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Effect of butaphosphan and cyanocobalamin on *postpartum* metabolism and milk production in dairy cows

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The aim of this study was to determine the effect of butaphosphan and cyanocobalamin (BTPC) supplementation on plasma metabolites and milk production in postpartum *dairy cows. A total of fifty-two Holstein cows were randomly assigned to receive either: (1) 10 ml of saline (NaCl 0.9%, control group); (2) 1000 mg of butaphosphan and 0.5 mg of cyanocobalamin (BTPC1 group); and (3) 2000 mg of butaphosphan and 1.0 mg of cyanocobalamin (BTPC2 group). All cows received injections every 5 days from calving to 20 days in milk (DIM). Blood samples were collected every 15 days from calving until 75 DIM to determine serum concentration of glucose, non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHB), cholesterol, urea, calcium (Ca), phosphorus (P), magnesium (Mg), aminotransferase aspartate (AST) and γ-glutamyltransferase (GGT). The body condition score (BCS) and milk production were evaluated from calving until 90 DIM. Increasing doses of BTPC caused a linear reduction in plasma concentrations of NEFA and cholesterol. Supplementation of BTPC also reduced concentrations of BHB but it did not differ between the two treatment doses. Milk yield and milk protein had a linear increase with increasing doses of BTPC. A quadratic effect was detected for milk fat and total milk solids according to treatment dose, and BTPC1 had the lowest mean values. Concentrations of glucose, urea, P, Mg, AST, GGT, milk lactose and BCS were not affected by treatment. These results indicate that injections of BTPC during the early* postpartum *period can reduce NEFA and BHB concentrations and increase milk production in Holstein cows.*

Keywords: butaphosphan, cyanocobalamin, *postpartum* dairy cows, non-esterified fatty acid

Implications

This study presents a new metaphylactic strategy aimed to reduce the intensity of negative energy balance (NEB) in *postpartum* dairy cows. Unlike previous studies, the current protocol consists of five injections of cyanocobalamin and butaphosphan at 5 days interval during the first 20 days in milk (DIM), the period of greatest metabolic challenge for dairy cows. The results of this experiment indicate that this protocol can improve metabolic adaptation in the early *postpartum* period, by reducing adipose tissue mobilization and increasing milk production.

Introduction

The transition from gestation to lactation is considered a critical period for high-producing dairy cows (Goff and Horst, 1997). The transition period is an important homeoretic event, in which several physiological modifications occur in a coordinated manner to support *postpartum* milk production (Drackley, 1999). During this period, the increase in dry matter intake (DMI) is not able to attend the rapidly increasing energy demand for maintenance and production (Vazquez-Anon *et al.*, 1994), leading to a state of negative energy balance (NEB) and the activation of catabolic pathways. As a consequence, there is an enhanced lipolysis in early lactation, that is, followed by an increase in plasma concentration of non-esterified fatty acids (NEFA; Adewuyi *et al.*, 2005). High plasmatic concentrations of NEFA might exceed the capacity of mitochondrial oxidation in the liver, resulting in the formation of ketone bodies, such as β-hydroxybutyrate (BHB), acetoacetate and acetone (Drackley *et al.*, 2001). Both NEFA and BHB can be used as markers to indicate NEB intensity during the *peripartum* period (Chung *et al.*, 2008).

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Most of the metabolic disorders occur *postpartum* (Drackley, 1999), decreasing the milk production, impairing the reproductive performance and increasing the risk of culling (Huzzey *et al.*, 2007). Several strategies have been attempted to reduce the effects of NEB, including supplementation with glycerol (DeFrain *et al.*, 2004), nicotinic acid (Pires *et al.*, 2007), *cis*-linoleic acid (Mosley *et al.*, 2007), fat (Moallem *et al.*, 2007), methionine (Preynat *et al.*, 2009), choline (Chung *et al.*, 2009), carnitine (Carlson *et al.*, 2007) and monensin (Duffield *et al.*, 2008). Overall, current treatments had limited success, and more research is needed. One potential alternative is the use of butaphosphan and cyanocobalamin (BTPC; vitamin B12) injections after calving, which have shown positive effects up to now (Furll *et al.*, 2010; Rollin *et al.*, 2010).

Cyanocobalamin is the synthetic form of vitamin B12. Methylmalonyl-CoA mutase, a mitochondrial enzyme, involved in the conversion of propionate to succinyl-CoA, is an important vitamin B12-dependent gluconeogenic substrate (Kennedy *et al.*, 1990). An inadequate supply of vitamin B12, especially in the early lactation, could possibly lead to decreased function of methylmalonyl-CoA mutase, and hinder energy production from propionate, thus leading to enhanced ketogenesis in the animal.

The butaphosphan is a compound used as an organic source of phosphorus for animal supplementation. In dairy cattle, the phosphorus content of liver tissue is decreased in early lactation (Grunberg *et al.*, 2009). Phosphorus plays an important role in hepatic carbohydrate metabolism, as several intermediates in the gluconeogenic pathway must be phosphorylated. Thus, the rates of gluconeogenesis and glycolysis are regulated by phosphorus availability (Berg *et al.*, 2006). In addition, phosphorus is an important component of nucleic acids, ATP and AMP (Cunningham, 2002), thereby it is also implicated in energy metabolism.

Recently, a study has shown that cows treated with BTPC at calving and 1 day later had lower incidence of ketosis (Rollin *et al.*, 2010). Another study also demonstrated that BTPC improved the energetic status of dairy cows during the *peripartum* period based on concentration of glucose, NEFA, BHB and cholesterol (Furll *et al.*, 2010).

The aim of this study was to test the effect of BTPC on plasma concentrations of metabolites and on milk production in *postpartum* dairy cows. Our results indicate that supplementation of BTPC during the first 20 days *postpartum* reduced the severity of NEB and increased milk production in a dose-dependent manner.

Material and methods

Animal welfare

The Committee for Ethics in Animal Experimentation from the Pelotas Federal University has approved all procedures performed in this experiment.

Animals, experimental protocol and treatments

For this study, 52 multiparous Holstein cows kept under semi-confinement management (concentrate and pasture fed) in a commercial dairy herd in southern Brazil (328.16′S, 528.32′E) were used. Cows calving between December 2008 and May 2009 were enrolled in the study. Cows were milked twice daily and received concentrate supplementation after every milking. The concentrate was formulated to meet the nutritional needs (National Research Council (NRC), 2001) of post-calving Holstein cows, composed of 35% of soybean hulls, 30% of sorghum, 17% rice meal, 13% of soybean meal, 4% of mineralized salt and 0.5% of urea. Between milkings, cows had *ad libitum* access to water and were kept under grazing of *Sorghum bicolor* L. Moench and *Lotus corniculatus* pasture. Immediately, post-calving cows were randomly assigned to one of three groups: (1) Control Group ($n = 16$), which received 10 ml of saline (i.m., NaCl 0.9%), every 5 days from calving to 20 days in milk (DIM); (2) 1000 mg of butaphosphan and 0.5 mg of cyanocobalamin (i.m., 10 ml of Catosal B12®, Bayer Health Care, São Paulo, Brazil; BTPC1 Group, $n = 18$) and (3) 2000 mg of butaphosphan and 1.0 mg of cyanocobalamin (BTPC2 Group, $n = 18$). Both, BTPC1 and BTPC2 groups received i.m. injections of BTPC every 5 days from calving to 20 DIM.

Body condition score (BCS) and blood sampling

At the beginning (0 DIM) and end of the experiment (75 DIM) BCS was determined by the same technician, based on a five-point scale, where obese equals 5 (Wildman *et al.*, 1982). Blood samples were collected from jugular vein every 15 days from calving to 75 DIM. Blood samples were collected in two heparinized 10 ml vacutainer tubes (Vacutainer Systems; Becton-Dickinson, Franklin Lakes, NJ, USA), either with EDTA or 15 mg NaF and 12 mg potassium oxalate. Blood samples were immediately centrifuged (1500 × **g** for 15 min). Plasma from tubes containing EDTA was frozen at −80°C for analysis of blood urea nitrogen, NEFA and BHB. Plasma from tubes containing NaF was frozen at −20°C for glucose assay.

Biochemical analysis

The concentrations of glucose, urea, calcium (Ca), phosphorus (P), magnesium (Mg), γ-glutamyltransferase (GGT), aminotransferase aspartate (AST) and cholesterol, were analyzed by enzymatic colorimetric assay quantified by a spectrophotometer (FEMTO 700 Plus, Femto Ind. e Com. de Instrumentos Ltda., São Paulo, Brazil). The reagents were handled according to the manufacturer's instructions (Labtest®, Lagoa Santa, Brazil). NEFA and BHB were analyzed by a commercial kit (Wako NEFA-HR, Wako Chemicals USA®, Richmond, EUA and Randox®, Randox Laboratories E.U.A.®, Oceanside, CA, USA, respectively), according to Ballou *et al.* (2009). Coefficients of variation were below 10% for all assays.

Milk production and composition

Milk production was evaluated daily (ALPRO® Windows, DeLaval, Kansas City, MO, USA) from 2 to 12 weeks *postpartum*, and weekly averages were generated for statistical analysis. Composite milk samples were collected every 15 days from 15 to 75 DIM to determine concentrations of

fat, lactose and protein by IR spectrophotometry (Bentley 20000, Bentley Instruments Inc., Chaska, MN, USA), and calculation of the total solids.

Statistical analysis

Data were analyzed using MIXED procedure of SAS. All independent variables were analyzed as repeated measures and considered cow within treatment as a random effect. Models included treatment, cow within treatment, time and the interaction between treatment and time. Additional covariables such as parity, month of parturition and BCS at calving were included when $P < 0.10$. Effects of supplementation (control *v.* BTPC1 + BTPC2) and effects of dose (BTPC1 *v.* BTPC2) were analyzed by orthogonal contrasts. Linear and quadratic relationships between treatments and independent variables were evaluated by orthogonal polynomial contrasts.

Results

The concentration of NEFA was higher ($P = 0.0008$) in the Control group compared with treated groups and had a linear decrease ($P = 0.0002$) with the increasing concentration of BTPC injected. The levels of NEFA increased on the 2nd week and remained elevated until the 6th week on BTPC1 and control group (Figure 1A). The concentration of BHB was only higher ($P = 0.03$) in control group compared with treated groups, with no dose effects. The peak of BHB concentration ($P < 0.001$) was on the 2nd week *postpartum* and reduced gradually up to 8 weeks *postpartum* (Figure 1B). Cholesterol also decreased ($P < 0.001$) as the dose injected increased. The BTPC1 and Control Groups had a gradual increase of cholesterol from 1 to 8 weeks *postpartum* (Figure 1). The concentration of glucose, urea, P, Mg, AST and GGT were not different among groups ($P > 0.05$).

Milk production was also linearly affected by treatment ($P = 0.006$), being higher ($P = 0.005$) for treated groups in comparison with control group (Table 1). Milk production peaked ($P < 0.001$) on the 6th week *postpartum* and decreased gradually on the subsequent weeks (Figure 2). Regarding the milk composition, a linear increase in protein content was observed ($P = 0.003$). For fat and total solids content, a quadratic effect of the supplementation ($P = 0.04$ and $P = 0.03$, respectively) was observed. The lactose content was not different among treatments. The BCS of cows ranged from 2.0 to 3.5, but did not significantly differ among groups ($P = 0.71$).

Discussion

The metaphylactic therapy used in this study, consisting of five injections of BTPC, reduced NEB intensity during the transition period, as indicated by the lower plasmatic concentrations of NEFA and BHB, and increased milk yield in treated cows. Although several studies had shown the positive effect of butaphosphan in reducing NEB intensity in high-producing dairy cows during the transition period (Furll *et al.*, 2010; Rollin *et al.*, 2010), its mode of action is not clear yet. What is known about the pharmacokinetics of this molecule is that it is rapidly eliminated from the organism after i.v. injection, with a half-life of 116 min in dairy cows (EMEA, 2000).

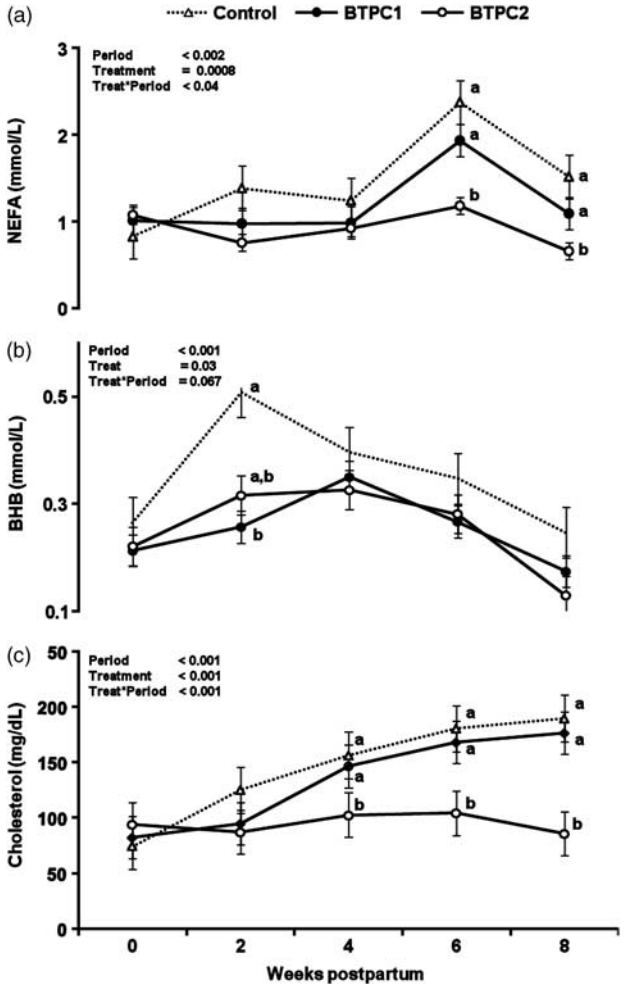

Figure 1 Plasma concentration of non-esterified fatty acids (NEFA) (a) β-hydroxybutyrate (BHB) (b) and cholesterol (c) of cows treated with placebo (Control Group; $n = 16$), 10 ml of butaphosphan and cyanocobalamin (BTPC1 Group; $n = 18$) or 20 ml of butaphosphan and cyanocobalamin (BTPC2 Group; $n = 18$) at 0, 5, 10, 15 and 20 days in milk. Different superscripts indicate differences at $P < 0.05$ between groups.

Some studies have indicated (Furll *et al.*, 2010; Rollin *et al.*, 2010) that the injection of butaphosphan (2 to 8 g/cow) and cyanocobalamin (1 to 4 mg/cow) in dairy cows, can reduce NEB intensity in a dose-dependent fashion with at least three injections of 2 g of butaphosphan and 1 mg of cyanocobalamin. Our results indicate that the use of 1 g of butaphosphan/cow, that is, half of the minimum dose used in other studies, is still able to induce positive effects on NEB and milk production.

During the *peripartum* period of dairy cows, there is an increase in serum NEFA concentration (Drackley, 1999; Douglas *et al.*, 2004), which leads to liver triacylglycerol (TAG) accumulation and is detrimental to milk production and reproductive performance (Grummer, 1993). The linear and dose-dependent reduction in the concentration of NEFA

Table 1 *Plasma metabolites, milk production and composition of* postpartum *dairy cows treated with placebo (Control group,* n = *16), with butaphosphan + cyanocobalamin (BTPC1 group,* n = *18; BTPC2 group,* n = *18)*

	Treatment[b]			Contrasts			
				Polinomial[c]		Orthogonal[d]	
Parameters[a]	Control	BTPC-1	BTPC-2	L	Q	Supplementation effect	Dose effect
Biochemical profile							
NEFA (mmol/l)	1.4 ± 0.07	1.1 ± 0.07	0.9 ± 0.08	0.0002	0.88	<0.0001	0.0005
BHB (mmol/l)	0.3 ± 0.02	0.2 ± 0.02	0.2 ± 0.02	0.22	0.02	0.02	0.16
Cholesterol (mg/dl)	145.3 ± 5.89	135.1 ± 5.34	94.8 ± 5.95	<0.001	0.01	<0.001	<0.001
AST (U/l)	33.8 ± 4.76	42.2 ± 4.70	31.5 ± 4.60	0.73	0.09	0.59	0.10
GGT (U/l)	39.4 ± 5.42	50.0 ± 5.26	40.9 ± 5.12	0.73	0.09	0.35	0.21
Ca (mg/dl)	9.6 ± 0.12	9.5 ± 0.12	9.2 ± 0.12	0.05	0.52	0.17	0.12
Mg (mg/dl)	2.0 ± 0.76	2.1 ± 0.75	3.3 ± 0.73	0.22	0.52	0.47	0.24
P (mg/dl)	5.3 ± 0.16	5.5 ± 0.16	5.4 ± 0.15	0.78	0.40	0.51	0.54
Glucose (mg/dl)	54.6 ± 0.87	55.7 ± 0.85	55.7 ± 0.83	0.32	0.59	0.26	0.97
Urea (mg/dl)	32.0 ± 1.09	32.4 ± 1.07	30.3 ± 1.03	0.27	0.35	0.64	0.17
Milk Production and composition							
Milk Production (kg)	23.9 ± 0.52	25.3 ± 0.54	25.9 ± 0.46	0.006	0.48	0.005	0.41
Fat	1.9 ± 0.25	1.4 ± 0.26	2.1 ± 0.27	0.64	0.04	0.53	0.04
Lactose	4.6 ± 0.03	4.6 ± 0.03	4.5 ± 0.03	0.10	0.23	0.40	0.06
Protein	2.8 ± 0.02	2.9 ± 0.02	3.0 ± 0.02	0.003	0.73	0.01	0.08
Total solids	10.3 ± 0.26	9.7 ± 0.26	10.5 ± 0.27	0.56	0.03	0.55	0.03

NEFA = non-esterified fatty acids; BHB = β-hydroxybutyrate; AST = aminotransferase aspartate; GGT = γ-glutamyltransferase; Ca = calcium; Mg = magnesium; P = phosphorus; DIM = days in milk.
[a]Plasma concentration of NEFA, BHB, cholesterol, AST, GGT, Ca, Mg, P, glucose, urea, milk production and milk composition (fat, lactose, protein and total solids) every 15 days from calving to 75 DIM.
[b]Treatments consisted on a five injections administered on days 0, 5, 10, 15 and 20 *postpartum*. Control = saline solution ($n = 16$); BTPC1 = 1000 mg of butaphosphan and 0.5 mg of cyanocobalamin ($n = 18$); BTPC2 = 2000 mg of butaphosphan and 1.0 mg of cyanocobalamin ($n = 18$).
[c]L = linear effect of the treatment; Q = quadratic effect of the treatment.
[d]Supplementation effect: Control *v.* BTPC; dose effect: BTPC1 *v.* BTPC2.

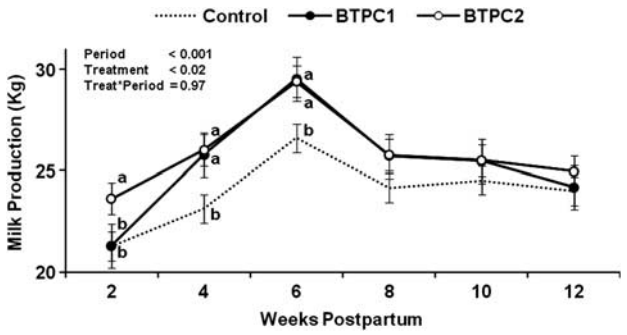

Figure 2 Milk production (kg) from week 2 to 12 *postpartum* of cows treated with placebo (Control Group; $n = 16$), 10 ml of butaphosphan and cyanocobalamin (BTPC1 Group; $n = 18$) or 20 ml of butaphosphan and cyanocobalamin (BTPC2 Group; $n = 18$) at 0, 5, 10, 15 and 20 days in milk. Different superscripts indicate differences at $P < 0.05$ between groups.

in cows treated with BTPC can be attributed to the ability of butaphosphan to improve ATP synthesis (Deniz *et al*., 2008). Moreover, cyanocobalamin, by acting as an enzymatic co-factor to methylmalonyl-CoA mutase enzyme, can also contribute to reduced circulating NEFA (Kennedy *et al*., 1990). The cyanocobalamin is a vitamin B involved in the synthesis of methionine, a donator of methyl for choline and carnitine. These two substances participate in the metabolism and transport of fat in the body (Rollin *et al*., 2010). Recently, a study showed that supplementation of dairy cows with folic acid and vitamin B increased glucose availability and reduced hepatic lipid accumulation during the transition period (Preynat *et al*., 2009).

As stated before, the concentrations of NEFA and BHB were linearly reduced in cows treated with BTPC. This indicates that the current protocol can help to control the incidence of subclinical ketosis. In agreement with those reported by Lohr *et al*. (2006), which detected reduced concentrations of BHB in cows that already developed secondary ketosis and were treated with BTPC. Furthermore, the injection of 2.5 mg of butaphosphan and 1.0 mg of cyanocobalamin, at calving day and one day later reduced the incidence of subclinical ketosis on the 1st week *postpartum* in multiparous cows (Rollin *et al*., 2010).

The current study demonstrated the effect of BTPC on lipid metabolism and on the ability of the liver to metabolize NEFA and reduce BHB formation. The reduction in cholesterol concentration was dose dependent for BTPC-treated cows and follows the same fashion, possibly because cholesterol can be transported with TAG in the bloodstream, and because hepatic secretion of lipoproteins is associated with NEFA conversion into TAG. Thus, high concentrations of NEFA increase the secretion of lipoproteins and consequently of circulating cholesterol (Grummer, 1993). The present study demonstrated that the dose of 2000 mg of butaphosphan and 1 mg of

cyanocobalamin can keep serum cholesterol concentration stable during early *postpartum* period.

Cows treated with BTPC had a linear increase in milk production. This result can be due to the detected reduction in NEB intensity that, in turn, can be associated with an increased DMI, and consequently, increased milk production. During intense NEB, when high serum NEFA concentration is detected, an excessive release of cytokines may occur and inhibit DMI and milk production (Allen *et al*., 2005). Thus, more studies are necessary to indicate whether BTPC can increase DMI or whether they have a direct effect on milk production.

In summary, the results of this study indicate that the metaphylactic injection of BTPC after calving increased milk production and reduced NEB intensity in Holstein cows, by reducing the plasma concentrations of NEFA and BHB.

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