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CORRELATION OF NIACIN, NAD AND NADP CONCENTRATION IN BLOOD ASSOCIATED WITH LIPOLYSIS IN PERIPARTAL DAIRY COWS

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Abstract

The aim of this study was to determine correlation between niacin application and concentration of NAD and NADP in blood of cow in peripartal period, also their relation with NEFA concentration was examined. Experiment included 30 cows (15 experimental and 15 animals in control group). Application of niacin was conducted 2 weeks before and after calving. Nicotinic acid was applicated *per os* with food, 120 grams per day per cow. Nicotinic acid was in rumen-unprotected form. The control group was not treated with nicotinic acid. Blood samples were taken before the application of niacin, then on the day of calving and last sampling was done first and second week after calving. Parameters such as NAD, NADP were determined. NAD and NADP were determined using colorimetric ELISA method from erythrocytes lysates. Relation between NAD, NADP and NAD/NADP thru experimental weeks was analyzed by Pearson's correlation coefficient and simple linear regression model. Correlation and regression between NAD, NADP and NAD/NADP with NEFA was calculated by same method. Results have showed that cows that had niacin application have higher concentrations of NAD and NADP in blood 1 and 2 week after calving, NEFA was statistically lower in niacin group compared to the control group in all weeks. Correlation between NAD and NADP was positive in niacin group and negative in control group but significant correlation wasn't founded between NAD and NADP and NEFA in control and niacin group.

Key words: Cow; niacin; NAD; peripartal period; lipolysis

1. INTRODUCTION

Niacin is water-soluble B vitamin, nicotinic acid and nicotinamide are the two most common forms of niacin. At pharmacological doses nicotinic acid has shown to inhibit lipolysis in cattle (Hristovska et al., 2017, Pires and Grummer, 2007), decrease the ketosis prevalence in early lactation (Havlin et al.,2017), reduce the heat stress (Wrinkle et al.,2012), had influence on insulin resistance during early lactation in cows (Hristoska et al.2017) Although less studied then other mechanisms of niacin, niacin is also an important precursor for nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) (Romani et al.,2019). Both forms of niacin (nicotinic acid and nicotinamide) are nutritionally equivalent and can be used for the synthesis of NAD (Carloson, 2005, Imai et al.,2000). NAD and NADP are actively involved in many essential redox reactions in cellular metabolism, in the metabolism of lipids and carbohydrates, whereas NAD protects the organism from oxidative stress (Bender, 2003; Cincović et al., 2018).

It has been well documented that nicotine acid applicated in pharmacological doses has potential to suppress lipolysis in dairy cows (Pires et al., 2007; Grummer, 2007; Niehoff et al.,2009; Hristovska et al., 2017). But there is a limited body of information on the NAD, NADP and niacin status response to niacin administration in dairy cows. Petrovic et al. 2022 concluded that NAD and NADP are good indicators of the ability of additional niacin source to create functional cofactors due to their concentration change.

One of the methods for assessing niacin status in humans is the measurement of the active coenzymes of niacin, NAD and NADP. Erythrocyte NAD and NADP concentration are more direct measure of functional niacin status and have been shown to respond to changes in niacin intake in humans (Fu et al, 1989; Jacobson and Jacobson,1997).

The aim of this study was to determine correlation between NAD and NADP concentration in blood in dairy cows after niacin application and their relation with product of lipolysis, non-esterified fatty acids (NEFA).

MATERIALS AND METHODS

For this study 30 clinically healthy, Holstein-Friesian cows were chosen. The cows were in second and third lactation (3 to 4 years old) without history of abortion and in normal body condition (scored from 3.30 to 3.55). On the clinical examination there were no signs of illness. Cows were divided into two groups, niacin and control groups, 15 cows in each group. The niacin group was treated with nicotinic acid for 2 weeks prior to the expected partus and 2 weeks after parturition. Nicotinic acid was applicated *per os* with food, 120 grams per day per cow, 28 day in row. Nicotinic acid was in rumen-unprotected form (Rovimix®Niacin, F. Hoffmann-La Roche AG, Switzerland). The control group was not treated with nicotinic acid. All the cows were fed and taken care of in a similar fashion, meeting daily NRC standards (2001).

Blood collection and analysis: Blood samples were collected before morning feeding by puncture of the coccygeal vein. Blood samples were taken four times in both groups. First time blood samples were taken before the application of niacin (week 0), second time on the day of calving and other times during first and second week after parturition (week 1, 2 ,3). Blood samples for analysis were collected in lithium heparin 3-mL tubes (BD Vacutainertubes®PST) and appropriately marked. The samples were kept on dry ice and protected from light until laboratory analysis. Samples were analyzed immediately after sampling.

Measurements of blood parameters: Parameters such as NAD, NADP were determined. NAD and NADP were determined using colorimetric ELISA method from erythrocytes lysates. The enzymes in the system specifically recognize NAD:NADP in an enzyme cycling reaction, there is no need to purify NAD:NADP from the simple mix. The enzyme cycling reaction significantly increases detection sensitivity. The concentration of NAD and NADP are expressed in pmol/10^6 erythrocytes. The resulting number is for erythrocytes in one μL blood, and when the results multiply with 100 it gets the value for NAD and NADP in one mL. Were used kits from manufactures Abcam. NEFA was determined by standard colorimetric assay (R33andox, UK) on Chemray spectrophotometer (Rayto, Ch).

The effects of niacin application on NAD, NADP values were analyzed during experimental weeks. GLM model was used for influence of group, week and interaction group×week. Posthock LSD test was used for determination of difference between experimental and control group in each week. Relation between NAD, NADP and NAD/NADP thru experimental weeks was analyzed by Pearson's correlation coefficient and simple linear regression model. Correlation and regression between NAD, NADP and NAD/NADP with NEFA was calculated by same methods. Statistical software SPSS 20.0 (IBM) was used for these purpose.

RESULTS

The application of niacin induced significant effects of NAD and NADP values in cows during peripartal period in all weeks of the experiment. The obtained results show that the cows that received niacin have significantly higher NAD and NADP concentration in all weeks of the experiment compared with the control group. In niacin group there was significant increase in

NAD and NADP value in week 0,1,2 (after niacin application) compared with week -1 (before niacin application). Results are presented in table 1 and 2.

Table 1. Influence of niacin application, week of sampling and group×week interaction on NAD

Parameter	Group/ Week	Average pmol/L	Standard deviation	Group	Week	Group× Week
NAD pmol/L	Control -1	898,30	183,30	p<0,001	p<0,01	p<0,01
	Niacin -1	862,30	90,20			
	Control 0	904,60	184,60			
	Niacin 0	1724,6	180,40			
	Control 1	889,30	181,40			
	Niacin 1	1968,6	207,10			
	Control 2	895,60	182,70			
	Niacin 2	1771,8	186,40			

Table 2. Influence of niacin application, week of sampling and group×week interaction on NADP.

Parameter	Group Week	Average pmol/L	Standard deviation	Group	Week	Group× Week
NADP pmol/L	Control-1	425,62	65,78	p<0,001	p<0,01	p<0,01
	Niacin -1	422,67	39,98			
	Control0	405,36	62,65			
	Niacin 0	704,45	66,64			
	Control1	385,09	59,52			
	Niacin 1	778,36	70,34			
	Control 2	397,25	61,40			
	Niacin2	796,18	75,77			

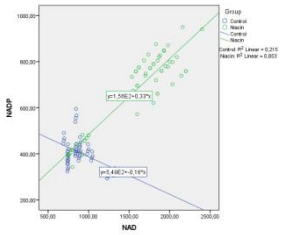
NEFA was statistically lower (p<0.01) in niacin group compared to the control group in week 0,1,2 (table 4).

Table 4. Influence of niacin application, week of sampling and group×week interaction on NEFA.

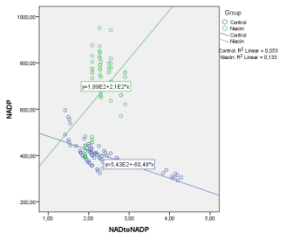
Parameter	Group Week	Average pmol/L	Stan.deviation	Group	Week	Group× Week
NEFA (mmol/L)	Control -1	0,22	0,05	p<0,001	p<0,01	p<0,01
	Niacin -1	0,24	0,04			
	Control 0	0,48	0,09			

	Niacin 0	0,31	0,09			
	Control 1	0,64	0,15			
	Niacin 1	0,35	0,10			
	Control 2	0,76	0,14			
	Niacin 2	0,39	0,11			

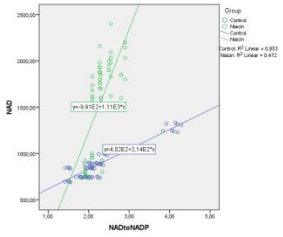
Correlation between NAD and NADP was negative in control group and positive in niacin group. NAD/NADP ration showed positive correlation with NAD in niacin and control group. NAD/NADP ration showed positive correlation with NADP in niacin group, and negative correlation in control group. Results are presented on graphic 1-3.



Graphic 1. Correlation between NAD and NADP

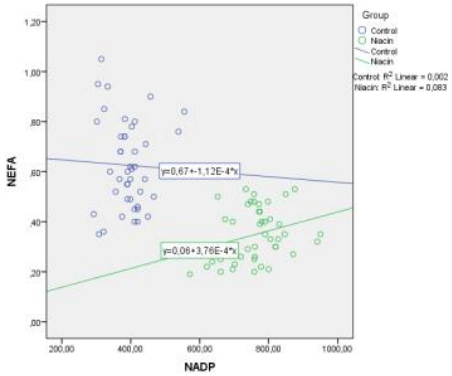


Graphic 2. Correlation between NADP and NAD/NADP ration

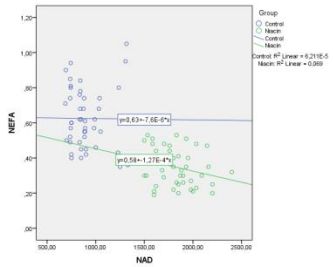


Graphic 3. Correlation between NAD and NAD/ NADP ration

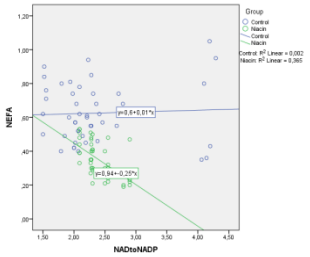
Statistically significant correlation wasn't founded between NAD and NADP and NEFA in control and niacin group. Statistically significant negative correlation was showed between NEFA concentration and NAD/NADP ratio in niacin, but not in control group. Results are shown on Graphic 4-6.



Graphic 4. Antilipolytic effect of niacin thru NADP



Graphic 5. Antilipolytic effect of niacin thru NAD



Graphic 6. Antilipolytic effect of niacin thru NAD/NADP ratio

DISCUSSION

According to literary data two weeks treatment of mice with high dosage of nicotine acid and nicotinamid (500 and 1000 mg/kg) has influence on NAD levels in many tissues. 40-60% higher concentration of NAD in liver and blood are founded (Jackson et al., 2005). That indicates that niacin is capable to stimulate synthesis of NAD in liver and blood and that nicotinamide can be converted in alternative form and increase bioavailability of nicotinamide and/ or treatment with nicotinamid can cause cellular adaptation that causes biosynthesis of NAD (Sauve et al., 2008). Jackson et al. (2005) showed that nicotine acid can increase NAD concentration in liver and blood, similarly like nicotinamide. Hara et al. (2007) showed that exogenous application of nicotine acid induces significant increase of cellular levels of NAD in human cells while nicotinamide added in the same concentration do not cause significant increase of NAD concentration. So, they concluded that nicotine acid is better substrate for NAD increase in cellular levels than nicotinamide is. But

up to our knowledge there are insufficient data about effect of niacin on NAD level in dairy cows. Our results showed that cows that received niacin have significantly higher NAD concentration in all weeks of the experiment compared with the control group, also in niacin group there was significant increase in NAD value in weeks after niacin application compared with week before niacin application. This results shows that niacin supplementation increased NAD level in blood in dairy cows.

Studies showed that application of pharmacology dose of nicotine acid decrease levels of NEFA in plasma by inhibiting lipolysis in cattle (Hristovska et al.,2017, Titgemeyer et al., 2011). Our results showed that cows that received niacin have significantly lower NEFA concentration in blood compared to the control group in all weeks. This antilipolytical potential of nicotine acid is probably developed across action on niacin receptor HCA_2 (GPR109A old name) (Gille et al., 2008; Offermanns, 2008;Chen et al.,2019). Recently was proved that HCA_2 antilipolytic pathway that is already described in other animals exists in functional form in cattle tissues in *in vitro* conditions (Kenez et al., 2014). In the other hand, nicotinamide have very low affinity towards HCA_2. Activation of HCA_2 by nicotine acid causes inhibition of adenyl-cyclase activity and reducescAMP concentration in the cells. Reduced cAMP levels in adipocytes cause inactivation of protein kinase A and reduction of phosphorilation of hormone sensitive lipase and lipase reduction (Gille et al., 2008). HCA_2 receptor is found in adipose tissue and immune cells. In cattle receptor has been found in muscles, brain and liver (Titgemeyer et al., 2011a). in cattle HCA_2 ligand- nicotine acid, nicotinamide and beta-hydroxybutirate (BHB) have showed different level of efficiency in induced antilipolysis in *in vitro* conditions. Nicotine acid reduces phosphorilation of hormone sensitive lipase and in that way decreases lipolytic response. Nicotinamide is not capable to suppress lipolytical activity in cows tissue in *in vitro* conditions.

In our study wasn't founded statistical significant correlation between NAD and NADP and NEFA in both groups, although there are researches that shows that anti-inflammatory drugs induce inhibition of lipolysis across NADPH oxydase (Vázquez-Meza et al., 2013) and NAD+ in adipose tissue has great significance on sirtuin and PARP balance (Jokinen et al., 2017). In our study statistically significant negative correlation was only showed between NEFA concentration and NAD/NADP ratio in niacin group, but statistically significant any correlation between NEFA concentration and NAD/NADP ratio was not found in control group.

To summarize, application of niacin in cows can induce increase of NAD and NADP in blood, NEFA was statistically lower in niacin group compared to the control group in all weeks. Correlation between NAD and NADP was positive in niacin group and negative in control group but significant correlation wasn't founded between NAD, NADP and product of lipolysis- NEFA in control and niacin group.

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