



## RESEARCH ARTICLE

### The investigation of the prevalence of subclinical ketosis in Sivas region dairy cows

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#### Özet

**Başbuğ O, Akar Y, Ercan N.** Sivas yöresindeki süt sığırlarında subklinik ketozis prevalansının araştırılması.

**Amaç:** Türkiye’de subklinik ketozis (SKK) prevalansının belirlenmesine yönelik sınırlı sayıda çalışma yapılmıştır. Bu çalışmada, Türkiye’nin Sivas ilindeki süt sığırları işletmelerinde bulunan Holştayn süt ineklerinde SKK’in prevalansının belirlenmesi ve serum  $\beta$ -hidroksibütirat (BHB) ile glikoneogenezisde rol alan glikoz, albümin ve total kolesterol konsantrasyonları arasındaki ilişkinin tespit edilmesi amaçlandı.

**Gereç ve Yöntem:** Çalışmada yaşları 3-6 arasında değişen, kuru dönem (40 baş) ve doğumdan sonraki 1-8 haftalar (160 baş) arasında bulunan toplam 200 baş holştayn inekler kullanıldı. Sistemik klinik muayenesi yapılan hayvanlardan kan örnekleri alındı. SKK’li ineklerin belirlenmesinde eşik değeri olarak 1.0 mmol/L BHB düzeyi alındı. Tüm ineklerin serum glikoz, albümin ve total kolesterol düzeyleri belirlendi.

**Bulgular:** Sivas yöresinde yetiştirilen Holştayn ırkı ineklerde SKK prevalansı %12 olarak tespit edildi. SKK’lı ineklerin kan BHB seviyelerinin laktasyonun ilk haftalarında daha yüksek seviyelerde seyrettiği, kan glikoz ve total kolesterol düzeyleri ile arasında negatif korelasyonun olduğu belirlendi.

**Öneri:** Süt verimi yüksek olan işletmelerde, periyodik aralıklarla BHB düzeylerinin belirlenmesi ve yüksek BHB’li ineklerde gerekli önlemlerin alınması yararlı olacaktır.

**Anahtar kelimeler:** Subklinik ketozis,  $\beta$ -hidroksibütirat, glikoz, inek

#### Abstract

**Basbug O, Akar Y, Ercan N.** The investigation of the prevalence of subclinical ketosis in Sivas region dairy cows.

**Aim:** In Turkey, there have been limited studies conducted to determine the prevalence of subclinical ketosis (SCK). The aim of this study is to determine the prevalence of SCK in Holstein dairy cattle of the commercial herds in Sivas province of Turkey and to define the relationship between serum  $\beta$ -hydroxybutyrate (BHB) and the glucose, albumin, total cholesterol concentrations which exist in gluconeogenesis.

**Materials and Methods:** In this study, ranging in age from 3 to 6, dry period (n:40) and 1-8 weeks after birth found among the 160 head of Holstein cows were used. Blood samples were collected from animals which had been subject to systematically clinical examination. In detecting the cows with SCK, the threshold value was BHB levels >1.0 mmol/L. Serum glucose, albumin and total cholesterol levels of all cows were determined.

**Results:** In Holstein cows of Sivas region the prevalence of subclinical ketosis is determined as 12%. It was seen that in those cows with SCK, blood BHB levels were higher during the early lactation and there was a negative correlation between blood glucose and total cholesterol level.

**Conclusions:** Commercial which are high yielding dairy cows, high BHB levels are periodically determined, in order to take necessary precautions will be useful.

**Keywords:** Subclinical ketosis,  $\beta$ -hydroxybutyrate, glucose, cows





## Introduction

Ketosis is a carbohydrate metabolism disorder which occurs due to the inadequacy of energy requirement increasing especially during the early stage of lactation in high yielding dairy cows (Grummer 1993, Açoğlu and Akgül 2012). It is characterized by the decrease in glucose level of blood, liver glycogen and depletion of other glucose reservoirs, decreasing gluconeogenetical activity, fatty degeneration of liver and increase of ketone bodies (acetoacetic acid, acetone, Beta-hydroxybutyrate) (Gul 2006). Ketosis is designated as clinical or subclinical by the clinical symptoms or by the concentrations of blood, urine and the ketone body in milk (Andersson 1984, Enjalbert et al 2001, Issı et al 2009). Subclinical ketosis (SCK) develops without clinical symptoms of ketosis and is widely detected especially in the early stage of lactation in high yielding dairy cows (Duffield 2000, Asl et al 2011, Suthar et al 2012). When not treated the SCK causes to decreasing in milk fertility, decreasing in unspesific immunity, breeding disorders. There also occur important economical loss as a result of predisposition against postpartum diseases such as metritis, abomasum depletion, retentio secundinarum, mastitis and clinical ketosis (Dohoo et al 1983, Andersson and Lundstrom 1984, Geishauser et al 2000, Sortorelli et al 2000, Walsh et al 2007, Suthar et al 2012). The studies conducted amongst high yielding dairy herds during puerperal period state that in the cows with SCK the pregnancy rate might fall to about 50% (Walsh et al 2007). Dohoo and Martin (1984) assigned that the SCK decrease the milk production down to between 4.4 and 6%. The prevalence of SCK is reported to be 9.6% in Canadian Holstein-Friesian dairy cows (Dohoo et al 1983), 11.5% in Finland cattles (Lindström et al 1984), 7.2% in Iran (Haghighat-Jahromi and Nahid 2011) and 16.39% in the cattles of Bursa region (Kennerman 1999).

Since BHB is the dominant keton body in SCK diagnosis; the measurements of serum, plasma and whole blood are made as golden standart tests (Oetzel 2004, Voyvoda and Erdoğan 2010). In some researchs, threshold values as 1.0-1.4 mmol/L are recommended to discriminate cows with SCK and the healthy ones (Whitaker et al 1983, Andersson 1984, Nielen et al 1994, Enjalbert et al 2001). In recent years, electronic hand held meters (Precision Xtra Meter, Abbott Diabetes Care, Abingdon, UK) have been gaining importance in milk cows' whole blood BHB measurements. Voyvoda and Erdoğan (2010) diagnosed the 1200  $\mu\text{mol/L}$  and more BHB concentrations in blood as SCK and stated that the sensitivity and specificity of electronic hand held device used in BHB measurement is 0.90 and 0.98% successively.

In this study, it was aimed to detect the prevalence of SCK in Holstein dairy cows of commercial herds in Sivas province of Turkey and also to determine the relationship between serum BHB and the glucose, albumin, total cholesterol concentrations which exist in gluconeogenesis.

## Materials and Methods

The materials of this study were 200 Holstein dairy cows in Sivas region between the years of 2012-2013. In study, these cows ranging in age from 3 to 6, dry period (n:40) and 1-8 weeks (n:160) after birth found among totally 200 head of Holstein cows were used. The ethical permission of this study was registered to the Local Ethics Committee of Cumhuriyet University with 06.10.2011 history and 83 numbers.

The cows which have been subject to a treatment or expressing any indication of a disease in last one month are not been included in this study. The animals in the study were divided into five groups; Group 1 (n:40): 1st week of lactation, Group 2 (n:40): 2nd week of lactation, Group 3 (n:40): 4th week of lactation, Group 4 (n:40): 8th week of lactation and Group 5 (n:40) dry period.

Blood samples were collected from all of the animals in the study 4 or 5 hours before feeding and in convenience with the technique, samples collected in steril tubes from taken from vena jugularis, centrifuged for 15 minutes in 3000 rpm and serum kept in  $-20^{\circ}\text{C}$ .

BHB analysis was promptly carried out with "Abbott Optimum Xceed Diabetes Monitoring System" introduced by Voyvoda and Erdoğan (2010). Threshold value to detect dairy cows with SCK is taken as BHB level  $>1.0$  mmol/L. Glucose, albumin and total cholesterol measurements were taken with Mindray BS-200 autoanalyzer (PRC).

In the evaluation process of the data, SPSS 14.0 (SPSS inc LTD Chicago USA) program was used. Comparisons of the differences between groups were made with Chi-square and Duncan test. Correlations among glucose, albumin, total cholesterol and BHB of all animal were made.  $P<0.05$  level was accepted statistically significance level.

## Results

The medians of biochemical examination results (glucose, albumin and total cholesterol levels) of all animals and the importance of the groups were given in Table 1. The correlation rates of BHB, glucose, albumin and total cholesterol levels were given in Table 2. Moreover, distributions of dairy cows with SCK by the ages inside the group were given in Graphic 1.

The comparisons of SCK prevalence of Holstein cattles in Sivas region and 5 groups divided as 1st, 2nd, 4th, 8th weeks of lactation and as dry stage are respectively 7.5, 15, 25, 12.5% and 0%. Also, the inter-group differences were found statistically important ( $P<0.05$ ).



Table 1. Biochemical examination results of healthy/sick cattles in the groups (Mean±SE).

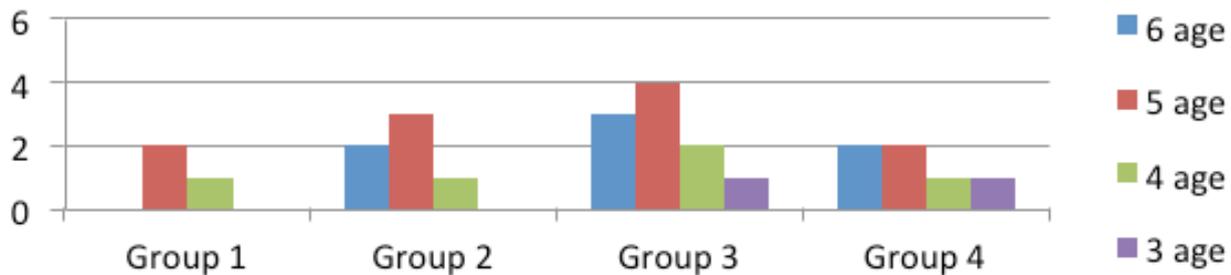
	Healthy					SCK			
	Group1 (n:37)	Group2 (n:34)	Group3 (n:30)	Group4 (n:34)	Group5 (n:40)	Group1 (n:3)	Group2 (n:6)	Group3 (n:10)	Group4 (n:6)
BHB mmol/L	0.65±0.02d	0.50±0.03e	0.42±0.02e	0.41±0.04e	0.26±0.01f	1.60±0.06a	1.53±0.03a	1.42±0.04b	1.20±0.05c
Glucose mg/dL	51.3±0.6c	53.3±0.7c	55.1±0.7bc	58.3±0.9b	65.6±0.9a	40.3±1.8e	41.2±0.7e	39.7±0.6e	45.8±1.2d
Albumin g/dL	3.15±0.03c	3.26±0.04bc	3.33±0.04abc	3.35±0.04abc	3.40±0.03ab	3.50±0.10a	3.49±0.12a	3.40±0.07 ab	3.22± 0.05bc
TC mg/dL	129±3.5b	132±2.4b	164±6.5a	169±6.6a	181±5.8a	98.3±4.2c	109±2.3bc	112±1.9bc	124±2.4bc

BHB:  $\beta$ -hydroxybutyrate, TC: Total cholesterol, a-f: The differences between groups of same line with different letters are important ( $P < 0.05$ ).

Table 2. Correlations amongst glucose, albumin, total cholesterol and  $\beta$ -hydroxybutyrate.

	Glucose	Albumin	Total cholesterol
BHB mmol/L	-0.801**	0.066	-0.579**
Total cholesterol mg/dL	0.532**	0.136	
Albumin g/dL	0.112		

BHB:  $\beta$ -hydroxybutyrate, \* $P < 0.05$ , \*\* $P < 0.01$ .



Graphic 1. Distribution of subclinical ketosis in groups by ages.

In the study, according to the dates given by dairy cow owners in Sivas region, in 1st, 2nd, 4th and 8th weeks after calving, daily median milk productions are determined as respectively 19.4±0.6, 21.5±0.8, 22.7±0.6 and 23.7±0.8 kg/per day.

## Discussion

Subclinical ketosis is an important metabolism disorder developing without absence of clinical symptoms of ketosis. It has been stated that SCK is ranging between 8.9% and 43% prevalence rate in the high yielding dairy cows during the early stage of lactation (Dohoo et al 1983, Duffield et al 1998, McArt et al 2012). It results in breeding disorders and serious economical losses due to increasing metabolic and immunological entities during postpartum period as well as a decreasing rate of milk yield (Andersson L 1984, Dohoo and Martin 1984, Geishauser et al 2000, Sortorelli et al 2000, Walsh et al 2007, Suthar et al 2012).

Different types of tests are being used to detect SCK prevalence. Amongst these test, detecting the keton bodies in blood, milk and urine has its place (Geishauser et al 2000, Carrier et al 2003). Since especially in recent years, it is highly easy to detect BHB in serum, plasma and whole blood in

SCK; in dairy cattle operations gold test standars are being used (Oetzel 2004, Voyvoda and Erdoğan 2010).

While some researchers claim that daily rate of BHB is changeable related to feeding and that is the reason why the most convenient time for sampling is 3 or 5 hours after morning feeding (Manston et al 1981, Whitaker et al 1993, Duffield 2000), others state that since BHB concentration may be affected by ration carbohydrate or glucogenic substrate levels; blood samples should be collected before feeding (Van Saun 2009, LeBlanc 2010). Hence, blood samples were collected 3 or 5 hours after morning feeding in this study. Moreover, serum was excluded due to the fact that the samples in which haemolysis was detected, might affect the results (Stokol and Nydam 2006). In literatures (Andersson 1984, Duffield 2000) threshold value for diagnosing SCK with BHB serum is determined as 1.000-1.400 mmol/L level and for clinical ketosis 2.6 mmol/L and more values are accepted.

Some researchers (Oetzel 2004, Walsh et al 2007, Duffield et al 2009) have identified certain increases of body ketons in especially early stages of lactation and this increase is related to the negative energy balance in dairy cows. In this study the low prevalence rate is probably related to gene factors or low





milk production as Haghghat-Jahromi and Nahid (2011) have stated. SCK prevalence has been reported to increase mostly between 2nd and 4th weeks of lactation. In this study the peak incidence rate was seen in the 4th week. This timing is probably feeding, gene or metabolic changes based.

It is stated that in dairy cows, there is a negative correlation between blood glucose level and keton body. It is also stated that decreasing glucose and BHB levels are reflecting the span and severity of negative energy balance (Gröhn et al 1983, Borrebaek et al 1990, Drackley 1999). And in this study it is in line with the researchers it is designated that there is a negative correlation ( $r=-0.803$ ,  $P<0.01$ ) between blood glucose level and BHB level (Sakha et al 2006, Gonzalez et al 2011). However Gröhn et al (1983) express that ketonemia might develop without a serious hypoglycaemia is developing.

Examining the glucose medians between groups, it is found that the medians of healthy cows especially in Groups 1, 2, 3 and 4 (Table 1) are relatively lower than Group 5. These decreases in glucose levels might be a response to having a low energy diet when there is an increasing glucose usage from mammary glands (Whitaker et al 1993, McGuire et al 1995, De Vries et al 1999). Serious decreases in serum glucose levels during early stages of lactation are reported in dairy cows with SCK (Sakha et al 2006, Gonzalez et al 2011). In this survey, serum glucose level is found lower in each of the four groups than the healthy ones. The requirement for glucose is met through gluconeogenesis in dairy cows. The decrease in the serum glucose level is reported to be related to the low energy amount in feeds, the inadequacy of liver functions and the increase in the requirement for glucose (Gröhn et al 1983, Aslan and Nizamlioglu 1985, Veenhuisen et al 1991, Duffield 2000) During the transitional lactation period, glucose requirement for lactose production is increasing in dairy milk cows which results in shaping the energy gap and decrease in serum glucose level due to gluconeogenesis (Brumby et al 1975).

Gonzalez et al (2011) have stated that in ketotic cows, albumin levels may decrease relating to inadequate synthesis of albumin which results from the fatty acids cumulating in hepatocyte hindering the liver from functioning and this cumulating occurs because of the hepatic changes. However Sevinc et al (1998) has found out that the number of cows with SCK in group 1 and 2 has increased in a statistically important way ( $P<0.001$ ) compared to other groups.

Total cholesterol levels of cows with SCK and healthy ones are inside the normal limits stated in sources. While Djokovic et al (2013) puts forward that there is a decrease in the serum cholesterol levels of puerpal ketotic cows which is the result of the increase in cholesterol and triglyceride cumulation in hepatocyte and which is related to the decrease in

liver synthesis of VLD stimulated probably beforehand; Can et al (1987) states that there might be an increase in serum cholesterol levels in ketotic cows and that this increase might be shaped by the usage of lipids instead of glucose when not available and providing the necessary lipids through mobilisation of lipids in body store. In the study it is determined that the cholesterol levels of the cows with SCK is having a statistically fundamental decrease comparing to the healthy cattle ( $P<0.001$ ). This is the case probably when the liver synthesis of VLD stimulated beforehand decreases and when cholesterol and triglyceride cumulation increases.

### Conclusions

As a conclusion; the prevalence of SCK in Holstein cows raised in Sivas region is determined as 12%. It is found out that the cows with SCK have higher total keton medians in the early stages of lactation and that there is a negative correlation between blood BHB levels and glucose and total cholesterol levels. It is estimated that the low glucose levels in the early stage of the lactation might be correlated with probably the low energy amount in feeds, inadequacy in functioning of the liver and the increased requirement for glucose. Moreover, the conclusion reached is that an appropriate diet programme should be made for the early and dry periods of the lactation in high production of Holstein dairy cows and that because of serious lacks of care and feeding, the efficiency of cows is less than their potential genetic capacities.

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