

## Comparison of Two Cow Side BHBA Tests For Diagnosis of Subclinical Ketosis

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**Abstract:** Ketosis is a disease of high yielding dairy cows caused by negative energy balance. Subclinical ketosis is a type of disease in which keton bodies in blood are elevated but not enough to cause clinical findings. Economic impact of subclincic ketosis makes disease one of the most important problems of dairy industry. Quick and accurate diagnosis of subclinical ketosis is crucial for economic development of a dairy herd thus in presented study two cowside BHBA test were compared according to two different blood BHBA cutoff values. Sensitivities were found to be 58%, 50% and specificities were 84%, 93% respectively for cut off points of 1.2 and >1.4mmol/L. overall specificity and sensitivity levels calculated indicates that Precision Xtra™ could be used for detection of blood BHBA levels in suspected cows at the same time Precision Xtra™ is quicker than Ketosite analysis takes 20 seconds per sample however with Ketosite one analysis takes about 2 minutes.

**Key Words:** BHBA, Subclincic ketosis, ketosis.

### Subklinik Ketozis'in Tanısı İçin İki BHBA Testinin Karşılaştırılması

**Özet:** Ketozis yüksek süt verimli ineklerde negative enerji dengesizliğine bağlı meydana gelen bir hastalıktır. Subklinik ketozis, kanda keton cisimciklerinin yükselmesiyle oluşan fakat klinik düzeyde semptom görülmemesiyle karakterize bir hastalıktır. Süt sektörünün en önemli hastalığından biri olan subklinik ketozisin ekonomik etkisi fazladır. Subklinik ketozisin hızlı ve doğru tanısı süt sığırcılığın ekonomik gelişimi için önemlidir, sunulan bu çalışmada iki BHBA testi kandaki iki farklı BHBA sınır değerine göre karşılaştırılmıştır. Sensitivite 1.2 mmol/L ye göre %58, 1.4mmol/L ye göre %50 ve spesifite 1.2 mmol/L ye göre %84, 1.4mmol/L ye göre %93 bulunmuştur. Hesaplanan spesifite ve sensivite değerleri gösteriyorki Precision Xtra™ şüpheli sığırların kanlarındaki BHBA düzeylerinin ölçümü için kullanılabilir aynı zamanda Precision Xtra™, Ketosite dan daha hızlıdır çünkü Precision Xtra™ analizi 10 saniyede Ketosite 2 dakikada analizi yapmaktadır.

**Anahtar Kelimeler:** BHBA, Subklinik ketozis, ketozis.

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## Introduction

Ketosis is a metabolic disease of mostly high yielding dairy cows caused by inability of cows to meet high dietary energy demands during postpartum period, thus resulting in negative energy balance and subsequent ketosis<sup>3</sup>. Subclinical ketosis is a condition in which keton bodies in blood are elevated but not enough to cause clinical symptoms<sup>2</sup>. Because of high yield demands of dairy industry, subclinical ketosis is now one of the most important problems of dairy industry, surpassing hypocalcaemia and ruminal acidosis in clinical significance<sup>5,1</sup>. Economic impact of disease is caused by decreased milk yield, treatment costs, increased herd removals and secondary diseases such as displacement of abomasum and hepatic lipidosis of which incidence is known to be higher in cows with ketosis<sup>7,4,11</sup>. Diagnosis of ketosis is based on evaluation of blood  $\beta$ -hydroxybutyric acid (BHBA) levels which is gold standard test for disease because of its higher stability in blood than other keton bodies like acetone and acetoacetate<sup>17,9,10</sup>. Therefore accurate and fast measurement of blood BHBA is a rewarding test for diagnosis of subclinical ketosis at field conditions. Precision Xtra had been used for detection of blood BHBA levels in a study and had been shown to have satisfactory sensitivity and specificity levels<sup>15,13</sup>.

The aim of this study was to determine the performance of blood BHBA test system using a human instrument marketed for diabetic patients (Precision Xtra™ blood glucose and ketone monitoring system, Abbott Diabetes Care Ltd. Range Road Witney, Oxon OX29 OXL UK) compared with serum BHBA (KetoSite™; GDS Diagnostics, Elkhart, Ind) and to evaluate its usefulness in a herd-screening process.

## Material and Methods

Animal material of the presented study consisted of 25 dairy cows from same herd at mean 23 days postpartum. All cows selected for the study had body condition scores (BCS) lower than 2.25. Mean milk yield of cows was 12.5 liters. Infertility and subclinical mastitis detected by California mastitis test (CMT) were two main problems of herd selected for the study. Blood samples were collected from all 25 cows by venipuncture 5 hours after feeding into 10 ml tubes without anticoagulant and into 10 mL

evacuated tubes containing EDTA (Ethylenediaminetetraacetic acid). Blood samples without anticoagulant were centrifuged at 3000 r.p.m. for 20 minutes and sera were separated and evaluated within 2 h of collection by KetoSite™ test. Blood samples collected to tubes containing EDTA were used for measurement of BHBA by Precision Xtra™. KetoSite™ test is made by dripping a drop of serum onto KetoSite™ test card, and measuring process takes 2 minutes per animal, however with Precision Xtra™ one drop of anticoagulated blood is used for the test and takes only 10 seconds to measure blood BHBA levels per cow. Sensitivity, specificity, positive and negative predictive values, odd ratio, positive and negative likelihood ratios were determined using 2x2 tables in cut off point of 1300  $\mu$ mol/L.

## Results

Commonly used blood BHBA cut off points for diagnosis of subclinical ketosis are 1.2 mmol/L and >1.4 mmol/L respectively. Due to different cut off point values stated in literature, in presented study tests were compared according to cut off points of 1.2 mmol/L and >1.4 mmol/L. In case where cut off point was determined 1.2 mmol/L number of positive results of KetoSite™ test were 12 however positive results detected by Precision Xtra™ were only 9. Nevertheless positive results were 9 and 6 respectively when >1.4 mmol/L was determined as cut off point. Sensitivity, specificity, accuracy, positive and negative predictive values and likelihood ratios were determined using 2x2 tables according to both cut off values mentioned above (table 1). Mean BHBA levels detected by KetoSite™ and Precision Xtra™ were 1.188 and 1.096 respectively.

## Discussion

Subclinical ketosis is one of the most important diseases of dairy herd industry that causes severe economic losses due to reduced milk yield, treatment costs and subsequent diseases like displacement of abomasums and hepatic lipidosis of which etiopathogenesis are known to be related with subclinical ketosis<sup>8</sup>. The first two weeks postpartum are the primary risk period for subclinical ketosis, defined by a serum BHBA concentration of 1400  $\mu$ mol/L or greater<sup>16,12</sup>. BHBA is the gold standard test for diagnosis of subclinical ketosis. BHBA is the most stable in blood compared to other keton

bodies, however blood BHBA levels are very variable according to feed intake; thus, all samples on a given farm should always be taken at the same time of day. In addition, hemolysis may elevate blood values of BHBA; therefore, hemolyzed samples should be not used in the determination of BHBA<sup>16</sup>. Disadvantages of serum BHBA analysis are the its cost (approximately \$5.00 per sample) and the laboratory turnaround time (minimum 24 hours). Therefore blood samples collected and evaluated for BHBA from suspected animals as quickly as possible provides a great advantage for realistic values.

**Table 1. Sensitivity (Se)(%), specificity (Sp)(%), predictive values of a positive (pV<sup>+</sup>)(%) and negative (pV<sup>-</sup>)(%) test, positive(LR<sup>+</sup>) and negative(LR<sup>-</sup>) likelihood ratios and accuracy (%) are given for Precision Xtra™**

**Tablo 1. Sensitivite (Se)(%), spesifite (Sp)(%), pozitif prediktif deđerler (pV<sup>+</sup>)(%) ve negative (pV<sup>-</sup>)(%), pozitif (LR<sup>+</sup>) ve negatif (LR<sup>-</sup>) olabilirlik ve dođruluk oranları (%) Precision Xtra™ için verilmiştir.**

Cut off point for subclinical ketosis	Se	Sp	PV <sup>+</sup>	PV <sup>-</sup>	LR (+)	LR (-)	Accuracy
1.2 mmol/L	58	84	77	68	3.62	0.50	72
>1.4 mmol/L	50	93	83	73	7.14	0.53	76

Economic impact caused by subclinical ketosis is reported to be approximately \$78 U.S./per cow<sup>11</sup>. Diagnosis of the disease and appropriate treatment plays a crucial role for economic productivity of a herd<sup>14</sup>. As mentioned above BHBA is the gold standard test for diagnosis of subclinical ketosis, because of its higher stability in blood compared to other keton bodies<sup>4,6</sup>. KetoSite™ is a widely used and accurate test for detection of blood BHBA levels in field, results of Precision Xtra™ were compared to blood BHBA levels determined by KetoSite™. Cut off points of 1.2 mmol/L and >1.4 mmol/L were assigned for diagnosis of subclinical ketosis. When 1.2 mmol/L was used as cut off point 3 of 12 cows were detected to be false negative by Precision Xtra™, also 3 of 9 cows were false negative when cut off point was >1.4 mmol/L. Sensitivities were 58%, 50% and specificities were 84%, 93% respectively for cut off points of 1.2 and >1.4mmol/L. Although specificities

detected in study presented were high, low sensitivity levels detected are relevant with high false negative counts detected by Precision Xtra™. But overall specificity and sensitivity levels calculated indicates that Precision Xtra™ could be used for detection of blood BHBA levels in suspected cows. Because of its ease of use and low costs per cow, Precision Xtra™ is a applicable test for diagnosis of subclinical ketosis in herd basis. However, because of low sensitivity level, use of Precise Xtra test to estimate herd prevalence must be done cautiously, especially when only a small number of animals are sampled.

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