Influence of niacin application on inflammatory parameters, non-esterified fatty acids and functional status of liver in cows during early lactation

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SUMMARY

Metabolic stress in periparturient period in cows is characterized by reduced food intake, negative energy balance, increased lipolysis, ketogenesis, insulin resistance and inflammation. Central metabolic organ in early lactation is liver. Lipid peroxidation and ketogenesis can trigger inflammatory response. Niacin has anti-lipolytic action and potential anti-inflammatory effect. The aim of this study was to determine influence of niacin application on inflammatory response and functional status of liver (expressed with liver functional index, LFI) in cows in early lactation. 30 Holstein-Friesian cows were included in the experiment. Niacin was applied to 15 cows two weeks before and two weeks after calving and 15 cows were included in negative control group. Blood samples were taken by venipuncture of *v. coccigea* before morning feeding, in the moment of calving and then at first and second week after calving. Reduction of TNF-α, haptoglobin, total bilirubin and NEFA concentration, but increase of albumin and cholesterol parameters was caused by niacin application. Fibrinogen concentration was unchanged. Increased values of albumin, cholesterol and reduced value of bilirubin followed by increase of LFI was noted after niacin application. That indicates improved liver hepatocytes function. Positive correlations between TNF-α and fibrinogen, NEFA, haptoglobin and negative with albumin were noted. That proves significance of albumin as a negative protein of acut phase and role of lipolysis in inflammation development in cows in early lactation. Negative correlation between LFI and TNF-α, haptoglobin and NEFA was noted. Correlation between LFI and NEFA and correlation of LFI and haptoglobin was controlled by TNF-α. This indicates that niacin can have dominant anti-inflammatory effect in liver protection. Besides anti-lipolytic effect, niacin has showed anti-inflammatory action that can be significant in hepoatocyte protection in early lactation in dairy cows. Significant influence of niacin on TNF-α was noted. This cytokine controls correlations of LFI, lipolysis and inflammatory response.

KEY WORDS

Cows, inflammation, liver, niacin, tumor necrosis factor alpha.

INTRODUCTION

Metabolic stress in periparturient period in cows is characterized by reduced food intake, negative energy balance, increased lipolysis, ketogenesis and insulin resistance development[1,2]. Increased lipolysis in cows can cause discharge of proinflammatory cytokines (adipokines) from fat tissue. The greatest role of them is given to the tumor necrosis factor alpha (TNF-α)[3].

Central metabolic organ in early lactation is liver. Liver processes increased lipid component. Lipid peroxidation and ketogenesis can trigger inflammatory response. Ohtsuka *et al.*[4] showed increased activity of tumor necrosis factor alpha in cows with mild and severe fatty liver syndrome. Haptoglobin and serum amyloid A concentrations are increased in blood plasma of cows that developed fatty liver[5]. Bertoni *et al.*[6] confirmed that cows classified as cows with high inflammatory index express higher values of bilirubin, AST, GGT and reduced levels of albumin and cholesterol. That indicates fatty liver biochemical profile. Trevisi *et al.*[7] confirmed that inflammatory mediators can directly cause metabolic changes.

Niacin expresses anti-lipolytic effect, reduces insulin resistance in early lactation and has a positive influence at functional status of hepatocytes[8,9]. Application of anti-inflammatory drugs in peripartal period causes reduction in lipolysis and improves hepatic functions[10]. Anti-lipolytic effect of nicotinic acid is mediated by nicotine receptor GPR109A. Nicotine-amid reduces bonding ability to GPR109A[11]. Activation of this receptor causes inhibition of adenylate-cyclase. After that protein-kinase A is inactivated and reduced phosphorilation of hormone sensitive lipase occurs, and consequently lipolysis is reduced. Considering that lipolysis triggers inflammatory response and niacin acts anti-lipolytic there is a possibility of anti-inflammatory action of niacin. It has been showed that nicotine receptor GPR109A can inhibit cytokine production[12] that indicates anti-inflammatory effect.

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The aim of this study was to determine niacin influence at inflammatory response and functional status of liver in cows in early lactation.

MATERIAL AND METHODS

Animals and management - 30 cows of Holstein- Friesian breed in second and third lactation were included in experiment. No health disorders were noted and milk production was 7000±500 L. Cows were kept in free system at deep rug. In transitional period cows were kept bounded in a maternity ward. Cows were fed with limited meals except in maternity yard were food was given *ad libitum*. Mixed meals that satisfied all individual needs were given to animals. Water was given *ad libitum.* Meal for cows in early lactation should contain proteins (17.5-19.5% crude proteins, 30-33% degradable proteins, 35-40% non-degradable proteins), carbohydrates (ADF min. 17-21%, NDF min. 28-31%, NDF from forage min. 18-23%, non-structure carbohydrates 35-42%, level of dry matter from forage min 40-45%), energy (NEL 7-7.4 MJ/kg meal dry matter) fats 5-7%.
Niacin application- Niacin was given mixed with foods *per os*. Fine granular powder of nicotinic acid (Rovimix®Niacin) was used at dosage that allows 6-12 g available in gastrointestinal tract. Dosage was applied daily (60-120 g/day in total mixed ration meal). In recent studies that dosage was suggested as optimal biologic concentration. Niacin was applied during two week period before and after calving.
Blood sampling- Blood was taken by venipunction of *v. coccigea* in cows before morning meal in order to avoid postprandial effect at metabolite values. Further blood was sampled at the moment of calving, then one and two weeks after calving. Samples for biochemical analyzes were taken in test tubes that contained gel separator (BD Vacutainer® SST II Advance, BD Plymoth, UK). Gel contained silicone that activates coagulation cascade and gel that represents barrier between coagulum and serum after centrifugation. Sampled blood was processed in laboratory in the shortest possible time.
Determination of blood parameters- Biochemical analyzes were taken by automatic spectrophotometer Chemray (Rayto, PRC). NEFA, albumin, cholesterol and bilirubin concentrations was determined by standard colorimetric kits (Biosystem, Spain). Tumor necrosis factor alpha (TNF-α), haptoglobin and fibrinogen were determined by standard kits of

Table 1 - Influence of niacin application at determined parameter values.

	Week	Niacin	Control	SEM	Niacin influence
TNF-α (ng/mL)	0	0.24	0.39	0.004	p<0.01
	1	0.28	0.52		
	2	0.27	0.61		
Haptoglobin					
(mg/dL)	0	0.39	0.76	0.012	p<0.01
	1	0.44	0.82		
	2	0.47	0.89		
Fibrinogen					
(g/L)	0	0.92	0.96	0.015	NS
	1	0.94	0.98		
	2	0.93	0.96		
Albumin					
(g/L)	0	39.1	32.2	0.42	p<0.01
	1	37.82	31.5		
	2	38.9	33.6		
T.Bilirubin					
(mol/L)	0	6.8	8.1	0.21	p<0.01
	1	7.2	9.5		
	2	8.1	10.2		
Cholesterol					
(mmol/L)	0	2.05	1.82	0.024	p<0.01
	1	2.19	1.93		
	2	2.41	2.08		
NEFA					
(mmol/L)	0	0.36	0.48	0.011	p<0.01
	1	0.35	0.77		
	2	0.35	0.45		
LFI	0	22.1	10.2	0.047	p<0.01
	1	24.2	11.2		
	2	25.9	12.7		

Table 2 - Correlation matrix of parameters in experiment.

	TNF-α	Haptoglobin	Fibrinogen	NEFA	ALB	LFI
TNF-α	1					
Haptoglobin	0.858**	1				
Fibrinogen	0.115	0.077	1			
NEFA	0.685**	0.608**	-0.001	1		
ALB	-0.531**	-0.452**	-0.046	-0.512**	1	
LFI	-0.464**	-0.398**	-0.091	-0.396**	0.963**	1

Cloud-Clone Corp (PRC) and ELISA reader (Rayto, PRC).
Liver function index (LFI)- This index was determined based on formula LFI = (ALB - 17.71) / 1.08 + (CHOL - 2.57) / 0.43 - (TBIL - 4.01) / 1.21 (Trevisi and Minuti, 2018)[13].
Statistical analyses- Influence of niacin application, week of sampling and their interaction at parameter values were determined by GLM model. Correlations between TNF-α, haptoglobin, fibrinogen, albumin, NEFA and LFI were determined by Pearsons correlation coefficient. By partial correlation was determined if TNF-α controls relation of LFI and other parameters with which showed significant correlation. Partial correlation was visualized by Graph of linear regression between non-standardized values obtained by analyzes of linear regression between determined parameters.

RESULTS

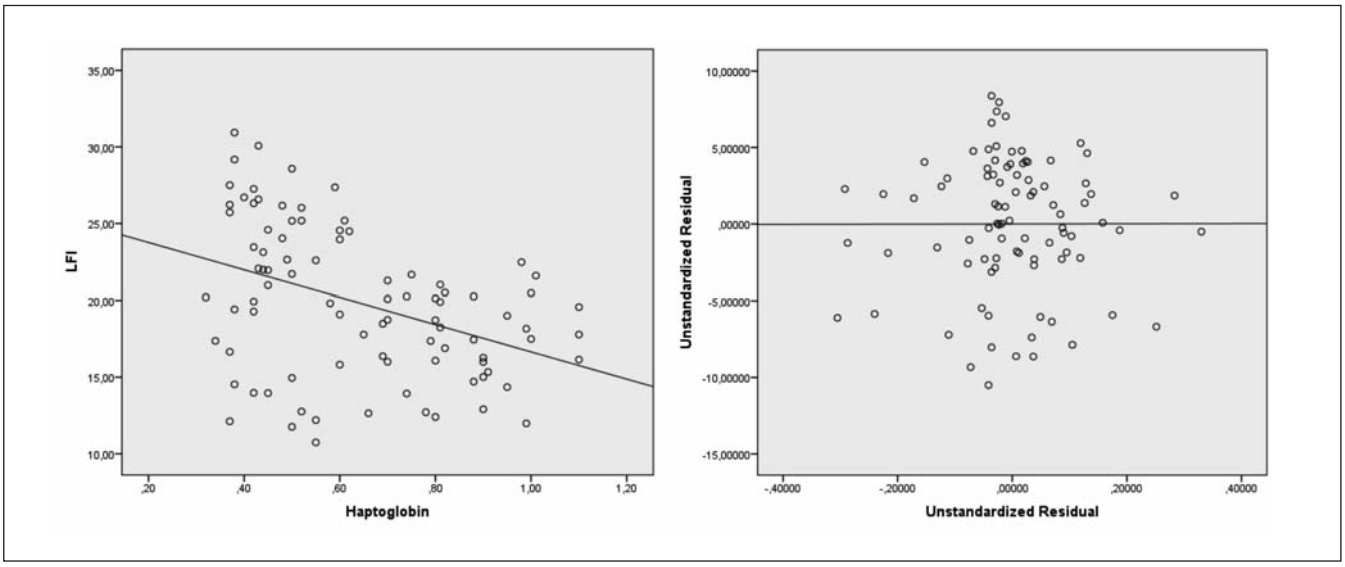
Reduction of TNF-α, haptoglobin, total bilirubin and NEFA concentration, but increase of albumin and cholesterol parameters was caused by niacin application. Fibrinogen concentration was unchanged. Increased values of albumin, cholesterol and reduced value of bilirubin followed by increase of LFI was noted after niacin application. That indicates improved liver hepatocytes function. Results are presented on Table 1. Positive correlations between TNF-α and fibrinogen, NEFA, haptoglobin and negative with albumin were noted (Table 2). That proves significance of albumin as a negative protein of acut phase and role of lipolysis in inflammation development in cows in early lactation. Negative correlation between LFI and TNF-α, haptoglobin and NEFA was noted (Table 3). Correlation between LFI and NEFA and correlation of LFI and haptoglobin was controlled by TNF-α (Figure 1-4). This indicates that niacin can have dominant anti-inflammatory effect in liver protection.

DISCUSSION

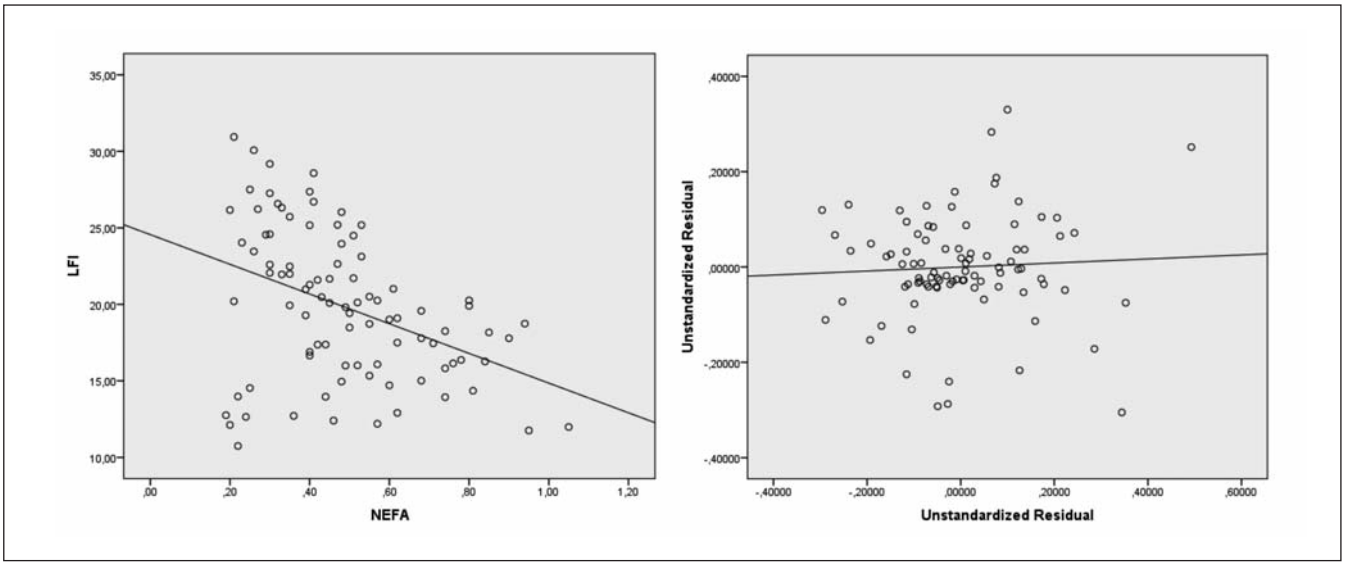
Lipolisis in dairy cows is in relation with ketogenesis and change in liver function[14]. Reduced lipolysis and inflammatory response in cows was showed in the results. Anti-lipolytic effect of niacin is well known and these results are showed in review study of Niehoff *et al.*[15]. Niacin expresses anti-inflammatory actions in fatty tissue. Wanders *et al.*[16] showed that in high fat meal fed mice niacin causes reduction in inflammatory response with increased expression of anti-inflammatory cytokines and reduction in expression of pro-inflammatory cytokines. Research of anti-inflammatory effect of niacin at human monocytes showed that niacin reduces secretion of TNF-α and other inflammatory cytokines[17]. Significant change in fibrinogen value wasn't noted in our research after niacin application, but haptoglobin value was reduced. Morey *et al.*[18] have not found statistically significant difference between haptoglobin value but tendency of niacin to reduce haptoglobin concentration in cows in early lactation was noted ($p<0.1$). At non-alcoholic fatty liver disease model was showed that niacin application directly affects lipid metabolism and indirectly affects inflammatory cascade showing protective role[19].
Liver showed immunologic and anti-inflammatory role by regulation of acute phase response[20]. During acute phase liver produces proteins of acute phase. They are great opsonins against bacteria and their toxins and they trigger complement reaction and other protective reactions[21]. For listed reasons, the main role of liver in albumin production is decreased due to competition between precursors. Albumins, many enzymes and apolipoproteins are known as negative proteins of acute phase. Presence of negative proteins of acute phase is crucial for maintaining metabolic integrity of the whole organism so their deficit is rapidly noticeable in cows that express health problems[22]. Aggregate index that combines, analyzes and

Table 3 - Summary of regression analysis for TNF-α, NEFA and haptoglobin variables predict LFI before (left) and after TNF-α exclusion (right part-partial) as a control parameter.

		Coefficient					Correlations		
	Model	B	SE	Beta	t	Sig.	Zero-order	Partial	Part
1	(Constant)	25.91	1.45		17.93	<0.01			
	Haptoglobin	0.06	4.1	0.003	0.015	NS	-0.398	0.002	0.001
	TNF-α	-18	7.14	-0.467	-2.52	<0.05	-0.464	-0.261	-0.240
2	(Constant)	26.4	1.37		19.22	<0.01			
	NEFA	-3.61	3.17	-0.147	-1.14	NS	-0.396	-0.121	-0.107
	TNF-α	-14.01	4.99	-0.363	-2.81	<0.01	-0.464	-0.288	-0.265



Graphs 1-2 - Correlation of haptoglobin and LFI before (left) and after exclusion of TNF-α as a control factor (right).



Graphs 3-4 Correlation of NEFA and LFI before (left) and after exclusion of TNF-α as a control factor (right).

compares negative proteins of acute phase and similar parameters was developed[23,24]. The main goal of this index is to reveal and determine cows with inflammatory processes and liver damage. Aggregate indexes calculate index of functional status of the liver (LFI) and index of liver activity (LAI). Using these indices allows early identification of cows with inflammatory processes in periparturient period even in subclinical states[25]. Generally poor LFI and LAI indicate reduced food intake and reduced digestive activity[26], greater lipid mobilization and reduced milk production[27]. Increased concentration of albumins and cholesterol and reduced levels of bilirubin followed by increased LFI after niacin application was noted in this research. That indicates improvement of liver hepatocytes function. It is not unusual that cows do not show clinical manifestations of metabolic disturbances and inflammatory processes until the calving and lactation. Calving triggers metabolic changes and their expression but some parameter in blood can be great indicators and signalize early development of metabolic disturbances and inflammation. The most commonly used are values of pro-inflammatory cytokines, positive proteins of acute phase and negative proteins of acute phase[28]. According to Trevisi *et al.*[29] the most significant biomarkers that indicate inflammatory processes and disturbances in cows 3-4 weeks before calving are positive proteins of acute phase. Positive proteins of acute phase are positively correlated with low LFI index and parameters included in calculation of LFI[30].

CONCLUSION

Negative correlation of LFI and TNF-α was noted in our research because niacin cause increase of LFI index while decreases TNF-α, NEFA and haptoglobin concentrations. Anti-lipolytic and anti-inflammatory action of niacin was noted. That is significant mechanism in liver hepatocytes protection in early lactation. Significant influence of niacin on TNF-α concentration was noted and that is very important because this cytokine controls correlations between indices of functional status of liver hepatocytes, lipolysis parameters and inflammatory response.

DISCLOSURE STATEMENT

No conflict of interest was reported by the authors.

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