.97 ± 3.59	7.48 ± 4.01	6.54 ± 0.93	0.52
	L. L.	5.16 ^A ± 0.62	4.92 ^A ± 0.75	6.59 ^B ± 1.02	0.18
Ca, mmol/L	L.P. V.G.	2.47 ^A ± 0.15	2.71 ^B ± 0.16	2.66 ^B ± 0.10	0.03
	L. L.	2.48 ± 0.23	2.41 ± 0.78	2.66 ± 0.14	0.08
Na, mmol/l	L.P. V.G.	150.83 ± 1.59	151.25 ± 3.05	152.08 ± 1.68	0.41
	L. L.	149.82 ^a ± 3.22	151.50 ^{ab} ± 7.28	152.17 ^b ± 1.89	0.44
K, mmol/l	L.P. V.G.	5.30 ± 0.35	5.23 ± 0.44	5.00 ± 0.32	0.06
	L. L.	5.03 ^A ± 0.37	5.33 ^A ± 0.28	4.75 ^B ± 0.22	0.07
Cl ⁻ mmol/l	L.P. V.G.	109.75 ^a ± 2.34	109.17 ^A ± 2.25	111.67 ^{Bb} ± 2.02	0.40
	L. L.	104.82 ± 1.94	106.75 ± 2.76	107.00 ± 1.95	0.06
SID, mmol/l	L.P. V.G.	46.38 ± 4.41	47.31 ± 2.58	45.53 ± 1.48	0.51
	L. L.	50.30 ± 3.33	50.00 ± 2.26	49.92 ± 2.74	0.46
z –value z –vrijed.	L.P. V.G.	0.32 ± 0.03	0.33 ± 0.02	0.31 ± 0.01	0.03
	L. L.	0.34 ± 0.02	0.40 ± 0.19	0.34 ± 0.01	0.02
BE _{tw}	L.P. V.G.	1.76 ± 0.91	1.90 ± 1.02	2.05 ± 0.55	0.14
	L. L.	1.55 ± 1.19	2.55 ± 2.07	2.31 ± 0.75	0.25
BE _{Cl⁻}	L.P. V.G.	-6.91 ± 3.54	-6.03 ± 2.08	-7.83 ± 1.34	0.42
	L. L.	-2.83 ± 2.37	-3.60 ± 2.22	-3.32 ± 2.15	0.37
HCO _{3⁻} , mmol/l	L.P. V.G.	27.29 ^A ± 2.09	27.03 ^A ± 2.05	24.30 ^B ± 1.99	0.40
	L. L.	25.96 ± 1.60	25.33 ± 1.48	25.62 ± 6.09	0.61
BE _{alb}	L.P. V.G.	-4.70 ± 3.89	-4.51 ± 2.55	-2.55 ± 3.35	0.49
	L. L.	1.16 ± 7.48	-2.09 ± 4.79	-4.04 ± 6.38	1.09

Mean = mean value; sd = standard deviation - standardna devijacija; SE = standard error - standardna pogreška; R.S. = reproduction status - reprodukcijski status; I. - control group – kontrolna skupina; II. - addition of selenized yeast – dodatak seleniziranog kvasca; III. - addition of sodium selenite – dodatak natrij selenita; a, b, P <0.05; A, B, P <0.01; L.P. - heavily pregnant; V.G. - visoki graviditet; L. - lactation; L - laktacija; pCO₂ – partial pressure of carbon dioxide – parcijalni tlak ugljičnog dioksida; pO₂ - partial pressure of oxygen – parcijalni tlak kisika; SID - strong ions difference – razlika jakih iona; BE_{tw} - Base excess free water – višak baza slobodne vode; BE_{Cl⁻} - Base excess chloride – višak baza klorida; HCO_{3⁻} - hydrogen carbonates - bikarbonati; AG - anion gap – anionski procijep Be_{alb} - Base excess albumin – višak baza albumina



Graph 1 Anion gap (AG) in the blood of heavily pregnant and lactating ewes

Grafikon 1. Anionski procijep (AG) u krvi visoko gravidnih ovaca i ovaca u laktaciji

The HCO_3^- content was the highest in the control group of heavily pregnant ewes, i.e. a significantly lower value ($P < 0.01$) was found in the III group of ewes, with the addition of an inorganic form of selenium in the feed mixture. A comparable HCO_3^- content in venous blood (24.61 mmol/l) was found by Sobiech et al. (2005). Increased lipid metabolism is a source of increased production of metabolically strong acids, which cause a shift in acid-base balance towards acidosis (Castillo et al., 1996). The respiratory system responds by hyperventilating to remove metabolic acids, causing a drop in pCO_2 . The decrease in pH results in an increase in the concentration of H^+ ions in the blood, and it leads to a decrease in the bicarbonate (HCO_3^-) content, which is due to its activity as a buffer to an increase in H^+ ions (Castillo et al., 1998). According to Fencel's method, metabolic acidosis is associated with significant changes in plasma osmolarity, which is evident in present research by an increase in BE_{fw} in heavily pregnant and lactating ewes. This indicator, in combination with the increase ($P < 0.05$) in Na^+ in lactating ewes supplemented with sodium selenite, indicates alkalosis because the lack of free water affects the increase in BE_{fw} , i.e. the increase in Na^+ concentration ac-

ording to Whitehair et al. (1995). The probable reason for the decrease in SID values in heavily pregnant and lactating sheep is the increase in Cl^- in the blood of ewes according to (Las et al., 2007). The mean Cl^- concentration of heavily pregnant ewes in the present research was highest in group III (111.67 mmol / L), i.e., significantly higher ($P < 0.01$; $P < 0.05$) compared to group II and group I (109.17: 109.75 mmol / L). According to Whitehair et al. (1995), a decrease in SID below normal values results in the occurrence of acidosis (H^+ increase), and an increase above normal values leads to alkalosis (decreased H^+). Figge et al. (1991) found that the value of the anion gap is correlated with the albumin concentration and that it increases due to the change in the HCO_3^- content as a dependent variable that participates in its calculation. The respiratory mechanism contributes to the return of normal pH value of blood by enhanced elimination of CO_2 , which contributes to a decrease in HCO_3^- (Tietz et al., 1994; Sivakumar et al., 2010) which is comparable to findings in the present research, especially in heavily pregnant ewes supplemented with sodium selenite where the significantly lower value of HCO_3^- in comparison to control ewes without selenium supplementation was determined. Sivakumar et al.

(2010) investigated the effect of selenium (sodium selenite) and vitamins C and E on acid-base balance in goats exposed to heat stress, and similar to present research, the addition of selenium and vitamin E affected the increase in pH compared to the control group of goats. According to the results of the present research, the authors found in the treated goats a significant decrease in $p\text{CO}_2$ ($P < 0.05$) compared to the control group (45.63: 55.25 mm Hg), and an increase in $p\text{O}_2$ (48.66: 40.36 mm Hg). Under physiological conditions, the bicarbonate buffer system maintains the HCO_3^- and $p\text{CO}_2$ content in a relatively constant ratio (20:1) which is not the case in this research on demanding physiological phases of ewes.

CONCLUSION

Demanding physiological states like heavy pregnancy and the beginning of lactation can compromise acid base balance in animals. Inorganic selenium has influenced the significant decrease of the $p\text{CO}_2$ in heavily pregnant and lactating ewes. Also, inorganic selenium affects a significant increase of $p\text{O}_2$ in lactating ewes. Significant growth of Ca concentration has been determined in heavily pregnant ewes supplemented with inorganic selenium in comparison to control, while the same growth of Na^+ concentration has been determined in lactating ewes. The concentration of Cl⁻ has significantly increased and HCO_3^- decreased in heavily pregnant ewes supplemented with inorganic selenium. Anion gap has significantly increased in heavily pregnant ewes supplemented with selenium. Most of the shown acid base balance indicators were in the reference range and supplementation with selenium especially inorganic helps move their values to reference and avoid acid base imbalance and disorders connected which can cause reduced productivity and economic results. Determined indicators are a sign of proper feeding of the ewes but also timely and good adjustments in demanding reproduction statuses.

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SAŽETAK

Cilj ovog istraživanja bio je utvrditi utjecaj dodatka selena (organskog, anorganskog) u obrocima kasno gravidnih ovaca i ovaca u laktaciji na pokazatelje acido-bazne ravnoteže. Istraživanje je provedeno na 30 gravidnih ovaca Merinolandschaf pasmine. Ovce su podijeljene u tri skupine po 10 životinja. Istraživanje je počelo u jesen, odabirom kasno gravidnih ovaca (90. dan gravidnosti) s jednim janjetom i nastavljeno tijekom proljeća sljedeće godine s istim ovcama nakon janjenja u laktaciji. Krmna smjesa kontrolne skupine ovaca (skupina I) bila je bez dodatka selena, krmna smjesa druge skupine ovaca (skupina II) bila je obogaćena dodatkom 0,3 mg/kg organskog selena, a krmna smjesa treće skupine ovaca (skupina III) bila je obogaćena dodatkom 0,3 mg/kg anorganskog selena. U krvi visoko gravidnih ovaca i ovaca u laktaciji utvrđeni su sljedeći pokazatelji: pH, plinovi ($p\text{CO}_2$, $p\text{O}_2$), elektroliti (Ca, Na^+ , K^+ , Cl i HCO_3^-), te izračunata z - vrijednost, BE_{fw} , BE_{Cl} , BE_{alb} , anionski procijep (AG) i jaka ionska razlika (SID). Većina pokazatelja acido bazne ravnoteže u sve tri skupine bila je unutar referentnih vrijednosti, a dodatak selena, posebno anorganskog oblika u obrocima ovaca pomaknuo je njihove vrijednosti prema referentnim i izbjegao acido-baznu neravnotežu i povezane poremećaje, koji mogu uzrokovati smanjenu produktivnost i ekonomske rezultate. Utvrđeni pokazatelji acido bazne ravnoteže znak su odgovarajuće hranidbe ovaca i njihove pravovremene i dobre prilagodbe u zahtjevnim reprodukcijским stanjima.

Ključne riječi: acido bazna ravnoteža, dodatak selena, ovce, visoka gravidnost, laktacija