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Metabolic markers and milk production by Holstein cows undergoing different protocols with cyanocobalamin and butaphosphan postpartum

Marcadores metabólicos e produção de leite de vacas da raça Holandesa submetidas a diferentes protocolos de aplicação de cianocobalamina e butafosfan no pós-parto recente

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Abstract: The objective of this study was to evaluate the effects of different protocols combining cyanocobalamin and butaphosphan on metabolic markers and milk production by Holstein cows postpartum. We used 154 multiparous cows housed in a free-stall system and divided into five groups, using the number of lactations and the probable date of calving as randomization criteria. The animals received intramuscular applications of a 100 mg/mL butaphosphan and 0.05 mg/mL cyanocobalamin combination. The treatment was delivered in a volume of 1 mL for every 20 Kg of body weight on varying treatment days as follows: treatment 1 (T1), on delivery day (day 0) (n=36); T2, days 0 and 3 (n = 31); T3: days 0 and 7 (n = 30); T4: days 0, 3 and 7 (n = 28). The control group (CG) received saline solution on days 0, 3 and 7 (n = 29). Blood samples were collected for metabolite evaluation on days 0, 7, 21 and 30. Milk production was recorded once a week for up to 98 lactation days. T4 elicited higher average milk production (25.87±0.34 kg/day; P < 0.001) than all other groups. Administering butaphosphan and cyanocobalamin on days 0, 3 and 7 postpartum increased milk production and improved energy and liver metabolism in the animals.
Keywords: negative energy balance; organic phosphorus; B12 vitamin

Resumo: O objetivo deste estudo foi avaliar os efeitos de diferentes protocolos de administração da associação de cianocobalamina e butafosfan no pós-parto recente de vacas da raça Holandesa sobre marcadores metabólicos e produção de leite. Foram utilizadas 154 vacas da raça Holandesa, multíparas, mantidas em sistema *Free-stall* e divididas em cinco grupos, utilizando como critérios de randomização o número de lactações e a data provável do parto. Os animais receberam aplicações por via intramuscular após o parto da associação de 100 mg/mL de butafosfan e 0,05 mg/mL de cianocobalamina, em volume de 1mL para cada 20 kg de peso vivo, variando apenas os dias de aplicação: **T1**: no dia do parto (dia 0) (n=36); **T2**: dias 0 e 3 (n = 31); **T3**: dias 0 e 7 (n = 30); **T4**: dias 0, 3 e 7 (n = 28). O grupo controle (**GC)** recebeu solução fisiológica nos dias 0, 3 e 7 (n = 29). As amostras de sangue foram coletadas para avaliação de metabólitos nos dias 0, 7, 21 e 30 pós-parto. A produção de leite foi registrada uma vez por semana até 98 dias em lactação. Observou-se que o grupo T4

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apresentou a média de produção de leite maior (25,87±0,34 kg/dia; P < 0,001) do que os demais grupos. O protocolo com a administração da associação de butafosfan e cianocobalamina aplicado nos dias 0, 3 e 7 pós-parto foi o mais eficiente em relação a produção de leite e contribuiu para um melhor metabolismo energético e hepático dos animais.
Palavras-chave: balanço energético negativo; fósforo orgânico; vitamina B12

1. Introduction

During the postpartum period, dairy cows undergo major physiological changes, such as uterine involution and lactation onset, which require a large supply of nutrients. This demand is generally not met by the diet leading to a negative energy balance (NEB) [(1)]. This intensifies lipid mobilization, increasing circulating concentrations of free fatty acids (FFA) and ketone bodies, such as β-hydroxybutyrate (BHB), to provide more energy [(2,3)]. Significant lipid mobilization predisposes the cow to metabolic and infectious diseases, such as ketosis, ruminal acidosis, and metritis [(4)].

To alleviate NEB, butaphosphan (organic phosphorus) and cyanocobalamin supplementation can be used with positive effects on metabolism [(5,6)]. Phosphorus has an important role in energy metabolism, acting in the synthesis of phosphoproteins and oxidative phosphorylation for the synthesis of adenosine triphosphate (ATP). In addition, it acts in hepatic metabolism by reducing the gene expression of enzymes related to ketogenesis and fatty acid oxidation [(7)]. Cyanocobalamin is the synthetic form of vitamin B12, which acts as a co-factor for methyl malonyl-CoA mutase, which transforms propionate into succinyl-CoA for ATP synthesis [(8,9)].

Studies have shown that the combined use of butaphosphan and cyanocobalamin postpartum can reduce FFA and BHB concentrations [(5)] and increase milk production [(5,10)]. There are several studies that demonstrate the positive effects of this association. Nonetheless, these studies employed different protocols, varying the number of doses and days postpartum, hindering their applicability in production systems [(5,10,11)]. Therefore, the objective of this study was to establish a treatment protocol using a butaphosphan+ cyanocobalamin combination in Holstein cows postpartum by measuring its effects on metabolic markers and milk production.

2. Materials and Methods

All procedures in this study were approved by the Animal Experimentation Ethics Committee of the *Universidade Federal de Pelotas* (protocol number 9378). The study was conducted on a commercial dairy farm, located in the south of Rio Grande do Sul, Brazil. We used 154 multiparous Holstein cows, with two to five lactations, housed in an intensive free-stall system. The cows were milked twice a day, using a mechanized system.

The animals received total mixed ration (TMR) after each milking (Table 1) and water *ad libitum*. The diet was formulated to meet the nutritional needs of dairy cows with average productivity postpartum (23). The diets were as follows: 71.87% corn silage, 10.27% pre-dried oats, 12.32% % citrus pulp, 4.54% soybean meal, 0.80% vitamin premix and 0.20% urea.

Table 1 Dietary ingredients in staple (SM) and dry matter (DM), in addition to nutrients in DM from the TMR of Holstein cows that received intramuscular cyanocobalamin and buafosphan postpartum.

Ingredients	Kg/SM	Kg/DM
Corn/silage > 34%	29,17	10,93
Pre-dried oat	4,17	1,52
Citric pulp	5,00	4,29
Soybean meal 44%	1,84	1,63
Urea	0,08	0,08
Vitamin pre-mix	0,33	0,33
Total	40,59	18,78

Nutrients (TMR)	Percentage (%)
Dry matter - (%)	46,31
NDT - (% DM)	69,17
Corn starch - (% DM)	17,36
Ether extract - (% DM)	3,29
Non-fibrous carbohydrates - (%)	39,80
Total protein - (% DM)	12,39
Degradable protein - (% DM)	8,43
Non-degradable protein - (% DM)	3,96
Acid detergent fiber (ADF) – (% DM)	23,51
Neutral detergent fiber (NDF) – (% DM)	39,61
Lignin - (% DM)	2,46
Calcium - (% DM)	0,62
Phosphorus - (% DM)	0,22
Magnesium - (% DM)	0,16
Potassium - (% DM)	1,32
Sodium - (% DM)	0,04
Chlorine - (% DM)	0,24
Sulfur - (% DM)	0,14
Ash - (% DM)	7,25
DCAD (mEq/100g)	20,44

The cows were divided into five groups using the number of lactations and probable calving date as randomization criteria. The treated groups received 100 mg/mL butaphosphan + 0.05 mg/mL cyanocobalamin (Catosal B12®; ELANCO, São Paulo, Brazil). The dosage administered was 1 mL for every 20 Kg of body weight, applied intramuscularly, varying only the days of application. The application schedules were as follows: treatment 1 (T1): on the day of delivery (day 0) (n=36); T2: days 0 and 3 (n = 31); T3: days 0 and 7 (n = 30); T4: days 0, 3 and 7 (n = 28). The control group (CG) received saline solution (0.9%) on days 0, 3 and 7 (n = 29), intramuscularly.

The body condition score (BCS) and weight of the animals were measured on days 0, 15, 30, 45, 60, 75, 90 and 105 postpartum. A five-point scale was used to assign the BCS, considering an obese animal with score 5 and a very lean animal with score 1 [12]. A graduated tape specific for dairy cattle was used for weighing [13]. Milk production was recorded once a week for up to 98 lactation days (LD) using the GEA Farm Technologies® system.

Blood samples were obtained on days 0, 7, 21 and 30 postpartum, through puncture of the coccygeal vein, using a vacuum system. Blood samples were retrieved in two tubes; one with sodium fluoride (4 mL Vacuplast® - Zhejiang, China) to evaluate glucose levels,

and another with silica (clot activator) (10 mL Vacuplast® - Shandong, China) to obtain serum and evaluate other metabolic parameters. Samples were centrifuged at 15,000 x g for 15 minutes. Serum and plasma were transferred to 1.5 mL microtubes, identified, and stored at -20ºC until analysis. All analyzes were conducted in the metabolism laboratory of the Center for Research, Education and Extension in Livestock (NUPEEC HUB), at the *Universidade Federal de Pelotas.*

Commercial kits were used to determine the concentrations of glucose, urea, magnesium, phosphorus (P), calcium (Ca), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT) (Labtest®, Lagoa Santa, Brazil), FFA (Wako NEFA-HR, Wako Chemicals, Richmond, USA) and BHB (Randox®, Randox Laboratories U.S.A., Oceanside, CA, USA) according to the manufacturers' instructions. The analyses were conducted using an automated biochemical analyzer (Labmax Plenno, Labtest®, Brazil). Paraoxanase-1 (PON) activity was determined using a commercial kit (ZeptoMetrix® Corporation, Buffalo, NY, USA), according to Schneider et al., [14].

Statistical analysis

All statistical analyses were performed using SAS 9.0 software (SAS® Institute Inc., Cary, NC, USA, 2004). Metabolite concentrations (Ca, P, glucose, magnesium, urea, AST, GGT, PON, FFA and BHB), weight, BCS, and milk production were evaluated using the MIXED MODELS procedure, considering treatment, period (in days), their interaction, and the animal as a random effect. The post-hoc analysis was conducted using Tukey-Krammer. Values of $P<0.05$ were considered statistically significant.

3. Results

In the present study, the T4 group presented a higher average milk production (25.87±0.34 Kg/day) than the T1, T2, T3, and CG groups (22.68±0.36, 23 .26±0.38, 23.44±0.36, and 23.29±0.33 Kg/day, respectively; $P < 0.001$), with no significant difference in the interaction between group and LD (Table 2; $P = 1.0$).

A significant difference was observed for BHB levels considering the interaction between group and period (Table 3). FFA concentrations in group T1 were lower than those in groups T2, T3 and T4 (Table 3; $P = 0.02$), not differing from the CG group. The GC group presented the highest concentration in AST compared to the T1, T2, T3 and T4 groups (Table 3; $P = 0.02$).

Animals in T4 and GC had lower urea concentrations than animals in T2 and T3 (Table 3; $P = 0.02$). T1 levels were similar to those of the groups. A significant difference was also observed in the group and period interaction (Table 3; $P = 0.02$). T1 and T3 presented higher mean Ca levels than the CG, while the T1 and T2 presented higher levels than T4 (Table 3; $P < 0.001$). A significant difference was also observed in the interaction between group and period (Table 3; $P =0.001$).

There was no difference between groups and in the interaction between group and period for magnesium, glucose, PON levels and GGT (Table 3). The BCS and body weight results are shown in Table 4.

Table 2 Mean milk production (Kg/day) in the different treatment groups of Holstein cows that received intramuscular cyanocobalamin and butaphosphan postpartum.

Days post-partum[1]	Groups[2]					Valor de P		
	T1	T2	T3	T4	CG	Group[2]	Period[3]	Group* Periodo[4]
7	20,66±1,22	22,81±1,25	21,85±1,27	25,03±1,40	21,62±1,25			
14	20,29±1,22	21,40±1,20	21,95±1,27	23,57±1,56	21,20±1,40			
21	22,00±1,25	21,45±1,25	23,00±1,30	23,33±1,52	21,46±1,40			
28	22,20±1,20	23,32±1,20	22,12±1,30	26,30±1,30	22,88±1,25			
35	23,02±1,22	23,23±1,22	24,74±1,33	25,51±1,33	24,12±1,33			
42	24,47±1,20	23,44±1,27	23,19±1,27	26,25±1,43	23,19±1,33			
49	22,01±1,27	24,30±1,22	23,98±1,36	26,19±1,47	23,37±1,30			
56	23,02±1,22	24,91±1,30	24,57±1,36	26,24±1,30	23,62±1,43	**<0.0001**	**<0.0001**	1.000
63	22,79±1,22	24,04±1,22	24,93±1,33	27,23±1,40	24,15±1,47			
70	23,73±1,25	23,13±1,25	21,65±1,36	26,64±1,47	23,83±1,36			
77	23,50±1,18	23,15±1,25	23,60±1,30	27,11±1,40	24,72±1,40			
84	23,35±1,27	22,90±1,30	23,97±1,43	26,40±1,47	23,74±1,33			
91	24,38±1,33	22,77±1,20	23,66±1,33	27,26±1,52	24,25±1,36			
98	22,05±1,73	24,74±1,22	25,00±1,56	25,12±1,40	23,94±1,43			

[1] Milk production on days 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, 77, 84, 91 and 98 postpartum.
[2] Group: the animals were divided into groups, which received the same treatment, intramuscularly, with 100 mg/mL butaphosphan and 0.05 mg/mL cyanocobalamin (1 mL for every 20 Kg of body weight), (Catosal® B12; ELANCO , São Paulo, Brazil), differing in the number of doses per group [T1, on the day of delivery (day 0) (n=36); T2, days 0 and 3 (n = 31); T3, days 0 and 7 (n = 30); T4, days 0, 3 and 7 (n = 28) and the control group (CG), days 0, 3 and 7 (n = 29) received saline solution].
[3] Period refers to the days on which milk production was measured.
*[4]Group*Period refers to the interaction of the group in relation to the days of measuring milk production. **P**<0.05 was considered significant.*

Table 3 Metabolic markers of Holstein cows that received intramuscular cyanocobalamin and butaphosphan postpartum.

Biochemical Parameters[1]	Period (days post-partum)[3]						P-value		
	Group[2]	0	7	21	30	Group[4] Mean	Group[2]	Period[3]	Group* Period[5]
BHB (mmol/L)	T1	0,19±0,08	0,44±0,08AC	0,31±0,08A	0,28±0,08	0,31±0,04	0,13	**<0,001**	**0,001**
	T2	0,19±0,08	0,87±0,08B	0,46±0,09AB	0,29±0,08	0,45±0,04			
	T3	0,27±0,08	0,61±0,08C	0,46±0,10AB	0,18±0,09	0,38±0,04			
	T4	0,21±0,08	0,43±0,08AC	0,68±0,09B	0,27±0,10	0,40±0,04			
	GC	0,15±0,08	0,28±0,09A	0,65±0,12B	0,20±0,12	0,32±0,05			
AGL (mmol/L)	T1	0,63±0,06	0,73±0,06	0,38±0,05	0,32±0,05	0,52±0,03a	**0,02**	**<0,001**	0,71
	T2	0,82±0,05	0,84±0,06	0,52±0,06	0,37±0,05	0,64±0,02b			
	T3	0,75±0,06	0,73±0,05	0,57±0,06	0,36±0,06	0,60±0,03b			
	T4	0,70±0,06	0,87±0,06	0,47±0,06	0,47±0,06	0,63±0,03b			
	GC	0,66±0,06	0,72±0,06	0,51±0,06	0,35±0,06	0,56±0,03ab			
AST (U/L)	T1	57,90±4,76	86,47±5,29	78,42±4,76	73,38±4,76	74,04±2,45a	**0,02**	**<0,0001**	0,88
	T2	66,09±4,65	91,75±5,45	80,65±4,88	73,85±4,76	78,08±2,47a			
	T3	70,05±4,88	89,85±4,76	76,65±4,88	76,55±7,27	78,27±2,77a			
	T4	62,63±5,00	93,75±4,88	79,10±5,00	77,38±5,14	78,21±2,50a			
	GC	77,73±5,00	90,47±5,29	88,93±5,45	86,60±5,63	85,93±2,67b			
Urea (mg/dL)	T1	28,94±2,01A	27,54±2,01AB	29,94±2,01A	27,68±2,01AB	28,53±1,00ab	**0,02**	0,09	**0,02**
	T2	31,56±2,01A	31,59±1,90A	27,20±2,20AB	28,64±2,01A	29,75±1,01a			
	T3	31,54±2,07A	26,38±2,13AB	28,27±2,20A	36,61±2,36B	30,70±1,09a			
	T4	23,24±2,07B	28,39±1,95AB	26,38±2,20AB	29,84±2,20A	26,96±1,05b			
	GC	29,87±2,13A	25,41±2,07B	21,18±2,84B	27,39±2,84A	25,96±1,25b			

Calcium (mg/dL)	T1	8,62±0,23	9.46±0,23A	10,32±0.22A	9,67±0,23A	9,52±0,11a	**<,0001**	**<,0001**	**0,001**
	T2	8.28±0,22	8.84±0,22B	10,23±0,23A	9,79±0,23A	9,28±0,11ab			
	T3	8,31±0,23	9,26±0,22AB	9,88±0,23ABC	8,80±0,25B	9,06±0,11ad			
	T4	8,67±0,23	8,61±0,23B	9,68±0,24C	8,42±0,26BC	8,84±0,12cd			
	GC	8,37±0,23	7,69±0,25AB	9,38±0,27B	9,23±0,28AB	8,67±0,13bc			
Phosphorus (mg/dL)	T1	4,59±0,34A	6,96±0,35A	7,6±0,37	7,61±0,37AC	6,59±0,18	0,58	**<,0001**	**0,03**
	T2	4,70±0,32A	6,95±0,34AB	7,53±0,38	7,36±0,36AB	6,63±0,17			
	T3	5,39±0,33AB	6,67±0,35AB	7,5±0,41	6,59±0,46BD	6,54±0,19			
	T4	5,89±0,35B	6,32±0,34B	6,76±0,39	6,22±0,37CD	6,30±0,18			
	GC	5,63±0,35B	6,41±0,36AB	6,85±0,48	6,38±0,42CD	6,32±0,20			
Magnesium (mg/dL)	T1	2,38±0,11	2,02±0,11	2,80±0,11	2,77±0,11	2,49±0,05	**0,05**	**<,0001**	0,30
	T2	2,28±0,10	2,13±0,11	2,92±0,11	2,66±0,11	2,50±0,05			
	T3	2,58±0,11	2,35±0,11	2,81±0,11	2,97±0,11	2,68±0,05			
	T4	2,46±0,11	2,45±0,13	2,95±0,11	2,60±0,11	2,61±0,05			
	GC	2,44±0,11	2,07±0,11	2,72±0,13	2,71±0,14	2,49±0,06			
Glucose (mg/dL)	T1	70,27±2,53	55,80±2,40	58,47±2,34	55,45±2,40	60,00±1,21	0,50	**<,0001**	0,05
	T2	76,00±2,40	53,14±2,34	55,45±2,40	62,77±2,53	61,84±1,21			
	T3	74,77±2,53	57,09±2,34	56,35±2,40	58,10±2,46	61,58±1,21			
	T4	83,95±2,40	55,80±2,77	54,21±2,46	58,77±2,53	63,18±1,27			
	GC	76,58±2,60	55,10±2,40	57,47±2,46	57,56±2,68	61,68±1,27			
GGT (U/L)	T1	24,75±1,88	27,27±1,98	24,89±1,93	26,21±1,93	25,78±0,96	0,15	**0,03**	0,32
	T2	25,68±1,79	26,86±2,17	30,20±1,88	26,33±1,83	27,27±0,96			
	T3	26,00±1,88	28,33±1,83	30,20±1,88	22,11±2,80	26,66±1,07			
	T4	24,31±1,93	29,42±1,93	27,23±2,04	28,82±2,04	27,44±0,99			
	GC	25,52±1,93	28,47±2,04	30,00±2,10	33,35±2,25	29,33±1,04			
PON1 (U/mL)	T1	87,34±6,06	63,79±6,06	104,76±6,21	110,73±6,06	91,65±3,04	0,36	**<0,0001**	0,21
	T2	88,84±5,92	85,08±6,21	112,03±6,21	111,22±6,06	99,29±3,05			
	T3	87,17±6,21	92,44±6,21	108,57±6,21	101,77±6,94	97,48±3,20			
	T4	84,08±6,37	84,51±6,21	98,70±6,37	103,98±6,54	92,82±3,18			
	GC	98,18±6,21	83,53±6,37	101,94±6,73	101,96±7,12	96,40±3,31			

[1]Clinical biochemical parameters: they were evaluated on delivery day (0), and on days 7, 21, and 30 postpartum.
[2] Group: the animals were divided into groups, which received the same treatment, intramuscularly, with 100 mg/mL butaphosphan and 0.05 mg/mL cyanocobalamin (1mL for every 20 Kg of body weight), (Catosal® B12; ELANCO , São Paulo, Brazil), varying the number of doses per group [T1, on the day of delivery (day 0) (n=36); T2, days 0 and 3 (n = 31); T3, days 0 and 7 (n = 30); T4, days 0, 3, and 7 (n = 28) and the control group (CG), days 0, 3 and 7 (n = 29) received saline solution].
[3] Period refers to the days on which the collections were conducted (delivery day (0), day 7, 21, and 30 postpartum.
[4] Group mean refers to the mean of the groups, and the significant difference was demonstrated with lowercase letters a–d.
*[5]Group*Period refers to the interaction of the group in relation to the sampling days, demonstrated by capital letters A–D between lines. **P**<0.05 was considered significant.*

Table 4. BCS and weight of Holstein cows that received intramuscular cyanocobalamin and butaphosphan postpartum.

Zootechnical Parameters[1]		Period (days post-partum)[3]									P-value		
	Group[2]	0	15	30	45	60	75	90	105	Group[4] Mean	Group[2]	Period[3]	Group* Period[5]
BCS	T1	3,10±0,08	2,81±0,09	2,79±0,09	2,80±0,09	2,77±0,09	2,74±0,09	2,77±0,09	2,77±0,09	2,88±0,03a	**0,02**	<0,001	0,99
	T2	3,01±0,09	2,78±0,09	2,61±0,09	2,74±0,10	2,74±0,09	2,74±0,09	2,84±0,10	2,79±0,10	2,78±0,03bc			
	T3	2,92±0,10	2,70±0,09	2,63±0,10	2,54±0,10	2,61±0,10	2,69±0,10	2,77±0,11	2,94±0,11	2,72±0,03b			
	T4	3,13±0,10	2,77±0,10	2,82±0,11	2,92±0,11	2,65±0,10	2,63±0,10	2,81±0,10	2,97±0,11	2,84±0,03ac			
	GC	3,12±0,09	2,76±0,09	2,63±0,11	2,68±0,10	2,80±0,10	2,70±0,11	2,90±0,11	3,00±0,12	2,82±0,03ac			

Weights	T1	574,4±13,1	550,6±14,8	527,1±14,0	527,0±14,0	546,7±14,2	537,0±13,7	544,0±13,7	539,9±14,5	543,38±4,9			
	T2	589,7±13,7	580,3±14,5	566,8±14,2	568,5±15,1	565,4±14,8	544,0±14,8	547,7±15,4	541,5±15,8	563,05±5,2			
	T3	578,3±15,4	559,4±15,1	550,2±16,1	546,0±15,8	538,6±15,8	538,6±15,8	546,1±17,0	555,0±17,4	552,56±5,7	0,07	0,001	0,99
	T4	560,8±14,8	563,3±15,8	560,4±16,5	559,4±16,5	535,7±15,8	526,6±15,4	536,6±15,1	553,7±17,4	549,60±5,6			
	GC	568,8±14,8	540,4±15,8	550,7±15,8	528,5±15,1	535,4±16,5	546,1±17,0	535,0±16,5	558,4±19,1	545,44±5,8			

[1] Zootechnical Parameters: Body condition score (BCS) and weight evaluated at 0, 15, 30, 45, 60, 75, 90, and 105 days postpartum.
[2] Group: the animals were divided into groups, which received the same treatment, intramuscularly, with 100 mg/mL butaphosphan and 0.05 mg/mL cyanocobalamin (1 mL for every 20 Kg of body weight) (Catosal® B12; ELANCO, São Paulo, Brazil), differing the number of doses per group [T1, on the day of delivery (day 0) (n=36); T2, days 0 and 3 (n = 31); T3, days 0 and 7 (n = 30); T4, day 0, 3 and 7 (n = 28) and the control group (CG), day 0, 3 and 7 (n = 29) received saline solution].
[3] Period refers to the sampling days: 0 (delivery), 7, 21 and 30 after delivery.
[4] Group mean refers to the general mean of the groups and the significant difference in this mean was demonstrated with lowercase letters a–d.
*[5]Group*Period refers to group interaction in relation to blood collection days. **P**<0.05 were considered.*

4. Discussion

There are several studies evaluating the use of a cyanocobalamin and butaphosphan combination in dairy cows postpartum [5,6,8,10], However, there is no standardization between studies, varying the number of doses and postpartum days. Therefore, this study sought to evaluate the best number of doses and interval between doses to yield positive effects on NEB and milk production. Schären et al. [15] and Pereira et al. [5] observed an increase in milk production in animals treated with six and four applications a cyanocobalamin and butaphosphan combination, respectively. In the present study, greater milk production was also observed in animals in the cows receiving three applications, consistent with the data by Pizoni et al., [10] who used the same protocol due to its reduced treatment costs and animal handling.

Increased milk production increases nutritional requirements and consequent lipid mobilization, as observed by the high levels of FFA and BHB in the blood [17]. Hepatic function is also affected as observed by higher levels of the AST enzyme [18]. Thus, animals with increased milk production can benefit metabolically when supplemented, as observed in the present study. Cows in the T4 group, had concentrations of FFA and BHB similar to those in the other groups, even though milk production increased. Therefore, they did not present a significant

NEB. In fact, the T4 group had lower levels AST enzyme than the GC group. Therefore, the T4 protocol improved energy and liver metabolism in the animals.

Animals that produce more milk tend to increase consumption to meet energy requirements [19], intensifying amino acid catabolism, with a likely increase in plasma urea levels [20]. However, in the present study, the T4 and GC groups exhibited reduced urea concentrations further highlighting the improvement in energy metabolism with this treatment. These data are consistent with those of Pereira Sheep. et al. [16] showing lower protein catabolism, since even with lower urea levels, greater milk production was maintained, without harmful changes in other biochemical parameters. Reduced urea levels in the GC group were likely related to lower milk production.

Treatment with a cyanocobalamin and butaphosphan combination can help in Ca homeostasis. In this study, the T4 animals did maintained Ca levels in the evaluated days, even though they produced more milk than those in the other groups. Organic phosphate constitutes energetic molecules, such as ATP and adenosine diphosphate (ADP), and participates in bone resorption. Adenylate cyclase is activated and synthesizes the second messenger (cyclic AMP) [21]. Therefore, increased availability of organic phosphorus induced bone mobilization, which is regulated by parathyroid hormone (PTH), contributing to calcium metabolism [22].

No significant difference was observed in the other parameters (P, magnesium, glucose, PON, GGT and weight), consistent with other studies using a cyanocobalamin and butaphosphan combination [10,5]. The difference in BCS between groups was not significant enough to affect body weight, with minimal variation between the treatment groups.

5. Conclusion

Supplementation with a cyanocobalamin and butaphosphan combination on days 0, 3, and 7 in Holstein cows postpartum increased milk production and improved energy metabolism and liver function in treated animals.

Conflict of Interest Declaration

The authors have no competing financial interests.

Author contributions

Conceptualization: Corrêa, M.N. *Data curation:* Barbosa, M.W.M and Londero, U.S. *Formal Analysis*: Londero, U.S. *Funding acquisition*: Corrêa, M.N. Investigation: Krusser, R.H. *Methodology*: Krusser, R.H; Barbosa, A.A and Carpinelli, N. *Project admnistration*: Corrêa, M.N. *Resources*: Krusser, R.H. *Software*: Barbosa, M.W.M. *Supervision*: Krusser, R.H; Barbosa, A.A. *Validation*: Da Silva, T.C. *Visualization*: Da Silva, T.C. *Writing – review & editing*: Da Silva, T.C; Feijó, J.O; Rabassa, V.R; Del Pino, F.A.B

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