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Effects of Rumen Protected Choline and Propylene Glycol on Metabolism in Dairy Cows with Low Body Condition

Hasan ATALAY
Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Balıkesir University
hasanatalay@balikesir.edu.tr, ORCID: https://orcid.org/0000-0002-5744-7538

Kudret YENILMEZ
Tekirdağ Namık Kemal University
kyenilmez@nku.edu.tr, ORCID: https://orcid.org/0000-0002-5532-0525

Halef DOĞAN
Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Tekirdağ Namık Kemal University
halefdogan@nku.edu.tr, ORCID: https://orcid.org/0000-0003-1365-1729

Yahya IŞIK
Kepsut Vocational School, Balıkesir University
yahya.isik@balikesir.edu.tr, ORCID: https://orcid.org/0000-0001-7654-1565

Abstract

Rumen protected choline (RPC), propylene glycol (PG) are used for preventing fatty liver disease and ketosis. The purpose of this study was to determine the effects of RPC, PG on metabolism in dairy cows with low body condition. The study included 32 multiparous Holstein cows (n=32) aged 3-6 years with a body condition score (BCS) of 2 to 2.5. The animals were divided into two groups using the random sampling method. The experimental group (n=16) received 60 g/d of RPC and 250 ml/d of PG by oral administration for one-week prepartum (7±5 days) and another week postpartum. The control group (n=16) received no RPC and PG. Blood samples were examined for calcium, Gamma-glutamyl transferase (GGT), Non-Esterified Fatty Acids (NEFA), β-hydroxybutyric acid (BHBA), urea, and triglycerides. All cows in the study received the same care and food routine. RPC and PG supplementation had a effect on blood concentrations of NEFA, urea, and triglycerides ($p<0.05$). Therefore, using RPC and PG could reduce the incidence of ketosis and fatty liver disease by significantly lowering blood triglyceride levels. Accordingly, it is thought that an increase in the NEFA and BHBA ratios could lead to fatty liver and ketosis.

Statistical analysis revealed that the oral supplementation of RPC and PG to dairy cows in the experimental group had a statistically significant effect on the blood triglyceride, NEFA concentrations. As a result, using RPC and PG in dairy cows could help to lower the risk of ketosis and fatty liver disease since blood triglyceride levels decreased significantly.

Keywords: Dairy Cows, Low Body Condition, Propylene Glycol, Rumen Protected Choline

INTRODUCTION

Ketosis is a common metabolic disease found in dairy cows in a negative energy balance, characterized by increased amounts of NEFA and ketone bodies (acetoacetate, BHBA, and acetone). Cows in ketosis have a lower live weight and milk yield than those not in ketosis (Li *et al.,* 2012). During negative energy balance (NEB), cows meet their energy needs by mobilizing body fat reserves. Mobilized fats are converted into fatty acids and glycerol. Glycerol is used in energy metabolism since it is a form of carbohydrate. Non-Esterified Fatty Acids that reach the liver are either esterified to triglycerides or oxidized to meet energy needs. As a result, the fatty liver and ketosis develop (De Vries and Veerkamp, 2000).

Choline is not considered a vitamin in the usual sense because it may be synthesized by cows. Cows require this nutrient in grams rather than milligrams or micrograms. It is believed that supplementing peripartum cows with a reliable source of RP choline which is absorbed in the intestines and does not degrade in the rumen will boost yield and maybe lower the incidence of some health issues. However, due to potential variability in commercial products and the fact that choline is not an essential nutrient (as it can be synthesized by the cow), the committee has not established a dietary requirement for it (NASEM, 2021).

Propylene glycol is either fermented in the rumen and degraded into propanol and propionate before entering the bloodstream, or it is absorbed directly into the bloodstream without first decomposing in the rumen. PG has no effect on the total concentration of volatile fatty acids. However, it decreases the acetate ratio while increasing the propionate ratio. Thus, it affects the acetate/propionate ratio. Propylene glycol is converted to lactate in the liver. The liver's uptake of PG has been found to be relatively low (Ruddick, 1972, Kristensen and Raun, 2007). The synthesis of milk fat requires NEFA. Increased NEFA levels lead to an excessive accumulation of

triglycerides in the liver, resulting in hepatic lipidosis and other adverse outcomes. Increased NEFA levels impair and reduce immune cell function (Contreras *et al.,* 2010, Ster *et al.,* 2012).

The severity of NEB is assessed by analyzing NEFA and BHBA levels two weeks before and two weeks after calving. During the transition period, NEB is typically severe. Individual variations during the transition period can have significant effects on herd structure. The non-standard distribution used in calculating the standard deviations of both NEFA and BHBA is insufficient for determining an objective threshold value for excessive NEB. Consumption of high energy roughage during the dry season increases BCS, and NEB occurs with an ineffective herd management system (Ospina *et al.,* 2013). There is a positive correlation between NEFA levels and BHBA, but a negative correlation between NEFA and both glucose and cholesterol (Taghipour *et al.,* 2010).

The purpose of this study was to assess the effects of feeding RPC and PG to dairy cows on their metabolism. Studies about the co-administration of PG and RPC to dairy cows are limited.

MATERIALS AND METHODS

The study was conducted at a modern dairy cattle breeding facility with a capacity of five hundred animals. The study included 32 multiparous Holstein cows (n=32) aged 3-6 years with a BCS of 2 to 2.5. The animals were separated into two groups using the random sampling approach. The experimental group (n=16) received 60 g/d of RPC (60% RPC, 36 g/d choline chloride) and 250 ml/d of PG via oral administration for a week prepartum (7 ± 5 days) and another week postpartum. The cows in the control group (n=16) received no RPC and PG. The cows were all fed the identical total mixed rations (TMR) ad libitum twice daily. Total mixed rations were prepared in the Animal Nutrition and Nutritional Diseases Laboratory of Balıkesir University, Faculty of Veterinary Medicine. The nutritional content of TMR was shown in Table 1. Blood samples were collected from all the animals twice via the coccygeal vein: on the seventh day before calving and on the seventh day after calving, and examined for calcium, Gamma-glutamyl transferase (GGT), Non-Esterified Fatty Acids (NEFA), β-hydroxybutyric acid (BHBA), urea, and triglycerides. Blood analyses were conducted using biochemical reagents with a Randox Imola biochemistry analyzer.

The power of the test for each variable was calculated to determine the required sample size for this study, targeting at least 80% power and a Type I error rate of 5%. The Shapiro-Wilk test was used to determine if the blood analysis measurements in the study were normally distributed. Nonparametric tests were used since the measurements did not follow a normal distribution. Descriptive statistics for continuous variables in the study were expressed as mean, standard deviation (SD), median, and range. Mann-Whitney-U test was used to compare blood analysis measurements between groups. The "prepartum and postpartum" blood analysis were compared separately within each group using the Wilcoxon test. Pearson correlation coefficients were used to assess the relationships between the data sets. The statistical analysis was conducted using SPSS (IBM SPSS for Windows, ver.26) with a significance level of $p<0.05$.

Table 1. TMR nutrient composition (% DM).

Dry Matter (DM)%	55
Crude Protein (CP)%	13,89
Crude Fat (CF)%	2,25
Crude Cellulose (CC)%	18,52
Crude Ash (CA)%	7,39
Starch %	27,5

RESULTS

Two-way comparisons of blood analysis results by group and by prepartum and postpartum periods were shown in Table 2.

Comparison of results between control and experimental groups

Table 2 shows that the experimental group had a higher prepartum NEFA value, with a significant difference ($p=0.028$). Similarly, the urea level was found to be higher in the control group throughout the prepartum period, with a difference ($p=0.044$). Again, the urea level was higher in the control group in the postpartum period, and the difference was statistically significant ($p=0.044$). Aside from the numbers given above, there was no difference in blood analysis findings between the control and experimental groups ($p>0.05$). Therefore, these values were similar between the two groups.

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Table 2. Two-way comparison of blood analysis values by group and by prepartum and postpartum periods.

	Control Group				Experiment Group				*p.
	Mean	Std. Dev.	Median	Range	Mean	Std. Dev.	Median	Range	
Calcium Pre (mg/dL)	10,05	0,87	10,18	3,75	9,67	1,37	9,91	5,18	*0,533*
Calcium Post (mg/dL)	9,95	0,89	10,09	2,87	9,34	1,23	9,50	4,53	*0,152*
**p.	*0,734*				*0,934*				
GGT Pre (U/L)	20,56	6,23	19,00	24,00	17,86	7,26	16,50	31,00	*0,150*
GGT Post (U/L)	21,25	16,58	17,50	60,00	20,33	9,86	22,50	32,00	*0,534*
**p.	*0,880*				*0,541*				
NEFA Pre (mmol/L)	0,09	0,09	0,04	0,31	0,18	0,16	0,14	0,54	***0,028***
NEFA Post (mmol/L)	0,35	0,39	0,15	1,21	0,31	0,30	0,17	0,86	*0,890*
**p.	**0,028**				*0,299*				
BHBA Pre (mmol/L)	0,50	0,16	045	0,54	0,41	0,16	0,37	0,54	*0,129*
BHBA Post (mmol/L)	1,10	1,32	0,52	4,15	0,44	0,12	0,42	0,39	*0,220*
**p.	*0,080*				*0,687*				
Urea Pre (mg/dL)	13,63	2,75	14,00	9,00	11,29	3,27	10,50	13,00	***0,044***
Urea Post (mg/dL)	14,50	3,46	14,50	11,00	12,00	3,93	11,00	16,00	***0,023***
**p.	*0,300*				*0,755*				
Triglycerides Pre (mg/dL)	14,38	5,38	13,55	24,14	14,03	5,45	13,76	21,16	*0,934*
Triglycerides Post (mg/dL)	8,19	3,25	7,97	9,97	7,95	2,89	7,97	11,77	*0,918*
**p.	***0,001***				***0,001***				

*p Significance levels according to Mann-Whitney-U test results (between groups→), Std. Dev.: Standard Deviation

**p Significance levels according to Wilcoxon test result (within group↓) Pre: Prepartum; Post: Postpartum, GGT: Gamma-glutamyl transferase, NEFA: Non-Esterified Fatty Acids, BHBA: β-hydroxybutyric acid

Comparison of results between prepartum and postpartum

In the control group, a statistically significant difference was observed between prepartum and postpartum NEFA values ($p<0.05$). Specifically, the NEFA value increased significantly after calving in this group. Similarly, in the control group, there was a difference between prepartum and postpartum triglyceride values ($p<0.05$). In particular, triglyceride levels decreased significantly

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after calving. The experimental group showed a difference in triglyceride levels between prepartum and postpartum ($p<0.05$), with triglyceride values decreasing significantly after calving.

Table 3 presents the results of the correlation analysis for the blood measurements of the control group. Significantly related correlation coefficients are marked with an asterisk (*). Table 3 shows a strong correlation between prepartum calcium and BHBA ($p<0.05$). Accordingly, calcium levels increased during prepartum, and so did BHBA. There was a significant negative correlation between postpartum calcium and BHBA ($p<0.05$). In this context, when postpartum calcium increased, BHBA decreased.

Table 3. Correlation analysis of blood values in the control group.

		Calcium Pre	Calcium Post	GGT Pre	GGT Post	NEFA Pre	NEFA Post	BHBA Pre	BHBA Post	Urea Pre	Urea Post	Triglycerides Pre	Triglycerides Post
Calcium Pre (mg/dL)	**R**	1											
	p.												
Calcium Post (mg/dL)	**R**	,132	1										
	p.	,626											
GGT Pre (U/L)	**R**	-,423	-,542	1									
	***p.**	,102	**,030**										
GGT Post (U/L)	**R**	-,376	,343	-,031	1								
	p.	,151	,193	,909									
NEFA Pre (mmol/L)	**R**	-,153	,224	-,013	,580	1							
	***p.**	,571	,405	,960	**,019**								
NEFA Post (mmol/L)	**R**	,173	-,400	,157	-,372	-,253	1						
	p.	,522	,125	,562	,155	,344							
BHBA Pre (mmol/L)	**R**	,571	-,061	-,207	-,309	-,151	,306	1					
	***p.**	**,021**	,823	,441	,245	,577	,248						
BHBA Post (mmol/L)	**R**	,099	-,538	,210	-,361	-,283	,871	,320	1				
	***p.**	,714	**,032**	,435	,170	,288	**,001**	,227					
Urea Pre (mg/dL)	**R**	,066	-,127	,413	-,059	-,224	,386	,193	,341	1			
	p.	,808	,640	,111	,828	,404	,139	,473	,196				
Urea Post (mg/dL)	**R**	-,015	-,316	-,002	-,161	-,346	,581	,016	,610	,468	1		
	***p.**	,957	,233	,995	,551	,189	**,018**	,952	**,012**	,067			
Triglycerides Pre (mg/dL)	**R**	-,002	-,204	-,060	-,155	-,659	,252	,029	,379	-,116	,186	1	
	***p.**	,994	,450	,827	,567	**,005**	,347	,916	,148	,668	,489		
Triglycerides Post (mg/dL)	**R**	,326	-,267	,035	-,483	-,116	-,041	,331	-,123	-,415	-,333	,042	1
	p.	,218	,318	,896	,058	,669	,879	,211	,649	,110	,208	,878	

r: Pearson correlation coefficients; p: Significance levels of the correlation coefficient; Pre: Prepartum; Post: Postpartum

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Similarly, a significant positive correlation was observed between postpartum NEFA and BHBA ($p<0.05$). In this case, BHBA increased along with NEFA during the postpartum period. Furthermore, a statistically significant positive correlation was found between postpartum NEFA and BHBA and postpartum urea ($p<0.05$). That is, as postpartum NEFA and BHBA levels increased, urea levels also increased. Additionally, a significant negative correlation was found between prepartum NEFA and triglyceride ($p<0.05$). Thus, as prepartum NEFA levels increased, triglyceride levels decreased.

There was a significant negative correlation between postpartum calcium and prepartum GGT ($p<0.05$). Specifically, prepartum GGT levels decreased as postpartum calcium levels increased. However, no significant correlations were found between the other blood analysis parameters ($p>0.05$).

Table 4. Correlation analysis of blood values in the experimental group.

		Calcium Pre	Calcium Post	GGT Pre	GGT Post	NEFA Pre	NEFA Post	BHBA Pre	BHBA Post	Urea Pre	Urea Post	Triglycerides Pre	Triglycerides Post
Calcium Pre (mg/dL)	r	1											
	p.												
Calcium Post (mg/dL)	r	-,543	1										
	*p.	**,045**											
GGT Pre (U/L)	r	,014	,004	1									
	p.	,961	,989										
GGT Post (U/L)	r	,597	-,602	,516	1								
	*p.	**,024**	**,008**	,059									
NEFA Pre (mmol/L)	r	-,531	-,113	-,326	-,206	1							
	p.	,051	,700	,256	,480								
NEFA Post (mmol/L)	r	,147	-,173	,332	,225	,083	1						
	p.	,616	,493	,246	,369	,777							
BHBA Pre (mmol/L)	r	-,592	,386	,119	-,459	,290	,114	1					
	*p.	**,026**	,173	,685	,099	,315	,698						
BHBA Post (mmol/L)	r	-,139	-,108	-,038	-,231	-,155	,399	,031	1				
	p.	,636	,669	,896	,357	,597	,101	,917					
Urea Pre (mg/dL)	r	-,095	,228	-,183	,039	,301	-,017	,065	-,284	1			
	p.	,746	,433	,531	,895	,295	,955	,824	,325				
	r	,133	-,138	-,176	-,056	-,347	-,106	-,631	,298	-,279	1		

Urea Post (mg/dL)	**p.**	,651	,585	,548	,825	,224	,677	,015	,229	,334			
Triglycerides Pre	**r**	-,026	,284	-,272	-,344	,174	,202	,239	,114	,262	-,449	1	
(mg/dL)	**p.**	,930	,325	,347	,229	,552	,488	,411	,698	,366	,108		
Triglycerides Post	**r**	-,017	-,311	-,464	-,090	,534	,534	,032	,236	,316	,172	,215	1
(mg/dL)	***p.**	,954	,210	,095	,723	**,049**	**,022**	,913	,346	,271	,495	,461	

r: Pearson correlation coefficients; p: Significance levels of the correlation coefficient.

Pre: Prepartum; Post: Postpartum

Table 4 presents the results of the correlation analysis between blood measurements in the experimental group. Correlation coefficients that are significantly correlated with each other are indicated with an asterisk (*). Prepartum and postpartum calcium levels showed a significant negative correlation ($p<0.05$) when Table 3 was examined.

Specifically, when prepartum calcium levels increased, postpartum calcium levels decreased. Again, a significant positive correlation was found between prepartum calcium and postpartum GGT ($p<0.05$). That is, when prepartum calcium levels increased, so did postpartum GGT.

Additionally, we found a significant negative correlation between prepartum calcium and BHBA levels ($p<0.05$). That is, as prepartum calcium levels increased, prepartum BHBA levels decreased. Postpartum calcium and GGT levels showed a significant negative correlation ($p<0.05$). Thus, as postpartum calcium increased, GGT decreased. There was also a strong correlation between prepartum and postpartum NEFA levels and postpartum triglycerides ($p<0.05$). In other words, as prepartum and postpartum NEFA levels increased, so did postpartum triglycerides. However, no significant correlation was identified between the correlation coefficients of other blood analysis measurements ($p>0.05$).

DISCUSSION

Dietary supplementation with RPC and rumen-protected methionine (RPM) has been shown to increase dry matter consumption, improve postpartum energy balance, reduce plasma concentrations of NEFA and BHBA, and enhance milk production in dairy cows (Sun *et al.,* 2016). Lima *et al.* (2024) found that RPC supplementation had no effect on plasma NEFA or BHBA concentrations. However, multiparous cows had a lower incidence of hyperketonemia. In the

presented study, rumen-protected choline supplementation increased lactation and metabolism in cows, but the advantages increased when the cows were fed prior to and following calving.

Feeding RPC to cows during the periparturient period reduced esterified fatty acids in the liver and increased hepatic glycogen levels (Piepenbrink *et al.,* 2003). Furthermore, RPC supplementation decreased hepatic triacylglycerol and increased glycogen content while having no effect on blood concentrations of fatty acids, BHB, glucose, triacylglycerol, or total cholesterol (Arshad *et al.,* 2023). Rumen-protected choline supplementation during the transition period improved lactation performance and health in dairy cows (Marques *et al.,* 2024).

Propylene glycol reduces the concentrations of NEFA and BHBA in the blood of cows during early lactation. It also reduces liver triglyceride levels and milk ketone body concentrations. Propylene glycol is a glycogenic substrate with antiketogenic effects. It has no effect on milk yield, feed intake, or palatableness. As a result, PG may reduce the risk of ketoacidosis and fatty liver disease (Nielsen *et al.,* 2004).

In the present study, prepartum PG treatment reduced plasma NEFA concentrations and hepatic triglyceride buildup. Given the natural decrease in dry matter intake prior to parturition and complications such as rumen acidosis resulting from an increase in non-structural carbohydrates in the diet, this reduction can be accomplished by improving energy balance (Studer *et al.,* 1993). Cows given PG prepartum consumed more dry matter throughout the dry and lactation seasons, whereas cows given PG postpartum consumed less dry matter. Prenatal and postnatal PG supplementation has been shown to improve the metabolic state in the blood by lowering fat mobilization in the body. There was no effect of prepartum or postpartum PG supplementation on milk yield (Van Soest *et al.,* 2023). The addition of PG to the diet as a dry product reduced plasma BHBA content, however PG sprinkled on top of the feed was more effective than PG added to the TMR. Rumen-protected choline increased milk yield linearly. The combination of dietary PG and choline showed no significant effect (Chung *et al.,* 2009).

Rumen-protected choline has been reported to support metabolic processes during the transition period, contributing to improved liver health, enhanced lipid metabolism, and increased milk yield (Huang *et al.,* 2023). Research indicates that supplementing the diets of transitioning cows with

choline is beneficial. Additionally, rumen-protected choline supplementation improved milk, fat, and protein yields. It has also been reported that the optimal amount of choline to be administered is more than 12.9 g per day (Arshad *et al.,* 2020). Although RPC supplementation increases dry matter intake and milk yield in lactating cows, there is no straightforward evidence that it improves health or reproduction (Humer *et al.,* 2019).

It has been observed that a cow with a higher BCS in the dry period mobilizes more body fat to meet the increasing energy requirement with the onset of milk production after calving. Cows prefer to build fat by eating more feed during the dry period and then use that fat for energy after calving (Winkelman *et al.,* 2008). Plasma NEFA and triacylglycerol concentrations increase after parturition. NEFAs are oxidized to ketone bodies.

Glucose consumption in the mammary gland significantly increases when lactation begins (Bell, 1995). As the concentration of NEFA in the blood rises, the liver's absorption rate also increases (Bell, 1981). Postpartum plasma glucose levels decrease, while the breakdown of glycogen to glucose increases after parturition (Drackley, 2001). Gluconeogenesis increases as glycogen stores in the liver are depleted. Glucose is synthesized through gluconeogenesis using amino acids as carbon sources. The glucose content in the blood of the cow determines the malonyl-CoA level. The proportion of NEFA absorbed by the liver is influenced by the concentration of malonyl-CoA. When carbohydrates are consumed in sufficient amounts, glucose and malonyl-CoA levels increase, and NEFA is prevented from being broken down by mitochondria and is esterified to triglyceride. When carbohydrate intake is low, glucose and malonyl-CoA levels decrease, and NEFA is converted into ketone bodies in mitochondria via carnitine palmityl transferase activity. Ketone bodies are released from the liver and used as an energy source (Mcgarry and Foster, 1979, Herdt, 2000). Rumen-protected choline supplementation during the transition period may increase the benefits of cow health and metabolism (Holdorf *et al.,* 2023).

CONCLUSION

As dry matter intake decreases with the onset of lactation and lactogenesis, energy needs increase, causing dairy cows to enter a NEB and experience energy deficiency. Cows with NEB try to meet

their energy needs by mobilizing body fat, which is the energy reserve in the body. This results in nutritional diseases, such as fatty liver disease and ketosis. When a cow is in ketosis, the energy deficit cannot be resolved by altering the ration alone. Consequently, conducting ration studies during ketosis is not useful. The cow in the NEB attempts to resolve the energy imbalance by increasing NEFA and BHBA levels.

In conclusion, it is believed that an increase in the NEFA and BHBA ratios could lead to fatty liver and ketosis. This study found that oral supplementation of RPC and PG to dairy cows in the experimental group had a statistically significant effect on blood concentrations of triglycerides and NEFA. Our findings suggest that using RPC and PG supplements in dairy cows may reduce the incidence of ketosis and fatty liver disease, as blood triglyceride levels significantly decreased.

Ethics approval

Tekirdağ Namık Kemal University Local Ethics Committee approved the study on August 1, 2024, with the document number T2024-2079.

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Conflict of interest

The authors state that there is no conflict of interest that could damage the impartiality of this experiment.

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