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# The effect of subclinical ketosis on activity at estrus and reproductive performance in dairy cattle

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## ABSTRACT

Our aims were to investigate the influence of subclinical ketosis (SCK) on physical activity at estrus using a neck accelerometer device and on future reproductive performance. Two hundred three Holstein-Friesian cows were studied on 3 dairy farms in Northwest England between September 2013 and March 2014. Seventeen percent (35 of 203) of the enrolled cows were affected with SCK between 7 and 21 d in milk, defined as a blood β-hydroxybutyrate concentration of 1.2 to 2.9 mmol/L. Time to event analyses and multivariable regression analyses were used to assess the effect of SCK on reproductive performance and activity at estrus. The SCK cows exhibited a lower peak activity (measured as the number of standard deviations above mean activity) and shorter duration in activity clusters associated with first estrus and first insemination postpartum, compared with non-SCK cows. Peak activity and cluster duration associated with the insemination that led to a pregnancy were not different between SCK and non-SCK cows. Calving to first estrus, calving to first insemination, and calving to pregnancy intervals were prolonged in SCK cows. First insemination was 4.3 times (95% confidence interval = 1.6 to 15.0) less likely to be successful in SCK cows compared with non-SCK cows. Adjusted mean number of inseminations per pregnancy was 2.8 for SCK cows and 2.0 for non-SCK cows. The current study confirms the long-lasting effects of SCK on reproductive efficiency. Furthermore, it is indicated that physical activity around estrus is reduced by SCK in early lactation, but this negative effect appears to diminish as cows progress through lactation.

**Key words:** subclinical ketosis, β-hydroxybutyrate, estrus activity

## INTRODUCTION

In early lactation, all dairy cattle undergo a period of negative energy balance (**NEB**), metabolic stress, and a degree of body condition loss due to mobilization of body reserves in response to increased energy requirements for lactogenesis (Drackley, 1999; Herdt, 2000). A delayed increase in DMI, genetic selection for greater milk production, and inappropriate diets can further augment the duration and magnitude of NEB (van Arendonk et al., 1991; Dechow et al., 2003; Ospina et al., 2010). This can potentially lead to development of ketosis, elevated levels of ketone bodies, such as acetone, acetoacetate, and BHB, found in body fluids (Enjalbert et al., 2001; Geishauser et al., 2001). Subclinical ketosis (**SCK**) is defined as high blood concentrations of ketone bodies without the signs that accompany clinical ketosis (Andersson, 1988).

More than 90% of SCK cases occur during the first and second months postpartum, with the former containing the peak prevalence (Duffield et al., 1997; Suthar et al., 2013). In the first 65 DIM, the prevalence of SCK ranged from 7 to 34%, with considerable between herd and study variation (Duffield et al., 2009; McArt et al., 2012; Suthar et al., 2013). The gold standard diagnostic test for SCK is the measurement of BHB in serum, plasma, or whole blood, as it is more stable than acetone or acetoacetate (Duffield et al., 1998; Oetzel, 2004). It is considered normal to have increased ketone bodies due to the natural metabolic response to the increase in energy demand in early lactation. However, postpartum blood concentrations of BHB above certain cut-off levels have been associated with poor reproductive performance, reduced milk yield, and increased risk of displaced abomasum (McArt et al., 2013). A blood BHB of 1.2 to 2.9 mmol/L has been described to identify cows with SCK and values ≥3.0 mmol/L indicate clinical ketosis (Oetzel, 2004; Duffield et al., 2009).

The effect of NEB during early lactation on later reproductive performance is well documented, acting via disruption of the hypothalamus-pituitary-ovary axis

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(Butler, 2003). Both the duration and magnitude of NEB have been associated with increased concentrations of growth hormone and decreased concentrations of insulin and IGF; directly reducing follicular competence and its response to circulating gonadotrophins (Lucy, 2001; Butler, 2003). Furthermore, NEB has been linked with delaying and reducing the magnitude of the LH surge, resulting in delayed resumption of luteal activity, increased incidence of cystic ovarian disease, and a lower probability of pregnancy to first insemination (Opsomer et al., 2000; Ospina et al., 2010; McArt et al., 2012).

In the modern high-yielding dairy cow, estrus detection and thus submission rates to insemination have decreased (Dobson et al., 2007). This is attributed to shorter periods of estrus with fewer behavioral signs exhibited together with a lower serum estradiol concentration, the major stimulus for estrus behavior (Lopez et al., 2004). Lameness, high SCC, low BCS, and high milk production can have detrimental effects on dairy cow fertility, reducing the release of GnRH, thus resulting in reduced LH pulsatility, which depresses estradiol production and disrupts estrus behavior (Dobson et al., 2007). However, the negative effect of NEB on estrus expression has not been investigated; it has been postulated that NEB leads to reduced pre-ovulatory estradiol concentrations, resulting in poor estrus expression (Lucy, 2000).

Increased activity has been shown to be associated with periods of estrus; activity monitors (accelerometers or pedometers) have recorded increases from 200 to 400% in animals exhibiting primary and secondary signs of estrus (Nebel et al., 2000; Firk et al., 2002). Many studies have identified that activity monitors with or without visual observation can accurately identify cows in estrus (Holman et al., 2011; Neves et al., 2012; Fricke et al., 2014). The main objective of the present study was to compare the physical activity at estrus, measured by neck accelerometer devices (Heatime, SCR, Netanya, Israel), of cows with SCK in early lactation to that of cows not affected with SCK in early lactation. A secondary objective was to compare the reproductive performance of SCK cows to that of non-SCK cows.

## MATERIALS AND METHODS

The study was performed under UK Animals (Scientific Procedures) Act 1986 project license (PIL 40/10876) for work on living animals and with the approval of the University of Liverpool Ethical Review Process. Assuming a mean calving to conception interval of 90 d, a 35-d SD, and a SCK prevalence of 20%, it was estimated that 185 cows would be adequate to detect a 20-d difference between SCK and non-SCK cows using an $\alpha$ of 0.05 and 80% power.

A prospective cohort study design and 203 Holstein-Friesian cows on 3 commercial dairy farms in northwest England were used. The study was conducted between September 2013 and March 2014. Herd size ranged from 190 to 350 cows with a mean 305-d milk yield of 9,786 kg (ranging from 9,846 to 10,136 kg). All 3 farms operated an all-year-round calving pattern, and cows were milked twice daily. The early lactation cows that were eligible for the study were housed in free stalls throughout the year and fed a partial mixed ration supplemented with in-parlor concentrate feeding according to milk yield.

Cows ranging from first to eighth parity were enrolled into the study. For analysis purposes, cows were subsequently categorized in 3 parity groups: group 1 for primiparous animals, group 2 for second-parity animals, and group 3 for animals in their third or greater parity. Eligible cows were between 7 and 21 DIM with no previous episodes of lameness or mastitis since calving. Each farm was visited weekly whereby the cows' reproductive tracts were examined at the time of enrollment by palpation per vagina, and rectal ultrasound using a 7.5-MHz linear array rectal probe (Easiscan 3, BCF Technology, Bellshill, UK). The incidence of metritis and vulval discharge (**VLD**) as described according to Sheldon et al. (2006) and the presence and size of any ovarian structures were recorded. The reproductive tracts of all the cows were again examined at 21 to 28 DIM with the addition of ovarian structures being noted and measured.

At enrollment, a single blood sample was collected by coccygeal venipuncture into lithium heparin tubes, and immediately analyzed for BHB using the Optium Xceed BHBA meter (Abbott, Maidenhead, UK) according to the manufacturer's instructions. This meter has been validated for BHB measurements in bovine blood samples (Iwersen et al., 2009). Cows that were found to have blood BHB concentration from 1.2 to 2.9 mmol/L were considered to have SCK (Oetzel, 2004). These cows were not treated and farmers were blinded to our measurements. However, cows found to have blood BHB concentration $\geq$3 mmol/L were considered to be clinically affected, were treated per farm protocol, and were not enrolled in our study.

Each cow was fitted with a neck accelerometer collar at enrollment. Mean individual cow activity was monitored and downloaded via infrared telemetry located in the milking parlor in 2-h blocks to the Heatime control box. The mean activity of an individual cow was generated from the equivalent 2-hourly block from the previous 8 d. The intervention level to indicate an activity cluster (potential estrus episode) was set at