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Research Article

Evaluation of the Effect of Different Doses of Butaphosphan and Cyanocobalamin Combination in Dairy Cattle with Subclinical Ketosis [1]

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Abstract

This study was conducted to assess the effects of different doses of butaphosphan-cyanocobalamin combination onbody condition score (BCS), beta-hydroxybutyrate (BHBA) and reproductive parameters in cows with subclinical ketosis (SCK). Holstein-Friesen cows (n=544) were checked for BHBA concentration. Cows with SCK (n=53, BHBA ranging from 1.00 to 3.00 mmol/L) were assigned randomly to receive saline (C0, n=18), 5 mL/100 kg BW (C5, n=18) or 10 mL/100 kg BW (C10, n=17) butaphosphan-cyanocobalamin combination. BHBA concentration was measured on d 0, 10, and 18 relative to treatment application. BCS was evaluated weekly until d 60 postpartum. For reproductive parameters cows were monitored until d 150 postpartum. The median reduction in blood BHBA concentrations was 28, 57, and 75% for C0, C5, and C10, respectively. NEFA and total bilirubin concentrations were significantly decreased in C10 group. The relative median change in BCS as compared to baseline was 17, 12, and 6% for C0, C5, and C10, respectively. Between d 15 and 25 postpartum uterine involution was completed in 44, 83, and 88% of cows in groups C0, C5, and C10, respectively. Interval from calving to first insemination in group C10 was shorter than control group. Overall pregnancy rate was not different among groups. In conclusion butaphosphan-cyanocobalamin combination decreased severity of hyperketonemia, stimulated uterine involution, shortened time to first insemination and increased pregnancy rate.

Keywords: *Beta hydroxybutyrate, Butaphosphan, Cyanocobalamin, Subclinical ketosis, Reproductive performance*

Subklinik Ketosisli Süt İneklerinde Farklı Dozlarda Butafosfan ve Siyanokobalamin Kombinasyonunun Etkisinin Değerlendirilmesi

Özet

Bu çalışma, subklinik ketozisli (SCK) süt ineklerinde farklı dozlarda butafosfan-siyanokobalamin kombinasyonunun vücut kondisyon skoru (VKS), beta-hidroksibütirat (BHBA) ve reprodüktif parametreler üzerine etkilerini değerlendirmek amacıyla gerçekleştirildi. Holştayn-Frizyan inekler (n=544) BHBA yönünden kontrol edildi. SCK'li inekler (n=53, 1.00-3.00 mmol/L BHBA); 4 gün süresince günlük salin uygulanan (C0, n=18) ve canlı ağırlığa 5 mL/100 kg dozunda (C5, n=18) ya da canlı ağırlığa 10 mL/100 kg dozunda (C10, n=17) butafosfan-siyanokobalamin kombinasyonunun uygulandığı gruplara rastgele olarak ayrıldı. BHBA düzeyleri; 0, 10 ve 18. günlerde ölçüldü. VKS postpartum 60. güne kadar haftalık değerlendirildi. Reprodüktif parametreler için inekler doğum sonrası 150. güne kadar takip edildi. Kan BHBA düzeyinde medyan azalma C0, C5 ve C10 grubunda sırasıyla %28, %57 ve %75 olarak gerçekleşti. NEFA ve total bilirübin düzeyleri C10 grubunda anlamlı şekilde azaldı. VKS'un relatif medyan değişimi baseline ile karşılaştırıldığında C0, C5 ve C10 grubunda sırasıyla %17, %12 ve %6 olarak belirlendi. Postpartum 15 ve 25. günler arasında uterus involüsyonu C0, C5 ve C10 gruplarında sırasıyla %44, %83 ve %88 tamamlandı. C10 grubunda buzağılama ilk tohumlama aralığı kontrol grubundan daha kısaydı. Ortalama gebelik oranı gruplar arasında farklılık göstermedi. Sonuç olarak butafosfan-siyanokobalamin kombinasyonu; hiperketonomi şiddetini azalttı, uterus involüsyonunu uyardı, ilk tohumlama zamanını kısalttı ve gebelik oranını arttırdı.

Anahtar sözcükler: *Beta hidroksibütirat, Butafosfan, Siyanokobalamin, Subklinik ketozis, Reprodüktif performans*

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INTRODUCTION

Subclinical ketosis is a consequence of transition period linked to negative energy balance (NEB), which leads lipolysis of body fat reserves and hyperketonemia [1]. Plasma BHBA greater than 1.2 mmol/L is considered ketosis [2]. However, definition of SCK is not consistent. In a study, SCK was considered whenblood concentration of BHBA higher than 1.00 mmol/L [3,4], while the other one indicated the SCK in the concentrations of BHBA above 1.40 mmol/L [5,6]. Negative energy balance is compensated by increased lipomobilization, which results in formation of BHBA produced by incomplete lipid oxidation in the liver immune system is adversely affected via hepatopathies-associated with ketotic metabolic status [7,8]. BCS loss resulting from fat mobilization is typical in ketotic cows [9]. This is often significant in cows with BCS greater than 3.25 during prepartum. These cows lose >0.75 BCS within 2 month after parturition [10,11]. Butaphosphan-cyanocobalamin combinationcontains both vitamin B12 and butaphosphan (alpha amino phosphonic acid). Researchers indicated that butaphosphan-cyanocobalamin combination could be supportive in correcting the metabolic status of high-producing cows [12,13] and controlling of SCK [14]. BCS loss was minimal in cows administered with 2 ml butaphosphan-cyanocobalaminon d 3 postpartum [12]. In other studies, injection of vitamin B12 [15] alone or in combination with butaphosphan [12] improved lactation yield. Cows with the high blood BHBA concentration between 2 and 15 day in milk (DIM) are more likely to reduce the first service conception rate and to yield less milk in the first 30 DIM [16] and to increase the odds of metritis, clinical ketosis, lameness and displaced abomasum [17]. Several reports indicate that this product had more likely positive effect to reduce blood BHBA concentration and to improve milk yield and general health status in animals suffering from SCK [18-20]. However, the short and long term impact of combined cyanocobalamin and butaphosphan treatment on reproductive measures parameters, such as uterine involution, ovarian function, days open and pregnancy rate has not been shown. Therefore, the objective of this study was to evaluate the effects of different dosages of combined cyanocobalamin and butaphosphan on BCS, blood BHBA concentration and reproductive parameters.

MATERIAL and METHODS

Ethical Approval

The ethics committee of the Ankara University (report no: 2009-45-214) approved the protocol used in this study.

Animals

This study was performed on 13 dairy farms in Turkey. All Holstein-Friesen cows were kept in loose housing with slatted floors and were milked twice daily. The milk yield was recorded after each milking. The feeding routine and composition in most part of the farms were generally standardized and calculated according to milk yield. The ingredients of ration for cows in lactation (kg/day/cow) were consisted of corn silage alfalfa hay, grass hay, straw, compound feed, barley grain, corn grain, calcium carbonate, salt, dicalcium phosphate, feed grade urea, and vitamin-mineral premix. The mean ME value of rations was 2600 kcal/kg DM. Water was provided *ad libitum*. The mean age in groups of C0, C5 and C10 were 4.83±2.13, 4.71±1.13, 4.82±1.24 respectively. The mean lactation number in groups of C0, C5 and C10 were also 2.5, 2.3 and 2.5, respectively.

Experimental Design

Five hundred and forty cows were screened with a hand-held meter (Precision Xceed, Abbott Diabetes Care®, Abingdon, UK) in whole blood from the coccygeal vein for BHBA concentration within 7-15 d postpartum *(Fig. 1)*. Of these, 53 cows with a blood BHBA concentration between 1.00-3.00 mmol/L were considered to have SCK at any point from d 7 to 15 following calving. They were randomly allocated into one of 3 study groups: group of cows administered intramuscularly with saline (10 mL/100 kg BW; C0, n=18) and with low (5 mL/100 kg/BW; C5, n=18) or high (10 mL/100 kg/BW; C10, n=17) dose of 10% butaphosphan and 0.005% cyanocobalamin combination (Catosal®, Bayer Animal Health, Leverkusen, Germany), every day for 4 days, starting from on d 11.4±2.79, 12.9±2.09 and 12.6±2.30 postpartum (pp) respectively, and it was not different among groups *(Fig. 1)*.

In addition, the concentrations of creatine kinase (CK), glutamate dehydrogenase (GLDH), cholesterol (CHO), aspartate amino transferase (AST) total bilirubin, indirect bilirubin (BID) (ERBA® Mannheim, Germany) and Non-esterified Fatty Acid (WAKO® Diagnostics NEFA-HR2) were measured spechtrophotometrically (ERBA® XL 600). The intra-assay and interassay CV for all the parameters were ≤3.4% and ≤7.8%, respectively. Blood samples were collected just before the first administration of butaphosphan and cyanocobalamin combinationand 10 and 18 d after the last administration. Animals diagnosed with clinical ketosis (BHBA >3 mmol/L) and secondary disease were not included to the study. Milk yield was recorded daily for 30 days. Body condition was evaluated by three person on d0, 10, 18, 30, 45 and 60.

The intervals from calving to first ovulation and from calving to morphologic uterine involution were categorized as d 15-25, 26-30, 31-35, 36-40, and ≥41 days due to weekly examination. The morphologic uterine involution was evaluated by rectal palpation if the uterus was returned to lie within the pelvic cavity [21]. Pregnancy diagnosis was performed twice by ultrasonographic examination 30 and 60 d after insemination. Cows that returned to estrus before the pregnancy check were re-inseminated.

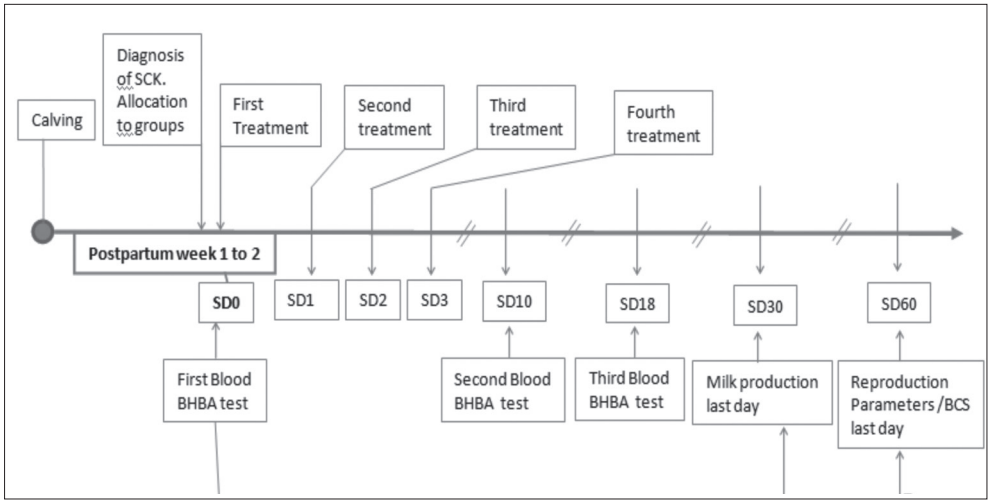

Fig 1. The experimental plan. Conception rate was observed up to 150 d postpartum. **SD:** Study day

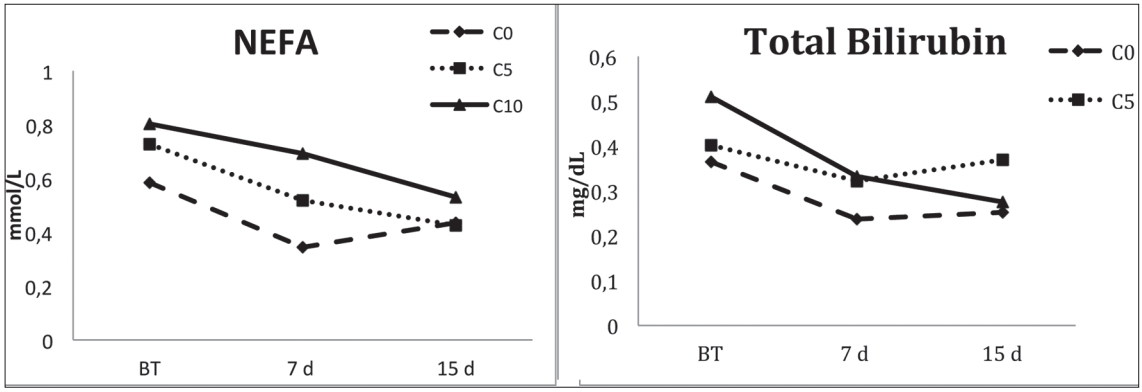

Fig 2. Effect of multiple injections of cyanocobalamin and butaphosphan administered on day 7 and 15 postpartum on the serum NEFA and Total Bilirubin concentrations of dairy cows. C0 = 10 mL/100 kg BW saline; C5 = 5 mL/100 kg/BW butaphosphan-cyanocobalamin combination; C10 = 10 mL/100 kg/BW butaphosphan-cyanocobalamin combination. **BT:** Before treatment, **7d:** Seven days after treatment, **15d:** Fifteen days after treatment **P*=0.002, ** *P*<0.05

The reproductive parameter information was obtained until d 150, which covered intervals from calving to first ovulation, morphologic uterine involution, interval from calving to first insemination, the number of insemination, days open, and pregnancy rate (%, number of animals pregnant/number of inseminated animals).

Statistical Analysis

All data were subjected to the Mean Procedures for descriptive statistics. The non-parametric Wilcoxon-Mann-Whitney-U-Test was used for group comparisons. All tests were performed two-sided with an α value of 0.05. The medical relevance of the differences between groups was quantified using the Mann-Whitney superiority measure (MW). The MW-measure (0.0 to 1.0) reflects the probability that a randomly selected animal of the test group is better off than a randomly selected animal of the control group, while 0.5 denoting equality. If the hole confidence interval(CI) is above the equality line in the graphic, it is considered that superiority is proven ($P<\alpha$). Wilcoxon-Mann-Whitney-U-Test also was applied for BHBA and BCS percent change from baseline without any LVCF-option.All statistical analysis were performed using the validated statistic program TESTIMATE Version 6.5 from IDV Datenanalyse und Versuchsplanung (Germany).

RESULTS

A total of 544 cows were screened for subclinical ketosis between d 7 and 15 postpartum. The SCK prevalence was 9.74% at the BHBA cut-off concentration of 1.0 mmol/L. The median and mean values of age, BW, and lactation numbers for cows with SCK were homogenous across the groups. The blood BHBA concentrations on the study days revealed insignificant alterations, except for decrease in BHBA for cows in C10 as compared to C0 on d 18 *(Table 1)*. The tendency to decrease in BHBA was highest in group C10, but was not different from that in C5 blood BHBA concentration for C0 did not change during the experiment. The percent change in blood BHBA concentration from baseline during sampling on d 10 and 18 for C5 (P=0.04 and P=0.005) and C10 (P=0.006 and P=0.00001) was significant. The serum NEFA and total bilirubin *(Fig. 2)* concentrations were lower in C10 group after treatment than C0 group

Table 1. *The median and mean blood BHBA levels (mmol/L) during experiment*

Groups *	d 0 Mean±SD (Min/Max) Median	d 10 Mean±SD (Min/Max) Median	d 18 Mean±SD (Min/Max) Median
C0 (n=18)	1.25±0.40 (1.0/2.6) 1.05	1.11±0.75 (0.2/3.0) 0.95	1.18±0.88 (0.4/3.0) 0.90
C5 (n=18)	1.76 ±0.74 (1.0/3.0) 1.40	1.07±0.85 (0.1/2.9) 0.75	0.85±0.78 (0.2/2.7) 0.60
C10 (n=17)	2.15±0.62 (1.2/3.0) 2.10	1.01±0.48 (0.2/1.8) 1.00	0.64±0.51 (0.2/2.4) 0.50**

**C0 = 10 mL/100 kg BW saline; C5 = 5 mL/100 kg/BW butaphosphan-cyanocobalamin combination; C10 = 10 mL/100 kg/BW butaphosphan-cyanocobalamin combination, ** P<0.05*

Table 2. *The median and mean blood parameters of liver function during experiment*

Groups *		GLDH (IU/L)			CHO (mg/dl)			AST (IU/L)			BID (mg/dl)		
		d0	d10	d18	d0	d10	d18	d0	d10	d18	d0	d10	d18
C0 (n=18)	Mean±SD (Min/Max) Median	24.19±15.38 (7.46/55.07) 18.60	32.21±19.65 (6.78/74.59) 21.07	22.54±17.86 (6.89/72.87) 18.37	128.53±42.29 (76/237) 131	150.06±39.86 (82/234) 150	166.33±43.38 (92/230) 166	99.81±44.98 (56.9/249.8) 88.5	133.61±185.41 (52.5/847.5) 93.1	85.35±23.66 (57.9/153) 82.7	0.15±0.09 (0.02/0.31) 0.19	0.12±0.06 (0.01/0.26) 0.11	0.14±0.08 (0.03/0.28) 0.14
C5 (n=18)	Mean±SD (Min/Max) Median	35.12±29.53 (5.74/89.20) 16.94	38.68±25.22 (4.71/75.50) 35.43	32.86±27.67 (7.41/89.92) 17.49	137.87±59.94 (63/301) 138	158.81±75.76 (77/385) 137	192.06±70.68 (71/289) 165.50	96.61±35.63 (58.30/198.20) 92.5	99.8±29.94 (55/171.60) 94.8	85.33±22.34 (50.5/132.8) 93.7	0.21±0.15 (0.06/0.47) 0.22	0.18±0.1 (0.02/0.32) 0.21	0.16±0.11 (0.01/0.37) 0.13
C10 (n=17)	Mean±SD (Min/Max) Median	37.95±27.49 (5.57/82.50) 27.39	43.77±28.41 (3.79/83.66) 42.44	49.37±28.75 (5.80/89.40) 52.71	119.36±33.61 (60/175) 117	154.07±65.64 (32/276) 157.50	193.21±64.76 (65/287) 198	102.65±39 (68.7/195.2) 85.5	96.17±33.02 (36.8/156) 101.15	88.1±20.72 (44.8/128.8) 85.55	0.22±0.12 (0.06/0.40) 0.17	0.19±0.09 (0.02/0.32) 0.21	0.18±0.11 (0.06/0.36) 0.17

** C0 = 10 mL/100 kg BW saline; C5 = 5 mL/100 kg/BW butaphosphan-cyanocobalamin combination; C10 = 10 mL/100 kg/BW butaphosphan-cyanocobalamin combination*

Table 3. *The median and mean values of milk production (first 30 d, kg)*

Groups*	Statistics**	Milk Production (kg)
C0 (n=18)	Mean±SD (Min/Max) Median (LQ/UQ)	640.8±201.2 (366/1182) 602.8 (532/720)
C5 (n=18)	Mean±SD (Min/Max) Median (LQ/UQ) W-Mann-Whit-U test (LB/UB)*** *P*<0.0096	863.2±201.2 (284/1313) 845.3 (714/1059.5) 0.75 (0.56/0.93)
C10 (n=17)	Mean±SD (Min /Max) Median (LQ/UQ) W-Mann-Whit-Utest (LB/UB)*** *P*<0.05	779.9±235.0 (302/1228) 811 (635.60/1002.50) 0.69 (0.50/0.87)

** **C0** = 10 mL/100 kg BW saline; **C5** = 5 mL/100 kg/BW butaphosphan-cyanocobalamin combination; **C10** = 10 mL/100 kg/BW butaphosphan-cyanocobalamin combination; ** **LQ** = Low Quarter; **UQ** = Upper Quarter; **LB** = Low Bar; **UB** = Upper Bar; *** **0.29/0.71** = large difference; **036/0.64** = medium sized difference; **0.44/0.56** = small difference; **0.50** = equality*

10 (*P*=0.002), 18 (*P*=0.02), 30 (*P*=0.02), 45 (*P*=0.0004), and 60 (*P*=0.0007) and in C5 on d 45 (*P*=0.01) and 60 (*P*=0.02). Percent change in BCS between C5 and C10 was different only on d 10 (*P*=0.05). The reproductive measures are summarized in *Table 5*. The percentage of uterine location into aperture pelvis at the interval 15-25 days pp was 83% and 88% in group C5 and C10, respectively, as compared to the control group. Morphologic uterine involution was notable at the pointed interval days pp (*P*=0.04 for C5 and *P*=0.007 for C10) in comparison with the control group.

The mean interval from calving to first insemination was lower by 15 days in group C10 than in group C0 (*P*=0.02) and by 7 days than in group C5 (*P*=0.19). There was no difference in the interval from calving to first ovulation among the groups. The mean days open for groups C5

Table 4. *The percent change in BCS relative to the baseline value during experiment*

Groups	SD-0	SD-10	SD-18	SD-30	SD-45	SD-60
C0 (n=18)						
Mean±SD (Min/Max)	3.08±0.31 (2.70/3.50)	-9.65±6.23 (-18.18/0)	-12.07±5.16 (-22.86/-3.57)	-13.83±7.83 (-28.57/0)	-19.12±10.54 (-42.86/-3.57)	-17.54±8.75 (-33.33/-3.57)
Median (LQ-UQ)	3.00 (2.80/3.50)	-10.00 (-14.29/-3.57)	-12.50 (-15.63/-7.41)	-14.29 (-18.18/-10.00)	-19.27 (-25.93/-10.00)	-16.67 (-24.24/-10.00)
C5 (n=18)						
Mean±SD (Min/Max)	3.05±0.52 (2.00/4.00)	-7.73±6.4 (-20/0)	-9.29±11.95 (-28.57/25)	-8.60±11.90 (-28.57/25)	-8.60±11.90 (-28.57/25)	-8.04±13.67 (-28.57/35)
Median (LQ/UQ)	3.00 (2.70/3.50)	-10.00 (-12.50/0)	-12.50 (-16.67 /0)	-12.50 (-16.67 / 0.00)	-12.50 (-16.67/0)	-12.50 (-16.67/0)
Man-Whit-U** (LB/UB)		0.40 (0.22/0.58)	0.45 (0.27/0.63)	0.37 (0.19/0.56)	0.26 (0.08/0.45)	0.28 (0.10/0.47)
P-Values*		0.3288	0.6542	0.2141	0.0155	0.0288
C10 (n=17)						
Mean±SD (Min/Max)	2.96±0.25 (2.50/3.50)	-2.02±6.97 (-16.67/11.11)	-4.20±10.95 (-16.67/20.00)	-4.94±12.59 (-28.57/20.00)	-4.39±12.30 (-28.57/20.00)	-4.39±12.30 (-28.57/20.00)
Median (LQ/UQ)	3.00 (2.80/3.00)	0.00 (-6.25/0)	-7.41 (-10.71/0)	-6.25 (-14.29/0)	-6.25 (-10.00/0)	-6.25 (-10.00/0)
Man-Whit-U** (LB/UB)		0.20 (0.01/0.38)	0.27 (0.08/0.45)	0.27 (0.08/0.46)	0.16 (0.00/0.35)	0.17 (0.00/0.37)
P-Values*		0.0015	0.0212	0.0223	0.0004	0.0007

** P value indicated the comparision with control group; ** Man-Whit-U = Wilcoxon-Mann-Whitney-U Test-Two -sided, **0.29/0.71** = largedifference; **036/0.64**= medium sized difference; **0.44/0.56** = small difference; **0.50** = equality. **LQ** = Low Quarter; **UQ** = Upper Quarter; **Min** = Minimum; **Max** = Maximum; **LB** = Low Bar; **UB** = Upper Bar; **SD** = StudyDay*

(P<0.05). In addition, the serum creatine kinase activity was significantly decreased in C10 group (P=0.047) with no differences between groups. There was no difference in the serum cholesterol, GLDH, AST and BID concentrations between groups *(Table 2)*. Treatment groups produced more milk than control group (*P*=0.01 for C5 and *P*=0.05 for C10; *Table 3*, but milk yield for C5 and C10 was not different (*P*=0.33). *Table 4* summarizes BCS during experiment and changes in BCS as compared to baseline. The cows in group C0 lost more BCS than those in group C10 on d and C10 were about 20 days shorter than that for C0 (*P*=0.07 for C5-C0; *P*=0.11 for C10-C0; and *P*=0.88 for C5-C10). The mean number of insemination and the overall pregnancy rate through 150 days DIM were similar across the groups.

DISCUSSION

The hyperketonemiain early lactation is a potential risk factor for general health status and the resumption of

Table 5. *Reproductive parameters in response to butaphosphan-cyanocobalamin combination administration*

Reproductive Measures	C0 (n=18)	C5 (n=18)	C10 (n=17)*	*P-value***
Interval from calving to first ovulation				
15 to 25 days pp	3/18 (16.67%)	8/18 (44.44%)	3/16 (18.75%)	
26 to 30 days pp	8/18 (44.44%)	4/18 (22.22%)	10/16 (62.50%)	
31 to 35 days pp	2/18 (11.21%)	4/18 (22.22%)	2/16 (12.50%)	
36 to 40 days pp	1/18 (5.56%)	2/18 (11.11%)	1/16 (6.25%)	
>41 dayspp	4/18 (22.22%)	-	-	
Morphologic uterine involution				
15 to 25 days pp	8/18 (44.44%)^{c}	15/18 (83.33%)^{a}	15/17 (88.24%)^{b}	^{a,c}0.04/^{b,c}0.007/1^{a,b}0.632
26 to 30 days pp	6/18 (33.33%)	1/18 (5.56%)	2/17 (11.76%)	
31 to 35 days pp	4/18 (22.22%)	1/18 (5.56%)	-	
36 to 40 days pp	-	1/18 (5.56%)	-	
Interval from calving to first insemination (days)	74.9±20.59^{c}	66.66 ±26.70^{a}	59.75±23.14^{b}	^{a,c}0.075/^{b,c}0.017/^{a,b}0.188
Daysopen (days, mean±SD)	95.00±32.61^{c}	74.16±35.76^{a}	75.17±34.33^{b}	^{a,c}0.066/^{b,c}0.106/^{a,b}0.876
No of insemination (mean±SD)	2.0±1.19^{c}	1.8±1.20^{a}	1.6±0.80^{b}	^{a,c}0.614/^{b,c}0.335/^{a,b}0,955
Overall pregnancy rate, %	64.71^{a}	66.67^{a}	70.59^{a}	^{a}1.000

** One cow did not ovulate; ** Wilcoxon-Mann Whitney-U Test, 2-sided was used; $^{a,b,c:}$ The differences between groups in the same line with different letters is significant (P<0.05)*

reproductive function. The increased NEFA in prepartum, advanced parity, birth of a male calf, calving difficulty, precalving high BCS are important predictors of having hyperketonemia at any time from 3 to 16 DIM [22]. The prevalence of SCK within 10 European countries using a threshold ≥1.2 mmol/L of blood BHBA was 21.8%, ranging from 11.2 to 36.6% between 2 and 15 d in milk in Turkey and Italy and ranged from 8.9% to 34% during postpartum 2 month [23,24]. Significant decrease of blood BHBA in the treatment groups entire study days after drug applications compared to the control group confirmed the efficacy of the treatment on the blood BHBA in ketotic dairy cows. Our findings are consistent with the previous studies focused on the use of combined butaphosphan and cyanocobalamin in hyperketonemic animals at early postpartum period [14,25]. The results of a recent mode of action study [26] revealed that the same drug combination at a dose of 10 mL/100 kg applied IV in nonketotic dairy cows for 3 consecutive days reduced significantly in the liver the mRNA abundance of acyl coenzyme A synthetase long-chain family member 1, involved in fatty acid oxidation and biosynthesis. This can explain that lower fatty acid synthesis in the liver can result in less circulation of BHBA in the blood. However, this may not explain why the milk production in the treatment groups C5 and C10 was higher than in the control group. It can only be speculated that animals received treatments and consequently normalized blood BHBA had better metabolic balance and general health status. Interestingly, why lower dosage of the combination tended to produce more milk in group C5 is unknown. Increased milk production was also reported after butaphosphan and cyanocobalamine application in dairy cows [12,18,26]. The parallel fall of serum concentration of NEFA and total bilirubin in C10 group was showed the evidence of the treatment efficiency. It was expected results because; NEFA and bilirubin are utilizing the common hepatic pathways [9]. Ketones have a glucose-sparing effect to compensate milk production until reaching severe hypoglycemia [27]. There was a negative relationship between elevated BHBA concentrations and decreased milk production during wk 1-2 postpartum and that the loss of milk production at first week was 1.8 kg/day when blood concentration of BHBA was 1.4 mmol/L [6]. When blood concentration of BHBA increased to 2.0 mmol/L, loss of milk production at wk 2 increased to 3.3 kg/day due to decreased DMI. Body condition reflects energy balance in dairy cows [28]. In the present study, groups C10 had lowest BCS lost. The obese cows have sluggish appetite and are predisposed to SCK [29]. The mean duration of uterus involution is reported to 23.7-30.0 d [30,31]. In the present study, morphologic involution of uterus was completed by 83.3 and 88.2% in groups C5 and C10, respectively, by d 15-25 postpartum. These high involution successes in treatment groups could be related to improvement in the smooth muscle function in the uterus, due to supported the calcium-phosphor homeostasis or by the avoidance of hypotonia in response to butaphosphan administration. Hypotonic effect of SCK on uterine muscle could be a risk factor for the endometritis and delayed endometrium restoration resulting from immune suppression. The resumption of ovarian function in postpartum dairy cows depends on the reproductive and metabolic hormones levels as well as metabolite concentration. The failure of ovarian function is the most common pathology associated with the increased blood NEFA and BHBA in postpartum dairy cows [32]. In the present study, time from calving to first ovulation was similar across the groups, but time from calving to first insemination was shorter for 15 d in treated groups than nontreated groups. This could be related to activation of the up regulation of ovarian follicular competition and hypothalamic feedback systems. The earlier resumption of estrus cyclicity in treatment groups could also be

linked to the increased systemic IGF-1 concentration and enhanced hepatic gluconeogenesis in liver [32]. These could positively affect to maintain BCS and reduce blood BHBA concentration. Althougha study [19] explained the possible action of butaphosphan on the metabolism of dairy cows, the direct effect of butaphosphan on phosphorus and calcium homeostasis in dairy cows is still unknown. But, it has been shown that serum, liver, or kidney levels of IGF-1 are not increased during phosphate depletion in normal rats and the reduction in auto phosphorylation reaction of IGF-1 receptor blocks theaction of IGF-1 at the level of cytoplasmic and nuclear in target cell. Therefore, the cross interaction of IGF-1 and phosphate could positively affect to support the calcium homeostasis, improve the uterine muscle function and resume the ovarian function. Unfortunately, we had no data to support this suggestion in dairy cows and the future experimentations are necessary to clarify a possible the interrelationship between IGF-1 and phosphate on phosphorus and calcium homeostasis and on energy metabolism. Although the decreased BCS after calving in dairy cows did not affect the number of insemination, it delayed intervals from calving to first insemination [1,33] and of calving to pregnancy [34] as well as increased risk for uterine disease [35]. The decreased BCS during first 5 wk was associated with prolonged intervals from calving to first insemination and days open through effecting the frequency of LH pulsation and intrafollicular metabolism [36,37]. Improved reproductive parameters in treated groups, thus, could be related to less BCS as compared to control group. Cows with SCK have poor reproductive performance [38-41]. In cows administered with butaphosphan-cyanocobalamin, days open was 20 d shorter than cows administered with saline. The overall pregnancy rate at 150 DIM was about 7% higher in treatment groups than control group. The faster uterine involution could reduce veterinary cost and increase health status through decreasing time to first insemination and pregnancy rate.

In conclusion, injection (IM) of 10% butaphosphan and 0.005% cyanocobalamin combination at the level of 10 mL/100 kg BW everyday for 4 days starting from DIM 7-15 decreased severity of hyperketonemia, which were accompanied by less BCS lost and more milk production. This administration also stimulated uterine involution and shortened time to first insemination/days open as well as increased pregnancy rate. Further studies coping with more metabolic parameters would help explain the action mode of butaphosphan-cyanocobalamin combination.

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