#### 한국가축위생학회지 제41권 제2호 (2018) Korean J Vet Serv, 2018, 41(2), 85-89 ISSN 1225-6552, eISSN 2287-7630 https://doi.org/10.7853/kjvs.2018.41.2.85

Korean Journal of Veterinary Service

Available online at http://kives.org

<Original Article>

# Cross-sectional study: prevalence of subclinical ketosis in dairy cattle at Chungnam province

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(Received 2 February 2018; revised 20 March 2018; accepted 20 March 2018)

#### **Abstract**

This study was conducted to demonstrate the prevalence of subclinical ketosis in dairy cattle based on days in milk and herd level. Four to five blood samples were collected in five different lactation periods and analyzed for  $\beta$ -hydroxybutyrate using a Portable Ketone Test Kit. Subclinical ketosis was observed in 12 herds at prevalence of  $6.3 \sim 57.1\%$  depending on herd level, with four herds showing more than 40% subclinical ketosis prevalence. The distribution of subclinical ketosis in the early lactation period, high yield lactation period, mid lactation period, late lactation period and dry period was 9.5%, 27.5%, 36.8%, 27.9% and 12.5%, respectively. Clinical ketosis was only observed during early lactation (9.5%) and mid lactation (2%) period.

**Key words**: β-hydroxybutyrate, Days in milk, Subclinical ketosis

# INTRODUCTION

Subclinical ketosis (SCK) is a common metabolic disease in which excess blood ketone bodies are produced during the transition period of dairy cattle. It's affect farm production by decreasing milk production, reducing reproductive performance and incurring treatment costs. In North America, approximately 40% of lactating cows experience SCK, although the incidence varies from herd to herd (McArt et al, 2012). Moreover, about 40-60% of the cases of SCK are found in cows subjected to repeated testing in different herds in Ontario, Canada (Duffield et al, 1998). Depending on the methods and frequency of screening, incidence rates of clinical ketosis (CK) are expected to be 2~15% in the first month of lactation, whereas 40% cumulative incidence of SCK is typical if cows are screened weekly during the same period (Duffield, 2000). The overall prevalence of SCK in dairy cows during early lactation is estimated to be 7.5%~14% (Geishauser et al, 2000; Enjalbert et al, 2001). However, the prevalence of SCK varies depending on herd factors, days in milk, breed, parity, and season (Andersson, 1988). SCK has also been associated with lower milk production and an increase in the average calving interval (Enjalbert et al, 2001). Cows with SCK are at increased risk of postpartum diseases such as displaced abomasum (DA), cystic ovarian disease and metritis (Duffield et al, 2009; Ospina et al, 2010).

Subclinical ketosis is characterized by elevated concentration of ketone bodies in the bloodstream without signs of CK (Andersson, 1988; Duffield, 2000). The major ketone bodies in the circulatory system are acetone, acetoacetate and beta-hydroxybutyrate, of which the latter predominates in ruminants (Duffield, 2000). CK and SCK both result in increased concentrations of ketone bodies in blood, milk and urine samples of the cows. Blood BHBA concentration has often been used for detection of SCK, and a cut-off point of 1.2 mmol/l

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was used to discriminate between healthy cows and animals with SCK (Duffield et al, 1997; Duffield et al, 1998; Geishauser et al, 1998; Jorritsma et al, 1998).

The prevalence of SCK has been recorded in many European countries, North America and Canada at different lactation stages of their dairy cattle. However, the prevalence of SCK at any interval post-calving has not been reported yet in Chungnam province, Republic of Korea. This is the first population-based study of the prevalence of SCK in this region. The goal of this study was to investigate the prevalence of SCK in dairy cattle based on days in milk (DIM) and herd level at Chungnam province.

#### MATERIALS AND METHODS

## Study design

A cross-sectional study was conducted among thirteen dairy herds at Chungnam province, South Korea, 2017. The individual cow was the experimental unit and the main outcome measurement was prevalence of SCK. The production level of each herd was high and all of them were Holstein Friesian cattle. To be selected, farms had to meet the following criteria: milking at least 50 cows, average level of milk production was 30 kg per cow per day during the study period, good management practices, and be willing to participate in the proposed ketosis testing. Whole blood samples were collected according to lactation periods (e.g., early lactation period 0~49 DIM, high yield lactation period 50~109 DIM, mid lactation period 110~219 DIM, late lactation period >220 DIM and dry period) of each herd.

#### Blood sampling and diagnosis of SCK

Four to five blood samples were collected from the animal during each lactation period. Approximately 5 mL of blood was collected from the coccygeal vein of each animal using a 19-gauge, 2.54 cm needle and a 10 mL syringe. β-Hydroxybutyrate (BHBA) testing was conducted according to the Portable Ketone Test (Precision Xtra® Abbott) instructions and performed immediately after blood collection. A ketone strip was attached to the Precision Xtra® meter until the "add blood" symbol appeared on the meter display. The lot number of the inserted ketone strip was then checked to ensure matching with the lot number displayed on the meter. For each cow test, a drop of blood was applied to the chamber of ketone test strip, and the meter indicated when the chamber was full. After 10 sec, the BHBA concentration was displayed on the meter and the value recorded. A blood BHBA between 1.2 and 2.9 mmol/l was diagnosed as SCK, and CK was diagnosed as BHBA ≥3.0 mmol/l (Oetzel, 2004).

### **RESULTS**

Subclinical ketosis was observed in 12 herds and its prevalence ranged from 6.3% to 57.1% based on herd level (Table 1). Four herds showed the prevalence of SCK was >40%, while 38.5% SCK was observed in two herds. The prevalence of SCK was  $20\sim25\%$  in another two herds and  $10\sim20\%$  in three herds. Interestingly, only one farm had no SCK. In case of CK, only three of the herds were positive, among them the highest prevalence of CK was 10% and lowest was 7.1%.

Based on the lactation period, the SCK level was highest during mid lactation (110~219 DIM), when it

Table 1. Estimated prevalence of Subclinical ketosis and Clinical ketosis among thirteen dairy herds

Ketosis stage -	Dairy herd*												
	A	В	С	D	Е	F	G	Н	I	J	K	L	M
Subclinical ketosis (%)	40	44.4	13.3	6.3	50	17.6	38.5	16.7	38.5	25	0	22.2	57.1
Clinical ketosis (%)	0	0	0	0	10	0	0	0	0	8.3	7.1	0	0

<sup>\*</sup>Alphabetic letter A, B, C....M represent different dairy herds.

Table 2. Frequency of Subclinical ketosis and Clinical ketosis in different stages of lactation

ketosis stage	Lactation period									
	Early (0~49 DIM)	High (50~109 DIM)	Mid (110~219 DIM)	Late (220~DIM)	Dry					
Subclinical ketosis (%)	9.5	27.5	36.8	27.9	12.5					
Clinical ketosis (%)	9.5	0	2	0	0					

was 36.8%. The lowest prevalence of SCK was 9.5%, which was observed in the early lactation period ( $0\sim49$  DIM). In case of high yield and late lactation period, the SCK prevalence was similar (about 27%), while it was 12.5% in the dry period (Table 2). CK during the early lactation and mid lactation period was observed to be 9.5% and 2%, respectively, while it was not observed during the high lactation, late lactation and dry period.

#### **DISCUSSION**

The prevalence of SCK in dairy cattle was investigated at herd level and in different lactation stages. The prevalence of SCK describes what proportion of the animals tested have SCK at the time of testing and can be used for herd monitoring over time, as well as an outcome indicator for changes in dry or fresh cow management. Determination of the level of BHBA in blood is considered as "gold standard" for identifying SCK and CK. A cutoff value of 1.2 to 2.9 mmol/l of BHBA in blood samples is used to distinguish between cows with and without SCK (Duffield et al, 1998; Asl et al, 2011; Seifi et al, 2011). The apparent prevalence of SCK in a population will vary with the cut point used for defining SCK.

The prevalence of SCK was develop from early lactation period in this study and this results is also similar in Holstein cattle at United States (McArt et al, 2012) because at the beginning of lactation, animals are faced with a sudden and drastic increase in energy demand. This demand is coupled with decreased feed intake and leads negative energy balance. Under these conditions, fat is mobilized from body stores in the form of non-esterified fatty acids (NEFA) to meet these energy requirements. During early lactation, homeorhesis proc-

esses also facilitate breakdown of body stores of fat and protein in excess of what would be allowed based on homeostatic regulation. This situation leads to a period of insulin resistance, which is nearly universal in early lactation animals. Milk production requires large amounts of glucose; however, because ruminants absorb only limited amounts of glucose from their diet, gluconeogenesis is required to meet this need (Herdt, 2000). This process is generally diminished in animals affected by ketosis in early lactation, which leads to hypoglycemia.

The prevalence of CK in the early lactation period was high by 9.5% although the prevalence of SCK (9.5%) in the early lactation period was lower than other lactation periods (Table 2). It would be explained that SCK was changed into CK due to the negative energy balance in the early stage of lactation period as the milk production rapidly increased during this period. Interestingly, farm producers did not recognize CK until the cattle were examined for blood ketone bodies in the present study. This emphasizes the importance of periodic ketone bodies screening for early stage detection of metabolic disease from blood, urine or milk sample based on their physical status and lactation period.

Several factors related to the nutritional management and genetic aspects could cause SCK (Uribe et al, 1995). Body condition score (BCS) at calving and the rate of BCS loss after calving contribute to the probability of metabolic diseases. Interaction between calving BCS (precalving level of feeding) and postcalving level of feeding on circulating ketone-body concentrations; plasma concentrations of BHBA were between 50% and 100% greater in cows that calved at BCS 3.0 and underwent a feed restriction postpartum than in cows that calved at BCS 2.75 and underwent the same feed restriction (Roche, 2007). These data represents the probability of ketosis was double with only a 0.25 BCS increase (Roche, 2007). Another study depicted that a

doubling of the risk of ketosis in dairy cows with a calving BCS of greater than 3.5 compared with those calving at BCS 3.25 (Duffield et al, 1998). Cows with excessive body condition (BCS  $\geq$ 3.5 on a 1 to 5 scale) during the transition period likely have a greater rate and extent of lipolysis than cows with lower BCS because they have a larger mass of adipose tissue that can be mobilized, and these prolonged lipolysis is associated with the formation of ketone bodies (Hotamisligil et al, 1993).

The results indicated that Herd-level prevalence of SCK ranged from 0 to 57.1%. Additionally, 0 to 60% SCK prevalence was observed at the herd level at  $7\sim$ 12 days after calving, while 0 to 24.4% SCK was observed at 35~40 days after calving (Compton et al, 2014). The higher prevalence of SCK at  $7 \sim 12$  days compared to 35~40 days post-calving suggests its categorization is more likely to be type II ketosis (Cook et al, 2006), which is mainly caused by excessive negative energy balance at pre-calving. The prevalence of SCK in dairy cows to be greatest between 2 and 6 weeks after calving, with peak prevalence occurring 2~4 weeks after calving (Compton et al, 2014). The prevalence of SCK from the high yield lactation period to the late lactation period ranged from 27.5%~36.8%, although CK was rarely seen during these lactation period. Another study reported that SCK prevalence was also varied from 8.9 to 43% in first 2 month of lactation (Duffield et al, 1998; McArt et al, 2012) and 50% of all lactating cows have a tendency to go through the stage of SCK in early lactation (Seifi et al, 2011). SCK is not easily recognized by clinicians and farm producer; however, it throughout the lactation period. This might be due to high milk production oriented farm management and early lactation centralized farm management relative to other lactation periods. The different prevalence of SCK and CK in these studies was considered to be due to variations in feeding management of each herd. Control of feeding management based on regular feed nutrient analysis and metabolic profile testing would reduce the prevalence of SCK.

#### **CONCLUSION**

In conclusion, this is a population-based report of the prevalence of SCK in Chungnam dairy herds. The results of this study inform dairy herd owners and their advisors that SCK is more prevalent in most of the herd and its frequency is vary from one herd to another herd. This might be presuming due to the differences in feed management on each farm and breeding for high milk production. The results of this study were based on a cross sectional study; therefore, further studies will be needed to determine the longitudinal changes of BHBA and cow herd level feeding management.

#### **ACKNOWLEDGEMENTS**

This study was supported by the Cooperative Research Program for Agriculture Science and Technology Development (PJ0126704), Rural Development Administration and Sunchon National University Research Fund in 2016, Republic of Korea.

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