

## INFLUENCE OF PROPYLENE GLYCOL TO ENDOCRINE AND METABOLIC CHARACTERISTIC IN COWS DURING PERIPARTURIENT PERIOD\*

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**SUMMARY:** *During periparturient period must be provided extra energy. Propylene glycol can rapidly supply transition dairy cows with energy and can be converted to propionic acid in the rumen and transport to liver, where it is converted in glucose. The aim of this study is to estimate influence of propylene glycol to metabolic and endocrine profile in periparturient period. We hypothesized that influence of propylene glycol in protection of energy metabolism in early lactation should be proven trough analysis of the relationship between metabolic and endocrine parameters with NEFA (level of lipolysis) and BHB (level of ketogenesis). Experiment involved 50 healthy holstein-frisian cows (25 – group supplemented with propylene glycol 100g per meal, twice daily, three weeks before calving and three week after calving and 25 – control group). Blood samples were collected one week before expected calving, at calving day and one week after calving by venipuncture of v.coccigea. Concentration of NEFA, BHB, insulin, IGF-I, glucose and bilirubin was measured. The results show that cows that received propylene glycol are less burdened with metabolic stress. Thus, cows treated with propylene glycol have higher concentrations of glucose, insulin, IGF-I and lower concentrations of NEFA, BHB and bilirubine. Post-hock analysis it can be concluded that cows in early lactation show these differences, but the differences in metabolite concentrations before calving were not significant. Cows in which was applied propylene glycol are less burdened by metabolic stress, which is reflected in lower proportion of cows with signs of metabolic stress. Efficacy of propylene*

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*glycol may be seen in the fact that propylene glycol is changing relationships and strength of links between metabolites. In the first week after calving, when there is most pronounced metabolic load was found a significant negative correlation between plasma NEFA or BHB with insulin, IGF-I and glucose concentration and positive correlation with bilirubin concentration. Correlation between metabolite was significantly lower or was absent in the group of cows that received propylene glycol. We performed the analysis of the regression model and tested the difference between the b parameters in cows that received propylene and control groups. For each unit of NEFA or BHB was found significantly lower decrease in the concentration of insulin, IGF-I and glucose and significantly lower increase in the concentration of bilirubin in relation to the test value of the control group. Energy added by propylene glycol changes metabolic characteristics of cows characterized by reduced lipid mobilization and ketogenesis with their reduced relationship with other metabolic parameters.*

**Key words:** dairy cows, propylene glycol, metabolic profile.

## INTRODUCTION

Negative energy balance in dairy cows during transition period (21 days before and 21 days after calving) leads to many metabolic changes. These metabolic changes occur as a result of the entry of the mammary gland in the metabolic processes. Negative energy balance is the result of decreased food intake, higher consumption of glucose in the udder and decrease insulin sensitivity. The consequence of mentioned metabolic changes is higher lipid mobilization from body depot with increased concentration of NEFA (nonesterified fatty acid). Higher NEFA mobilization in liver leads to higher concentration of ketone body in blood, especially BHB (beta-hydroxybutyrate) (Ingvarsen and Andersen, 2000). Lipomobilisation and ketogenesis burden hepatocytes with lipide infiltration as consequence. Cows with liver lipidosis show higher bilirubine and lower IGF-I concentration (Bobe et al, 2004). The ability of the hepatocytes to use NEFA and BHB for energy purpose is depends of propionate from rumen. But, decreased dry mater intake in transition period reduces propionate concentration. Therefore during periparturient period must be provided extra energy. Propylene glycol can rapidly supply transition dairy cows with energy and can be converted to propionic acid in the rumen and transport to liver, where it is converted in glucose (Nielsen and Ingvarsen, 2004). In many experimental study cows supplemented with propylene-glycol showed decreased lipide mobilization (lower NEFA concentration) and ketogenesis (lower BHB concentration) with change in hormonal status, milk production and reproductive efficiency (Studer et al., 1993; Formigoni et al., 1996; Rizos et al, 2008; Hoedemaker et al, 2004).

The aim of this study is to estimate influence of propylene glycol to metabolic and endocrine profile in periparturient period. We hypothesized that influence of propylene glycol in protection of energy metabolism in early lactation should be proven trough analysis of the relationship between metabolic and endocrine parameters with NEFA (level of lipolysis) and BHB (level of ketogenesis).

## MATERIAL AND METHODS

Experiment involved 50 healthy holstein-frisian cows. Cows were divided in two groups with 25 cows: group supplemented with propylene glycol (100g per meal, twice daily, three weeks before calving and three week after calving) and control group. Blood samples were collected one week before expected calving, at calving day and one week after calving by venipuncture of v.coccigea. Concentration of insulin and IGF-I was measured using a standard ELISA procedures (Cusabio). Concentration of glucose, NEFA, BHB and bilirubin were measured using a standard kit (Randox, UK). The samples were analyzed immediately after the taking by automatically analyzer (Rayto).

Statistical procedures included t-test for difference between mean value in experimental and control group for each examined parameters in blood. Reduction of metabolic stress by propylene glycol was examined using a t-test for difference in proportion of cow burden with metabolic stress in experimental and control group. Criteria for metabolic stress included: insulin  $<0.5$  ng/ml, IGF-I  $<25$  ng/ml, glucose  $<2$ mmol/l, NEFA  $>0.6$ mmol/l, BHB  $>0.9$ mmol/l and bilirubin  $>9$ μmol/l. Correlation between NEFA and BHB and insulin, glucose, IGF-I and bilirubin in first week after calving was analysed using a t-test for Pearson correlation coefficient separately in experimental and control group. Change in metabolic profile in relation to value of NEFA and BHB in first week after calving was detected using a test for b parameters in regression analysis, separately in experimental and control group.

## RESULTS AND DISCUSSION

The results show that cows that received propylene glycol are less burdened with metabolic stress. Thus, cows treated with propylene glycol have higher concentrations of glucose, insulin, IGF-I and lower concentrations of NEFA, BHB and TBIL (Table 1). Post-hock analysis it can be concluded that cows in early lactation show these differences, but the differences in metabolite concentrations before calving were not significant. Bojković-Kovacević et al. (2011) in their observation concluded that the application of propylene glycol leads to an increase in glucose concentration above the normal range, while the concentration of BHB and TBIL was significantly lower. Our findings are in the spirit of these results. The higher the concentration of insulin during the application of propylene was found in the work of Lien et al. (2010), but it did not exist at any time of sampling, but only in the first days after parturition (4-6), where we also performed blood sampling. Prepartum application of propylene glycol from 21 d before calving to the d of parturition reduced prepartum plasma concentrations of NEFA and BHBA and increased prepartum plasma concentrations of glucose and insulin (Juchem et al., 2004; Miyoshi et al., 2001; Pickett et al., 2003; Stokes and Goff, 2001). Also, peripartum application of propylene glycol leads to decrease blood concentrations of NEFA and BHBA and increases blood concentrations of glucose, insulin and cholesterol (Butler et al., 2006; Formigoni et al., 1996; Hoedemaker et al., 2004). Our results are consisted with previously results. The application of propylene glycol leads to an increase in IGF-I in the weeks before and the first week after calving (Hoedemaker et al, 2004), which was confirmed by our results. In one experiment on a large group of cows has been shown that the application of propylene glycol has no effect on the levels of insulin, glucose, IGF-I and NEFA

(Lomander et al., 2012). However, in this study, the propylene was administered only postpartum, with a significant effect of the herd of cows which are encouraged, and we conclude that the significant effect of propylene is absent as a result of a heterogeneous group. Lower serum bilirubin levels and lower activity of liver enzymes may be related to reduce triglyceride accumulation in hepatocytes, which was found in cows that received propylene glycol (Studer et al, 1993). Also, the application of propylene glycol has a positive effect on cholesterol (Formigoni et al, 1996). It is known that a higher value implies higher cholesterol VLDL lipoproteins that transport fats from hepatocytes. Cows in which was applied propylene glycol are less burdened by metabolic stress, which is reflected in lower proportion of cows with signs of metabolic stress (Table 2).

Efficacy of propylene glycol may be seen in the fact that propylene glycol is changing relationships and strength of links between metabolites. In the first week after calving, when there is most pronounced metabolic load was found a significant negative correlation between plasma NEFA or BHB with insulin, IGF-I and glucose concentration and positive correlation with bilirubin concentration. The existence of a correlation is consistent with previously obtained results (Wathes et al, 2007). Correlation between metabolite was significantly lower or was absent in the group of cows that received propylene glycol (Table 3). We performed the analysis of the regression model and tested the difference between the b parameters in cows that received propylene and control groups. For each unit of NEFA or BHB was found significantly lower decrease in the concentration of insulin, IGF-I and glucose and significantly lower increase in the concentration of bilirubin in relation to the test value of the control group (Table 4). The above results indicate that application of propylene glycol reduces the impact of lipid mobilization and ketogenesis (NEFA and BHB) on metabolic parameters. Lipid mobilization and ketogenesis are the most important consequences of negative energy balance, which could affect many aspects of metabolic and functional status of hepatocytes in cows (González et al, 2011; Cincović et al, 2012; Đoković et al, 2013). High concentrations of NEFA and BHB in cows imply high risk of periparturient disease (Ospina et al., 2010; Cincović et al, 2012a) and our early obtained results show a significant strong negative correlation between NEFA and insulin levels in diseased cows (Cincović et al, 2012b).

Table 1: Prepartum and postpartum metabolic profile in control and cows supplemented with propylene glycol

	Control			Propylene			SEM	Week	Propylene
	-1	0	+1	-1	0	+1			
Insulin <i>ng/ml</i>	0,72	0,42	0,55	0,89	0,67	0,71	0,11	<0,01	<0,05
IGF-I <i>ng/ml</i>	51,2	30,1	29,5	62,6	49,9	38,8	5,5	<0,05	<0,01
Glu <i>mmol/l</i>	2,71	2,19	2,9	2,94	2,69	3,32	0,14	<0,05	<0,05
NEFA <i>mmol/l</i>	0,69	0,84	0,43	0,41	0,54	0,28	0,11	<0,01	<0,05
BHB <i>mmol/l</i>	0,59	0,99	0,56	0,51	0,64	0,33	0,21	<0,01	<0,05
BIL <i>μmol/l</i>	8,2	10,1	7,05	6,5	7,23	5,89	1,13	<0,05	<0,05

Table 2: Proportion of cows with suboptimal metabolic status

	Control			Propylene			Week	Propylene
	-1	0	+1	-1	0	+1		
Insulin <i>ng/ml</i>	4	12	8	4	8	4	<0,05	<0,05
IGF-I <i>ng/ml</i>	4	16	12	4	8	4	<0,01	<0,01
Glu <i>mmol/l</i>	8	24	12	2	16	4	<0,01	<0,01
NEFA <i>mmol/l</i>	4	20	16	0	12	12	<0,01	<0,05
BHB <i>mmol/l</i>	4	32	8	4	12	4	<0,01	<0,01
BIL <i>μmol/l</i>	8	20	16	0	16	8	<0,01	<0,05

Tabela 3: Influence of propylene glycol supplementation to relationship between NEFA, BHB and other metabolites in first week after calving

	NEFA		P Difference	BHB		P Difference
	Propylene	Control		Propylene	Control	
Insulin <i>ng/ml</i>	-0.28 <0.05	-0.49 <0.01	<0.05	0.30 <0.05	0.56 <0.01	<0.05
IGF-I <i>ng/ml</i>	-0.14 NS	-0.31 <0.05	<0.05	0.33 <0.05	0.47 <0.01	NS
Glu <i>mmol/l</i>	-0.22 NS	-0.51 <0.01	<0.01	0.24 NS	0.55 <0.01	<0.05
BIL <i>μmol/l</i>	0.26 NS	0.32 <0.05	NS	0.33 <0.05	0.52 <0.01	<0.05

Table 4: Regression analysis – testing of b parameters which means change in metabolic value for each unit of NEFA or BHB in control group and group supplemented with propylene glycol in first week after calving

	NEFA		P Difference	BHB		P Difference
	Propylene	Control		Propylene	Control	
Insulin	- 0.05±0.008	- 0.09±0.007	<0.01	- 0.07±0.01	- 0.09±0.013	<0.05
IGF-I	- 0.32±0.11	- 0.44±0.09	<0.05	- 0.37±0.09	- 0.51±0.10	<0.01
Glu	- 0.08±0.02	- 0.11±0.03	<0.01	0.11±0.09	0.17±0.11	NS
Bil	0.52±0.27	0.63±0.31	NS	0.47±0.12	0.6±0.15	<0.05

## CONCLUSION

Cows treated with propylene glycol have higher concentrations of glucose, insulin, IGF-I and lower concentrations of NEFA, BHB and TBIL, especially in early lactation. These cows are less burdened by metabolic stress, which is reflected in lower proportion of cows with signs of metabolic stress. Energy added by propylene glycol changes metabolic characteristics of cows characterized by reduced lipid mobilization and ketogenesis with their reduced relationship with other metabolic parameters.

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## UTICAJ PROPILLEN GLIKOLA NA ENDOKRINE I METABOLIČKE KARAKTERISTIKE KRAVA U PERIPARTALNOM PERIODU

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### Izvod

Tokom peripartalnog perioda kravama se mora obezbediti dodatna energija za početak laktacije. Propilen glikol može veoma brzo dati energiju koja je potrebna kravama tako što biva konvertovan u propionsku kiselinu u buragu, koja biva transportovana u jetru gde se dalje konvertuje u glukozu. Cilj ovog istraživanja je da se ispita uticaj dodavanja propilen glikola u hrani na metabolički i endokrini profil krava u peripartalnom periodu. Predpostavka je da se zaštitini efekat propilen glikola može dokazati analizom korelacije između metaboličkih i endoktrinih parametara sa koncentracijom NEFA (neesterifikovane masne kiseline, pokazatelj lipolize, u vezi sa insulinskom rezistencijom) i BHB (beta-hidroksibutirat, pokazatelj ketogeneze, u vezi sa masnom jetom). U eksperiment je uključeno 50 krava holštajn-frizijske rase: 25 krava je dobijalo propilen glikol 100g/obrok/krava, dok je 25 krava bila kontrolna grupa i nije dobijala ovaj energetski suplement. Krv je uzorkovana u nedelji pre teljenja, nedelji u kojoj se krava otelila i u nedelji potom venepunkcijom *v.coccigea*. Merena je koncentracija NEFA, BHB, insulina, IGF-I (insulinu sličan faktor rasta I) glukoze i bilirubina. Dobijeni rezultati pokazuju da su krave koje su dobijale propilen glikol mnogo manje opterećene metaboličkim stresom u ispitivanom periodu. Tako, ove krave pokazuju višu koncentraciju glukoze, insulina, IGF-I i nižu koncentraciju NEFA, BHB i bilirubin. Ove razlike su postojale u nedeljama posle teljenja, ali ne i pre teljenja. Manje opterećenje metaboličkim stresom kod krava koje su dobijale propilen glikol odlikuje se i značajno manjom proporcijom krava koje pokazuju znake metaboličkog stresa (indikatori metaboličkog stresa: insulin <0.5 ng/ml, IGF-I <25 ng/ml, glukoza <2mmol/l, NEFA >0.6mmol/l, BHB >0.9mmol/l i bilirubin >9μmol/l). Efikasnost propilen glikola može da se objasni činjenicom da aplikacija ovog energetskog prekursora menja relacije između metabolita. U prvoj nedelji posle teljenja, kada je metaboličko opterećenje krava najveće, postoji značajna negativna korelacija koncentracije NEFA ili BHB sa insulinom, IGF-I i glukozom i pozitivna korelacija sa koncentracijom bilirubina. Kod krava koje su dobijale propilen glikol ove korelacije izostaju. Regresionom analizom je pokazano da za svaku jedinicu NEFA odnosno BHB postoji značajno manji pad vrednosti insulina, IGF-I i glukoze i značajno slabiji porast koncentracije bilirubina u grupi krava koje su dobijale propilen glikol u odnosu na kontrolnu grupu. Energija dobijena iz propilen glikola smanjuje stepen lipidne mobilizacije i ketogeneze i smanjuje njihov uticaj na metaboličku adaptaciju koja se ogleda u kretanju vrednosti ostalih ispitanih metaboličkih parametara.

**Ključne reči:** mlečne krave, propilen glikol, metabolički profil.

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