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

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We investigated the impact of subclinical ketosis on future reproductive performance and the intensity of estrus behavior as measured by a commercially available neck accelerometer device. Cows with subclinical ketosis exhibited a lower peak activity and shorter activity cluster duration associated with first estrus and first insemination postpartum, compared to non-affected cows. This suggests that subclinically ketotic animals do not exhibit as great an increase in activity and are less likely to trigger accelerometer based activity monitoring systems comparing to non ketotic counterparts. Calving to first estrus, calving to first insemination and calving to pregnancy intervals were prolonged in SCK cows.

SUBCLINICAL KETOSIS AND ESTRUS BEHAVIOR

The impact 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 and three Holstein-Friesian cows were studied on three dairy farms in Northwest England between September 2013 and March 2014. Seventeen per cent (35 of 203) of the enrolled cows were affected with SCK between 7 and 21 days in milk (DIM), defined as a blood β-hydroxybutyrate (BHBA) concentration of 1.2 to 2.9 mmol/L. Time to event analyses and multivariable regression analyses were used to assess the impact 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 post-partum, compared to 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 to 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 (SCK), β-hydroxybutyrate (BHBA), 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 mobilisation of body reserves in response to increased energy requirements for lactogenesis (Drackley, 1999; Herdt, 2000). A delayed increase in dry matter intake (**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; Opsina et al. 2010). This can potentially lead to development of ketosis, elevated levels of ketone bodies, such as acetone, acetoacetate and BHBA, found in body fluids (Geishauser et al., 2000; Enjalbert et al., 2001). Subclinical ketosis (SCK) is defined as high blood concentrations of ketone bodies without the signs that accompany clinical ketosis (Anderson, 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 BHBA 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, post-partum blood concentrations of BHBA 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 BHBA 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 (Butler, 2003). Both the duration and magnitude of NEB have been associated with increased concentrations of growth hormone and decreased concentrations of insulin and insulin like growth factor; 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 somatic cell count, low body condition score 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 impact 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 licence (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 days, 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 α of 0.05 and 80% power.

A prospective cohort study design and 203 Holstein-Friesian cows on three 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-day milk yield of 9,786 kg (ranging from 9,846 to 10,136 kg). All three 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-parlour concentrate feeding according to milk yield.

Cows ranging from first to eighth parity were enrolled into the study. For analysis purposes cows were subsequently categorised in three 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 vaginam* and rectal ultrasound using a 7.5MHz 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 - 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 analysed for BHBA using the Optium Xceed BHBA meter (Abbott, UK) according to the manufacturer's instructions. This meter has been validated for BHBA measurements in bovine blood samples (Iwersen et al., 2009). Cows that were found to have blood BHBA 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 BHBA concentration ≥ 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 parlour in two-hour blocks to the Heatime control box. The mean activity of an individual cow was generated from the equivalent two-hourly block from the previous eight days. The intervention level to indicate an activity cluster (potential estrus episode) was set at the manufacturer's default setting of a value ≥ 5 SD above the mean weighted activity for a minimum of 2 consecutive 2 hour blocks. Peak activity was measured in SD above the mean activity but a maximum of 15 SD could be recorded by the software version supplied (PC Heatime Monitor v4.51). The duration of each activity cluster was measured in hours and defined as the time interval between the first and last 2 hour period of increased activity above 5 SD.

Artificial insemination was conducted twice daily (a.m. and p.m.) by each farm's trained inseminator, with cows receiving a single AI using non-sexed semen from Holstein-Friesian sires. Each farm used their standard criteria for estrus identification with detection being primarily observational supplemented with detection of increased activity. No farmer would inseminate cows solely on Heatime data. Each farm's inseminator was given a data capture form for each enrolled cow to record primary (standing to be mounted) and secondary (mounting other cows, restlessness, mucus discharge, sniffing and licking the vulva of other cows) signs of estrus behavior and any activity cluster within 24 hours. Production and fertility data as well as the incidence of retained fetal membranes (**RFM**), information regarding dystocia, and information regarding sires' Predicted Transmitting Ability (**PTA**) for milk yield were collected via The Cattle Information Services milk recording organisation (Rickmansworth, UK). Heatime data were downloaded at monthly intervals from the control box using the accompanying software to a personal computer. All cows were monitored until either removed from the herd (culled or died), confirmed pregnant or the end of the study period had been surpassed, whichever occurred first. Pregnancy diagnosis occurred from four weeks post AI using transrectal ultrasonagraphy with a positive diagnosis based on the presence of an embryo or fetus surrounded by clear amniotic fluid with a detectable heartbeat.

Statistical Analysis

Data were analysed using JMP Pro 11 (SAS Institute Inc., Cary, NC). Mixed effects multivariable regression models were built in order to assess the association between SCK and each activity cluster duration or peak activity for the first estrus, estrus that led to the first insemination, and estrus that led to pregnancy. Activity cluster duration and SD above mean peak activity were continuous outcome variables. The following independent variables were entered into the model as fixed effects: diagnosis of SCK at 7 to 21 DIM (0/1), a categorical variable characterizing each evaluated event (1 = first observed estrus, 2 = estrus that led to the first insemination, 3 = estrus that led to pregnancy), parity (groups 1 to 3), sire PTA for milk yield, dystocia, RFM, metritis, and VLD. The interaction between SCK and each event (1 = first observed estrus, 2 = estrus that led to the first insemination, 3 = estrus that led to pregnancy) was also offered in the models. Additionally, the random effect of the cow nested within farm was fitted in the models. Variables were removed from the models manually in a stepwise manner and only variables with $P < 0.05$ were kept in the final model. The Akaike's information criterion (**AIC**) was used to evaluate models' goodness of fit.

Cox proportional hazard analysis with right censoring was originally used in order to explore the effect of SCK on the intervals from calving to first observed estrus, from calving to first insemination and from calving to pregnancy. Potential confounding variables offered to these models were: parity, RFM, farm, dystocia, metritis and VLD. None of these confounding variables were found to be important ($P > 0.05$) with the exception of an effect of metritis on the interval from calving to pregnancy. However, removing metritis from the model did not change the hazard ratio estimates for SCK. In addition, Cox proportional hazard analysis exploring the effect of SCK on the interval from calving to pregnancy suggested a significant SCK with parity interaction. Therefore simpler, Kaplan-Meier time to event analyses with right censoring were eventually used to explore the effect of SCK on the intervals from calving to first observed estrus, from calving to first insemination and from calving to pregnancy. Analysis for the effect of SCK on the interval from calving to pregnancy was conducted for each different parity group separately.

Multivariable logistic regression models were built in order to assess the association between SCK and pregnancy risk at the first AI (0 = unsuccessful, 1 = successful), presence or absence of VLD, or the presence or absence of a CL at 21 - 28 DIM. A multivariable linear regression model was built in order to assess the association between SCK and number of inseminations per pregnancy. The following independent variables were offered to all these models: diagnosis of SCK (0/1), farm, parity, dystocia, RFM, metritis. Presence or absence of VLD was offered to all the models except for the model that had VLD as an outcome variable. Variables were removed from the models manually in a stepwise manner and only variables with $P < 0.05$ were kept in the final models. The AIC was used to evaluate models' goodness of fit.

RESULTS

Descriptive characteristics of enrolled cows and SCK prevalence by herd are presented in Table 1. Twelve cows were removed from their herd within the first 28 days DIM and were only included in the time to event analyses. Reasons for early exiting were: death (3), displaced abomasum (4), mastitis (4) or unknown (1). A further 11 cows were removed from their herd later in the study (greater than 28 days DIM) due to non-reproductive reasons of mastitis (4), lameness (5), displaced abomasum (1), or death (1); their data were included in the time to event analyses, and in the estrus behavior analysis until the time of their exit.

Overall, subclinically ketotic cows, compared to non-SCK cows, were active for shorter periods of time (least square mean ± SE of 10.7 ± 0.7 and 12.5 ± 0.3 h respectively, $P = 0.02$) and had a lower peak activity at estrus (least square mean ± SE of 9.3 ± 0.5 and 14.3 ± 0.2 SD increase at mean activity respectively, $P < 0.001$). The interaction between SCK and each different event (first observed estrus, estrus that led to the first insemination, estrus that led to pregnancy) suggested that SCK cows exhibited lower peak activity and shorter duration in activity clusters associated with first observed estrus compared to non-SCK cows. Additionally, SCK cows exhibited lower peak activity in activity clusters associated with the estrus that led to the first insemination compared to non-SCK cows. The least square means for cluster duration and peak activity in non-SCK and SCK cows associated with first observed estrus, estrus leading to first insemination and estrus leading to pregnancy are presented in Figure 1.

Kaplan-Meier time to event analysis with right censoring showed that cows with SCK identified at 7 to 21 DIM had a delayed interval from calving to first observed estrus compared with non-SCK cows, Figure 2. In addition, the calving to first insemination interval and the calving to pregnancy interval were prolonged in SCK cows, Figure 3 and Figure 4A, respectively. Kaplan-Meier time to event analysis exploring the effect of SCK on the interval from calving to pregnancy was also conducted for each different parity group separately. Subclinical ketosis did not have an effect on the interval from calving to pregnancy in primiparous cows (median of 98 and 108 for non-SCK cows and SCK cows respectively, $P = 0.69$). However, the interval from calving to pregnancy was prolonged in SCK second parity cows, and in SCK cows in their third or greater parity, Figure 4B and Figure 4C, respectively.

By the end of March 2014, 180 of the animals (88% of the non-SCK cows and 91% of the SCK cows) had been inseminated and 168 were diagnosed as pregnant (95% of non-SCK cows and 84% of SCK cows that were inseminated). First insemination was 4.3 times (95% CI = 1.6 to 15.0) less likely to be successful in SCK cows compared to non-SCK cows. All the other variables examined were not found to be associated with pregnancy risk at first insemination.

Adjusted mean number of inseminations per pregnancy (± SE) was 2.8 ± 0.3 for SCK cows and 2.0 ± 0.1 for non-affected with SCK ones ($P < 0.05$). Dystocia was found to affect number of inseminations per pregnancy with adjusted mean number of inseminations per pregnancy (± SE) being 2.7 ± 0.3 for cows that had dystocia comparing to 2.0 ± 0.1 for cows that did not have dystocia. Presence or absence of VLD, and the presence or absence of a CL at 21 - 28 DIM were not associated with SCK.

DISCUSSION

To the best of our knowledge, this is the first study to show that SCK is associated with reduced intensity and duration of estrus activity. Itle et al. (2014) recently showed differences in standing behavior in the week before and on the day of calving between clinically ketotic and healthy cows, but an effect of SCK on estrus behavior was not addressed.

The prevalence of SCK across the 3 study herds was 17%, it being at the mid to lower end of the range published in the literature (Oetzel, 2004; Macrae et al., 2012; McArt et al., 2012). Higher prevalences are routinely described in higher yielding dairy herds from North America; these animals may have a greater genetic potential for high milk production that can further exacerbate the degree of NEB and lead to SCK in early lactation. Some studies also use a lower blood BHBA threshold to define SCK and sample cows in the first week postpartum; both of which could increase prevalence estimates (Macrae et al., 2012; McArt et al., 2012; McArt et al., 2013). A dairy cow has the potential to develop and resolve a case of SCK within five days, therefore by only testing once during the period between 7 and 21 DIM there is a potential for SCK cases to be underdiagnosed (Valergakis et al., 2012; McArt et al., 2012). Additionally, McArt et al. (2012) described the peak prevalence of SCK at 5 DIM while in the described here study cows were sampled at 7 DIM or later, similarly to other previously published studies (Berge and Vertenten, 2014; Vanholder et al., 2015). Ideally, we would have sampled the studied animals repeatedly during the first month after calving to decrease the chances of misclassification bias. Unfortunately, this was not possible due to time and budget limitations. However, several studies have followed a similar sampling regime to show useful information regarding SCK risk factors or SCK effects on health and reproductive performance (Ospina et al., 2010; Suthar et al., 2013; Vanholder et al., 2015). It should also be stated that misclassification would bias our results towards the null hypothesis and in that case presented results could be considered conservative with the true differences being even greater than what we report here.

Cluster duration and mean peak activity increase in non-SCK cows were comparable throughout the study and there were no differences as lactation progressed. The mean duration of all increased activity clusters in non-SCK is comparable to the mean durations reported for cows monitored for estrus by visual observation of estrus behavior (Roelofs et al., 2005; Dobson et al., 2007) and estrus quantified by an accelerator system (Roelofs et al., 2005; Aungier et al., 2012; Valenza et al., 2012). The present study shows that SCK in early lactation has a profound effect on cluster duration and mean peak activity at first observed estrus and estrus leading to first insemination. This negative effect appears to diminish as cows progress through lactation. The cluster duration and mean peak activity increase in the estrus leading to pregnancy in SCK cows were comparable to that of non-SCK cows.

In addition, estrus cluster duration and mean peak activity increase in SCK cows did not seem to improve until over 100 DIM; the underlying mechanisms behind this are not fully clear. One interpretation is that for the cow to establish a pregnancy the physiology of the animal needs to progress to a state of positive energy balance whereby DMI is matching metabolic demands. Only then can the cow fully express behavioral estrus, evident as normal cluster duration and mean peak activity increase. It is likely this return in normal estrus behavior is directly related to estradiol production; a recent study by Morris et al. (2011) reported that lower circulating estradiol levels in lame cows was affecting behavioral expression of estrus. This may be further augmented by a reduction in insulin like growth factor -1 and inadequate progesterone priming, without which the hypothalamus has diminished responsiveness to estradiol (Dobson et al., 2007); both of these conditions have been linked with NEB (Opsomer et al., 2000; Reist et al., 2000; Butler, 2003). It has also been shown that developing follicles are compromised when exposed to similar concentrations of glucose, nonesterified fatty acids and BHBA experienced by dairy cows in NEB with these follicles exhibiting reduced sensitivity to circulating LH leading to reduced estradiol production. If the compromised follicles reach dominance, ovulate and are fertilised they exhibit a reduced ability to cleave into a blastocyst and when coupled with poor CL formation and function, thus increasing the chance of embryo death (Leroy et al., 2006). Further research is required to fully understand which body systems are involved and how they interact to reduce physical activity at estrus in SCK cows.

The current study confirms the negative effects of SCK on reproductive efficiency shown previously by other studies (Butler, 2003; Walsh et al., 2007; Ospina et al., 2010; McArt et al. 2012). Studies from North America use a large repertoire of ovulation synchronisation protocols and typically only demonstrate the effects of SCK on reproductive parameters such as pregnancy risk in response to first insemination and the likelihood of removal from the herd in the first 30 DIM (Ospina et al., 2010; Chapinal et al., 2012; McArt et al., 2012). Few smaller scale studies (not utilising synchronisation protocols) have also demonstrated an association between SCK in early lactation and reduced reproductive efficiency (Reist et al. 2000; Cook et al., 2001; Walsh et al., 2007). In the present study no synchronisation protocols were used and the calving to first observed estrus and calving to first insemination intervals were both extended in cows suffering from SCK. This is comparable to the findings by Reist et al. (2000), where it was shown that cows in NEB and with increased blood concentrations of ketone bodies in early lactation had an increased calving to first service interval of 10 days compared to their non-affected counterparts. Ospina et al. (2010) highlighted a reduced pregnancy risk in cows with SCK and Walsh et al. (2007) established that SCK cows were 20 to 50% less likely to be pregnant at first insemination; these findings are in concurrence with our study's findings. We also report here that SCK cows take longer to establish a viable pregnancy than non-SCK cows. This is in agreement with Walsh et al. (2007) where an increased calving to pregnancy interval in SCK cows was reported. Previous studies have attributed this reduced reproductive performance to a delay in return to cyclicity due to reduced GnRH and LH pulsatility which is essential for follicular development and ovulation (Butler, 2003). Interestingly, SCK in our study had a more profound effect on the interval from calving to pregnancy in multiparous cows, with SCK cows in their third or greater parity requiring 63 more days to establish a pregnancy comparing to non-affected third or greater parity cows.

CONCLUSION

The current study confirms the long-lasting effects of SCK on reproductive efficiency. Calving to first estrus, calving to first insemination and calving to pregnancy intervals were prolonged in SCK cows. Furthermore, it is indicated that physical activity around estrus can also be reduced by SCK in early lactation. The SCK cows exhibited a lower peak activity (measured as the number of standard deviations above mean activity) and shorter cluster duration in activity clusters associated with first estrus and first insemination post-partum, compared to non-SCK cows.

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Table 1. Descriptive statistics of 305 days milk yield and prevalence of subclinical ketosis (SCK) by herd. Number of cows in each different parity group (Group 1 for primiparous animals, Group 2 for second parity animals, and Group 3 for animals in their third or greater parity) by herd is also provided

Herd	Milk Yield ± SD	n	Parity Group (n)			SCK prevalence (%)
			1	2	3	
1	9,733 ± 1,626	56	20	12	24	20
2	10,106 ± 1,079	74	25	12	37	11
3	10,518 ± 1,680	73	23	14	36	22
Total		203	68	38	97	
Overall Mean	10,128 ± 1,478					17

Milk Yield = mean 305 days milk yield (kg)

Figure 1. The least square means (± 95% CI) for 191 cows from three herds are reported for increased activity cluster duration (A) and peak activity increase (B) in subclinically ketotic (SCK, n = 34) and Non-SCK (n = 157) cows associated with first recorded estrus, estrus leading to first insemination and estrus leading to pregnancy. Least square means not connected by the same letter are significantly (P < 0.05) different (Tukey HSD test). One hundred and ninety one cows from three different herds were included in this analysis.

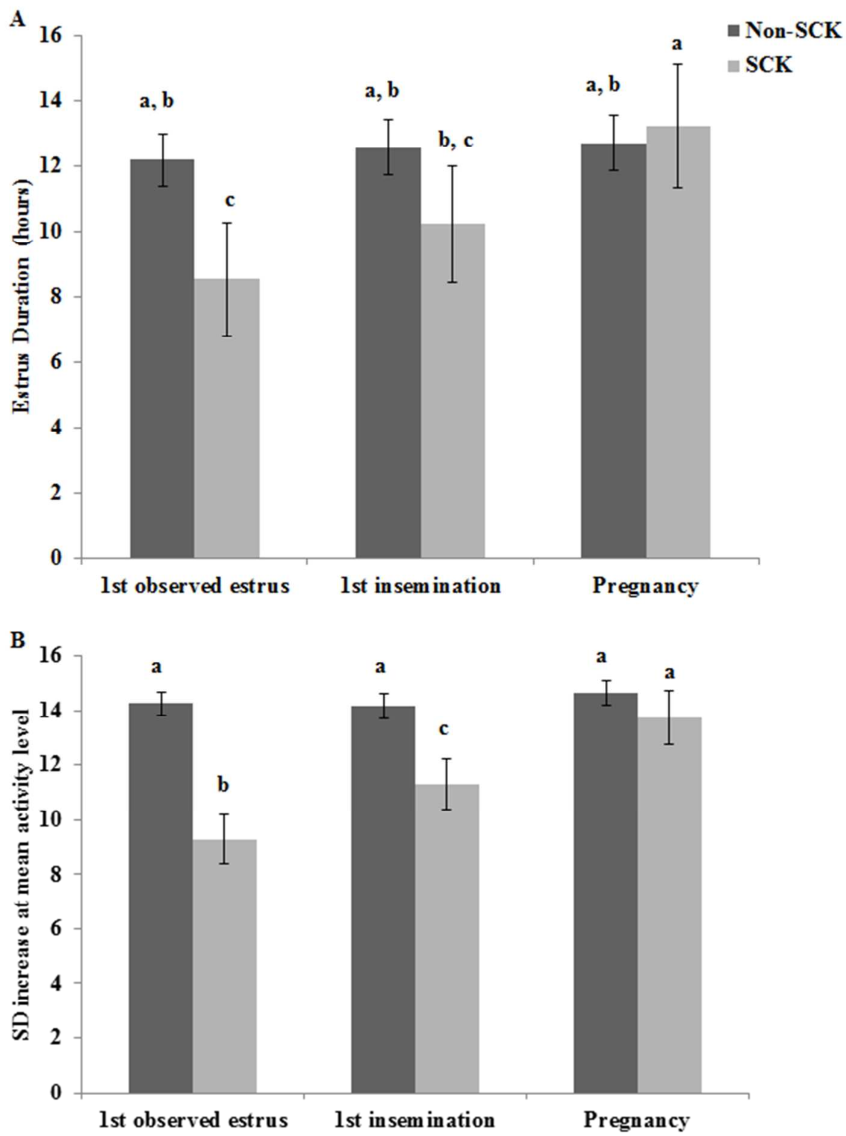


Figure 2. Kaplan-Meier time to event plot demonstrating the effect of subclinical ketosis (SCK) on the number of days from calving to first observed estrus event for 203 cows from three herds. Median time to event was 51 and 42 days for SCK (dashed lined, n = 35) and non-SCK (solid line, n = 168) cows respectively ($P = 0.01$).

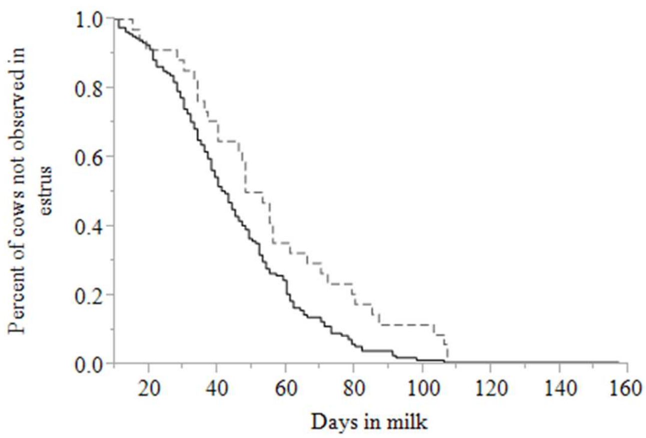


Figure 3. Kaplan-Meier time to event plot demonstrating the effect of subclinical ketosis (SCK) on the number of days from calving to first insemination for 203 cows from three herds. Median time to event was 71 and 61 days for SCK (dashed lined, n = 35) and non-SCK (solid line, n = 168) cows respectively ($P = 0.02$).

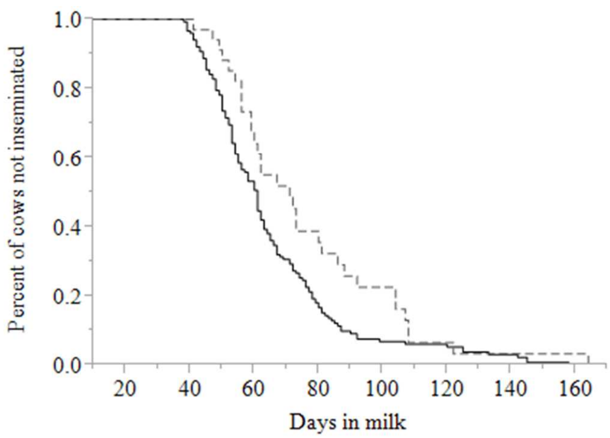
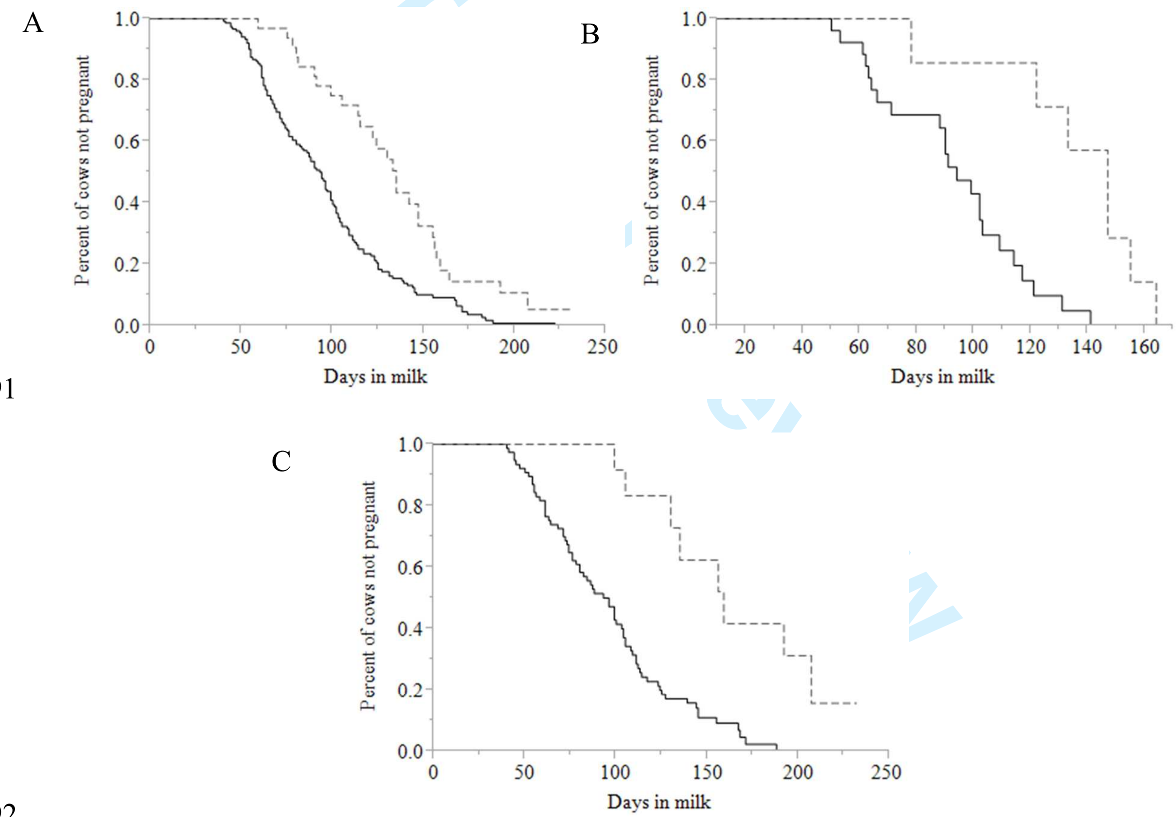


Figure 4. Kaplan-Meier time to event plots demonstrating the effect of subclinical ketosis (SCK) on the number of days from calving to pregnancy for A) all studied cows (n = 203) from three herds, B) 38 second parity cows from three herds, and C) 97 third and greater parity cows form three herds. Dashed lines represent SCK cows and solid lines non-SCK cows. Median time to event was: A) 93 and 135 days for non-SCK and SCK cows respectively, ($P < 0.001$), B) 94 and 147 for non-SCK second parity cows and SCK second parity cows respectively, ($P < 0.001$), and C) 96 and 159 days for non-SCK third and greater parity cows and SCK third and greater parity cows respectively ($P < 0.001$).



Subject: JDS-15-10154.R2 The impact of subclinical ketosis on activity at estrus and reproductive performance in dairy cattle

05-Feb-2016

Dear Dr. Georgios Oikonomou:

Experts in the field have carefully reviewed your manuscript and I am happy to report that they recommended only minor revisions of your attention before it can be accepted for publication. Accordingly, I invite you to respond to the reviewers comments and recommendations. Please make sure to label all changes to facilitae tracing back what you actually changed. A decision on acceptability of your manuscript will be made only after the revised version has been reevaluated.

Please refer to the current Instructions for Authors(http://www.journalofdairyscience.org/content/inst-auth) when revising your manuscript. A checklist has also been attached to this letter to help you with your revision.

Your revision is due by 18-Mar-2016.

You will be unable to make your revisions to the originally submitted version of the manuscript. Instead, revise your manuscript using a word processing program and save it on your computer. Once the revised manuscript is prepared, you can upload it and submit it through your Author Center.

To upload your revised manuscript, Click this link https://mc.manuscriptcentral.com/jds?URL_MASK=c0fd527aee8c43a4ae9a6f09194a68f9 which will direct you to your author center. Your manuscript number will be appended to denote a revision.

When submitting your revised manuscript, you will be able to respond to the comments made by the reviewer(s) in the box below the decision letter. You can use this space to document any changes you make to the original manuscript. In order to expedite the processing of the revised manuscript, please be as specific as possible in your response to the reviewer(s).

PLEASE PROVIDE YOUR RESPONSES AND(OR) REBUTTAL TO THE REVIEWER'S COMMENTS BY PASTING IN THEIR COMMENTS FOLLOWED BY YOUR RESPONSE.
PREFACE YOUR RESPONSE BY "AU:" AND HIGHLIGHT CHANGES IN YELLOW IN THE REVISION (IF REASONABLE).

PLEASE RESPOND IN A LINE-BY-LINE MANNER SO I CAN CORRELATE EACH OF YOUR RESPONSES TO EACH OF THE REVIEWER'S COMMENTS OR CRITICISMS.

IMPORTANT: Your original files are available to you when you upload your revised manuscript. Please delete any redundant files before completing the submission.

Unless we hear from you about this paper within 6 weeks, we will presume you have WITHDRAWN the manuscript from consideration by the Journal of Dairy Science.

Once again, thank you for submitting your manuscript to the Journal of Dairy Science and I look forward to receiving your revision.

Sincerely,

Dr. Helga Sauerwein
Section Editor, Journal of Dairy Science sauerwein@uni-bonn.de

Reviewer(s)' Comments to Author:
Reviewer: 1

Comments to the Author
General comments:
Good revision – manuscript much improved. I have only minor comments for suggestion:

Line 28: Consider removing the ( ) around "between 7 and 21 days in milk (DIM)" so as to include it in the larger part of the sentence.
AU: Done

Lines 33 and 216: These sentences might read better if the word "cluster" associated with "cluster duration" is removed.
AU: Done

Lines 53 and 54: Since acetone and acetoacetate are only mentioned twice in the manuscript (here and in line 62), I suggest not defining them as Ac and AcAc but rather writing them out both times.
AU: Done

Lines 59 and 60: Consider changing to "considerable between herd and study variation".
AU: Done

Line 89 and throughout: Be mindful about removing the space after a number and before the % sign (here as 400 % should read 400%). This happens quite often throughout the manuscript.
AU: Corrected throughout the manuscript

Lines 152 and 153: Please bold RFM and PTA the first time they are used.
AU: Done

Lines 226-228: Consider removing the results and changing the sentence to read "In addition, the calving to first insemination interval and the calving to pregnancy interval were prolonged in SCK cows, Figure 3 and Figure 4, respectively." These results are already in Figures 3 and 4 and thus do not need to be repeated in the results section.
AU: Done

Lines 231-235: As for the previous comment, please remove the results and refer the reader to Figure 4.
AU: Done

Line 243: Please remove the italics from "< 0.05".
AU: Done

Table 1:
- Even though it is mentioned in the caption, I would put Milk Yield +/- SD as the column title instead of Yield.
- Add "," for milk yields when >999 kg.
- I don't think "n" needs to be described as a footnote.

- Consider Parity Group (n) with subheadings: 1, 2, 3, total

AU: Done

Figures 1-3: Consider rewording first sentence to read (for example using Figure 1) “The least square means (+/- 95% CI) for 191 cows from three herds are reported for increased activity cluster duration (A) and peak activity increase (B) in subclinically ketotic (SCK, n = 34) and non-SCK (n = 157) cows associated with …”

AU: Done

Figure 4: Same comment as above for the first sentence with addition at the end to read “for A) first parity, B) second parity, and C) third and greater parity cows. Dashed lines represent SCK cows and solid lines non-SCK cows.” Then list median time to events for each graph. This will shorten the caption and make it easier to follow.

AU: Done