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Use of Butaphosphan with Cyanocobalamin in High Producing Cows and Associations with Milk Yield and Dry Matter Intake

Uso de Butafosfan com Cianocobalamina em Vacas de Alta Produção e Associações com Rendimento de Leite e Consumo de Matéria Seca

Uso de Butafosfan con Cianocobalamina en Vacas de Alta Producción y Asociaciones con Producción de Leche y Consumo de Materia Seca

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Abstract
This study aimed to verify the effect of butaphosphan combined with cyanocobalamin (B+C) on dry matter intake (DMI) and milk yield in high producing dairy cows. Eighteen multiparous Holstein cows managed in a compost barn

system were enrolled on a calving date and remained under observation until 28 days in milk (DIM). The B+C group administered at 2500 mg of butaphosphan and 1.25 mg of cyanocobalamin (25 mL/cow/day, n = 9), or the control group (NaCl 0.9% administered at 25 mL/cow/day, n = 9,) receiving injections at calving, and at day 3 and 7 post-partum. The DMI, feed efficiency, and DMI/% body weight were evaluated until 21 DIM and the milk yield, rumination, activity, and lying time until 28 DIM. Various metabolites were evaluated at 0, 3, 7, 14, 21, and 28 DIM. DMI did not change with treatment. Milk yield was more significant in the B+C group than in the control group, with an increase of 3.66 kg/milk/d. The maintenance of DMI and the greater milk yield in the B+C group may suggest that the use of B+C can improve feed efficiency. No treatment effect was observed for concentrations of serum glucose, NEFA, BHB, and acetone, however, albumin was higher in B+C than in control. No effect was observed on milk composition. Our results suggest that B+C improves milk yield and feed efficiency by modulating the DMI.
Keywords: Dry matter intake; Organic phosphorus; Postpartum cows.

Resumo
Este estudo teve como objetivo verificar o efeito do butafosfan combinado com cianocobalamina (B+C) no consumo de matéria seca (CMS) e na produção de leite em vacas leiteiras de alta produção. Dezoito vacas Holandesas multíparas manejadas em sistema de compost barn foram inscritas na data do parto e permaneceram em observação até 28 dias em lactação (DIM). O grupo B+C administrado a 2500 mg de butafosfano e 1,25 mg de cianocobalamina (25 mL/vaca/dia, n = 9), ou o grupo controle (NaCl 0,9% administrado a 25 mL/vaca/dia, n = 9,) recebendo injeções no parto e nos dias 3 e 7 pós-parto. Avaliaram-se o CMS, eficiência alimentar e CMS/% peso corporal até os 21 DIM e a produção de leite, ruminação, atividade e tempo de repouso até os 28 DIM. Diversos metabólitos foram avaliadas em 0, 3, 7, 14, 21 e 28 DIM. O DMI não se alterou com o tratamento. A produção de leite foi mais significativa no grupo B+C do que no grupo controle, com aumento de 3,66 kg/leite/d. A manutenção do CMS e a maior produção de leite no grupo B+C podem sugerir que o uso de B+C pode melhorar a eficiência alimentar. Nenhum efeito do tratamento foi observado para as concentrações de glicose sérica, AGNE, BHB e acetona, no entanto, a albumina foi maior em B+C do que no controle. Nenhum efeito foi observado na composição do leite. Nossos resultados sugerem que B+C melhora a produção de leite e a eficiência alimentar modulando o CMS.
Palavras-chave: Consumo de matéria seca; Fósforo orgânico; Vacas no pós-parto.

Resumen
Este estudio tuvo como objetivo verificar el efecto del butafosfano combinado con cianocobalamina (B+C) sobre el consumo de materia seca (CMS) y la producción de leche en vacas lecheras de alta producción. Dieciocho vacas Holstein multíparas manejadas en sistema de compostaje fueron enroladas en una fecha de parto y permanecieron en observación hasta los 28 días en leche (DEL). El grupo B+C administrado a 2500 mg de butafosfano y 1,25 mg de cianocobalamina (25 mL/vaca/día, n = 9), o el grupo control (NaCl 0,9% administrado a 25 mL/vaca/día, n = 9,) recibiendo inyecciones al parto y al tercer y séptimo día posparto. Se evaluó la CMS, eficiencia alimenticia y CMS/% peso corporal hasta las 21 DEL y la producción de leche, rumia, actividad y tiempo de reposo hasta las 28 DEL. Se evaluaron las concentraciones de varios metabolites a los 0, 3, 7, 14, 21 y 28 DEL. DEL no cambió con el tratamiento. La producción de leche fue más significativa en el grupo B+C que en el grupo control, con un aumento de 3,66 kg/leche/d. El mantenimiento de DEL y la mayor producción de leche en el grupo B+C pueden sugerir que el uso de B+C puede mejorar la eficiencia alimenticia. No se observó ningún efecto del tratamiento para las concentraciones de glucosa sérica, NEFA, BHB y acetona, sin embargo, la albúmina fue mayor en B+C que en el control. No se observó ningún efecto sobre la composición de la leche. Nuestros resultados sugieren que B+C mejora la producción de leche y la eficiencia alimenticia al modular el CMS.
Palabras clave: Consumo de materia seca; Fósforo orgánico; Vacas posparto.

1. Introduction

The transition period for dairy cows comprises of 3 weeks before and after calving and is a phase of great demand (Drackley, 1999, Grummer, 2007, Girma et al.,2019). During this period, important nutritional, immune, and metabolic changes occur, which can reduce dry matter intake (DMI) by up to 30% at the end of gestation (Janovick & Drackley 2010, Drackley & Cardozo, 2014), requiring a high level of adaptation (Drackley, 1999, Chapinal et al., 2011, Goff et al., 2014). A decrease in DMI, followed by an increase in energy and mineral demands due to the start of lactation, leads to a negative energy balance (NEB) in the cow reducing milk yield and, depending on the severity, harming productive potential throughout lactation (Coleman, 2021, Grummer et al., 2004, Janovick & Drackley 2010).

Negative energy balance, if not controlled, increases lipid mobilization and consequently, non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHB) levels, leading to ketosis (Drackley, 1999, Grummer et al., 2004, French, 2006).

Ketosis, in turn, is correlated with other postpartum diseases, such as displaced abomasum and metritis, in addition to reduced fertility and an increased culling rate (Ospina et al., 2010, Chapinal et al., 2011, McArt et al., 2011). Raboisson et al. (2014) reported that cows with ketosis were more likely to have clinical mastitis. High NEFA and BHB values have also been associated with reduced immune capacity in cows during the postpartum period (Suriyasathaporn et al., 2000, Hammon et al., 2006).

To minimize the negative effects of NEB by increasing DMI and optimizing the productive potential of cows, different strategies have been studied (Yuan et al., 2012, Gordon et al., 2013, Vailati-Riboni et al., 2017). In this context, the use of an organic macro-mineral, composed of a source of phosphorus associated with cyanocobalamin, has been studied for more than 10 years (Rollin et al., 2010) and has shown to reduce NEFA and BHB, thus reducing NEB (Pereira et al., 2013, Nuber et al., 2016). Additionally, it reduced the expression of ketogenesis-related genes, which are genes related to the greater predisposition of cows to develop ketosis (Kreipe et al., 2011) and milk yield (Kreipe et al., 2011, Pereira et al., 2013, Gordon et al., 2017). However, it is still unknown whether this increase in milk production is associated with greater DMI in the postpartum period.

This study hypothesizes that postpartum dairy cows supplemented with butaphosphan combined with cyanocobalamin have a higher DMI, with improved metabolic status and lower occurrence of postpartum diseases in comparison to non-supplemented cows. This study aimed to verify the effect of butaphosphan with cyanocobalamin on DMI and milk yield on high producing dairy cows.

2. Methodology

The experiment was performed in a commercial dairy farm in Southern Brazil (32°16' S, 52° 32' W) from May 4, 2019, to July 28, 2019, with approval from the Animal Experimentation Ethics Committee of the Federal University of Pelotas (Universidade Federal de Pelotas), code 0102025-2017.

Animals

Eighteen multiparous Holstein cows maintained in a compost-barn system were used. The cows were fed a total mixed ration (Table 1) with water ad libitum and were milked twice a day, and. Aiming to keep homogeneity of groups, cows were randomly allocated into two groups with 9 animals each, according to body weight (B+C = 698.67 ± 23.55, CON = 737.56 ± 23.55) and body condition score (BCS) (B+C= 2.9 ± 0.11, CON=2.6 ± 0.10). Also, for recruitment, animals should meet the following criteria: locomotion scored 1 by the veterinarian (Sprecher et al., 1997), similar circulating values of NEFA and BHB at the time of delivery (complementary Fig. 1), same drying process in the previous lactation, same pre-delivery management, average yield over 30 liters per day during the last lactation, no significant difference in BW, BCS and previous milk production before the experiment, and, absence of mastitis in the previous lactation.

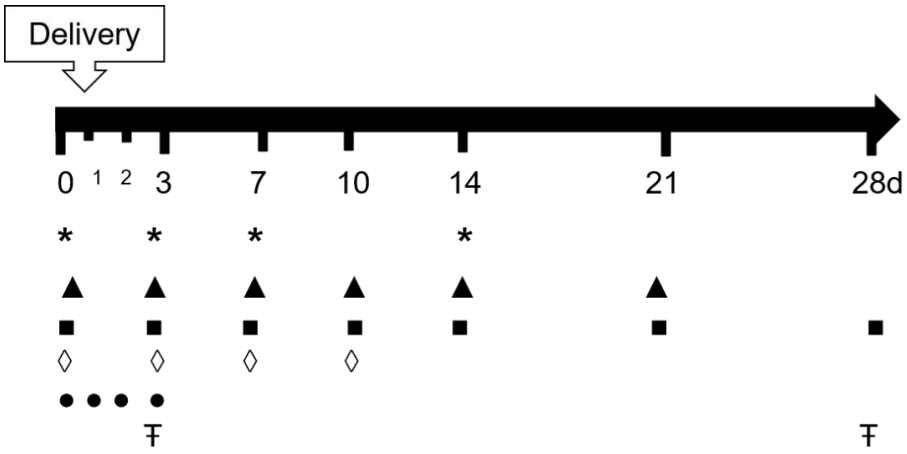
The first group (B+C) received three intramuscular injections of 2500mg of butaphosphan associated with 1.25 mg cyanocobalamin (25 mL per cow per day, Catosal B12, Bayer Animal Health, Germany), at 0, 3, and 7 days in milk (DIM). The control group received three intramuscular injections of saline solution (NaCl 0.9%, 25 mL per cow per day) at 0, 3, and 7 DIM. No animals were excluded from the experiment.

Table 1. Ingredients and chemical composition of experimental diets (DM basis).

Item	
TMR composition (% of DM)	
Corn silage	49.25
Soybean meal	10.36
Soybean hulls	9.49
Buffering	7.42
Rice bran	6.85
High moisture grain silage	5.70
Ground corn	3.65
Rice residue	3.54
Ryegrass baleage	3.60
Mycosorb	0.03
Chemical composition analysis[1]	
DM (%)	51.20
OM (%)	94.03
CP (% of DM)	14.85
NDF (% of DM)	37.40
ADF (% of DM)	21.40
TDN (% of DM)	72.42
Starch (% of DM)	18.84

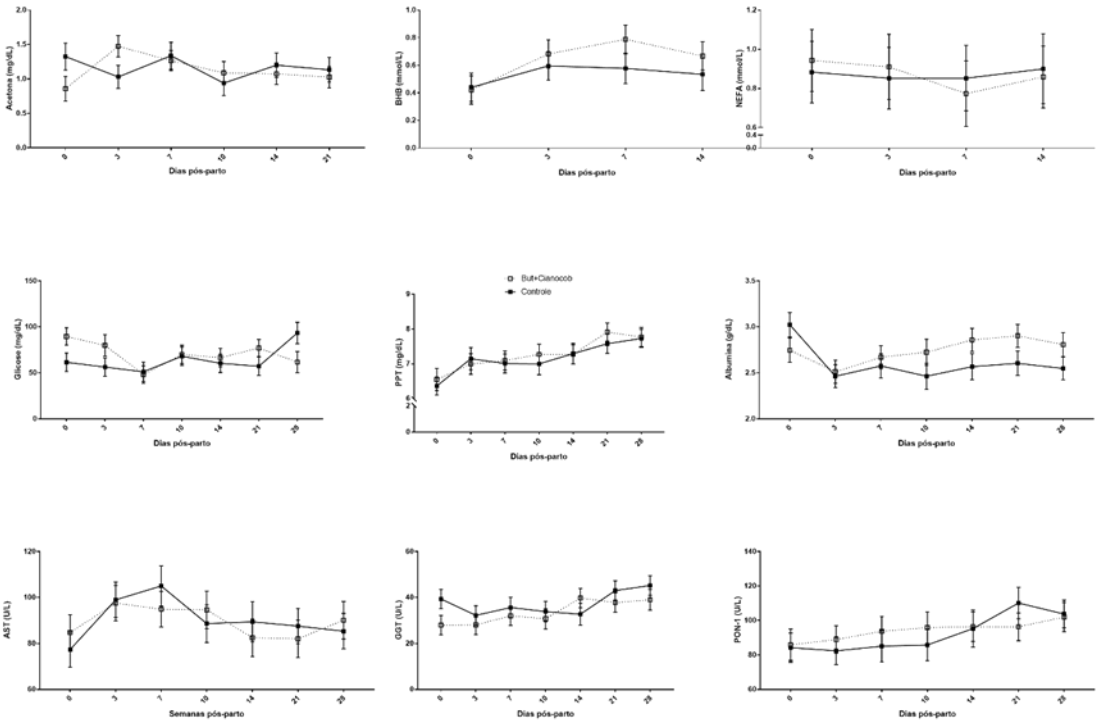
[1]DM = dry matter, OM = organic matter, CP = crude protein, NDF = neutral detergent fiber, ADF = acid detergent fiber, TDN= total digestible nutrient. Source: Authors.

Figure 1. Experimental design of the blood collection and metabolite analysis.



Source: Authors.

Complementary Figure 1 Metabolic parameters of multiparous cows in the butaphosphan + cyanocobalamin or control group from calving up to 4 weeks in milk.



Source: Authors.

Clinical Assessments

After calving, cows were monitored during the first 28 DIM. The occurrence of diseases was observed through a complete clinical examination performed weekly (Jain, 1993) and the evaluation of metabolite parameters at specific days. The main criteria used to characterize the postpartum diseases were: total serum calcium inferior to 5mg/dL for clinical hypocalcemia when, serum calcium was between 5 mg/dL and 8 mg/dL, including at least one blood sample with these calcium concentrations in 0, 1, 2 and 3 days after calving, for subclinical hypocalcemia (Goff et al., 2014), time superior than 12 h after the delivery to consider retained fetal membranes (Gohary and LeBlanc, 2018), presence of lumps in the "dark-bottomed mug" test for clinical mastitis, somatic cells count (SSC) > 200x1000 cell/mL for subclinical mastitis (Harmon, 1994), circulating BHB levels superior than 1 for clinical ketosis, circulating BHB levels between 1.2mmol/L and 1.4 mmol/L including evaluated in 0, 3, 7, 10 and 14 days after calving for subclinical ketosis (Oetzel, 2004, Ospina et al., 2010), fetid vaginal discharge, with or without pus and blood accompanied by fever and apathy up to 21 days after calving for metritis, as described by (Sheldon et al., 2006), uterine cytology using a cytobrush in 28 days after calving, in which polymorphonuclear cells (PMN) were evaluated according to Wagener et al., (2017), and abomasal displacement when the abomasum was displaced from its original location. All the animals diagnosed with clinical diseases were treated following the protocols of the farm. No animal was dropped from the study despite the occurrence of diseases.

Performance Assessments

After parturition, all the cows were monitored weekly for 4 weeks for body live weight (BW) using a weighing tape (Heinrichs et al., 2007) and considering a BCS from 1 to 5 (Edmonson et al., 1989). Consumption and eating behavior was assessed individually by monitoring the total diet consumption (kg) (Intergado®, Brazil) (Chizzotti et al., 2015) until 21 DIM. Based on cows' feed intake and weight data, it was possible to calculate their DMI in kg by body live weight % in kg (DMI/BW %) (Pérez-Báez et al., 2019). Feed efficiency was also calculated, including milk yield (kg/DMI) (Moallem, 2016).

Energy balance was calculated according to the NRC (2001) and other studies (Moallem, 2016, Osorio et al., 2016), using the following equations:

NEc = (NEL by kg of DM) × DMI,

NEm = BW 0.75 × 0.08 × 1.1,

NEmilk = milk (kg)×{ [0.0929×(fat %)] +[0.0547×(prot. %)] + [0.0395×(lact.%)] },

EB = NEc – (NEm + NEmilk)

where NEc = net energy consumed, NEm = net energy maintenance, NEmilk = net energy milk, EB = energetic balance.

Animal behavior was assessed through individual tracking collars (ChipInside®, Brazil), which indicates the daily cow activity time, rumination, and idleness (min/day) (Leiber et al., 2016).

Samples of total mixed ration (TMR) were collected daily from each feeder immediately after feeding. Pre-dried matter was measured by sampling the experimental diet, it was dried in a forced-air oven at 55 °C for 72 h for DMI calculation. Dry matter (DM) content and mineral matter (MM) were obtained in accordance with Easley et al. (1965) and the AOAC (1975), methods 22.010 and 7.010, respectively. Organic matter (OM) content was calculated as DM-MM. The fraction of neutral detergent fiber (NDF) and acid detergent fiber (ADF) content was determined according to Van Soest and Robertson (1985). Crude protein (CP) was determined using the Kjeldahl method (Method 984.13) (AOAC, 1997). Total digestible nutrients (TDN) were calculated according to the equation: TDN = 87.4 - (0.7 × % ADF), as described by Teixeira and Teixeira (1998).

Cows were milked twice a day by mechanical milking, and the Milk yield was measured daily from calving until the 28 DIM. Milk samples were collected weekly to assess the composition and occurrence of subclinical mastitis. Based on milk composition data, a calculation was performed for energy correction of milk yield, adjusting fat to 4% (NRC, 2001), in which:

FCM = (0.4 × milk yield, kg) + (fat, kg × 15), where FCM = fat corrected milk.

Energy corrected milk (ECM) was calculated, also considering protein correction (Osorio et al., 2016), with the following formula:

ECM = (12.82 × fat, kg/milk) + (7.13 × protein, kg/milk) + (0.23 × milk yield, kg)

Blood Collections and Analyses

Blood samples were collected from the coccygeal vein using the Vacutainer system (BD Diagnostics, São Paulo, Brazil) at 0, 1, 2, 3, 7, 10, 14, 21, and 28 DIM. Blood was centrifuged at 1800 × g for separating serum (vial without anticoagulant 10 mL) and divided into two 1.5 mL microtubes, in addition to plasma (vial with sodium fluoride 4 mL), stored in a microtube, afterward, they were frozen at -80 ºC for further metabolite analysis.

To assess the energy status, NEFA and BHBA were analyzed at 0, 3, 7, and 14 DIM, acetone was analyzed at 0, 3, 7, 10, 14, and 21 DIM, and glucose was analyzed at 0, 3, 7, 10, 14, 21, and 28 DIM. For mineral assessment, phosphorus was measured at 0, 3, 7, and 10 DIM, and calcium was measured at 0, 1, 2, and 3 DIM. For hepatic and acute-phase protein assessment, aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), total protein (TP), paraoxonase-1 (PON-1), and albumin (Alb) were analyzed throughout the follow-up period. Cholesterol and bilirubin were also analyzed at 3 and 28 DIM to calculate the liver functionality index (LFI), according to Bertoni and Trevisi (2013), in which LFI = [(IAlb - 17.71) / 1.08 + (IChol - 2.57) / 0.43 - (I - 6.08) / 2.17)] , IAlb and IChol = 0.5_D3 + 0.5_(D28-D3), and IBil= 0.67_D3 + 0.33_(D28-D3) when D3 e D28 are days after calving. Fig. 1 shows the collection times for each metabolite. The metabolites were analyzed using commercial kits (Labtest, Brazil) in a LabMax Plenno automatic analyzer (Labtest, Brazil), except PON-1 and acetone. Both were analyzed using a previously described kinetic method (Browne et al., 2007) and NMR spectroscopy (NMR

Bruker 400 MHz, UFPel-Brazil), respectively.

Statistical Analysis

Statistical analysis was performed using the Statistical Analysis System Studio (SAS University Edition2019). All variables were analyzed using the Shapiro-Wilk test (P > 0.90), those without normal distribution were transformed using LOG 10 and, those with normal distribution were analyzed using the PROC MIXED test.

As independent variables were used: cow, group, and weeks/ days of collection. As dependent variables considering collection days, Ca, P, NEFA, BHB, Acetone, AST, GGT, Bilirubin, Albumin, Cholesterol, TP, PON-1, DMI, parameters of feeding behavior, feeding efficiency, energy balance, milk production (kg), rumination time, leisure and activity were analyzed. The dependent variables considering the weeks were: ECM, FCM, milk composition, weight, ECC, and clinical examination. Comparison between groups considered points from all collections, depending on the analyzed variables (0, 1, 2, 3, 7, 10, 14, 21, and 28 DIM or 0, 1, 2, 3 e 4 weeks) with the model: $Yijk = \mu + \upsilon i + Pj + Tk + PTjk + \varepsilon ijk$, where Yijk is the dependent variable, μ is the general mean, υi is the random effect of the cow, Pj is the fixed effect of the period, Tk is the fixed effect of the treatments, PTjk is the interaction between treatment and period, and εijk is the random residual error.

The analysis was used to compare the main effects, and their interactions were included in the models. P values below 0.05 were deemed significant. Descriptive statistics were used to assess the occurrence of diseases. The multivariable selection of the model was performed using a manual backward step-by-step procedure. The variables were retained in the model if they had P < 0.1 or if their removal resulted in a change of ≥10% in the treatment coefficient. Precision was assessed by analyzing the same sample six times on the same day (intra-assay precision) and different days (inter-assay precision). Precision was assumed when the coefficient of variation was 5%. The variance of the components' structure was used because of the lower values in the Bayesian information criterion for all variables.

3. Results

Regarding milk yield (Table 2), it was observed that there was a higher milk production (3.66 kg, P < 0.05) in the B+C group than in the control group (Figure 2A). There was also a higher milk yield in the B+C group for 3.5%-corrected fat (8.18 kg) (P < 0.01) and ECM (Figure 2B) (8.07 kg) (P < 0.01) over 4 weeks. There was no difference in milk composition during the experimental period (P > 0.05).

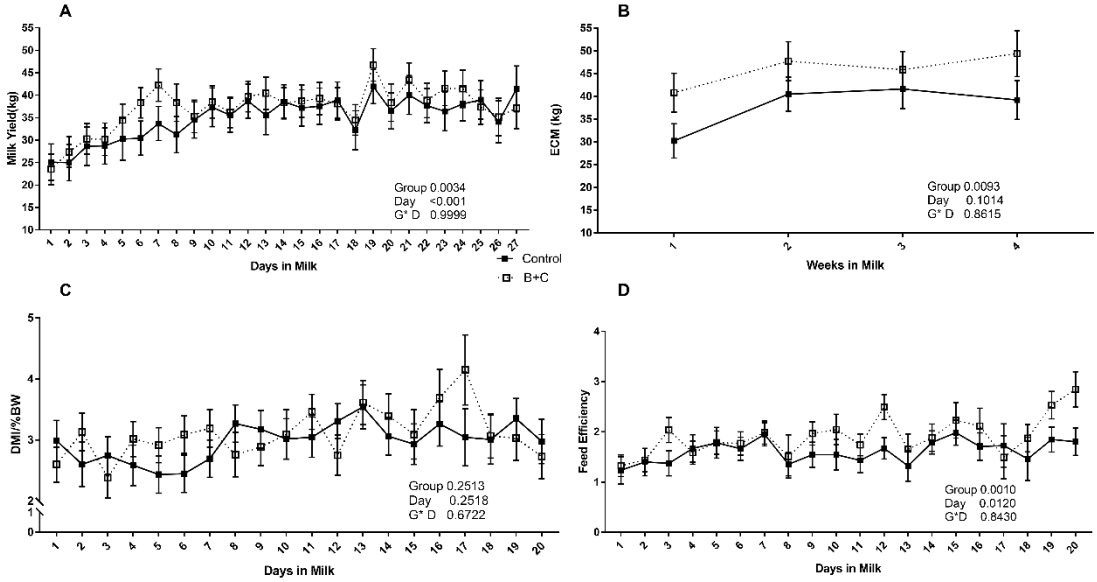
Table 2. Mean and standard error of milk yield and milk composition of multiparous cows of the butaphosphan + cyanocobalamin or control group since calving up to 4 weeks in milk.

Parameter	B+C[3]	Control	*P*-value[4,5]		
			Treatment	Week (day)	T*W(D)
Milk yield, kg/day	37.53 (0.85)	33.87 (0.86)	<0.01	<0.01	0.99
3.5% FCM[1]	43.97 (2.21)	35.79 (2.01)	<0.01	0.10	0.85
ECM[2]	45.95 (2.20)	37.88 (2.01)	<0.01	0.10	0.86
Fat, /kg of milk	1.85 (0.10)	1.61 (0.10)	0.11	0.14	0.77
Protein, /kg of milk	1.28 (0.05)	1.14 (0.05)	0.11	0.07	0.31
Fat, %	4.78 (0.19)	4.56 (0.17)	0.42	0.41	0.52
Protein, %	3.30 (0.07)	3.26 (0.07)	0.66	0.01	0.16
Lactose, %	4.40 (0.04)	4.47 (0.04)	0.21	0.61	0.32
Total solids, %	13.29 (0.22)	13.13 (0.21)	0.58	0.27	0.60

[1]3.5 % FCM = (0.4255 × kg milk) + (16.425 × kg milk fat). [2]ECM = (12.82 × fat, kg/milk) + (7.13 × protein, kg/milk) + (0.23 × milk production, kg). [3]butaphosphan + cyanocobalamin. [4]*P* < 0.05 represents statistical difference. [5]*P* < 0.09 – 0.06 represents trend. Source: authors.

There was no difference between groups regarding intake (Table 3) (P > 0.05) or the DMI/BW% ratio (Figure 2C) (P > 0.05), thus, associated with milk yield, the feed efficiency (Fig. 2D) was higher in the B+C group (P < 0.05). Assessing the animals' behavior, a lower frequency of consumption was observed in the B+C group (P < 0.05) along with a tendency for longer meals (P < 0.06). There was no difference between groups with respect to rumination time (P > 0.05). Still, the B+C group presented a tendency for lower activity (P = 0.09) and, therefore, a longer idle time compared to the control (P < 0.05) (Table 3).

Figure 2. (A) Milk Yield, (B) ECM, (C) DMI/%BW, (D) Feed efficiency of multiparous cows in the butaphosphan + cyanocobalamin or control group from calving up to 4 weeks in milk.



Source: Authors.

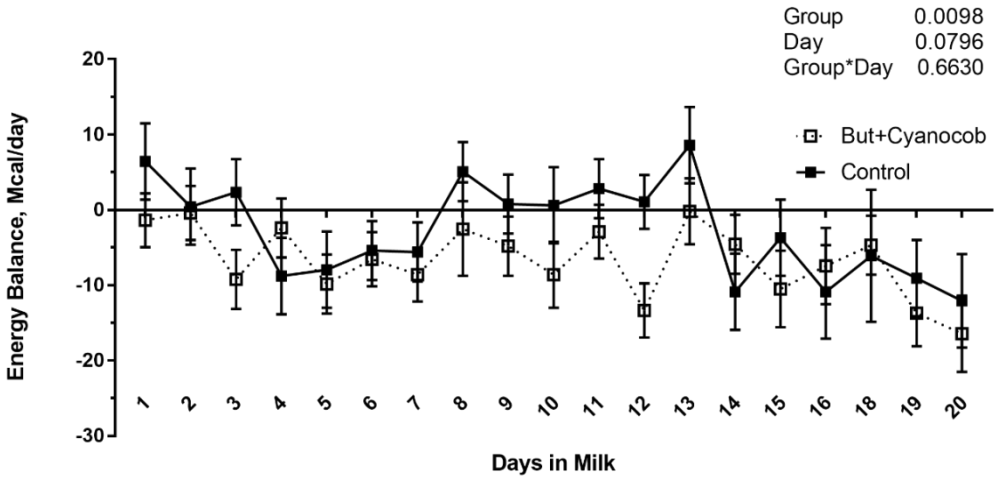
Table 3. Mean and standard error of the intake, feed efficiency and behavior of multiparous cows of the butaphosphan + cyanocobalamin or control group since calving up to 4 weeks in milk.

Parameter	B+C[3]	Control	*P*-value[4,5]		
			Treatment	Week (day)	T*W(D)
DMI[1], kg	21.06 (0.53)	20.86 (0.50)	0.77	0.73	0.73
DMI/%BW	3.10 (0.08)	2.97 (0.07)	0.25	0.25	0.67
Feed Efficiency, milk/DMI	1.91 (0.06)	1.61 (0.06)	<0.01	0.01	0.84
Total time, min/day	148.40 (3.60)	144.22 (3.64)	0.41	<0.01	0.23
Eating time, min/day	139.82 (3.54)	136.63 (3.58)	0.52	<0.01	0.32
Meal frequency, meals/day[2]	16.80 (0.76)	20.13 (0.76)	<0.01	0.97	0.97
Eating rate, %	15.48 (0.004)	16.12 (0.004)	0.33	0.47	0.88
Meal size, kg/meal	1.18 (0.11)	1.31 (0.10)	0.40	0.96	0.99
Meal duration, min	9.33 (0.11)	8.19 (0.43)	0.06	0.15	0.96
Rumination, min/day	625.44 (9.82)	636.46 (9.58)	0.42	0.68	0.99
Activity, min/day	179.52 (9.15)	200.66 (8.92)	0.09	0.93	0.99
Lying, min/day	635.03 (6.64)	602.88 (9.40)	0.01	0.85	0.88

[1]DMI= dry matter intake, DMI/%PW = dry matter intake per body weight percent. [2]meal ≥ 0.250kg. [3]butaphosphan + cyanocobalamin. [4]*P* < 0.05 represents statistical difference. [5]*P* < 0.09 – 0.06 represents trend. Source: Authors.

In the EB assessment (Figure 3), we observed that the B+C group had a higher NEB compared to the control group (P < 0.05), but it was not severe enough to change NEFA and BHB values (P > 0.05). Regarding metabolic parameters (Table 4), albumin concentration was higher in the B+C group (P < 0.05). The other parameters showed no difference between the groups (P > 0.05).

Figure 3. Energy balance (Mcal/day) of multiparous cows of the butaphosphan + cyanocobalamin or control group up to 20 days in milk.



Source: Authors.

Table 4. Mean and standard error of metabolic parameters of multiparous cows of the butaphosphan + cyanocobalamin or control group since calving up to 4 weeks in milk.

Parameter	B+C[2]	Control	*P*-value[3,4]		
			Trearment	Day	T*D
Acetone, mg/dL	1.13 (0.06)	1.16 (0.07)	0.76	0.53	0.18
Albumin, g/dL	2.74 (0.04)	2.60 (0.05)	0.04	0.09	0.29
AST[1], U/L	89.49 (3.01)	90.32 (3.05)	0.84	0.16	0.89
BHB[1], mmol/L	0.63 (0.05)	0.53 (0.05)	0.17	0.08	0.73
Calcium, mg/dL	7.75 (0.23)	7.53 (0.22)	0.50	0.21	0.43
EB[1], Mcal/d	-6.74 (0.97)	-2.75 (1.16)	<0.01	0.01	0.66
GGT[1], U/L	33.63 (1.61)	37.42 (1.64)	0.10	0.06	0.57
Glucose, mg/dL	70.38 (3.86)	63.97 (3.90)	0.24	0.16	0.11
LFI[1]	-9.07 (2.92)	-9.19 (2.40)	0.93	-	-
NEFA[1], mmol/L	0.87 (0.08)	0.87 (0.08)	0.99	0.64	0.96
PON-1[1], g/L	94.14 (3.23)	92.37 (3.40)	0.70	0.15	0.85
Phosphorus, mg/dL	6.58 (0.29)	6.32 (0.31)	0.54	0.72	0.87
TP[1], g/dL	7.26 (0.11)	7.15 (0.11)	0.48	<0.01	0.98

[1]AST= aspartate aminotransferase, BHB= β-hydroxybutyrate, EB= energy balance, GGT= gamma glutamyl transferase, LFI= liver function index, NEFA= non esterified fatty acids, PON-1= paraoxonase 1, TP= total protein. [2]butaphosphan + cyanocobalamin. [3]$P < 0.05$ represents statistical difference. [4]$P < 0.09 – 0.06$ represents trend. Source: Authors.

There was no difference (P > 0.05) in the clinical parameters (Table 5), as well as weight, weight loss (Fig. 4), and BCS during the study. Concerning the occurrence of diseases, 23 clinical and subclinical cases were found, and it is worth noting that the same cow may have been affected by one or more diseases. In the assessment of subclinical diseases, nine cases of hypocalcemia and seven cases of mastitis were detected. Concerning clinical diseases, seven cases were diagnosed: one case of retained fetal membrane, one case of clinical mastitis, and five cases of grade-3 clinical metritis. As for Citobrush neutrophils (PMN cells), it was not possible to observe any difference between groups (P > 0.05). The values found were lower compared to those used in the literature to characterize subclinical endometritis (5 to 18% of PMN cells with 28 postpartum days) (Wagener et al., 2017), with no occurrence of this disease in our study.

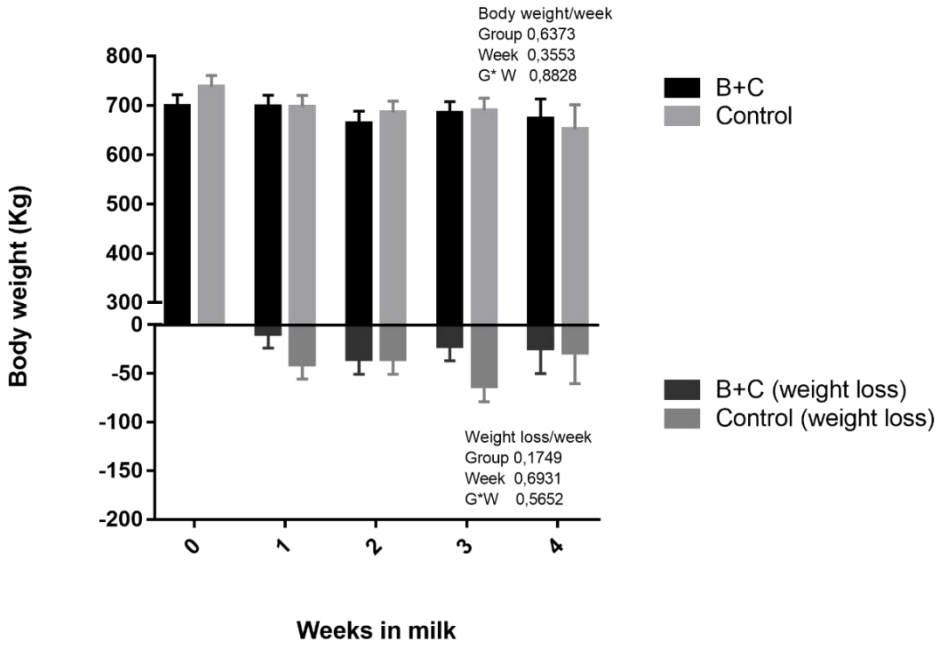
Moreover, 34.78% of all diseases, clinical or subclinical, occurred in the B+C group, and 65.21% were found in the control group. Mean for the leukogram values of the animals also did not show any difference (P > 0.05) and were within physiological limits (Jain, 1993).

Table 5. Mean and standard error of clinical and performance assessments of multiparous cows of the butaphosphan + cyanocobalamin or control group since calving up to 4 weeks in milk.

Parameters	B+C[2]	Control	*P*-value[3,4]		
			Treatment	Week	T*W
Heart rate, bpm	79.51 (2.12)	83.59 (2.35)	0.20	0.06	0.81
Respiratory rate, mpm	42.75 (2.04)	43.95 (2.27)	0.69	0.30	0.88
Ruminal movements, x/2min	2.06 (0.10)	1.90 (0.11)	0.31	0.22	0.67
Body temperature, ºC	38.64 (0.08)	38.61 (0.08)	0.79	<0.01	0.95
Body weight, kg	683.51 (12.57)	692.37 (13.83)	0.63	0.35	0.88
Weight loss, kg	-22.38 (9.31)	41.70 (10.48)	0.17	0.69	0.56
BCS[1]	2.66 (0.05)	2.59 (0.05)	0.43	0.24	0.06
SCC[1], X1000/mL	483.17 (0.19)	354.97 (0.18)	0.78	0.94	0.53
% Citobrush neutroplhis	2.33 (1.34)	1.91 (1.13)	0.6259	-	-
Subclinical hypocalcemia	3/9	6/9	-	-	-
Subclinical mastitis	3/9	4/9	-	-	-
Clinical mastitis	1/9	0/9	-	-	-
Clinical metritis	1/9	4/9	-	-	-
Retained fetal membranes	0/9	1/9	-	-	-

[1]BCS= body condition score, SCC= somatic cell count. [2]butaphosphan + cyanocobalamin. [3]$P < 0.05$ represents statistical difference. [4]$P < 0.09 – 0.06$ represents trend**.** *SCC with standard transformation error for log10. Source: Authors.

Figure 4. Body live weight and weight loss of multiparous cows in the butafosfan + cyanocobalamin or control group since calving up to 4 weeks of lactation.



Source: Authors.

4. Discussion

We observed in the present study that cows supplemented with butaphosphan combined with cyanocobalamin produced an extra 3.66 kg of milk/day than cows in the control group. The increase in milk yield shown in the present investigation is explained by the fact that phosphorus and cyanocobalamin activate the Krebs cycle (Rollin et al., 2010), thus improving energy re-synthesis through gluconeogenesis (Mcdowell, 1992) and milk yield in the mammary gland (Cuteri et al.,

2008, Pereira et al., 2013, Tabeleão et al., 2017). Other authors have already been reported this effect in cows with a more pronounced NEB, that is, with a high concentration of NEFA and BHB (Kreipe et al., 2011, Pereira et al., 2013, Gordon et al., 2017). This evidence supports the benefits of the active ingredients in challenged cows at the risk of developing ketosis. Unlike other studies, cows in our experiment did not present subclinical or clinical ketosis, nor did they develop severe NEB, which may explain the significant increase in milk production observed in the current study. Similar results were found in a study with sheep (Mohammadi Barminanloo, 2021), in which the administration of bustaphosphan and cyanobalamin decreased the risks of metabolic disorders.

Although the B+C group presented a higher NEB associated with higher milk yield, it is important to consider that the energy increase provided by the compound is not included in the EB calculation. Also, weight loss, glucose, BCS, NEFA, and BHB, which are important markers of NEB severity (Heuer et al., 2000, Grummer, 2008), remained similar between groups. This observation could mean that NEB was not a challenge with the potential to induce ketosis in the animals but a physiological event of the postpartum period (Ingvartsen, 2006). Therefore, the compound helped in maintaining metabolic homeostasis, which is a strategy that can also be adopted in animals with a lower metabolic challenge.

In cows with severe NEB, a lack of energy is responsible for a cascade of events that culminate in reduced milk yield as a consequence of reduced DMI (NRC, 2001). Nevertheless, in our study, NEB was less severe, without a change in the NEFA, BHB, and acetone concentrations. Therefore, it is possible to speculate that, similar to mid-lactation cows (Moallem, 2016), which are less metabolically challenged, mammary gland requirements are responsible for determining DMI, and not the opposite. This may explain the reason behind the unchanged DMI. Thus, the more significant energy input supplied to the B+C group through the compound could be used by the mammary gland, leading to greater productive efficiency, without the need to increase DMI.

As for feed efficiency, cows in the B+C group had the same DMI as control cows, with higher production and greater feeding efficiency. Greater feed efficiency is an indispensable factor for the system's profitability since costs associated with feeding can account for up to 50% of the farm revenue (Connor, 2015). Moreover, the ethology of cows in the B+C group showed that they needed fewer visits to the feeders since they were more efficient. However, there was a tendency for longer meals, which has already been described as negatively correlated with rumination time since cows cannot ruminate while eating (Schirmann et al., 2012). The smaller number of visits to the feeders in the B+C group explains their lower activity and, consequently, their longer idle time, which can be beneficial since a longer idle time has already been positively correlated with more frequent periods of rumination (Schirmann et al., 2012).

Our observations contradict those described by Harder et al. (2019), they proposed that it is better to compromise on greater feeding efficiency in the postpartum period to not aggravate NEB and render cows more susceptible to diseases. As already discussed in our study, although more efficient cows had a more severe NEB, the latter could not change their metabolic homeostasis or increase weight loss compared to the control group. This fact led us to infer that when there is no change in lipolysis markers, NEB is a metabolic process often necessary for maximum efficiency.

Regarding the occurrence of diseases, a lower incidence was observed in postpartum cows from the B+C group. This may occur because of an improvement in the immune system since phosphorus is a fundamental element for cellular growth, differentiation, and integrity (Berg et al., 2006). Additionally, it plays an essential role in the ADP/ATP cycle (Cunningham, 2002), which is one of the most important source of cellular energy, including the cells of the immune system (Zhang et al., 2006).As already noted by Eisenberg et al. (2014), the depletion of phosphorus can decrease the number and survival of granulocytes, leaving the animal more prone to diseases. Moreover, the use of butaphosphan in mice showed a tendency toward the greater activity of the myeloperoxidase enzyme (Mattei, 2017), indicating an increase in the functionality of neutrophils, principal cells of the first-line host defense against infectious pathogens, and a renewal of blood cells through an

increase in hematopoiesis (Jacometo et al., 2016). As a result, cows with more active defense cells become less predisposed to developing diseases in the recent postpartum period (reference). However, the number of animals evaluated in the current study is a limiting factor for the health parameters.

The higher albumin concentration in the B+C group can be attributed to an indirect effect of the B+C compound. Albumin has a high capacity for binding with various substances, such as metals and hydroxyls, released into the blood, generating reactive oxygen species (ROS) responsible for increased oxidative stress (Halliwell, 1988, Peters, 1995). The B+C group experienced these indirect effects since the antioxidants reduce lipid mobilization, which is followed by a rise in ROS production (Gaal et al., 2006). Albumin is associated with a better liver condition, as described by Trevisi et al. (2012), who showed that cows with better liver function had an increase in albumin concentration after calving. Further studies must be conducted to investigate this possible potential.

The hepatic and mineral profiles remained within the physiological limits and were similar between groups, showing that such markers did not influence the results. Phosphorus levels also remained unchanged between groups, a fact also noted by other authors (Rollin et al., 2010, Pereira et al., 2013). Nevertheless, Fürll et al. (2010) found an increase in phosphorus concentration in their postpartum research, with applications being performed early in the prepartum period. They argue that the rise in serum phosphorus is not an immediate effect of the applications, which would explain why we did not observe this increase in our investigation. Another possible explanation is that phosphorus is directed to the mammary gland because milk yield has significant importance in draining the mineral (Fürll et al., 2010).

5. Conclusion

We can conclude that butaphosphane combined with postpartum cyanocobalamin injections increases milk production without altering DMI. However, further studies are needed to better elucidate the combination's pathways of action, as well as possible other effects.

References

AOAC (1975). Association of Official Analytical Chemists, *Official Methods of Analysis*, 12th ed, Washington.

AOAC (1997). Association of Official Analytical Chemists, *Official Methods of Analysis*, (16th ed.), Washington.

Berg, J. M., Tymoczko, J. L. & Stryer, L. (2006). Glycolysis and gluconeogenesis, In: *Biochemistry*, 6th ed. W. H. Freeman, 433-474.

Bertoni, G. & Trevisi, E. (2013). Use of the liver activity index and other metabolic variables in the assessment of metabolic health in dairy herds. *Veterinary Clinics of North America: Food Animal Practice* 29, 413-431.

Browne, R. W., Koury, S. T., Marion, S., Wilding, G., Muti, P. & Trevisan, M. (2007). Accuracy and biological variation of human serum paraoxonase 1 activity and polymorphism (Q192R) by kinetic enzyme assay. *Clinical Chemistry* 53, 310-317.

Chapinal, N., Carson, M., Duffield, T. F., Capel, M., Godden, S., Overton, M., Santos, J. E. P. & LeBlanc, S. J. (2011). The association of serum metabolites with clinical disease during the transition period. *Journal of Dairy Science* 94, 4897-4903.

Chizzotti, M. L., Machado, F. S., Valente, E. E. L., Pereira, L. G. R., Campos, M. M., Tomich, T. R., Coelho, S. G. & Ribas M. N. (2015). Technical note: Validation of a system for monitoring individual feeding behavior and individual feed intake in dairy cattle. *Journal of Dairy Science*. 9, 3438–3442.

Coleman, D. N., Alharthi, A. S. & Liang, Y. (2021). Multifaceted role of one-carbon metabolism on immunometabolic control and growth during pregnancy, lactation and the neonatal period in dairy cattle. *J Animal Sci Biotechnol* 12, 27 (2021). https://doi.org/10.1186/s40104-021-00547-5

Connor, E. E. (2015). Invited review: Improving feed efficiency in dairy production: challenges and possibilities. Animal 9, 395-408.

Cunningham, J. G., 2002. *Textbook of Veterinary Physiology*, (3rd ed.) W.B. Saunders Co, Philadelphia.

Cuteri, V., Nisoli, L., Attili, A.R., Tejeda, A.R., Preziuso, S. & Fruganti, A. (2008). Clinical field evaluation of a butafosfan+ vitamin B12 compound (Phosphorum B12/Catosal) in the treatment of subclinical ketosis in dairy cows. *Hungarian Veterinary Journal* 130, 16-17.

Drackley, J. K. . (1999). Biology of dairy cows during the transition period: the final frontier? *Journal of Dairy Science* 82, 2259-2273.

Drackley, J. K. & Cardoso, F. C. (2014). Prepartum and postpartum nutritional management to optimize fertility in high-yielding dairy cows in confined TMR systems. *Animal* 8, 5-14.

Easley, J. F., McCall, J. T., Davis, G. K. & Shirley, R. L. (1965). Analytical methods for feeds and tissues. Nutrition Laboratory, Departament of Animal Science, University of Florida, Gainesville.

Edmonson, A. J., Lean, I. J., Weaver, L. D., Farver, T. & Webster, G. (1989). A body condition scoring chart for Holstein dairy cows. *Journal of Dairy Science* 72, 68-78.

Eisenberg, T., Schroeder, S., Andryushkova, A., Pendl, T., Küttner, V., Bhukel, A., Mariño, G., Pietrocola, F., Harger, A. & Zimmermann, A. (2014). Nucleocytosolic depletion of the energy metabolite acetyl-coenzyme a stimulates autophagy and prolongs lifespan. *Cell Metabolism* 19, 431-444.

French, P.D. (2006). Dry matter intake and blood parameters of nonlactating Holstein and Jersey cows in late gestation. *Journal of Dairy Science* 89, 1057-1061.

Fürll, M., Deniz, A., Westphal, B., Illing, C. & Constable, P.D. (2010). Effect of multiple intravenous injections of butaphosphan and cyanocobalamin on the metabolism of periparturient dairy cows. *Journal of Dairy Science* 93, 4155-4164.

Gaal, T., Ribiczeyne-Szabo, P., Stadler, K., Jakus, J., Reiczigel, J., Kover, P., Mezes, M. & Sumeghy, L. (2006). Free radicals, lipid peroxidation and the antioxidant system in the blood of cows and newborn calves around calving. Comparative Biochemistry and Physiology. Part B, *Biochemistry and Molecular Biology* 143, 391-396.

Girma, D. D., Ma, L., Wang, F., Jiang, Q. R., Callaway, T. R., Drackley, J. K. & Bu1, D. P. (2018). Effects of close-up dietary energy level and supplementing rumen-protected lysine on energy metabolites and milk production in transition cows. Journal of Dairy Science. 102, 7059–7072.Goff, J.P., Liesegang, A., Horst, R.L. (2014). Diet-induced pseudohypoparathyroidism: A hypocalcemia and milk fever risk factor. Journal of Dairy Science 97, 1520-1528.

Gohary, K. & LeBlanc, S. J. (2018). Cost of retained fetal membranes for dairy herds in the United States. *Journal of the American Veterinary Medical Association* 252, 1485-1489.

Gordon, J. L., LeBlanc, S. J. & Duffield, T. F. (2013). Ketosis treatment in lactating dairy cattle. *Veterinary Clinics of North America: Food Animal Practice* 29, 433-445.

Gordon, J. L., LeBlanc, S. J., Kelton, D. F., Herdt, T. H., Neuder, L. & Duffield, T. F. (2017). Randomized clinical field trial on the effects of butaphosphan-cyanocobalamin and propylene glycol on ketosis resolution and milk production. *Journal of Dairy Science* 100, 3912-3921.

Grummer, R. R. (2007). Strategies to improve fertility of high yielding dairy farms: management of the dry period. *Theriogenology* 68, S281-S288.

Grummer, R. R. (2008). Nutritional and management strategies for the prevention of fatty liver in dairy cattle. *The Veterinary Journal* 176, 10-20.

Grummer, R. R., Mashek, D. G. & Hayirli, A. (2004). Dry matter intake and energy balance in the transition period. *Veterinary Clinics of North America: Food Animal Practice* 20, 447-470.

Halliwell, B. (1988). Albumin an important extracellular antioxidant? *Biochemical Pharmacology* 37, 569-571.

Hammon, D. S., Evjen, I. M., Dhiman, T. R., Goff, J. P. & Walters, J. L. (2006). Neutrophil function and energy status in Holstein cows with uterine health disorders. *Veterinary Immunology and Immunopathology* 113, 21-29.

Harder, I., Stamer, E., Junge, W. & Thaller, G. (2019). Lactation curves and model evaluation for feed intake and energy balance in dairy cows. *Journal of Dairy Science* 102, 7204-7216.

Harmon, R. J. (1994). Symposium-mastitis and genetic evaluation for somatic-cell count-physiology of mastitis and factors affecting somatic-cell counts. *Journal of Dairy Science* 77, 2103-2112.

Heinrichs, A. J., Erb, H. N., Rogers, G. W., Cooper, J. B. & Jones, C. M. (2007). Variability in Holstein heifer heart-girth measurements and comparison of prediction equations for live weight. *Preventive Veterinarie Medicine* 78, 333-338.

Heuer, C., Van Straalen, W. M., Schukken, Y. H., Dirkzwager, A. & Noordhuizen, J. P. T. M. (2000). Prediction of energy balance in a high yielding dairy herd in early lactation: model development and precision. *Livestock Production Science* 65, 91-105.

Ingvartsen, K. L. (2006). Feeding-and management-related diseases in the transition cow: Physiological adaptations around calving and strategies to reduce feeding-related diseases. *Animal Feed Science and Technology* 126, 175-213.

Jacometo, C. B., Zhou, Z., Luchini, D., Trevisi, E., Correa, M. N. & Loor, J. J. (2016). Maternal rumen-protected methionine supplementation and its effect on blood and liver biomarkers of energy metabolism, inflammation, and oxidative stress in neonatal Holstein calves. Journal of Dairy Science 99, 6753-6763.

Jain, N.C. (1993). *Essentials of veterinary hematology*. Lea & Febiger, Philadelphia.

Kreipe, L., Deniz, A., Bruckmaier, R.M. & van Dorland, H.A. (2011). First report about the mode of action of combined butafosfan and cyanocobalamin on hepatic metabolism in nonketotic early lactating cows. *Journal of Dairy Science* 94, 4904-4914.

LeBlanc, S., (2010). Monitoring metabolic health of dairy cattle in the transition period. *Journal of Reproduction and Development* 56 Suppl, S29-35.

Leiber, F., Holinger, M., Zehner, N., Dorn, K., Probst, J. K. & Neff, A. S. (2016). Intake estimation in dairy cows fed roughage-based diets: an approach based on chewing behaviour measurements. *Applied Animal Behaviour Science* 185, 9-14.

Mattei, P. (2017). Efeitos do butafosfan sobre o processo inflamatório em camundongas desafiadas com endotoxina bacteriana. *Master Thesis*, Master, Federal Univesrity of Pelotas.

McArt, J. A. A., Nydam, D. V., Ospina, P. A. & Oetzel, G. R. (2011). A field trial on the effect of propylene glycol on milk yield and resolution of ketosis in fresh cows diagnosed with subclinical ketosis. *Journal of Dairy Science* 94, 6011-6020.

Mcdowell, L. R. (1992). Minerals in animal and human nutrition: comparative aspects to human nutrition. *Elsevier*, Amsterdam.

Moallem, U. (2016). Future consequences of decreasing marginal production efficiency in the high-yielding dairy cow. Journal of Dairy Science 99, 2986-2 Mohammadi Barimanloo, A., Chalmeh, A., Pourjafar, M., & Mirzaei, A. (2002). Effects of intravenous butaphosphan and cyanocobalamin to late pregnant ewes on the metabolic indices around parturition and weight gain of their lambs after birth. *Veterinary Medicine and Science*, 00, 1– 11. https://doi.org/10.1002/vms3.687995.

NRC (2001). Nutrient requirements of dairy cattle, 7th ed. *Academies Press*, Washington.

Nuber, U., van Dorland, H. A. & Bruckmaier, R. M. (2016). Effects of butafosfan with or without cyanocobalamin on the metabolism of early lactating cows with subclinical ketosis. *Journal of Animal Physiology and Animal Nutrition* 100, 146-155.

Osorio, J. S., Trevisi, E., Li, C., Drackley, J. K., Socha, M. T. & Loor, J. J. (2016). Supplementing Zn, Mn, and Cu from amino acid complexes and Co from cobalt glucoheptonate during the peripartal period benefits p/ostpartal cow performance and blood neutrophil function. *Journal of Dairy Science* 99, 1868-1883.

Oetzel, G. R. (2004). Monitoring and testing dairy herds for metabolic disease. Veterinary Clinics of North America: Food Animal Practice 20, 651-674. Ospina, P. A., Nydam, D. V., Stokol, T. & Overton, T. R. (2010). Evaluation of nonesterified fatty acids and beta-hydroxybutyrate in transition dairy cattle in the northeastern United States: Critical thresholds for prediction of clinical diseases. *Journal of Dairy Science* 93, 546-554.

Pereira, R. A., Silveira, P. A., Montagner, P., Schneider, A., Schmitt, E., Rabassa, V. R., Pfeifer, L. F., Del Pino, F. A., Pulga, M. E. & Correa, M. N. (2013). Effect of butaphosphan and cyanocobalamin on postpartum metabolism and milk production in dairy cows. *Animal* 7, 1143-1147.

Pérez-Báez, J., Risco, C. A., Chebel, R. C., Gomes, G. C., Greco, L. F., Tao, S., Thompson, I. M., Amaral, B. C., Zenobi, M. G. & Martinez, N. (2019). Association of dry matter intake and energy balance prepartum and postpartum with health disorders postpartum: Part I. Calving disorders and metritis. *Journal of Dairy Science* 102, 9138-9150.

Peters, T. (1995). All about albumin: biochemistry, genetics, and medical applications. *Academic press*, San Diego.

Raboisson, D., Mounié, M. & Maigne, E. (2014). Diseases, reproductive performance, and changes in milk production associated with subclinical ketosis in dairy cows: A meta-analysis and review. *Journal of Dairy Science* 97, 7547-7563.

Rollin, E., Berghaus, R. D., Rapnicki, P., Godden, S. M. & Overton, M. W. (2010). The effect of injectable butaphosphan and cyanocobalamin on postpartum serum beta-hydroxybutyrate, calcium, and phosphorus concentrations in dairy cattle. *Journal of Dairy Science* 93, 978-987.

Schirmann, K., Chapinal, N., Weary, D. M., Heuwieser, W. & Von Keyserlingk, M. A. G. (2012) . Rumination and its relationship to feeding and lying behavior in Holstein dairy cows. *Journal of Dairy Science* 95, 3212-3217.

Sheldon, I. M., Lewis, G. S., LeBlanc, S. J. & Gilbert, R. O. (2006). Defining postpartum uterine disease in cattle. *Theriogenology* 65, 1516-1530.

Shirley, J. E. (2006). Feed efficiency is an important management tool for dairy producers, In: *High Plains Dairy Conference*, Kansas, pp. 63-67.

Suriyasathaporn, W., Heuer, C., Noordhuizen-Stassen, E. N. & Schukken, Y. H. (2000). Hyperketonemia and the impairment of udder defense: a review. *Veterinary Research* 31, 397-412.

Tabeleão, V. C., Pereira, R. A., Prietsch, R. d. F., Feijó, J. O., Bondan, C., Mattei, P., Schmitt, E., Del Pino, F. A. B. & Corrêa, M. N. (2017). Butafosfan e cianocobalamina: efeitos indiretos na recuperação da glândula mamária de vacas leiteiras após mastite clínica. *Science and Animal Health* 4, 238-254.

Teixeira, J. C., Teixeira, L. F. A. C. (1998). Do alimento ao leite: entenda a função do rúmen. *UFLA - FAEPE*, Lavras.

Trevisi, E., Amadori, M., Cogrossi, S., Razzuoli, E. & Bertoni, G. (2012). Metabolic stress and inflammatory response in high-yielding, periparturient dairy cows. *Research in Veterinary Science* 93, 695-704.

Tyrrell, H. F. & Reid, J. T. (1965). Prediction of the energy value of cow's milk. *Journal of Dairy Science* 48, 1215-1223.

Van Soest, P. & Robertson, J. B. (1985). Analysis of forages and fibrous foods. *Cornell University*, Ithaca.

Vailati- Riboni, M., Zhou, Z., Jacometo, C. B., Minuti, A., Trevisi, E., Luchini, D. N. & Loor, J. J. (2017). Supplementation with rumen-protected methionine or choline during the transition period influences whole-blood immune response in periparturient dairy cows. *Journal of Dairy Science* 100, 3958-3968.

Weaver, S. R., Laporta, J., Moore, S. A. E. & Hernandez, L. L. (2016). Serotonin and calcium homeostasis during the transition period. *Domestic Animal Endocrinology* 56, S147-S154.

Yuan, K., Shaver, R. D., Bertics, S. J., Espineira, M. & Grummer, R. R. (2012). Effect of rumen-protected niacin on lipid metabolism, oxidative stress, and performance of transition dairy cows. *Journal of Dairy Science* 95, 2673-2679.

Zhang, X., Wu, X. Q., Lu, S., Guo, Y. L. & Ma, X. (2006). Deficit of mitochondria-derived ATP during oxidative stress impairs mouse MII oocyte spindles. *Cell Research* 16, 841-850.