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Investigation of Ketosis Prevalence in Dairy Cows in Bingöl Province of Turkey

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ABSTRACT

The aim of this study is to investigate the prevalence of ketosis in 7 different villages (Alibir, Ardıçtepe, Çavuşlar, Garip, Sudüğünü, Yamaç, and Yeşil) of Bingöl province. A total of 411 dairy cows, including Simmental, Holstein and crossbred breeds, were used in the study during the 3-week period after birth. Ketosis was diagnosed by measuring blood beta-hydroxy-butyric acid (BHBA) concentration. Blood BHBA concentrations were analyzed using the FreeStyle Optium Neo H (Abbott Diabetes Care Ltd., Witney, UK). Blood BHBA ≥2.6 mmol/L was determined as clinical ketosis (CK), BHBA≥1.2 mmol/L as subclinical ketosis (SCK), and BHBA<1.2 mmol/L as healthy (H). In a total of 411 dairy cows, 76 were found to be SCK (17.88%), 16 were CK (7.06%), and 319 were H (75.1%). Determining management and feeding strategies that can reduce the prevalence of ketosis is important for the region. In future studies, it would be beneficial to study a larger population and examine different variables such as secondary diseases, milk yield and racial differences together with ketosis.

Türkiye'nin Bingöl İlindeki Süt İneklerinde Ketozis Prevalansının Araştırılması

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ÖZ

Bu çalışmanın amacı Bingöl ilinin 7 farklı köyünde (Alibir, Ardıçtepe, Çavuşlar, Garip, Sudüğünü, Yamaç ve Yeşil) ketozis prevalansını araştırmaktır. Araştırmada doğumdan sonraki 3 haftalık süreçte Simmental, Holstein ve melez ırklardan oluşan toplam 411süt ineği kullanıldı. Ketoz tanısı kandaki beta-hidroksi-bütirik asit (BHBA) konsantrasyonunun ölçülmesiyle konuldu. Kan BHBA konsantrasyonları FreeStyle Optium Neo H (Abbott Diabetes Care Ltd., Witney, UK) kullanılarak analiz edildi. Kanda BHBA≥2,6 mmol/L klinik ketozis (KK), BHBA≥1,2 mmol/L olması subklinik ketozis (SKK) ve BHBA<1,2 mmol/L olması ise sağlıklı (S) olarak belirlendi. Toplam 411 süt ineğinin 76'sının (%17,88) SKK, 16'sının (%7,06) KK, 319'unun (%75,1) S olduğu belirlendi. Ketozis prevalansını azaltabilecek yönetim ve besleme stratejilerinin belirlenmesi bölge için önemlidir. Gelecek çalışmalarda daha geniş bir popülasyonda çalışılarak ikincil hastalıklar, süt verimi, ırk farklılıkları gibi farklı değişkenlerin ketozis ile birlikte incelenmesi yararlı olacaktır.

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INTRODUCTION

The transition period in dairy cows is defined as 3 weeks before calving and 3 weeks after calving. A number of important changes occur in cows during this period in terms of physiological, metabolic, immunological, and reproductive aspects (1). Cows in the transition period are prone to metabolic disorders due to the reasons described (2). These metabolic diseases, which are commonly seen in dairy cows, include ketosis, hypocalcemia, fatty liver syndrome, mastitis, metritis, laminitis, and retention secundinarum (1). Ketosis is one of the most important metabolic diseases encountered by cows in transition. The most important reason underlying the formation of this disease is the development of negative energy balance (NED) as a result of not meeting the animal's energy needs (3, 4). Depending on NED, the process of ketone formation called ketogenesis begins in the body. In this process, a significant portion of the free fatty acids that undergo oxidation in the liver turn into ketone bodies (acetone, acetoacetic acid, and beta-hydroxybutyric acid (BHBA)) (5, 6). Ketosis occurs in two forms: subclinical ketosis (SCK) and clinical ketosis (CK). The SCK form is defined as an increase in circulating ketone bodies without clinical symptoms of the disease (4, 7). However, even if no clinical signs of the disease are noticed, it has a negative effect on metabolism, reproduction and milk production (1). In the initial phase of CK, clinical symptoms such as loss of appetite, decrease in milk yield and live weight, pica, reluctant movement, and hard, dry, mucus-covered stools are observed, while in the final stage of the disease, various neurological findings such as seizures, aggressive behavior, and an increase in the amount of saliva occur (8, 9).

There are different methods for diagnosing and monitoring ketosis. These tests are based on the detection of ketone bodies (acetoacetate, acetone, BHBA) in urine or milk samples. dipsticks are for the detection of acetoacetate and acetone in urine, providing semi-quantitative results based on the intensity of the color change (1, 5). Similarly, milk-based tests are used to determine BHBA levels in milk. In these tests, the color change becomes more pronounced with increasing concentrations of ketone bodies, allowing the tests to be evaluated semi-quantitatively in practice (5). BHBA level measurement is accepted as the gold standard in the diagnosis of ketosis because it is more stable than acetone or acetoacetic acid in the blood (10).

High BHBA concentrations in cows with ketosis predispose them to diseases such as retention secundinarum, abomasum displacement, metritis, and mastitis (11). Ospina et al. (12) found that when plasma BHBA concentrations exceed the threshold of 1.0 mmol/L after birth, the risk of metritis in cows is 2.3 times higher and the risk of abomasum displacement is 6.9 times higher. Likewise, McArt et al. (7) found that the probability of developing abomasum displacement in cows with BHBA concentration at the upper limit of the SCK range (2.4 mmol/L) was 3 times, and the probability of being removed from the herd was 50 times. The prevalence of ketosis has been reported to be 9.6% in Holstein-Fresian dairy cows in Canada, 11.5% in dairy cows in Finland, 7.2% in Iran, and 16.39% in Bursa province of Turkey (13-16). It is reported that the average total cost of hyperketonemia is 289 USD per case, depending on the differences in each region and inputs (17). For this reason, when the direct effects of ketosis on cows as well as the cost of diseases associated with hyperketonemia are evaluated, it is stated that the actual economic loss for the livestock sector is greater (11, 17).

It is important to know the regional prevalence of ketosis, as ketosis is a common metabolic disease that negatively affects businesses in terms of treatment costs by increasing their predisposition to other diseases. The prevalence of ketosis in dairy cattle in Bingöl province is unknown. For this reason, the presented study aimed to investigate the prevalence of ketosis in dairy cattle in Bingöl.

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MATERIALS AND METHODS

Before starting this study, approval was obtained from XX Animal Experiments Local Ethics Committee (Meeting number: 2024/1, Decision Number: 01/11)

Animal Selection

The study was carried out in a total of 7 different villages, namely Alibir, Ardıçtepe, Çavuşlar, Garip, Sudük, Yamaç, and Yeşil, in the Bingöl province of Turkey, located between 41º 20 and 39º-56º eastern longitudes and 39º-31 and 36º-28º northern latitudes. A total of 411 dairy cows, including Simmental, Holstein and crossbred breeds, were used in the study during the 3-week period after birth. While the average age of cows with SCK and CK was 4.25 years old, the age of the healthy (H) group was determined as 5 years old. In addition, it was determined that the average number of lactations in the SCK and CK groups was 4, while the average lactation number in the S group was 4.80. The cows used in this study were selected from both farms and family businesses. In this study, groups with SCK, CK, and H were created from animals in the first 21 days postpartum.

BHBA Analysis and Grouping

Blood BHBA concentrations were analyzed using the FreeStyle Optium Neo H (Abbott Diabetes Care Ltd., Witney, UK) device and disposable β-ketone test strips (FreeStyle Optimum β-Ketone, Abbott Diabetes Care Ltd.). We calibrated the device before starting the analysis by attaching a special calibrator kit to the FreeStyle Optium Neo H device. After the calibration process, 1 drop of blood from the jugular vein was read into the device in accordance with the technique, and the results obtained after 10 seconds were recorded. Cows were divided into three subgroups according to BHBA concentration. Blood BHBA≥2.6 mmol/L was determined as CK, BHBA≥1.2 mmol/L as SCK, and BHBA<1.2 mmol/L as H (18, 19). Animals with secondary ketosis diseases such as abomasum displacement, mastitis, metritis, and lameness were excluded from the study.

Statistical analysis

Statistical analysis of the data was performed using SPSS 26 (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Whether the data were parametric or not was evaluated with the Kolmogorov-Smirnov test. Then, Kruskall Wallis and Mann Whitney U were used to detect intra- and inter-group differences, respectively. Data are presented as mean ± SD, minimum and maximum values. Statistical significance was accepted as $p<0.05$ for all values.

Funding

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RESULT and DISCUSSION

In the study, the number of animals with SCK, CK, and H and the disease percentage distribution in the 21-day postpartum period in Alibir, Ardıçtepe, Çavuşlar, Garip, Sudüğünü, Yamaç, and Yeşil villages of Bingöl province are presented in **Table 1**. According to the blood BHBA concentration of a total of 411 dairy cows in these villages, 76 were found to be SCK (17.8%), 16 were CK (7.06%), and 319 were H (75.1%).

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Table 1. Distribution of SCK, CK and H according to different villages of Bingöl province

Village	SCK	CK	H
Alibir	24.07 % (n:13)	-	75.93 % (n:41)
Ardıçtepe	21.9% (n:14)	9.4% (n:6)	68.8% (n:44)
Çavuşlar	23.1 % (n:12)	9.6 % (n:5)	67.3% (35)
Garip	12.5% (n:8)	7.8 % (n:5)	79.7 % (n:51)
Sudüğünü	14.2 % (n:7)	-	85.8% (n:42)
Yamaç	17.8% (n:11)	-	82.2 % (n:51)
Yeşil	17.8% (n:11)	-	82.2 % (n:55)
Total	17.8% (n:76)	7.06% (n:16)	75.1 % (n:319)

SCK: Subclinical ketosis CK: Clinical ketosis H: Healthy, n: Number of animals

The mean ± SD, minimum and maximum values of BHBA concentration and postpartum day variables according to different villages of Bingöl province and the statistical differences between the groups are presented in **Table 2**. In the villages of Ardıçtepe ($p<0.001$), Çavuşlar ($p<0.001$) and Garip ($p<0.001$) the concentrations BHBA the H group were found to be significantly lower than in the SCK and CK groups. The concentration of BHBA in these villages was also found to be significantly higher than in the SCK group ($p<0.001$).

In Alibir village, it was determined that the number of postpartum milking days in the group with SCK was significantly lower than the group with H ($p<0.035$). In Çavuşlar village, it was determined that the number of postpartum milking days of both SCK ($p<0.001$) and CK ($p<0.001$) groups was significantly lower than the H group. In Garip village, it was found that both the SCK ($p<0.003$) and the CK ($p<0.005$) groups were significantly lower than the H group in the number of postpartum milking days. A significant difference was found between the H group and the SCK ($p<0.006$) and CK ($p<0.001$) groups in the variable of postpartum milking days in Sudüğünü village. Additionally, a statistically significant difference was detected between the SCK and CK groups ($p<0.008$). It was determined that there was a statistically significant difference between the H group and the SCK and CK ($p<0.044$) groups in the variable of postpartum milking days in Yeşil village. Additionally, a significant difference was seen between the SCK and CK ($p<0.041$), SCK and H ($p<0.033$) groups.

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Table 2. Statistical significance between groups of BHBA concentrations, number of lactations, postpartum milking days, and age variables according to different villages of Bingöl province.

Village	Variables	SCK (Mean±SD) (Min-Max)	CK (Mean±SD) (Min-Max)	H (Mean±SD) (Min-Max)	p
Alibir	**BHBA**	1.56±0.08[a] (1.20-2.10)	-	0.59±0.03[b] (0.30-1.10)	0.001
	Postpartum Milking Day	13.53±1.12[a] (6.00-21.00)	-	16.26±0.53[b] (6.00-18.00)	0.035
Ardıçtepe	**BHBA**	1.73±0.10[a] (1.20-2.50)	3.86±1.15[b] (2.90-6.10)	0.60±0.03[c] (0.20-1.10)	0.001
	Postpartum Milking Day	15.00±0.97 (9.00-21.00)	15.00±1.84 (9.00-21.00)	16,81±0.45 (5.00-18.00)	0.113
Çavuşlar	**BHBA**	1.56±0.09[a] (1.20-2.10)	5.12±0.95[b] (3.20-7.40)	0.61±0.04[c] (0.20-1.10)	0.001
	Postpartum Milking Day	12.08±1.17[a] (7.00-21.00)	11.40±1.93[a] (7.00-18.00)	15.62±0.12[b] (14.00-17.00)	0.001
Garip	**BHBA**	1.70±0.13[a] (1.30-2.40)	3.02±0.19[b] (2.70-3.70)	0.60±0.03[c] (0.10-1.10)	0.001
	Postpartum Milking Day	10.62±1.16[a] (12.00-21.00)	9.00±0.70[a] (7.00-11.00)	13.50±0.07[b] (13.00-14.00)	0.001
Sudüğünü	**BHBA**	1.78±0.20[a] (1.20-2.40)	-	0.66±0.04[b] (0.10-1.10)	0.001
	Postpartum Milking Day	14.57±1.42[a] (9.00-21.00)	-	11.28±0.19[b] (9.00-13.00)	0.001
Yamaç	**BHBA**	1.63±0.11[a] (1.20-2.40)	-	0.49±0.03[c] (0.10-1.10)	0.001
	Postpartum Milking Day	15.09±1.04 (9.00-21.00)	-	12.76±0.53 (7.00-21.00)	0.071
Yeşil	**BHBA**	1.75±0.12[a] (1.20-2.40)	-	0.49±0.03[b] (0.10-1.10)	0.001
	Postpartum Milking Day	14.81±0.99[a] (11.00-21.00)	-	12.12±0.49[b] (5.00-20.00)	0.044

[a,b,c]: Different letters on the same row indicate statistically significant differences. SCK: Subclinical ketosis, CK: Clinical ketosis, H: Healthy, BHBA: Beta-hidroksi-bütirik asit.

The transition of dairy cows from the dry period to the transition period involves important changes in body homeostasis (19). The lactation process starts with the birth of the cows and if the energy homeostasis of the cows is impaired in this process, NED develops in the animals. With the development of NED, the level of ketone bodies in the circulation of animals increases and ketosis occurs (20, 21). Ketosis, creates an important predisposition to various transitional diseases (11). As a result, ketosis causes significant economic losses in livestock farms, such as veterinarian and treatment fees, decrease in milk production and worsening of reproductive performance (1, 17, 20). Therefore, it is of great importance to determine and closely monitor the incidence of SCK and CK in dairy cows in a region. Determining the prevalence of SCK and CK will lead livestock enterprises to take the necessary precautions against this disease, thus preventing significant economic losses for animal welfare.

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In this study, it was aimed to investigate the prevalence of ketosis and the effect of the number of postpartum milking days in dairy cows in different settlements of Bingöl province (Alibir, Ardıçtepe, Çavuşlar, Garip, Sudüğünü, Yamaç and Yeşil). In this study, it was determined that 76 of a total of 411 dairy cows were SCK (17.88%), 16 were CK (7.06%) and 319 were H (75.1%). It was also determined that the number of postpartum milking days in ketosis cows in these villages varied significantly.

BHBA is considered the gold standard method in the diagnosis of ketosis as it is relatively stable compared to other ketone bodies in whole blood, plasma or serum both in vivo and in vitro (22, 23). In many studies, BHBA is considered to be ≥1.2-1.4 mmol/L in the diagnosis of SCK (1, 10, 17, 20, 24), while BHBA is ≥3.0-2.6 mmol/L in the diagnosis of CK (19-21) is widely accepted. In this study, which is presented in accordance with previous literature reports, BHBA ≥1.2 mmol/L was taken as reference in the diagnosis of SCK, and BHBA ≥2.6 mmol/L was taken as reference in the diagnosis of CK.

The CK form can be identified more easily than SCK due to the detection of hyperketonemia in urine, milk and blood and the appearance of clinical findings. Additionally, studies have reported that a significant portion of high milk yielding cows are at risk of SCK (10, 17, 20, 24, 25). In a study investigating the rate of ketosis in 8902 dairy cows from 12 countries in South and Central America, the average prevalence of SCK was found to be 24.1% (8.3%–40.1%) (11). In Turkey, Ayvazoğlu and Gökçe (26) found CK 1%, SCK 10%, Başbuğ et al. (27) determined SCK as 12%. In parallel, Şentürk et al. (24) reported SCK 12.9% in the Aegean Region, 24.6% in the Marmara Region, and 10.8% in the Mediterranean region. In this study, SCK was determined to be 17.9% in Bingöl province, and Başbuğ et al. (27), Şentürk et al. (24) and Ayvazoğlu and Gökçe (26) are similar to the study results. However, the finding of 7.06% in CK in this study is consistent with Ayvazoğlu and Gökçe (26) and Başbuğ et al. (27) is higher than the rate found. The possible reason for this high rate may be related to care and feeding errors and the difference in the animal population in the study.

The primary risk period for ketosis is the first month of laciation, and high rates of ketosis are in the first two weeks (28). It is also that the highest prevalence of ketosis occurs between 6 and 7 days after birth, and decreases after the 30 day period (29). Garro et al. (10) found that the prevalence of SCK was 10.3% between 4-19 days after birth, but this rate decreased after the first 30 days. Asl et al. (30) reported that 97% of the cows in a study group with repeated breeding developed SCK at least once in the first 6 weeks after birth. In another study, it was stated that the prevalence of ketosis remained high from day 0 to day 30 after birth and gradually decreased from day 30 to day 65 (31). In this study, since the fresh period covers the postpartum period of 3 weeks and the disease is common in this period, dairy cows in the first 21 day period were included in the study and ketosis was monitored after this period (1, 2). In this study, it was determined that the number of days of postpartum milking was significantly reduced in the villages of Alibir ($p<0.035$), Çavuşlar ($p<0.001$), Garip ($p<0.001$), Sudüğünü ($p<0.001$) and Yeşil ($p<0.044$). The possible reason for this may be related to the development of NED status in the first month period, the increase in ketone bodies and the decrease in feed intake in the periods close to birth (29).

CONCLUSIONS AND RECOMMENDATIONS

The presented study is the first study to investigate the prevalence of SCK and CK in dairy cattle in Bingöl province. Although its effect is variable in each region, it was concluded that the postpartum milking day variable should be considered as an important risk factor in the prevalence of ketosis. Determining management and feeding strategies that can reduce the prevalence of

ketosis is important for the region. In future studies, it would be useful to examine different variables such as secondary diseases, milk yield and breed differences along with ketosis.

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