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Effect of Calcium Propionate Administration in First Week Postpartum of Dairy Cows on Subclinical Ketosi

Çağdaş KARA[1], Abdülkadir ORMAN[2], Hıdır GENÇOĞLU[1], H. Melih YAVUZ[1]

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Abstract: This study was conducted to evaluate the effect of calcium propionate administrations on serum ß-hydroxybutyrate (BHBA) concentration and subclinical ketosis occurrence in dairy cows. Twenty four multiparous Holstein cows were sorted by parity, body condition score and dry matter intake in close-up period, average milk production of previous lactation and season of calving and assigned to one of the three groups. Each group consisted of eight cows. The cows in group 1 (G1) received two drenches at calving and 24h after calving. The cows in group 2 (G2) received three drenches at calving, 24h after calving and 7 days after calving. The cows in group 3 (G3) were the control (no drench). Each drench contained 0.68kg of calcium propionate. There were no statistical differences for serum BHBA concentrations at all times among groups ($P>0.05$), except for the difference at calving between G1 and G3 ($P<0.05$). Two drenches of calcium propionate tended to decrease serum BHBA concentration and the incidence of subclinical ketosis during first 4 weeks of lactation.

Key Words: Calcium Propionate, Serum BHBA, Subclinical Ketosis, Dairy Cow.

Süt Sığırlarının Doğumdan Sonraki İlk Haftalarında Kalsiyum Propiyonat Uygulamasının Subklinik Ketozis Üzerine Etkisi

Özet: Bu çalışma süt sığırlarında kalsiyum propiyonat uygulamasının serum BHBA konsantrasyonu ve subklinik ketozis oluşumu üzerine etkisini değerlendirmek için yapılmıştır. Yirmi dört baş Holstein Irkı süt sığırı buzağılama sayısı, yakın kuru dönemdeki vücut kondisyon skoru ve kuru madde tüketimi, önceki laktasyondaki ortalama süt verimi ve buzağılama mevsimi göz önünde bulundurularak sınıflandırılmış ve birbirine benzer üç grup oluşturulmuştur. Her bir grup sekiz hayvandan meydana gelmiştir. Grup 1 (G1)'de yer alan sığırlar buzağılama zamanı ve buzağılamadan 24 saat sonra olmak üzere iki kalsiyum propiyonat uygulaması almıştır. Grup 2 (G2)'de yer alan sığırlar buzağılama zamanı, buzağılamadan 24 saat sonra ve buzağılamadan 7 gün sonra olmak üzere üç kalsiyum propiyonat uygulaması almıştır. Grup 3 (G3)'te yer alan sığırlar hiç kalsiyum propiyonat uygulaması almamıştır. Her bir uygulama 0.68kg kalsiyum propiyonat içermiştir. G1 ve G3'ün buzağılama zamanı serum BHBA konsantrasyonları arasındaki fark ($P<0.05$) hariç, tüm zamanlarda gruplar arasında serum BHBA konsantrasyonları bakımından istatistiksel bir fark bulunamamıştır ($P>0.05$). Kalsiyum propiyonatın iki uygulamasının laktasyonun ilk dört haftası boyunca serum BHBA konsantrasyonunu ve subklinik ketozisin görülme sıklığını azaltma eğiliminde olduğu görülmüştür.

Anahtar Kelimeler: Kalsiyum Propiyonat, Serum BHBA, Subklinik Ketozis, Süt Sığırı.

[1] University of Uludag, Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Diseases, 16059 Gorukle, Bursa, Turkey, cagdaskara@uludag.edu.tr

[2] University of Uludag, Faculty of Veterinary Medicine, Department of Zootechnics, 16059 Gorukle, Bursa, Turkey.

Introduction

The transition period, from 3 weeks before to 3 weeks after parturition, is critically important to health, production, and profitability of dairy cows[4]. It is characterized by tremendous metabolic and endocrine adjustments that the cows must experience from late gestation to early lactation[3,5]. Most health disorders such as ketosis, milk fever, retained fetal membranes, metritis and displaced abomasum occur during this time[4].

Most dairy cows in early lactation go through a period of negative energy balance, due to a metabolic priority for milk production and an increase in body fat mobilization[2]. Negative energy balance and excessive body fat mobilization cause ketosis[8]. Ketosis is defined as a metabolic disease characterized by high levels of ketone bodies in blood, milk and urine. The ketone bodies, acetone, acetoacetate, and ß-hydroxybutyrate (BHBA), are formed in the liver during oxidation of fatty acids (nonesterified fatty acids-NEFA). Ketosis affects dairy cows in the period from parturition to 6 weeks postpartum[14,16].

Ketosis may be clinical or subclinical. Subclinical ketosis is defined as a preclinical stage characterized by elevated blood ketone body concentrations without clinical signs such as loss of appetite, hard feces, or dullness[6,14]. Subclinical ketosis can cause economic losses through decreased milk production, impaired reproductive performance, increased risk of displaced abomasums, and higher risk of clinical ketosis[11].

The gold standard diagnostic test for subclinical ketosis is the measurement of BHBA in serum or plasma because its stability[6,9]. Serum BHBA measurement is useful for examining individual cows and evaluating herd health and monitoring feeding management practices[11].

Ruminal propionate is the single most important substrate for gluconeogenesis[5]. However, depressed dry matter intake at parturition is likely limiting ruminal propionate supply to the liver[3]. Nutritionally, propionate can be supplied orally in the form of sodium propionate or calcium propionate. Calcium propionate has been studied as a calcium-energy substrate for dairy cows[13]. Calcium propionate is a gluconeogenic precursor as well as a source of calcium[7] and it can be used in prevention and / or treatment of ketosis[10,12,14]. Calcium propionate was administered to dairy cows as two oral drenches at calving (680 g) and again 24h after calving (680 g)[19] for preventing and / or treating subclinical ketosis. However, effects of three oral drenches on subclinical ketosis were not studied. The objective of this study was to evaluate the effect of calcium propionate administrations as two (at calving and 24h after calving) or three oral drenches (at calving, 24h after calving and seven days after calving) on serum BHBA concentration and subclinical ketosis occurrence in dairy cows.

Materials and Methods

Cows and Management

This study was carried out at a commercial dairy farm in Bursa, Turkey. Twenty four Holstein cows, with calvings between July 10 and October 16, 2007, which were entering their second or greater lactation, were sorted by parity, body condition score and dry matter intake in close-up period, average milk production of previous lactation and season of calving and assigned to one of the three groups in the study. Each group consisted of eight cows.

The cows were fed the dry diet described in Table 1 during dry period. After calving, the cows were moved into the lactation pen and fed the lactation diet described in Table 1. Diets were formulated for a 600kg cow consuming 10.5kg and 19.3kg of dry matter per day for dry and lactation period, respectively, and producing 29kg of milk per day containing 3.5% fat according to recommendations by NRC[15]. Rations were delivered as total mixed ration.

Experimental Design

Group 1 (G1): The cows received two drenches of calcium propionate; the first drench at calving and the second at 24 h after calving.

Group 2 (G2): The cows received three drenches of calcium propionate; the first drench at calving, the second at 24h after calving and the third at 7 days after calving.

Group 3 (G3-control): The cows did not receive calcium propionate.

Calcium propionate (Lunapik®, Luna Chemical Materials Firm, İstanbul/TURKEY) used in this study was a white crystalline powder and calcite form containing 68% propionate. Each drench contained 0.68kg of calcium propionate. All drenches were delivered into the esophagus via an esophageal feeder tube.

Table 1. Ingredients and chemical compositions of dry and lactation diets

Tablo 1. Kuru dönem ve laktasyon dönemi rasyonlarının bileşimi ve besin maddesi içeriği

Dry Diet	
Ingredient	% Dry matter
Wheat straw	58.02
Commercial concentrate mixture[1]	41.98
Chemical composition	% Dry matter
Neutral detergent fibre	60.20
Acid detergent fibre	35.30
Crude protein	11.61
Ether extract	3.05
Ash	7.80
Non-fibre carbohydrates	17.34
Calcium	0.64
Phosphorus	0.38
Lactation Diet	
Ingredient	% Dry matter
Wheat straw	2.44
Oats hay	24.38
Alfalfa hay	15.70
Commercial concentrate mixture[2]	56.70
Sodium bicarbonate	0.78
Chemical composition	% Dry matter
Neutral detergent fibre	43.60
Acid detergent fibre	24.41
Crude protein	16.08
Ether extract	4.81
Ash	8.00
Non-fibre carbohydrates	27.51
Calcium	0.90
Phosphorus	0.65

[1]Dry Period Concentrate Mixture, 43.27 % barley, 22.03 % wheat, 10.42 % soybean meal, 22.38 % sunflower meal, 1.58 % lime stone, 0.10 % vitamin-mineral premix, 0.22 % salt on dry matter basis

[2] Lactation Period Concentrate Mixture, 27.48 % barley, 33.64 % wheat, 22.94 % soybean meal, 13.48 % sunflower meal, 2.02 % lime stone, 0.10 % vitamin-mineral premix, 0.34 % salt on dry matter basis

Sampling and Chemical Analyses

Blood samples were collected from the jugular vein in 10ml volume serum separator tubes at calving (prior to first drench), 4h after first drench, 4h after second drench, 4h after third drench, day 10 postpartum and week 4 postpartum. The samples were centrifuged at ambient temperature for 10 min at 3000 x g (NF 615, Nüve Industrial Materials Firm, Ankara/TURKEY). Serum samples were stored in a freezer at -20°C for BHBA analysis. BHBA concentrations were measured with STAT-Site meter (GDS Diagnostic, 25235 Leer Drive Elkhart, 46514, IN) by using KetoSite diagnostic kit (Stanbio Laboratory Boerne, Texas, 78006, USA).

Subclinical ketosis starts at serum BHBA concentration $\geq$ 1.0 mmol/l[6,21,22]. In the present study, we used the BHBA cut-off value of 1.0 mmol/l as an indication of subclinical ketosis and recorded cows with subclinical ketosis.

The chemical analyses (crude protein, ether extract, ash, calcium, phosphorus) of diets were performed according to AOAC[1], NDF and ADF analyses were performed according to Van Soest et al.[20].

Statistical Analysis

Serum BHBA concentration was analyzed by analysis of variance using the Kruskal-Wallis procedure and the significance controls of the differences among the groups were determined by Mann-Whitney U test. SPSS 13[18] computer program package was used for the statistical analyses. Significance was accepted at $P<0.05$.

Results

Serum BHBA concentrations at calving (prior to first drench) were similar between G1 and G2 ($P>0.05$) but G3 had a lower concentration of serum BHBA than G1 at same time ($P<0.05$) (Table 2). At calving, there was no statistical difference for serum BHBA concentrations between G2 and G3 ($P>0.05$) (Table 2). There were no statistical differences for serum BHBA concentrations at 4h after first, second and third drenches, day 10 and week 4 postpartum among groups ($P>0.05$) (Table 2).

There were 2 cows with subclinical ketosis in each group at calving (Table 3). At week 4 postpartum, while 3 of 8 cows were subclinical ketosis in G3, there was 1 cow with subclinical ketosis in both G1 and G2 (Table 3).

Discussion

Propionate is produced in the rumen after fermentation of starch, fiber, and protein[14]. Propionate is the major substrate used for gluconeogenesis[14] and suppresses NEFA mobilization[4]. Propionate is antiketogenic in the liver[4]. Depressed feed intake at parturition is likely limiting ruminal propionate supply to the liver[3]. Feeding propionate decreases serum BHBA concentration[7,17]. Serum BHBA concentration is a direct indicator in diagnosis of subclinical ketosis[6,9]. We investigated whether calcium propionate administered as a source of propio-

nate in first week postpartum was affected on serum BHBA concentration. At week 4 postpartum compared with calving time (prior to first drench), while serum BHBA concentrations for G1 (two drenches) and G2 (three drenches) were decreased, serum BHBA concentration for G3 (no drench-control) was slightly increased. In addition, while G1 and G2 had higher serum BHBA concentrations compared with G3 at calving (prior to first drench), those had lower serum BHBA concentrations than G3 at week 4 postpartum. However, There were no statistical differences for serum BHBA concentrations at all times among groups (P>0.05), except for the difference at calving between G1 and G3 (P<0.05) (Table 2).

Table 2. Comparison of serum BHBA concentrations (mmol/l) among groups

Tablo 2. Serum BHBA konsantrasyonlarının (mmol/l) gruplar arası karşılaştırılması

	G1[1] $\bar{X} \pm S\bar{X}$	G2[2] $\bar{X} \pm S\bar{X}$	G3[3] $\bar{X} \pm S\bar{X}$	P
Calving (prior to first drench)	$0.97^{a} \pm 0.46$	$0.93^{ab} \pm 0.48$	$0.84^{b} \pm 0.21$	0.05
4 h after first drench	0.93 ± 0.45	0.82 ± 0.34	0.84 ± 0.25	NS
4 h after second drench	0.74 ± 0.26	0.67 ± 0.22	0.72 ± 0.24	NS
4 h after third drench	0.68 ± 0.20	0.63 ± 0.21	0.78 ± 0.24	NS
Day 10 postpartum	0.65 ± 0.16	0.60 ± 0.16	0.78 ± 0.23	NS
Week 4 postpartum	0.76 ± 0.16	0.68 ± 0.17	0.89 ± 0.31	NS

a-b: indicates significant differences in the same row among treatments (P<0.05)
NS: not significant (P>0.05)
[1]Drenches were administered at calving and 24h after calving.
[2]Drenches were administered at calving, 24h after calving and day 7 after calving.
[3]The cows did not receive calcium propionate.

Table 3. Subclinical ketosis occurrences

Tablo 3. Subklinik ketozis vakaları (adet)

	G1[1]	G2[2]	G3[3]
Calving (prior to first drench)	2	2	2
4 h after first drench	3	1	3
4 h after second drench	2	1	2
4 h after third drench	-	1	2
Day 10 postpartum	-	-	2
Week 4 postpartum	1	1	3

[1]Drenches were administered at calving and 24h after calving.
[2]Drenches were administered at calving, 24h after calving and day 7 after calving.
[3]The cows did not receive calcium propionate.

Stokes and Goff[19] reported that two drenches of calcium propionate at calving and 24h after calving (0.68kg per drench) were not affected on serum BHBA concentration in Holstein cows. The result of our study was in agreement with Stokes and Goff[19]. Calcium propionate was administered orally to dairy cows as two doses at calving (0.342kg) and 12h after calving (0.342kg) by Goff et al.[7]. In addition, Melendez et al.[13] administered two drenches of calcium propionate (0.51kg) plus propylene glycol (0.40kg) to dairy cows within 12h after calving and at 24h after calving. Calcium propionate administered by above-mentioned different amount, time and blend did not also affect serum BHBA concentration in dairy cows.

We observed that there was no difference in respect of decreasing serum BHBA concentration between two and three drenches of calcium propionate and two drenches tended to decrease serum BHBA concentration with time (Table 2).

Subclinical ketosis (serum BHBA concentration ≥ 1.0 mmol/l) was diagnosed by measuring individual serum BHBA concentration periodically during the study. The number of cows with subclinical ketosis (2 of 8 cows) was same in G1 (two drenches), G2 (three drenches) and G3 (no drench-control) at calving (prior to first drench). However, at week 4 postpartum compared with calving time (prior to first drench), the incidences of subclinical ketosis were decreased in the groups receiving either two or three drenches of calcium propionate (G1; 1 of 8 cows and G2; 1 of 8 cows) while the incidence of subclinical ketosis for control group (G3) was increased (3 of 8 cows) (Table 3). We observed that there was no difference in respect of decreasing the incidence of subclinical ketosis between two and three drenches (Table 3).

The study of Hernández et al.[10] demonstrated that oral treatment with 0.70kg of calcium propionate within 2h after calving decreased the incidence of subclinical ketosis in first 3 weeks of lactation. The result of Hernández et al.[10] showed that 0.70kg of calcium propionate as one drench was enough to decrease the incidence of subclinical ketosis. Similar to our study, Stokes and Goff[19] reported that calcium propionate administration (two drenches; 0.68kg per drench) decreased the incidence of subclinical ketosis despite of the fact that it was not markedly affected on serum BHBA concentration.

Conclusion

Our results showed that two drenches of calcium propionate tended to decrease serum BHBA concentration and the incidence of subclinical ketosis during first 4 weeks of lactation. These findings will be useful to help further explore the frequency, timing, and amount of calcium propionate administration to dairy cows. More studies must be conducted to evaluate effects of three drenches of calcium propionate on blood metabolites and subclinical ketosis.

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