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EFFECTS OF NIACIN ON BLOOD INDICATORS OF LIPOMOBILIZATION AND HEPATIC FUNCTION IN DAIRY COWS DURING EARLY LACTATION

Talija Hristovska[1*], Marko Cincovic[2], Petar Dodovski[1], Nikola Karabolovski[1], Natasha Pejcinovska[1], Pance Dameski[1], Igor Zdravevski[1], Mimi Ristevski[1]

[1]Veterinary Faculty, University of St. Kliment Ohridski, Prilepska bb, 7000 Bitola, Macedonia
[2]Department of Veterinary Medicine, Faculty of Agriculture, Trg Dositeja Obradovic 8, 21000 Novi Sad, Serbia

[*]e-mail: talija.hristovska@uklo.edu.mk

Abstract

During the first 2 weeks of lactation dairy cows experience a negative energy balance. As a response to the negative energy balance high degree of lipid mobilization from the stored body fat occurs. When niacin is administered in pharmacological doses, it has an antilipolytic effect and reduces non-esterified fatty acids release from adipose tissue. The present study aimed to investigate the effect of pharmacological doses of niacin on blood indicators of lipomobilization and hepatic function in dairy cows during early lactation.

The experiment included 30 Holstein-Friesian dairy cows. The cows were allocated into two groups 15 cows in each (niacin and control group). Niacin was administered *per os* with food in the dose of 120 grams per day per cow, for 4 weeks, 2 weeks before and after calving. Blood samples were taken three times: on the day of calving, 7 and 14th day after calving. Metabolic parameters non-esterified fatty acids, beta-hydroxybutyrate, glucose, triglyceride, cholesterol, total protein, albumin, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyltransferase, total bilirubin and urea were determined using colorimetric reaction according to the manufacturer's instruction colorimetric test kits and were measured using a semi-automatic analyzer. Differences in metabolite concentration between the niacin and control group of cows were determined. Two-way ANOVA, Least Significant Difference, and Pearson correlation were used for statistical analysis.

Correlation coefficients among the blood indicators of lipomobilization (non-esterified fatty acids and beta-hydroxybutyrate) and the blood indicators of hepatic lesion and function (aspartate aminotransferase, alkaline phosphatase, gamma-glutamyl transferase, glucose, cholesterol, albumin, total protein, urea, and triglyceride) were calculated. Significant correlations ($p < 0.01$) were observed in most of the parameters. We found significantly lower aspartate aminotransferase concentration in the niacin group compared to the control group and this result indicates that cows that received niacin had lower liver lesions and damage because of decreased lipomobilization. A positive correlation between indicators of lipomobilization and hepatic enzymes was found.

Pharmacological doses of niacin supplemented daily for 4 weeks suppress lipolysis and improve hepatic function in dairy cows during the peripartal period.

Key words: *Niacin, Niver, Dairy cows, Peripartal period.*

1. Introduction

Niacin belongs to the water-soluble vitamin B-group and this term is used for the two forms of vitamers - nicotinamide and nicotinic acid. It has been shown that niacin acts as a precursor for the coenzymes nicotinamide adenine dinucleotide NAD(H) and nicotinamide adenine dinucleotide phosphate NADP(H), and they are included in numerous metabolic processes [1]. Niacin is required in very small amounts to maintain cellular metabolism [2], but when used in pharmacological doses it suppresses lipolysis [3, 4, 5, and 6]. Niacin inhibits adipose tissue lipolysis when binding to its receptor hydroxyl-carboxylic acid-2 receptor (HCA_2), formerly also known as GPR109A [7].

During the first 2 weeks of lactation, dairy cows experience a negative energy balance (NEB) [8]. NEB occurs due to limited feed intake and increased milk production. As a response to the NEB high degree of lipid mobilization from the stored body fat occurs [9, 10]. Increased lipolysis leads to high concentrations of non-esterified fatty acids (NEFA) in blood [11]. The release of NEFA into the blood provides energy for the tissue, however, the bovine liver has a limited capacity to completely oxidize NEFA. When the influx of NEFA to the liver is greater than its oxidative capacity, the result is an accumulation of NEFA as triglyceride (TG) [9]. Acetyl CoA (resulting from oxidation of the NEFA) that is not utilized in the Krebs cycle is converted into ketone bodies (acetone, acetoacetate, and beta-hydroxybutyrate - BHB) which may appear in the blood, milk, and urine [12]. So the main blood indicators of lipomobilization in ruminates are beta-hydroxybutyrate (the most important ketone body) and non-esterified fatty acids (NEFA). Circulating NEFA represents the extent of mobilization of fat from the adipose tissues [13], while the BHB level reflects the completeness of oxidization of fat in the liver [14].

The main indicators of hepatic lesion and function are the enzymes: asprtate transaminase (AST), gamma-glutamyltransferase (GGT), glutamate dehydrogenase (GLDH), and the metabolites: glucose, cholesterol, and albumin [15]. Excessive accumulation of triglycerides (TG) in the liver impairs its normal function [16]. The synthesis and accumulation of TG in the liver are related to the concentration of NEFA in the blood, therefore cows with lipolysis are at high risk of developing fatty liver syndrome [17, 18]. The fatty liver infiltration and hepatocyte degeneration involve cell membrane damage and hepatocyte destruction coupled with the release of cytoplasm enzymes (AST, GGT, and GLDH) [19, 20]. AST values are more sensitive than GGT as indicators of hepatic lipidosis in cows during early lactation in dairy cows [10].

The present study aimed to investigate the effect of pharmacological doses of niacin on blood indicators of lipomobilization and hepatic function in dairy cows during early lactation. The correlation between indicators of lipomobilization and hepatic function was examined.

We hypothesized that pharmacological doses of niacin during the peripartal period may improve hepatic function due to the antilipolytic effect of niacin.

2. Materials and Methods

2.1 Experiment design

The experiment included 30 Holstein-Friesian dairy cows from a commercial dairy farm in Serbia. The selected cows were clinically healthy and in normal body condition, and they were in their second and third lactation, without a history of abortion. The cows were allocated into two groups: 15 cows in the experimental, and 15 cows in the control group. The first group of cows was treated with niacin (niacin group), and the second group was not treated with niacin (control group). Niacin was administered *per os* with food in the pharmacological dose of 120 grams per day per cow. Niacin was administered for 4 weeks, starting 2 weeks before the expected calving date, and ending 2 weeks after parturition. Nicotinic acid was in the rumen-unprotected form (Rovimix®Niacin, F. Hoffmann-La Roche, Switzerland). The dose of niacin was determined according to the following characteristic: the bioavailability of rumen-unprotected nicotinic acid is about 5% (120 g/d of rumen-unprotected niacin provides an estimated 6 g/d of bioavailable niacin). The cows in late pregnancy were fed with a diet consisting of 6 kg lucerne hay, 15 kg maize silage (30% dry matter - DM), and 3 kg concentrate (18% crude proteins - CP). The cows in early lactation were fed with a diet consisting of 7 kg lucerne hay, 20 kg maize silage (30% DM), and 5 kg concentrate (18% CP).

Blood samples were taken three times: on the day of calving (day 0), on day 7, and on day 14 after calving. Blood samples were collected from the coccygeal vein in the morning before feeding using a 10 mL sterile vacutainer with a gel separator for biochemical analyses (BD Vacurainer® SSTII, Advance Plymouth, UK). All samples were marked appropriately.

2.2. Laboratory analyses

After centrifugation of the blood samples at 4,000 x g for 10 minutes, the blood plasma was collected. Metabolic parameters NEFA, BHB, glucose, triglyceride, cholesterol, total bilirubin, total protein, albumin, aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), total bilirubin, and urea were determined using colorimetric reaction according to the manufacturer's instruction colorimetric test kits (Randox, UK, and Pointe Scientific, USA), and were measured using a semi-automatic analyzer (AnalyzernRayto RT-1904cv, Rayto Electronic, China).

2.3 Statistical analyses

Differences in metabolite concentration between the niacin and control group of cows were determined during the first 14 days after parturition (0, 7, and 14). The effect on the groups was determined by two-way ANOVA analysis and LSD test. The correlation between NEFA and metabolic parameters and BHB and metabolic parameters was evaluated by Pearson correlation analysis. Software SPSS version 19.0 for Microsoft Windows was used to perform the statistical analysis. Differences were accepted as statistically significant when P values were below 0.05.

3. Results and Discussion

Indicators of lipomobilization, NEFA, and BHB, were statistically lower ($p < 0.01$) in the niacin group compared to the control group (Figures 1 and 2). The results of serum biochemical analysis for both groups of cows are given in Table 1. Activities of serum AST, GGT, and ALP were lower ($p < 0.01$) in the niacin group compared to the control group. The serum concentrations of glucose, albumin, cholesterol, and TG of the niacin group were significantly higher in the niacin group than those of the control group ($p < 0.01$). The niacin group showed a significantly lower concentration of total bilirubin compared to the control group.

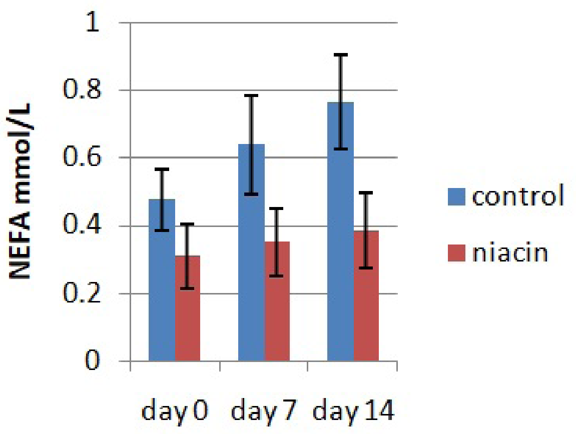

Figure 1. NEFA concentration at 0, 7, and 14 days postpartum in niacin-treated and control cows

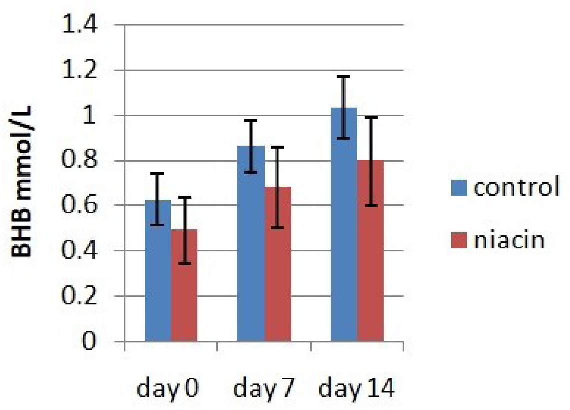

Figure 2. BHB concentration at 0, 7, and 14 days postpartum in niacin-treated and control cows

We calculated the correlation coefficients among the blood indicators of lipomobilization (NEFA and BHB) and the blood indicators of hepatic lesion and function (AST, ALP, GGT, glucose, cholesterol, albumin, total protein, urea, and TG). Significant correlations ($p < 0.01$) were observed in most of the parameters. Significantly positive correlations were observed between NEFA and hepatic enzyme AST, BHB, and AST. Significantly negative correlations were observed between NEFA and glucose, BHB and glucose, NEFA and cholesterol, and BHB and cholesterol. No significant correlation was observed between NEFA and total protein. Results are given in Table 2.

Table 1. Influence of niacin application on blood indicators of hepatic function in early lactation

Day postpartum	Niacin	Control	p
TG mmol/L			
0 day	0.12 ± 0.009	0.10 ± 0.01	< 0.01
7 day	0.11 ± 0.009	0.09 ± 0.01	
14 day	0.10 ± 0.008	0.08 ± 0.007	
Cholesterol mmol/L			
0 day	2.53 ± 0.18	2.04 ± 0.13	< 0.01
7 day	2.41 ± 0.17	1.94 ± 0.12	
14 day	2.02 ± 0.14	1.68 ± 0.10	
Glucose mmol/L			
0 day	3.08 ± 0.36	2.53 ± 0.30	< 0.01
7 day	2.73 ± 0.32	2.10 ± 0.25	
14 day	2.51 ± 0.35	1.9 ± 0.23	
Total protein g/L			
0 day	77.0 ± 4.5	78.8 ± 4.9	NS
7 day	72.4 ± 4.2	74.0 ± 4.6	
14 day	71.0 ± 4.2	72.6 ± 4.5	
Albumin g/L			
0 day	41.7 ± 6.1	34.6 ± 3.5	< 0.01
7 day	38.0 ± 5.5	31.4 ± 3.2	
14 day	37.0 ± 5.4	30.7 ± 3.1	
Urea mmol/L			
0 day	3.54 ± 0.6	5.10 ± 1.1	<0.01
7 day	3.36 ± 0.6	4.85 ± 1.1	
14 day	3.53 ± 0.6	4.67 ± 1.0	
Total bilirubin µmol/L			
0 day	6.36 ± 1.21	8.34 ± 0.96	< 0.01
7 day	7.89 ± 1.27	10.85 ± 1.25	
14 day	9.18 ± 1.32	13.02 ± 1.5	
AST U/L			
0 day	83.1 ± 11	93.3 ± 7.9	< 0.01
7 day	101.6 ± 10	121.1 ± 9.8	
14 day	106.7 ± 10	130.8 ± 10	
GGT U/L			
0 day	15.26 ± 2.9	18.6 ± 2.2	< 0.01
7 day	17.55 ± 3.3	21.3 ± 2.6	
14 day	18.25 ± 3.4	22.2 ± 2.7	
ALP U/L			
0 day	68.1 ± 9	99.0 ± 14	< 0.01
7 day	72.9 ± 10	106.0 ± 15	
14 day	74.3 ± 10	108.1 ± 15	

Legend: NS - Non-Significant.

Table2. Pearson's correlation coefficients for the biochemical parameters with the main blood indicators of lipomobilization NEFA and BHB

Parameters	NEFA	BHB
Glucose	-0.7267*	-0.7185*
TG	-0.7609*	-0.5660*
Cholesterol	-0.7136*	-0.6579*
T. Bilirubin	0.7118*	0.6229*
AST	0.6636*	0.6802*
ALP	0.6528*	0.4168*
GGT	0.5735*	0.6401*
T.Protein	-0.1415	-0.2531°
Albumin	-0.5261*	-0.4801*
Urea	0.5277*	0.2887°

Legend: *$p < 0.01$; °$p < 0.05$.

Fatty liver (i.e. hepatic lipidosis) is very often a major metabolic disorder in dairy cows during early lactation and is associated with decreased health status and reproductive performance [21]. In dairy cows, fatty liver occurs primarily in the weeks after calving [8] when up to 50 % of all cows have some accumulation of TG in the liver [22]. Biochemical parameters (NEFA, BHB, glucose, TG, total cholesterol, bilirubin, and AST) may be used as important biochemical indicators in determining the functional status of the liver in dairy cows during the transition period [23].

When niacin is administered in pharmacological doses, it has an antilipolytic effect and reduces NEFA release from adipose tissue [24]. In the present study, the results have shown significantly lower NEFA and BHB concentrations in the niacin group compared to the control group in all weeks of the experiment, confirming the antilipolytic effect of niacin. NEFA concentration > 0.40 mmol/L indicates problems with energy balance and subsequent intensive lipomobilization [25]. In this study cows that received niacin had NEFA concentration lower than 0.40 mmol/L in all weeks of the experiment. Reduced NEFA concentration leads to a lower TG accumulation and ketone body formation in the liver [26]. When TG accumulates in the liver cells, their circulating values decrease [27]. We found a significantly negative correlation between NEFA and TG ($r = -0.7609$).

The process of lipomobilization can cause liver lesions in one-third of the early lactation cows which can be detected by increased AST activity in serum, which is considered the most sensitive indicator for diagnosing fatty liver in cows [10, 20]. We found significantly lower AST concentration in the niacin group compared to the control group and this result indicates that cows that received niacin had lower liver lesions and damage because of decreased lipomobilization. Accordingly, we found a positive correlation between indicators of lipomobilization (NEFA, BHB), and hepatic enzymes (AST, ALP, and GGT).

Another biochemical marker that increases in cows during the peripartal period is bilirubin. Liver damage induces an increase in the total bilirubin and bilirubin is considered a sensitive indicator for liver injury [20]. In our research cows that have received niacin had lower bilirubin concentration compared to the control group. Also, we found a significantly positive correlation between NEFA and bilirubin, indicating improved excretory function of hepatocytes in cows that have received niacin.

Serum levels of glucose, triglyceride, albumin, total protein, cholesterol, and urea are indicators of hepatic function [28, 29, and 30]. Decreased concentrations may be due to their reduced synthesis in fat infiltrated liver of cows with high lipomobilization [10]. In our study niacin treated cows had a significantly higher concentration of glucose, TG, albumin, and cholesterol compared to the control group suggesting that niacin application during the peripartal period improves hepatic function *via* reduced lipolysis in adipose tissue and decreased infiltration of lipids in the liver. Higher plasma glucose concentration in cows treated with niacin was found in previous research [4, 31], and the mechanism by which niacin increases plasma glucose concentration is unknown but may involve an increase in hepatic glucose production and greater gluconeogenic activity in the liver. Gluconeogenic activity in the liver can be impaired by fat infiltration which lowers blood glucose and decreases insulin secretion [8]. A significantly negative correlation between NEFA and glucose ($r = -0.7267$), and BHB and glucose ($r = -0.7185$) was observed in this study.

In previous research, it was found that different dosages of niacin administrated during the peripartal period had various effects on metabolic parameters. Chamberlin [32], demonstrated that niacin supplementation (98 mg/kg) did not alter plasma AST concentration. Another study of oral application of 12 g per day of rumen-unprotected niacin has failed to prevent fatty liver [33]. In our study, we used pharmacological doses of 120 g/d of rumen-unprotected niacin, which provides 5% bioavailability of nicotinic acid [34]. Morey *et al.*, [2], used 24 g/d of rumen-protected form of niacin. Rumen-protected niacin has 40% bioavailability, and he reported that treated cows have lower plasma NEFA concentration and showed a tendency to have less TG in the liver than control cows during the first week of lactation.

A recent study found that supplementation of very high doses of rumen-protected niacin to dairy cows in the periparturient period may induce a systemic inflammation-like condition in the liver [35]. Also, research from human medicine has reported that long use of niacin may lead to liver damage and increased liver enzymes in blood [36]. Gautam *et al.*, [37], found that HCA_2 activation by different concentrations of niacin may exert dual responses in the same cells, namely, anti-inflammatory responses and cell apoptosis. Chen *et al.*, [38], concluded that nicotinic acid could suppress lipolysis and inflammation in *in vitro* studies but effects found *in vivo* are different and the dosage of niacin supplementation and ruminal degradation of free nicotinic acid may partly explain differences between findings.

4. Conclusions

- Based on the results obtained from this study, we found that pharmacological doses of niacin (120 g/d days of rumen unprotected niacin, providing an estimated 6 g/d of bioavailable nicotinic acid) supplemented daily for 4 weeks suppresses lipolysis and improves hepatic function in dairy cows during peripartal period.
- Considering inconsistencies in the reference therapeutic doses of niacin and its effects, further research is needed to establish the beneficial therapeutic doses of daily and total niacin in high-yielding dairy cows.

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