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Serum Vitamin B_{12} and Phosphorus Concentrations in Hyperketonemic Postpartum Dairy Cows

Ömer DENİZ [1, a]
Kenan Çağrı TÜMER [1, b]

[1] Kastamonu University, Faculty of Veterinary Medicine, Department of Internal Medicine, Kastamonu, TÜRKİYE

[a] ORCİD: 0000-0002-2981-2032
[b] ORCİD: 0000-0002-2861-0236

Ketosis is a common and significant metabolic disorder in dairy cows during the postpartum period. In affected animals, commercial preparations containing vitamin B_{12} and phosphorus are frequently used as part of treatment protocols. However, there is limited research regarding serum vitamin B_{12} and phosphorus concentrations in hyperketonemic cows. This study aimed to evaluate the concentrations of phosphorus and vitamin B_{12} in the serum of hyperketonemic dairy cows. Blood samples were collected from cows on multiple farms in the Kastamonu district. A total of 25 Holstein cows were utilized in the study, 12 of which exhibited normal serum BHBA levels (H) and 13 of which presented with hyperketonemia (HK). Blood samples were taken within 21 days postpartum and between 4–6 hours after morning or evening feeding. Serum levels of vitamin B_{12} and phosphorus were then measured. No statistically significant differences were found between the HK and control groups for serum Vit B_{12} (p=0.327) or phosphorus (p=0.314) concentrations. These findings suggest that despite their altered metabolic state, B_{12} and phosphorus levels in ketotic cows appear to be independent of serum BHBA concentrations.

Key Words: *Metabolic disorders, negative energy balance, postpartum period*

Hiperketonemik Postpartum Süt İneklerinde Serum Vitamin B_{12} ve Fosfor Konsantrasyonları

Ketozis, postpartum dönemindeki süt ineklerinde sık görülen önemli bir metabolik hastalıktır. Etkilenen sığırlara tedavide vitamin B_{12} ve fosfor içeren ticari preparatlar yaygın bir şekilde kullanılmaktadır. Ancak hiperketonemik sığırlarda serum vitamin B_{12} ve fosfor konsantrasyonları ile ilgili sınırlı sayıda çalışma bulunmaktadır. Bu çalışmada, hiperketonemik süt ineklerinde serum vitamin B_{12} ve fosfor konsantrasyonlarının değerlendirilmesi amaçlanmıştır. Çalışma, Kastamonu bölgesindeki farklı çiftliklerde yetiştirilen, serum BHBA konsantrasyonlarına göre sınıflandırılmış 13 hiperketonemik (HK) ve 12 sağlıklı (H) olmak üzere toplam 25 Holstein inek üzerinde gerçekleştirilmiştir. Tüm ineklerden kan örnekleri, doğum sonrası 21 gün içerisinde ve sabah veya akşam yemlemesinden 4-6 saat sonra toplanmıştır. Elde edilen serum örneklerinden vitamin B_{12} ve fosfor konsantrasyonları ölçülmüştür. Serum Vit B_{12} (p=0.327) ve serum P (p=0.314) konsantrasyonlarında HK ve K grupları arasında istatistiksel olarak anlamlı bir fark bulunmamıştır. Bu bulgular, ketozisli ineklerde B_{12} vitamini ve fosfor seviyelerinin, metabolik durumlarına rağmen BHBA düzeyinden bağımsız olduğunu göstermektedir.

Anahtar Kelimeler: *Metabolik bozukluklar, negatif enerji dengesi, postpartum dönemi*

Introduction

Ketosis is a prevalent metabolic disorder in dairy cows, particularly during the postpartum period from late gestation to early lactation. It is marked by increased β-hydroxybutyrate (BHBA) concentrations in the blood and rises from a negative energy balance (NEB) caused by inadequate energy consumption and increased energy demands during late gestation and the early lactation (1, 2).

The postpartum period entails heightened metabolic stress and elevates the likelihood of both subclinical and severe ketosis. Subclinical ketosis (BHBA ≥1.2 mmol/L) typically presents no overt symptoms; however, it adversely impacts milk production and reproductive performance (3, 4). Clinical ketosis is characterized by a BHBA level exceeding 3.0 mmol/L and is associated with anorexia, weight loss, and diminished performance (5). Effective management necessitates the surveillance of essential metabolic indicators, including BHBA, non-esterified fatty acids (NEFA), glucose, and vital minerals such as vitamin B_{12} (vitB_{12}) and phosphorus (6, 7). Vitamin B_{12}, produced by rumen bacteria in the presence of cobalt, is crucial for energy metabolism via glucose production and fatty acid oxidation. A deficiency in this vitamin may aggravate ketosis by hindering hepatic metabolism (8).

Though much is unknown, the interactions between phosphorus, vitB_{12}, and BHBA in postpartum cattle are significant. Even if a deficiency of phosphorus or vitB_{12} might aggravate energy metabolism issues, limit nutrient absorption. Feeding management that directly targets these interactions might reduce the frequency of ketosis and increase production performance (9, 10). This study aims to investigate the serum vitB_{12} and phosphorus concentrations in postpartum hyperketonemic dairy cows.

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Correspondence

Ömer DENİZ
Kastamonu University, Faculty of Veterinary Medicine, Department of Internal Medicine, Kastamonu – TÜRKİYE

odeniz@kastamonu.edu.tr

Materials and Methods

Research and Publication Ethics: All methods involving cattle in this study received approval from the Kastamonu University Institutional Animal Care and Use Committee, protocol 22-2025.

Samples From the Case Group and Control Group: Serum samples for this research were collected as part of an earlier study carried out on farms in Kastamonu region between October 2023 and December 2024.

All the sampled cattle were in the postpartum period. The median postpartum time for sampled cattle was 10 days (range= 1 to 14 days). Blood samples were colleceted from the coccygeal vein using vacuum tubes and incubated for a maximum of one hour at room temperature. After the incubation period, blood samples were centrifuged at 1000 × g for 10 minutes. The resulting serum was then transferred into eppendorf tubes and stored at –20°C until analysis. Serum vitB_{12} concentrations (pg/mL) were determined using an automated immunoassay analyzer (ADVIA Centaur XPT Immunoassay System, Siemens, Germany). Serum β-hydroxybutyrate (BHBA) and phosphorus levels were measured using a chemistry analyzer (Respons® 910Vet, DiaSys Diagnostic Systems GmbH, Holzheim, Germany).

Statistical Analysis: Statistical analyses of the obtained data were performed using the statistical software MedCalc (Ostend, Belgium). The normality assumption of the measured parameters was assessed using the Shapiro-Wilk test. Changes in serum BHBA, vitB_{12} and phosphorus concentrations between groups were analyzed using the Mann Whitney U test. Graphs were created using GraphPad software. A *p*-value of <0.05 was considered statistically significant.

Results

The study analyzed blood levels of BHBA, vitamin B_{12}, and phosphorus in samples from 13 hyperketonemic cows and 12 healthy cows (Table 1).

Biochemical analysis revealed clear metabolic distinctions between the hyperketonemic cows group and control groups. In the hyperketonemic cows group, blood BHBA levels were significantly higher, averaging 5.30±1.33 mmol/L, compared to 0.45±0.24 mmol/L in the control group (p=0.001). When looking at vitamin B_{12} concentrations the hyperketonemic cows group showed a noticeable elevation (355.6±235.9 pg/mL) relative to the healthy group (218.3±76.26 pg/mL). Vitamin B_{12} levels were higher in the hyperketonemic group, but this difference was not statistically significant (p=0.327), suggesting that any potential difference could not be confirmed with the available data. Phosphorus levels, while not statistically different (p=0.314), tended to be lower in the hyperketonemic cows group (5.46±1.81 mg/dL) than in controls (6.00±1.81 mg/dL) (Figure 1).

Tablo 1. Includes data on β-hydroxybutyrate (BHBA, mmol/L), Vitamin B_{12} (pg/mL), and phosphorus (mg/dL) concentrations in the hyperketonemic cows and control groups.

Parametres	Hyperketonemia (n=13)	Control (n=12)	*p-value*
BHBA (mmol/L)	5.30±1.33 (4.49-6.10) [a]	0.45±0.24 (0.30-0.61) [b]	0.001
Vit B_{12} (pg/mL)	355.6±235.9 (213.1-498.2) [a]	218.3±76.26 (169.8-266.7) [a]	0.327
Phosphorus (mg/dL)	5.46±1.81 (4.36-6.56) [a]	6.00±1.81 (4.36-6.56) [a]	0.314

Values are expressed as mean ± standard deviation (SD) and 95% confidence interval (CI) for each group. A statistically significant difference exists between groups denoted by different letters in the same row (p<0.05). There is no substantial distinction between groups denoted by the same letter. The *p* values at the table's conclusion denote the statistical significance of the differences across groups.

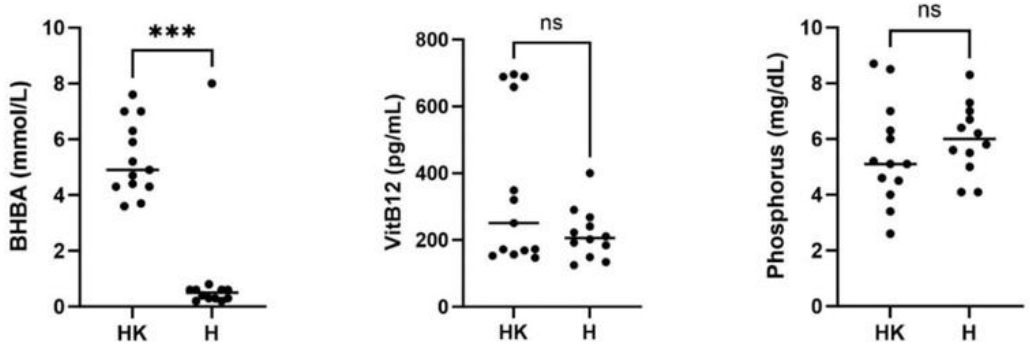

Figure 1. Blood concentrations of beta-hydroxybutyrate (BHBA), vitamin B_{12}, and phosphorus in hyperketonemic (HK) and healthy cows (H). BHBA levels were significantly higher in the hyperketonemic group (p=0.001), confirming ketosis. Vitamin B_{12} levels were also higher in this group, but the difference was not statistically significant (p=0.327). Phosphorus levels showed a non-significant decrease in hyperketonemic cows (p=0.314), indicating a possible trend toward mineral imbalance.

Discussion

In this study, serum vitB_{12} and phosphorus concentrations were investigated in hyperketonemic postpartum dairy cows. Our results indicated that hyperketonemic cows had similar serum vitB_{12} and phosphorus concentrations compared to healthy cows.

The diagnosis of ketosis is mostly based on the measurement of blood BHBA concentrations, as this parameter has substantial correlations with vitamin and mineral metabolism. The observed sixfold rise in BHBA is in aggreement with other studies of metabolic abnormalities associated with clinical ketosis (11, 12). BHBA values of ≥1,400 µmol/L within the first two weeks postpartum are strongly indicative of clinical ketosis onset. Our analysis revealed that the average BHBA level in the hyperketonemic group was 5.30 mmol/L (5.300 µmol/L), indicating a severe state of ketosis. This result corresponds with the established threshold values and the findings that BHBA levels over 0.54 mmol/L on postpartum day 10 signify hyperketonemia (13, 14).

The significant increase in BHBA concentration may be due to variations in vitamin B_{12} levels. A recent study suggests that the metabolic state may have an impact on the bioavailability of vitamins generated by rumen bacteria (15). Even if this trend did not reach statistical significance, it is crucial to take into account the potential biological significance of the reported rise in vitamin B_{12} levels in hyperketonemic cows. Previous research on the relationship between vitamin B_{12} and hyperketonemia has produced contradictory findings. Duplessis et al., (16) found no association between plasma or milk vitamin B_{12} concentrations and hyperketonemia (defined as BHBA ≥1.2 mmol/L), while Korpela and Mykkänen (17) found that the serum vitamin B_{12} levels of ketotic cows were significantly lower than those of healthy cows (268 ± 64 pg/mL vs. 362±154 pg/mL; p<0.02). Similarly, Corse and Elliot (18) found that the vitamin B_{12} levels were lower in cows with spontaneous ketosis in late pregnancy. However, Obitz and Fürll (19) found that the postpartum vitamin B_{12} levels of the affected mice dropped less precipitously. These contradictory results suggest that vitamin B_{12} dynamics in ketotic states are complex and may be influenced by several factors, including the level of metabolic imbalance and the lactation stage. Therefore, even in the lack of statistical significance, the increasing trend in our study might suggest an early shift in vitamin B_{12} metabolism in response to metabolic stress or a compensatory physiological response. This finding is supported by a prior study demonstrating that increased plasma free fatty acid (FFA) levels in cows were associated with elevated vitamin B_{12} concentrations in both milk and plasma (3). This study indicates that lipolysis, occurring during negative energy balance, may indirectly influence B_{12} metabolism by altering the rumen microbiota or the kinetics of vitamin absorption. However, as serum FFA concentrations were not evaluated in our study, it is hard to determine whether the observed increase in vitamin B_{12} levels may be ascribed to such causes. Thus, our interpretation relies solely on previously published data, lacking corroborative metabolic evidence from our own research. The absence of FFA data is a considerable constraint of the present investigation, and we advocate for future research to include the evaluation of serum FFA levels to clarify this possible biological connection. Similar to our results increased serum vitB_{12} concentrations were reported in hyperketonemic goats and cattle with tropical theileriosis (20- 22).

Blood BHBA levels primarily dictate the diagnosis of ketosis, since this test exhibits substantial relationships with mineral and vitamin metabolism. Current reports indicate that butafosfan and cyanocobalamin (B_{12}) injections may reduce BHBA levels and lower the occurrence of subclinical ketosis, particularly in cows with three or more lactations (23). As no supporting medication was administered in our investigation, it is believed that the elevation in vitamin B_{12} levels may only pertain to metabolic reaction.

The evaluation of phosphorus should be considered in relation to the increase in BHBA. Despite elevated BHBA levels, no statistically significant difference in phosphorus concentrations was seen between the groups (control: 6.01 mg/dL; hyperketonemic: 5.46 mg/dL). The lack of significance may be partly due to individual variability; nonetheless, the observed decline in the hyperketonemic group, despite its statistical insignificance, may still hold biological relevance. Djoković et al., (24) suggest that ketosis may affect mineral metabolism, potentially altering phosphorus absorption and mobilization. Our findings correspond with those of Zhang et al., (25), which demonstrated that phosphorus homeostasis typically remains constant during the early stages of ketosis. This suggests that compensatory mechanisms such as adequate dietary phosphorus consumption or physiological regulation may help maintain serum phosphorus levels within normal limits, especially during the early stages of metabolic stress. Therefore, while the fluctuations in phosphorus levels lacked statistical significance, the observed pattern may suggest subtle metabolic adaptations that require more investigation.

As a conclusion, the results of this study showed that hyperketonemia induce negligible changes in serum vitB_{12} concentrations and it has limited interaction with serum phosphorus status.

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