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Sodium butyrate supplementation and the effect on glucose levels and lipid metabolism of dairy cows

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RESUMEN

The aim of this study was to evaluate the effect of sodium butyrate supplementation on plasma glucose levels and lipid metabolism of the cow in the mid-lactation. For this, ten dairy cows (four Holstein and six Jersey, multiparous, 150 days in milk (DIM) were used and randomly divided into two groups: Control Group (CG, n = 5), which received the standard diet; Treatment Group (TG, n = 5), which received the standard diet plus 1.0 g/kg of body weight per day of butyrate over a 8 days period. Individual blood samples were collected once daily throughout the experimental period for analysis of β-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA), triglycerides, cholesterol and glucose. It can be observed that in TG the BHB blood levels were higher (0.64 ± 0.03 mmol/l and 0.91 ± 0.03 mmol/l, for CG and TG, respectively, P=0.001) and the glucose levels were lower than the CG (55.5 ± 0.84 mg/dL and 51.1 ± 0.75 mg/dL, for TG and CG, respectively, P<0.0001). The results show that supplementing cows with sodium butyrate may regulate the rate of lipolysis and the concentrations of NEFA improving the health status of the animal.

Suplementação de butirato de sódio e os efeitos nos níveis de glicose e no metabolismo lipídico de vacas leiteiras

Palavras chave adicionais
Ácido graxo de cadeia curta.
Lipólise.
ß-hidroxibutirato.
Vacas lactantes.

RESUMO

O objetivo desse estudo foi avaliar os efeitos da suplementação com butirato de sódio nos níveis de glicose plasmática e no metabolismo lipídico de vacas no meio da lactação. Para isso, foram utilizadas 10 vacas (4 da raça Holandês e 6 da raça Jersey), multíparas, com 150 dias em lactação (DEL) e aleatoriamente divididas em 2 grupos: Grupo Controle (GC, n = 5), que recebeu uma dieta padrão; Grupo Tratamento (GT, n = 5), que recebeu a dieta padrão mais 1,0g/kg de peso vivo por dia de butirato de sódio por um periodo de 8 dias. Amostras individuais de sangue foram coletadas diariamente durante o periodo experimental para análise de β-hidroxibutirato (BHB), ácidos graxos não esterificados (AGNE), triglicerídeos, colesterol e glicose. Pode ser observado que no GT os níveis de BHB plasmático foi maior (0,64 ± 0,03 mmol/l e 0,91 ± 0,03 mmol/l, para GC e GT, respectivamente, P = 0.001) e os níveis de glicose foram mais baixos que o GC (55,5 ± 0,84 mg/dL e 51,1 ± 0,75 mg/dL, para GT e GC, respectivamente, P < 0.0001). Esses resultados mostram que a suplementação de vacas com butirato de sódio pode regular a taxa de lipólise e as concentrações de AGNE, melhorando o estado de saúde do animal.

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INTRODUCTION

In dairy cows, ketone bodies (acetoacetate, acetone and β-hydroxybutyrate), a physiological products of lipid metabolism represent one of the main sources of energy used to maintain and produce milk (Zhang et al., 2016), most pronounced in the peripartum period, wherein is usually characterized by a negative energy balance (NEB) state. In this period, the insufficient carbohydrate intake, in view of the high-energy demand required for milk synthesis, causes the body to increase the rate of lipolysis, resulting in high plasma levels of non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHB).

The increased levels of NEFA and BHB during NEB are indicated as one of the main factors of immunosuppression in dairy cows (Lacetera et al., 2005). According to Ospina et al. (2010), serum concentrations of NEFA in pre and postpartum greater than 0.3 and 0.6

mmol/L, respectively, are associated with an increased risk for abomasal displacement, clinical ketosis, placenta retention, and metritis. However, in ketogenic diets already studied and used in human medicine, it is used to treat different disorders, being proven that the exogenous use of ketone bodies, mainly BHB, is related to glucose and NEFA rates and, it can be used to treat patients with hyperglycemia and hyperlipidemia (Beylot et al. 1994; Mikkelsen et al., 2015).

According to Metz and Van Den Bergh (1972) and Metz (1977), exogenous sources of BHB result in reduction of the lipolysis rate in adipose tissue from cows in the peripartum, leading to a decrease in NEFA levels. In this sense, it is possible that the use of BHB precursors in the diet of lactating cows, such as sodium butyrate may be an alternative to control NEFA levels, improving the energy status of the animal. Thus, the hypothesis is that elevate plasma BHB levels could cause a change in glucose levels and on lipid metabolism in lactating cows. The aim of this study was to evaluate the effect of β-hydroxybutyrate on plasma glucose levels and lipid metabolism in cows supplemented with sodium butyrate.

MATERIAL AND METHODS

This study was conducted at the Centro Agropecuário da Palma, located in the city of Capão do Leão, Rio Grande do Sul, Brazil. All the procedures performed were accordance with the protocol approved by the Ethics Committee on Animal Experimentation at the Federal University of Pelotas (protocol 9379-2016).

Ten dairy cows, four Holstein and six Jersey with a body condition score (BCS) of 2.0 and body weight (BW) of 619.75 ± 10.96 kg; 391 ± 32.23kg, respectively, and with 150 days in milk (DIM) were selected. The animals were milked twice a day, producing 9.15 ± 3.57 liters of milk per day. The animals were randomly divided into two groups: Control Group (CG, n = 5, two Holstein and three Jersey, BW: 454, 6 ± 81.58kg), which received the standard diet (NRC, 2001) and the Treatment Group (TG, n = 5, two Holstein and three Jersey, BW: 470.4 ± 105.9kg), which received the standard diet plus sodium butyrate (CM3000® - 30% Sodium Butyrate - Microencapsulated, Vetanco, Brazil). The treatment consisted of 1.0 g of butyrate per kg of BW / day (1.26 g of sodium butyrate per kg of body weight / day), as suggested by (Herrick et al., 2017) were applied as a top dress on TMR during an experimental period of 8 days, with sodium butyrate divided into two daily offerings (**Table I**).

The animals were kept during the milking intervals in a forage paddock. After each milking, they were fed with a basal TMR with or without sodium butyrate in a free-stall barn where they were kept until eating all TMR containing the treatment, then they were moved to the paddock again. The forage intake was estimated by the mass of forage before grazing subtracted by the mass after grazing divided by the number of cows in the area, which was 10. The basal TMR consisted of corn silage (2.37 kg of DM/day/cow, whole plant corn silage with 35% DM) more concentrate (2.80 kg of DM/day/cow). The concentrate was composed by soybean meal, wheat bran, ground whole corn, vegetable fat and wheatgrass, with 18% of crude protein and, 3.5% of ethereal extract. The daily dry matter intake could not be recorded for the statistical analysis since the cows were in an extensive system, thus the forage intake was not accurate.

Table I. Diet offered to the control (CG) and treatment (TG) groups (Dieta oferecida aos grupos de controle (CG) e tratamento (TG)**).**

Item	Groups	
	CG	TG
Concentrate (kg DM/day)[1]	2.80	2.80
Corn silage (kg DM/day)[2]	2.37	2.37
Butyrate 3% (g/ kg BW/day)[3]	-	1,50
Forage (kg DM/day)[4]	11	10

DM: dry matter; [1]Composed by soybean meal, wheat bran, ground whole corn, vegetable fat, wheat, crude protein (18%), ethereal extract (3.5%); [2] Whole plant corn silage with 35% of DM; [3]CM3000® – Sodium Butyrate 30% – Microencapsulated, Vetanco, Brazil; [4] Estimated forage consumption (native pasture).

According to the energy calculation performed (NRC, 2001), for a cow with 470 kg of BW producing on average of 10 liters of milk per day, the supply of available feed at the farm did not adequately meet the energy requirements to the animals, which means, the animals were in negative energy balance (NEB), with a deficit of 18.2 Mcal / day. Sodium butyrate was offered to the cows before TMR allocation, after mixing with small amount of concentrate (1kg) to ensure its entire intake.

The profile of short-chain fatty acids present in corn silage were analyzed by the Near-Infrared Spectroscopy (3rLab, Lavras – Minas Gerais, Brasil) to minimize the error. Once the incorrect silage conservation, with presence of oxygen, can increase the proliferation of unwanted bacteria (e.g.: *Clostridium tyrobutyricum*) (Dinic et al., 2010) which are capable to ferment lactic acid to butyric acid, resulting in a greater concentration of butyric acid in the diet of dairy cows. However, in the present study, the presence of butyric acid was not detected in the silage offered to the cows (**Table II**).

Blood collection was performed daily in the morning after the end of feeding (approximately one hour) through the coccygeal vascular complex puncture. The tubes were identified after the collections and placed in a thermal box at a temperature of 4ºC until processing

Table II. **Profile of volatile fatty acids from the corn silage offered to the animals** (Perfil de ácidos graxos voláteis da silagem de milho oferecido aos animais).

Fermentation products	Concentration
Dry Matter (%)	23.29
Lactic Acid (%DM[1])	0.57
Acetic Acid (%DM)	4.78
Butyric Acid (%DM)	-

[1]DM: dry matter.

in the laboratory. The blood was centrifuged at 1800xG for 15 minutes (SIEGER centrifuge, Sirius 4000) for complete serum separation, which was transferred to Eppendorf type microtubes and immediately analyzed. The plasma from the collection tube with glycolytic inhibitors was separated and frozen at -20 ° C for later analysis of glucose, NEFA, BHB and triglycerides.

The analyzes were performed with the automatic biochemical equipment (Lambax-Plenno-LABTEST MG, Brazil) and included BHBA concentration (commercial Kit - RANDOX Brazil Ltda, SP, Brazil), glucose (commercial Kit - LABTEST, MG, Brazil), non-esterified fatty acids (NEFA) (commercial Kit - Wako Diagnostics, CA, USA), and triglycerides (Commercial Kit - LABTEST, MG, Brazil). Data were analyzed as repeated measures with the ANOVA procedure of NCSS (2005) using treatment (butyrate or control), time (days), and their interactions as fixed effects, and *cow nested within treatment as random effect*. The normality of the data were analyzed using the Shapiro-Wilk normality test. Statistical significance was declared at $P \leq 0.05$.

RESULTS AND DISCUSSION

Over the years, the genetic selection of dairy cows focused on milk production culminated in a constant increase in this process, making these animals more efficient and consecutively more challenged, thus making the negative energy balance (BEN) a relatively common event. Some studies report that supplementation with sodium butyrate in ruminants results in a significant increase in the plasma BHB levels. Approximately 75% of the butyrate produced in the rumen environment is oxidized to BHB in the epithelium of the digestive tract by the enzyme Butyryl-CoA synthetase (Huhtanen et al., 1993a); (Vicente et al., 2014), and the surplus is metabolized at the hepatic level, which can be redirected to the synthesis of ketone bodies or Krebs cycle via acetyl-coA (Miettinen and Huhtanen, 1996); Kozloski et al. 2011; Santos, 2011; Mahrt et al. 2014).

The use of butyrate in animal nutrition has been studied with several hypothesis. Some of these studies were based on the evaluation of butyrate on young calves' performance, as demonstrated by (Guilloteau et al., 2010) which observed positive effects on the intestinal health of calves supplemented with butyrate. Besides that, (Kowalski et al., 2015)) carried out an investigation on the effect of supplementation on dairy cows in the transition period and concluded that supplementation of butyrate in the diet of cows in this period can enhance a better growth of papillae and adaptation of the rumen in pre-calving diets, with no changes on the performance of animals during lactation.

The blood biomarkers are presented in **Table III**. There was a treatment effect ($P < 0.001$) for BHB, which resulted in greater concentrations in TG animals compared to CG. A treatment effect for lower NEFA ($P < 0.001$) and lower glucose ($P < 0.001$) was observed in the TG group when compared with CG. Cholesterol and triglycerides were not affected by treatment.

β-hydroxybutyrate is an important precursor of energy used by the muscle in times of energy deficit (Newman and Verdin, 2014), and is intensively used by the mammary gland for fat synthesis (Huhtanen et al., 1993a); (Miettinen and Huhtanen, 1996); (Mahrt et al., 2014). Thus, it is likely that the BHB levels of animals in the present study were lower than expected, due to possible use by the tissues and mammary gland, once the cows a low BCS and the feed did not adequately meet the energy requirements.

The increase in BHB and the decrease in NEFA can be contrasted with the hypothesis raised by some studies (Metz and van den Bergh, 1972); (Metz S. G., 1977), that BHB has a negative feedback on the rate of lipolysis in adipose tissue, in order to avoid intoxication by excess ketone bodies and NEFA in the circulation. Corroborating with this hypothesis (Herrick et al., 2017), observed that only one hour of butyrate supplementation was enough to alter the levels of NEFA, where the control group had greater concentrations compared to cows given 1 and 2 g / kg BW of butyrate infusion.

Metz et al. (1974), evaluating the effect of adipose tissue incubation at doses of 0 - 10 mmol / L BHB, observed a significant reduction in NEFA levels with increasing of BHB levels in recent postpartum cows' adipocytes. In this same sense, (van der Drift et al., 2013), studied the effect of the incubation of adipose tissue at 3 mmol / L BHB and observed a reduction in the lipolysis rate of 47% and 65%, with the tissue samples coming from cows in the first and second weeks of lactation, respectively. Thus, it is likely that in this study, the high concentration of BHB in the GT, caused a

Table III. Means ± standard error of plasma β-hydroxybutyrate, glucose, non-esterified fatty acids, triglycerides and cholesterol levels of dairy cows supplemented with sodium butyrate (média ± erro padrão de plasma β-hidroxibutirato, glicose, ácidos graxos não esterificados, triglicérides e níveis de colesterol de vacas leiteiras suplementadas com butirato de sódio).

Metabolite	Groups		*P-Value*		
	CG[1]	TG[2]	Group	Days	G x D
BHB[3] (mmol/L)	0.64 ± 0.03	0.91 ± 0.03	0.001	0.005	0.75
Glucose (mg/dL)	55.50 ± 0.84	51.10 ± 0.75	0.00002	0.001	0.24
NEFA[4] (mmol/dL)	0.30 ± 0.03	0.16 ± 0.01	0.00004	0.47	0.66
Cholesterol (mg/dL)	140.23 ± 3.68	142.24 ± 3.64	0.70	0.94	0.94
Triglycerides (mg/Dl)	18.57 ± 0.85	16.44 ± 1.04	0.14	0.58	0.95

[1]CG: Control Group receiving diet calculates based on NRC (2001); [2]TG: Treatment group receiving diet plus 1.0g / kg of body weight of butyrate; [3] β-hydroxybutyrate; [4] non-esterified fatty acids.

reduction in the synthesis of NEFA and, consequently, reduced the plasma concentrations of this metabolite.

In addition, these levels indicate the relevance of NEB in animals under conditions of high glucose demand and efficiency in the use of mobilized fatty acids in the lipolysis process (Caldeira, 2005). BHB levels can also be related to BSC, knowing that BSC is a subjective measure evaluated visually and allows to evaluate the deposition and mobilization of fat in ruminants, variations in BHB levels are interpreted with greater accuracy, where studies show that BHB levels have a negative correlation with BSC, where the drop in BSC is associated with an increase in BHB in the blood (RIBEIRO and MATTOS, 2004), which is in agreement with the results found for BHB and BSC of the animals in the present study.

Even so, recent studies (Kemper et al., 2015) and (Caminhotto et al., 2017) demonstrate that BHB acts in the regulation of cholesterol levels in rats and humans, wherein BHB levels are related to the decrease in the concentration of total cholesterol, LDL and with the increase in HDL levels, in ruminants, the cholesterol is linked, mainly, the very low-density lipoproteins of VLDL. There is a metabolic relationship between NEFA and cholesterol, which indicates the intensity of lipolysis in relation to meeting the energy demand in cattle, the levels of BHB, NEFA and triglycerides are directly related to the energy balance of the animal (Fernandes et al., 2012).

However, in the present study, although both groups had plasma levels above the physiological limits applied by Kaneco et al. (2008), there was no effect of treatment under these markers, with the physiological levels of cholesterol (80.0 to 120.0 mg / dL) and triglycerides (0.0 to 14.0 mg / dL), this increase can be related with the variation in cholesterol concentration, being useful with a nutritional condition of lactating cows (Fernandes et al., 2012).

These results show that there is a negative relationship between the supplementation of precursors of ketone bodies, mainly BHB and lipid metabolism, such as the rate of lipolysis and NEFA synthesis. However, the metabolic pathways involved in these processes are not yet known. According to (Kemper et al., 2015), a possible route would be the decrease in key enzymes, as shown by the results found by these authors, where rats submitted to the ketogenic diet for 36 days had a 17% and 50% reduction in liver concentrations of mevalonate, a biomarker of cholesterol synthesis, and malanoyl-coA, an enzyme that regulates NEFA synthesis, respectively.

NEFA is known to be closely related to metabolic disorders and immunosuppression in dairy cows (Lacetera et al., 2005). (Hoeben et al., 2000), observed a negative relationship between neutrophil immune function and plasma NEFA levels in cows. Therefore, the reduction in NEFA concentrations by using BHB precursors, such as butyrate in the present study, may be an interesting alternative in regulating the rates of NEFA lipolysis and synthesis, thus improving the animal NEFA synthesis, respectively thus improving the animal immune status.

In swine, these organic compounds have been tested in the diet as a possible alternative to chemotherapy and antibiotics with positive effects in terms of digestibility and growth performance. The results show that pigs supplemented with butyrate obtain a high average coefficient of apparent digestibility of gross energy (Anderson et al. 1999).

Some studies have observed that the product may have decreased the number of microorganisms that produce toxins adhered to the intestinal epithelium, being a mode of action that maintained the integrity of the enterocytes, not causing irritation in the intestinal epithelium (Costa, 2009; Guilloteau et al., 2010). Costa, (2009) also concluded that pigs that receive supplementation with sodium butyrate in their diets tended to present greater daily weight gain and greater height of intestinal villi, configuring themselves as growth promoters for this species.

Thus, although the species have different digestive tracts, it seems possible that cattle can reproduce similar results with the use of sodium butyrate.

In relation to glucose levels, other researches evaluating the effect of butyrate supplementation observed similar decrease in glucose levels (Huhtanen et al., 1993; Herrick et al., 2018). Herrick et al., (2017) observed that with the increase in plasma of levels BHB after the intraruminal infusion of sodium butyrate, there was a decrease in glucose concentrations but the explanation for the decrease in glucose levels against a rise in plasma levels BHB has not been well elucidated yet. It is speculated that this change is not related to insulin levels but to glucagon levels, since a reduction in the levels of this hormone was observed in animals with BHB infusion, therefore, it is assumed that the infusion would cause an inhibition in the gluconeogenesis by reducing glucagon levels (Zarrin et al., 2013; Zarrin et al., 2014). The glucagon is a hormone responsible for increasing the concentration of glucose by gluconeogenesis and glycogenolysis, resulting in a partial replacement of glucose by BHB as a source of energy for tissues (Jones et al., 2012). In addition to the latter, other studies which observed decrease in plasma glucose (Aiello et al., 1989; Herrick et al., 2018; Rice et al., 2019) in response to butyrate infusion hypothesized that BHB may affect gluconeogenic pathways, resulting in alterations in glucose metabolism. Aiello et al., (1989) reported that an increase of 1.25 mM of butyrate reduced around 46% of the propionate uptake in sheep hepatocytes, which resulted in blood glucose alteration.

CONCLUSIONS

The results of this study show that supplementing cows with sodium butyrate may regulate the rate of lipolysis and the concentrations of NEFA improving the health status of the animal. However, the supplementation with sodium butyrate also affects the glucose concentrations, which may be related to the potential of butyrate to affect various pathways related to glucose metabolism.

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