Prepartum walking and eating activity of dairy cows as possible early indicators of a postpartum ketosis attention on the milk production registration (MPR) in the Netherlands

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Abstract

Ketosis in dairy cattle is defined as an increase in the circulating ketone bodies. It has negative effects on milk production and is a risk factor for many diseases related to the transition period. In the Netherlands, a relatively new method of detecting acetone in milk recently became included in the regular milk production registration (MPR). The objective of this study was to determine if prepartum walking and eating activity of dairy cattle could be used as an early indicator of receiving a ketosis attention on the MPR result form in the first 60 days postpartum. The prepartum walking activity (steps/d) and eating activity (h/d) of respectively 302 and 310 dairy cows, monitored by Nedap SmartTag sensors during the last four weeks prepartum, was analysed. The dairy cows were divided in a ketosis group and a no ketosis group. The means, regression coefficients and residual standard deviations of three prepartum periods (first period d -28 to -15; second period d -14 to -3; third period d -3 to 0) were used as independent variables in logistic regression analysis (backward model reduction). The mean walking activity of the total prepartum period (steps/d ± SD; d -28 to 0) differed significantly between the ketosis group and the no ketosis group ($\bar{x}_{k}$ = 2665 ± 902; $\bar{x}_{no}$ = 3000 ± 819). In logistic regression, the mean (steps/d x1000) of the first period (p = 0.001) and the residual standard deviation of the third period (p = 0.037) were found to be significant model parameters. The odds ratios and the corresponding confidence intervals of the two model parameters were respectively 0.473 [0.299, 0.749] and 1.473 [1.025, 2.119]. In contrast to walking activity, the mean eating activity of the total prepartum period (h/d ± SD; d -28 to 0) did not differ significantly (p = 0.087) between the ketosis and the no ketosis group ($\bar{x}_{k}$ = 5.89 ± 1.29; $\bar{x}_{no}$ = 6.22 ± 1.04). The remaining model parameters in logistic regression were the residual standard variation of the first period (p = 0.055) and the mean of the second period (p = 0.093). The odds ratios were respectively 0.292 [0.083, 1.027] and 0.812 [0.637, 1.035]. With respect to the 95% confidence interval, neither of the remaining parameters of prepartum eating activity had a statistically significant association with postpartum ketosis attentions, although a clear trend was established. The sensitivity and specificity of both prepartum walking and prepartum eating activity are not yet high enough to be useful as early indicators of a ketosis attention postpartum. However, the trends that were established in this study between both prepartum walking and eating activity and postpartum ketosis attentions, might contribute to further research into prevention and control of transition period related diseases such as ketosis.

Introduction

Ketosis is often referred to as a disease of modern high-producing dairy cows. It is reported worldwide with a prevalence that ranges from 7% up to 40%[1-6]. Ketosis in dairy cattle is defined as an increased level of circulating ketone bodies. Ketone bodies include beta-hydroxybutyrate (BHB), acetoacetate and acetone[7]. The increased level of ketone bodies is due to a high demand of glucose for milk production and a parallel insufficient supply of glucose. Hence, ketosis is mainly of importance during early lactation, when a negative energy balance is experienced by most high-producing dairy cows[7-9].

Ketosis can be divided into clinical and subclinical ketosis by the presence of clinical signs. Signs of clinical ketosis (CK) include abrupt loss of appetite, decrease in milk production, and rapid loss of body condition[8]. The majority of the cows are apathetic. Although some cows can become excitable, among other signs of nervous dysfunction[8,10]. However, only a small percentage of the cows with ketosis show clinical signs[11]. In most cows, there are no clinical signs. This condition is often referred to as subclinical ketosis (SCK). It is a condition that, if remaining undetected and untreated, has many negative effects. Not only does it have a negative effect on milk production[8], it is also a risk factor for many diseases related to the transition period. SCK is associated with, among others, an increased risk of fatty liver[12], left displaced abomasum[13] and decreased fertility[14,15]. Hence, the economic consequences of both CK and SCK on the dairy industry vary greatly. The average costs in the Netherlands are estimated at €150 per cow[16].

Ketone bodies are present in blood, urine and milk[9] and several methods exist for detection. Measurements of ketone bodies in blood serum is widely used to determine the degree of ketosis. Varying definitions of ketosis are used, up to 2.6 mmol BHB/ L blood serum, and it is tested up to 65 days in milk[6,9,17-22]. During the past decades, the protein to fat ratio in milk was used to define a cow as having a higher risk of ketosis. Recent studies concluded that this ratio was not very accurate compared to the detection of acetone in milk with a combined method of Fourier transform infrared (FTIR) spectroscopy and chemical analysis (serial testing)[1,22,23]. This method of detecting acetone in milk of individual cows recently became a readily available test in the Netherlands for dairy farmers that are subscribed to the milk production registration (MPR) every 3, 4, 5 or 6 weeks[24]. If a test-day milk sample is tested positive for acetone, the cow receives a 'ketosis attention' on the MPR result form.

The detection of cows with ketosis has already improved. However, it is impractical and economically disadvantageous to collect and assay blood or milk samples. Studies have shown that by measuring walking activity postpartum[25,26], an impression might be gained of the NEB status of individual cows. In the study of Edwards and Tozer (2004) ketosis was detected 7 to 8 days earlier, based on a lower walking activity in early lactation[25]. Not only walking activity has been shown to be correlated with ketosis. The study of Goldhawk *et al.* (2009), among others, showed that changes in feeding behaviour were also correlated with the development of ketosis[27-29]. Social and competitive behaviour at the feed bunk, which subsequently influences feeding behaviour[30], have also been identified as important health factors[27,31]. The question remains, however, whether detection as early as prepartum and, hence, prevention of ketosis postpartum is possible. Walking and eating activity can easily be monitored and possibly contribute to early detection. Early detection should subsequently lead to implementing preventive management adjustments and thereby reducing the chance of developing ketosis.

In the present study, both walking and eating activity of dairy cattle during the last four weeks prepartum have been evaluated as possible early indicators of receiving a ketosis attention on the MPR result form in the first 60 days postpartum.

Material and Methods

Dairy cows from 11 Dutch dairy farms were included in the study. All the dairy farms were subscribed to the milk production registration (MPR). They were freestall barns with different types of beddings. The included dairy farms either had a conventional or an automatic milking system. The dairy farms milked from 110 up to 300 dairy cows. Cows enrolled were late in pregnancy, healthy and were mainly Holstein-Friesian dairy cows. Between August 2015 and May 2016, the activity from these cows was measured by Nedap SmartTag sensors. Two sensors were attached to the cows, one around a front leg (a pedometer) and one around the neck (an accelerometer). The sensors measured the neck and leg movements. The rough data of the movements were converted by Nedap into data of individual cows in number of steps per day (steps/d) and time spent eating in hours per day (h/d).

The dairy farms received result forms of the milk production registrations (MPR) every 3, 4, 5 or 6 weeks, supplied by CRV holding BV. A ketosis attention on the MPR was given to cows of which the test-day milk samples had been tested positive for acetone by a combined method of Fourier transform infrared (FTIR) spectroscopy and chemical analysis. Only the ketosis attentions given in the first 60 d postpartum were included as such in the study and referred to as postpartum ketosis attentions. Since the data of this study were based on the presence of acetone in the milk without measuring for other symptoms, no distinction between clinical and subclinical ketosis was made and the term ketosis was used.

Some of the processed data were incomplete or contained errors in the activity of individual cows. This was mostly due to administrational errors or to the sensors being temporarily out of reach in grazing periods. These data were removed from the dataset. The cows included in the study had either complete walking activity, eating activity, or both, during the period of four weeks prepartum (d -28 to 0; d 0 is the day of calving). The remaining data led to a total of 302 dairy cows of which the walking activity (steps/d) was known and a total of 310 cows of which the eating activity (h/d) was known. Of these cows was checked on the MPR whether they had received a postpartum ketosis attention. The ketosis attention determined whether the cows belonged to the ketosis group (walking activity n_{walk} = 58; eating activity n_{eat} = 49), or the no ketosis group (n_{walk} = 244; n_{eat} = 261).

The total prepartum period of four weeks was split up into three periods, based on the patterns of the mean walking and eating activity of both the ketosis and no ketosis group. The means and regression coefficients of individual cows were determined for each period. Thereafter, the corresponding residual standard deviations were calculated. Differences between the groups were analysed using logistic regression. The means, the regression coefficients and the residual standard deviations of all three periods were used as independent variables to analyse the dependent variable, a postpartum ketosis attention. The walking activity variables were divided by 1000 to be able to interpret the odds ratio. Model parameters were estimated with the maximum likelihood ratio method (backward model reduction). Results were checked visually with deviance residuals. Data analysis was performed with IBM SPSS Statistics 24.

To further evaluate prepartum walking and eating activity as early indicators of a postpartum ketosis attention, the sensitivity and specificity were calculated. If a cow had a mean walking or eating activity below the optimum cut-off point, they were defined as being at risk of a postpartum ketosis attention. The optimum cut-off point was defined as the cut-off point where the sum of the calculated sensitivity (Se) and specificity (Sp) was maximum. The results were calculated for the mean walking and eating activity of the total prepartum period (d -28 to 0) and the first prepartum period (d -28 to -15).

Results

Walking activity

The prepartum period was divided into three periods, based on the pattern of the mean walking activity (steps/d x1000). Figure 1 shows the mean walking activity from d -28 to 14 for both the ketosis group and the no ketosis group (n_k = 58; n_{no} = 244). During the first (d -28 to -15) and second period (d -14 to -3) the mean walking activity of both groups rose slowly (first period $\bar{x}_k$ = 2405, $\bar{x}_{no}$ = 2796; second period $\bar{x}_k$ = 2684, $\bar{x}_{no}$ = 3014). The mean walking activity of the ketosis and the no ketosis group appeared parallel during the first period, while the means started to approach each other during the second period. During the third period (d -2 to 0) the mean walking activity of both groups rose quickly and reached their maximums around calving (ketosis group) and the day after calving (no ketosis group).

Table 1 shows the results of the two-tailed t-test comparing the mean of the ketosis group with the mean of the no ketosis group (d -28 to 0). The results are shown for both walking and eating activity. The mean walking activity of the ketosis group ($\bar{x}_k$ = 2665 ± 902) differed significantly from the mean of the no ketosis group ($\bar{x}_{no}$ = 3000 ± 819), even though the standard deviation of the mean was quite large. The distribution of the mean walking activity is shown in Figure 2. The histogram illustrates that the data were a little positively skewed. In further calculations, the data were accepted as being normally distributed.

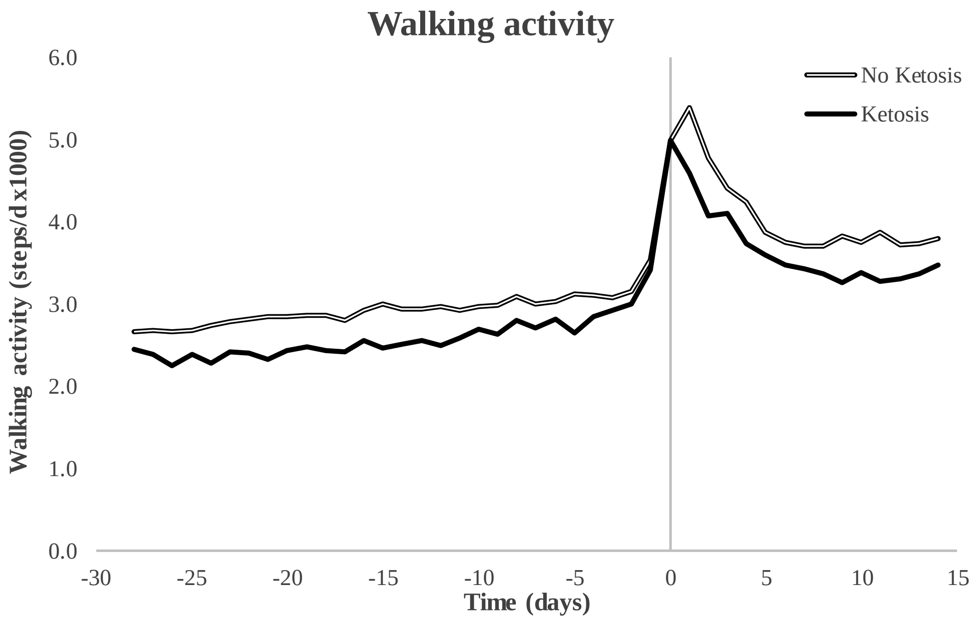

Figure 1. Line graph showing the mean walking activity (steps/d) of the ketosis group (n = 58) and the no ketosis group (n = 244) from d -28 to 14

Table 1. Two-tailed t-test comparing the mean of the total prepartum period (d -28 to 0) of the ketosis group with the mean of the no ketosis group of both walking and eating activity

		N	Mean	SD	Sig.
Walking activity (steps/d x1000)	Ketosis	58	2.67	0.90	0.006
	No Ketosis	244	3.00	0.82	
Eating activity (h/d)	Ketosis	49	5.89	1.29	0.087
	No Ketosis	261	6.22	1.04	

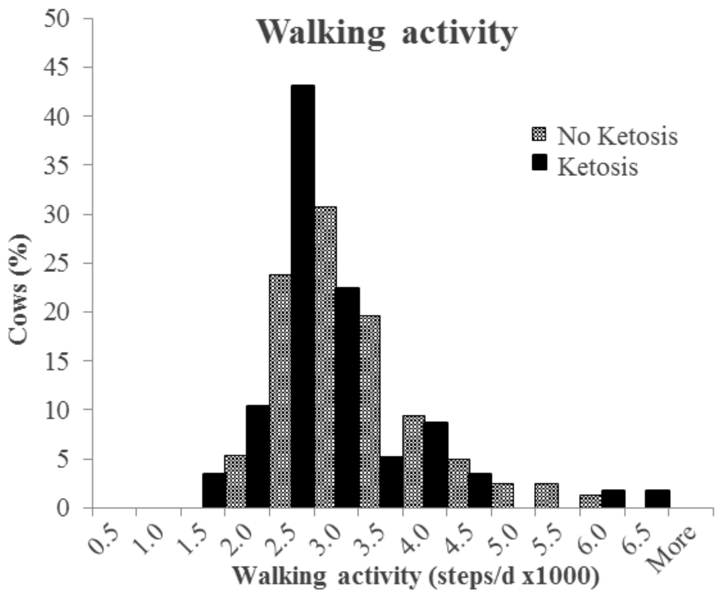

Figure 2. Histogram showing the distribution of the mean walking activity (steps/d x1000) of 302 dairy cows (n_k = 58; n_n = 244) of the total prepartum period (d -28 to 0)

The mean (steps/d x1000) of the first period (p = 0.001) and the residual standard deviation of the third period (p = 0.037) were found to be significant using the maximum likelihood ratio method in logistic regression analysis (backward model reduction). Table 2 shows the results of the analysis. The odds ratio of the mean of the first period was 0.473 with a 95% confidence interval of [0.299, 0.749]. The odds ratio of the residual standard deviation of the third period was 1.473 [1.025, 2.119]. The predicted probability of receiving a postpartum ketosis attention was calculated using the constants (β) from Table 2 in the following formula:

$$\pi = \frac{e^{\beta_0+\beta_1 x_1+\beta_2 x_2}}{1+e^{\beta_0+\beta_1 x_1+\beta_2 x_2}}$$

The value of constant β_0 was provided by the logistic regression analysis (β_0 = 0.135). The results of predicted probabilities are presented in Table 3 for a relevant range of values of x_1 (mean steps/d x1000 of the first period) and x_2 (residual standard variation of the third period). The associations presented by the odds ratios were similar to the association presented in Table 3. An increase in the mean number of steps/d in the first period resulted in a decrease in the predicted probability of receiving a ketosis attention postpartum. On the contrary, an increase in the residual standard variation in the third period resulted in an increase in the predicted probability of receiving a ketosis attention postpartum.

The quality of prepartum walking activity as an early indicator of a postpartum ketosis attention was further evaluated by the sensitivity and specificity. Table 4 presents the results that would be achieved when an early warning, an alert, would be given to every cow that had a mean number of steps/d below the optimum cut-off point. The results were calculated for both the total prepartum period and the first prepartum period. The optimum cut-off point, with the maximum sum of the sensitivity and specificity, of the total prepartum period was determined at 87% (2610 steps/d) of the mean of the no ketosis group ($\bar{x}_{no}$ = 3000 steps/d). This led to 38 cows that got an alert at d 0, the last day of the total prepartum period, out of all 58 cows that received a ketosis attention postpartum (Se = 66%). The optimum cut-off point of the first prepartum period was determined at 90% (2516 steps/d) of the mean of the no ketosis group ($\bar{x}_{no}$ = 2796 steps/d). This resulted in 43 cows (Se = 74%) that got an alert at the end of d -15. On the other hand, of all cows that did not receive a postpartum ketosis attention (n = 244), 167 cows did not get an alert at the end of the total prepartum period (Sp = 68%) and 136 cows did not get an alert at the end of the first period (Sp = 56%).

Table 2. The results of the estimated model parameters when logistic regression analysis (backward model reduction) was used; the results show the two walking activity parameters that are used best to predict the probability of receiving a ketosis attention postpartum

	β^1	Sig.	OR	95% C.I. for OR Lower	95% C.I. for OR Upper
Mean[2] (first period)	-0.748	0.001	0.473	0.299	0.749
Residual standard deviation[2] (third period)	0.387	0.037	1.473	1.025	2.119

[1]Constant; values were used to calculate the predicted probabilities (Table 3)
[2]Values were divided by 1000 to be able to interpret the odds ratio

Table 3. Calculated values of the predicted probability[1] (π) of receiving a ketosis attention postpartum, using varying values for x_1, the mean of the first period (d -28 to -15) and x_2, the residual standard variation of the third period (d -2 to 0)

Residual standard variation of third period (d -2 to 0; x_2)	Mean (steps/d x1000) of first period (x_1): 1	2	3	4	5	6
0	0.351	0.204	0.108	0.054	0.026	0.013
1	0.444	0.274	0.152	0.078	0.038	0.019
2	0.540	0.357	0.208	0.111	0.056	0.027
3	0.634	0.450	0.279	0.155	0.080	0.039
4	0.718	0.547	0.363	0.213	0.113	0.057
5	0.790	0.640	0.457	0.285	0.158	0.082

[1]Formula used to calculate the values of π is: $\pi = \frac{e^{\beta_0+\beta_1 x_1+\beta_2 x_2}}{1+e^{\beta_0+\beta_1 x_1+\beta_2 x_2}}$ with the constants β_i based on the results of the logistic regression analysis (backward model reduction);
β_0 = 0.135 (constant);
β_1 = -0.748 (constant of mean of first period);
β_2 = 0.387 (constant of residual standard variation of third period);
x_1 = value of mean of first period;
x_2 = value of residual standard variation of third period

Table 4. Illustration of the values that would be achieved when an alert would be given to all cows that had a mean walking activity (steps/d) below the cut-off point. The cut-off point was determined at the percentage of the mean of the no ketosis group, where the sum of the sensitivity and specificity was maximum. This led to a cut-off point of 87% of the mean of the no ketosis group of the total prepartum period (2610 steps/d), and 90% of the first period (2516 steps/d).

	Period (days prepartum)					
	Total (d -28 to 0)			First period (d -28 to -15)		
	Ketosis	No Ketosis	Total	Ketosis	No Ketosis	Total
Alert	38	77	115	43	108	151
No alert	20	167	187	15	136	151
Total	58	244	302	58	244	302

Eating activity

Figure 3 shows the mean eating activity (h/d) of all cows during the period from d -28 to 14 (n_k = 49; n_{no} = 261). Like walking activity, the prepartum eating activity (d -28 to 0) was divided into three periods. During the first period (d -28 to -15) the mean eating activity of the ketosis and the no ketosis group appeared parallel. In contrast to walking activity, the mean eating activity of the ketosis and the no ketosis group slowly diverged during the second period (d -14 to -3). During the third period (d -2 to 0) the mean eating activity of both groups quickly rose towards their maximums on d 0.

During the complete prepartum and postpartum period the mean eating activity of the ketosis group was smaller than the mean of the no ketosis group. The peak at d 0 was possibly created by a feature in the system as a result of the cow licking its calf. This movement could have been registered by the Nedap SmartTag Neck sensor as 'eating movement', which was consequently processed into time spent eating. The histogram (Figure 4) shows the distribution of the mean of total prepartum period (d -28 to 0) for both the ketosis and the no ketosis group. It illustrates that the data was normally distributed.

Even though a clear trend was visible from Figure 3, the prepartum eating activity mean of the ketosis group ($\bar{x}_k$ = 5.89 ± 1.29) did not differ significantly of the mean of the no ketosis group ($\bar{x}_{no}$ = 6.22 ± 1.04), as presented in Table 1 (p = 0.087). This p-value is still quite low, however, since it suggests that there is only an 8.7% chance of wrongly rejecting the null hypothesis that prepartum eating activity is not useful as an early indicator of a postpartum ketosis attention.

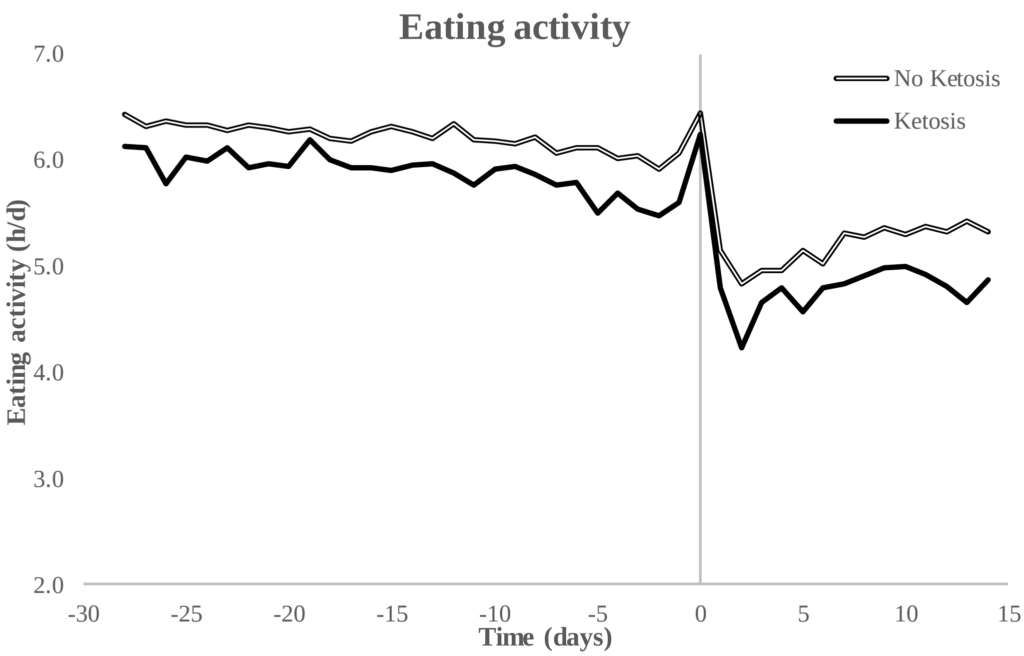

Figure 3. Line graph showing the mean eating activity (h/d) of the ketosis group (n = 49) and the no ketosis group (n = 261) from d -28 to 14

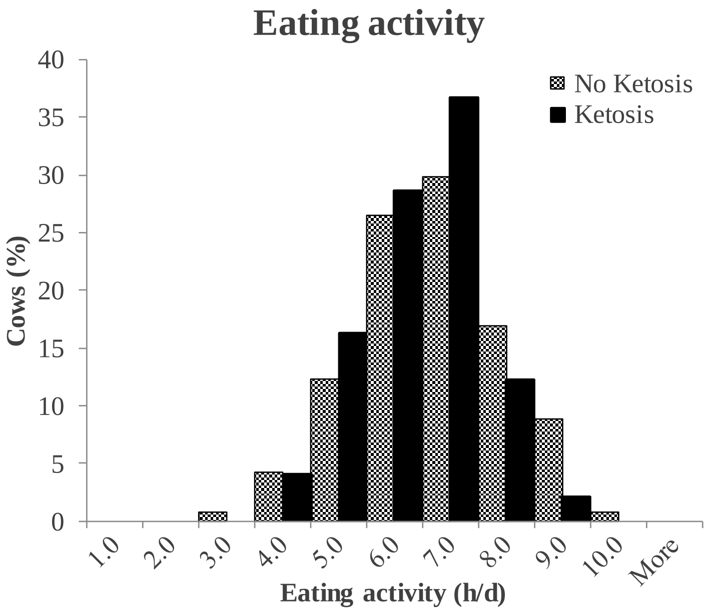

Figure 4. Histogram showing the distribution of the mean eating activity (h/d) of 310 dairy cows (n_k = 49; n_n = 261) of the total prepartum period (d -28 to 0)

The trend between prepartum eating activity (h/d) and a postpartum ketosis attention was analysed using the maximum likelihood ratio method in logistic regression analysis (backward model reduction). The results are shown in Table 5. Of all entered independent variables (the regression coefficient, residual standard variation and mean of the first, second and third period), the residual standard variation of the first period and the mean of the second period remained as parameters in the last model. The results did not appear significant. More importantly, the odds ratios were respectively 0.292 [0.083, 1.027] and 0.812 [0.637, 1.035]. Given the 95% confidence interval, neither of the parameters had a unilateral association with receiving a ketosis attention postpartum.

Eating activity (h/d) was extremely variable among individual cows. The graph in Figure 5 illustrates this extreme variability as the standard deviation of the mean (h/d) of the second period. These largely overlapping standard deviations, among other reasons, are likely to have contributed to the fact that a significant association was not found between prepartum eating activity and a postpartum ketosis attention, even though a trend was established from the data.

Table 6 presents the values that would be achieved when the prepartum eating activity data of the present study were used to detect cows at risk of a postpartum ketosis attention. The optimum cut-off points were determined at 87% of the mean eating activity of the no ketosis group of the total prepartum period ($\bar{x}_{no}$ = 6.22 h/d) and at 94% of the mean eating activity of the first prepartum period ($\bar{x}_{no}$ = 6.30 h/d). If a cow had a mean eating activity below the cut-off point, it suggested that the cow was at risk of a postpartum ketosis attention postpartum and, hence, would lead to an alert being given to that cow at the end of the last day of the period, d 0 and d -15 respectively. The sensitivity (Se) and specificity (Sp) were calculated from the results in Table 6. The optimum cut-off point of the total prepartum period (5.42 h/d) resulted in a sensitivity of 33% and a specificity of 76%. The optimum cut-off point of the first period (5.92 h/d) resulted in a sensitivity of 49% and a specificity of 61%.

Table 5. The results of the estimated model parameters when logistic regression analysis (backward model reduction) was used; the results show the two eating activity parameters that are used best to predict the probability of receiving a ketosis attention postpartum

				95% C.I. for OR	
	β	Sig.	OR	Lower	Upper
Residual standard variation (first period)	-1.230	0.055	0.292	0.083	1.027
Mean (second period)	0.208	0.093	0.812	0.637	1.035

Table 6. Illustration of the values that would be achieved when an alert would be given to all cows that had a mean eating activity (h/d) below the cut-off point. The cut-off point was determined at the percentage of the mean of the no ketosis group, where the sum of the sensitivity and specificity was maximum. This led to a cut-off point of 87% of the mean of the no ketosis group of the total prepartum period (5.42 h/d), and 94% of the first period (5.92 h/d).

	Period (days prepartum)					
	Total[1] (d -28 to 0)			First period[2] (d -28 to -15)		
	Ketosis	No Ketosis	Total	Ketosis	No Ketosis	Total
Alert	16	63	79	24	102	126
No alert	33	198	231	25	159	184
Total	49	261	310	49	261	310

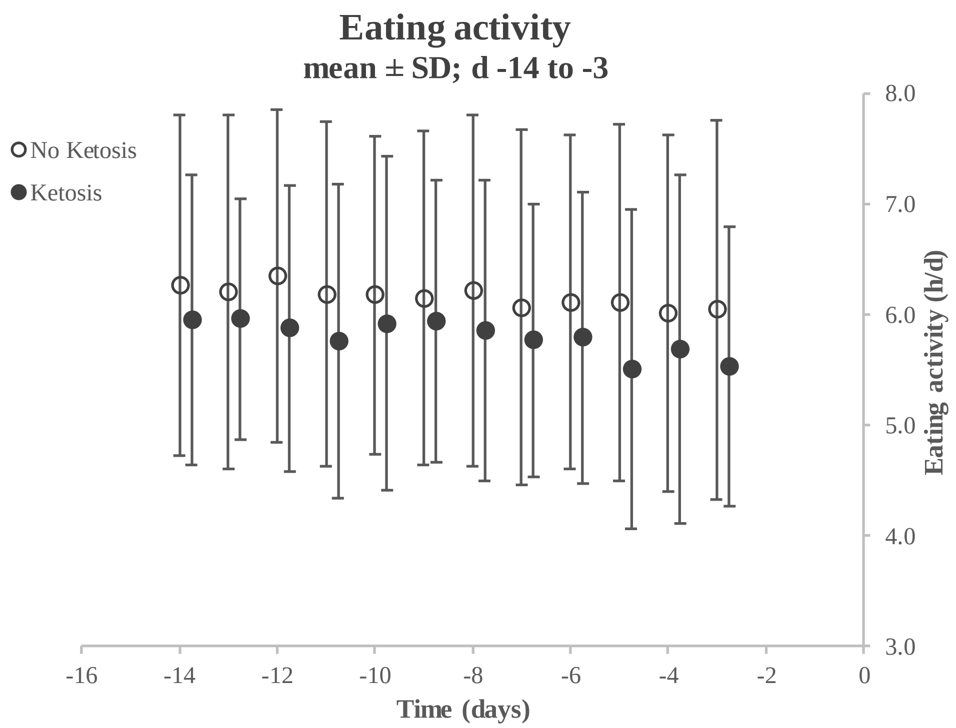

Figure 5. Scatterplot showing the mean eating activity (h/d) ± the standard deviation (SD) of the ketosis group (n = 49) and no ketosis group (n = 261) of the second period prepartum (d -14 to -3); the means ± SD of the ketosis group are intentionally placed a quarter of a unit to the right, to increase the readability of the graph

Discussion

The incidences of ketosis attentions in both the samples in this study were dependent on the methods used to detect the ketone bodies and the threshold value used to give a ketosis attention on the MPR. The threshold value was defined in the thesis of van der Drift (2013) as the value where the sum of the sensitivity and specificity was maximum (Se = 82.4%; Sp = 83.8%)[23]. In the present study, the incidences of the ketosis attentions were 19.2% in the walking activity sample and 15.8% in the eating activity sample. These incidences are not identical, since cows with a complete set of data in the walking activity sample, did not necessarily have a complete set of data in the eating activity sample. This resulted in two different samples, with different incidences of ketosis attentions. It should be noted that a ketosis attention is not to be interpreted as a clinical diagnosis. It does, however, give a good impression of the prevalence of ketosis on a farm[23]. The reported incidences are comparable to the overall prevalence in the Netherlands (16%)[23,32].

For a variable to be useful as an early indication of the development of a disease, it must precede the disease and be characteristic for it[29]. Hence, these aspects were investigated for prepartum walking and eating activity. The walking and eating activity monitored during a period of four weeks prepartum preceded all postpartum ketosis attentions. Furthermore, postpartum walking and eating activity were reported by foregoing studies to be characteristic for ketosis[25-27,29,33]. The objective of this study was to determine whether prepartum walking and eating activity were also characteristic for ketosis and, hence, possibly useful as early indicators. It should be kept in mind that walking and eating activity are not solely characteristic for ketosis, but also for other transition period related diseases[9,33]. However, many of these diseases are again associated with the development of ketosis, either as a cause or a consequence[12-15].

The pattern of the mean walking activity (Figure 1) showed a peak around calving (ketosis group) and the day after calving (no ketosis group). This could be due to the agitation around calving and to the moment of introducing the cows into the herd after calving respectively. Both situations lead to stress and subsequently result in a higher number of steps/d. Overall, the mean walking activity of the ketosis group is lower during the total illustrated period than the mean walking activity of the no ketosis group. During the total prepartum period the means differed 11% ($\bar{x}_{k} = 2665 \pm 902$; $\bar{x}_{no} = 3000 \pm 819$), which is a substantial difference.

The results of Adewuyi *et al.* (2006) showed that postpartum walking activity of dairy cattle was normally distributed with a large standard deviation, because of the high maximum and very low minimum activities of individual cows[26]. The present study provided similar results for prepartum walking activity (Figure 2). The large standard deviation could be the result of, among other reasons, the differences in management on the different dairy farms. The data used to describe the mean walking activity (Figure 1), came from 11 different Dutch dairy farms. The farms were larger than average in the Netherlands, but they were a representative sample. No distinction was made between factors on the farms such as the type of floor, type of freestalls, number of milking and feeding times per day, grazing outdoors and overcrowding. These factors all contributed to the overall behaviour of cows, i.e. walking, eating, lying, ruminating. Moreover, factors such as social stress due to mixing up groups of cows, cows in heat, lameness and parity also contributed to the great variation of individual cow behaviour[34-36]. Since no distinction was made between all these factors, the large standard deviation was not surprising. Despite the large standard deviation, the prepartum mean walking activity of the ketosis group and the no ketosis group differed significantly ($p = 0.006$). This confirmed a strong association between prepartum walking activity and postpartum ketosis attentions.

The data of the present study was used to determine whether prepartum walking activity was useful to detect cows at risk of receiving a ketosis attention postpartum. The sensitivity and specificity of such a system must be taken into consideration when evaluating walking activity (steps/d) as being a useful early indicator. The system would give an alert to cows at risk of receiving a ketosis attention postpartum, based on their mean walking activity (Table 4). The sensitivity and specificity of both the total prepartum period (Se = 66%, Sp = 68%) and the first prepartum period (Se = 74%, Sp = 56%) were lower than the sensitivity and specificity of the method which was used to give out the ketosis attentions on the MPR (Se = 82.4%; Sp = 83.8%)[23]. The sensitivity of the first period seemed relatively high, which led to more cows correctly receiving an alert. However, this was accompanied by a low specificity, which resulted in half of the sample receiving an alert (n = 151). If the mean walking activity was used to determine the incidence of cows at risk, it would look like 50% of the herd was at risk of receiving a ketosis attention postpartum. However, only 43 cows out of the 151 were alerted correctly. The practical and economic consequences that this entails should be taken into consideration when evaluating prepartum walking activity as an early indicator. As described earlier, considering the impact of management factors of a dairy farm on prepartum walking activity could lead to a smaller standard deviation of the mean. A monitoring system that takes these factors into account would, therefore, lead to a higher sensitivity and specificity.

The association provided by the results of this study might contribute to the development of an early warning system, which helps to detect cows at risk of a postpartum ketosis attention. Since pedometers are already used worldwide to detect cows in heat, prepartum walking activity could be implemented into a parallel system, using the same sensors. By implementing early warnings based on walking activity, cows at risk of developing ketosis can possibly be detected. The earlier cows at risk are detected, the earlier management adjustments can be achieved to help those cows through the transition period. However, the large variation in means and patterns still make it difficult to predict the probability of receiving a ketosis attention for individual cows. Nonetheless, an early warning system could still be useful to determine the prepartum incidence of cows at risk of receiving a ketosis attention postpartum. If the incidence is high at a dairy farm, the reason could very well lie in a causal management factor. Outside the scope of this study, the impact of management factors on prepartum walking and eating activity needs to be established. Subsequently, their possible impact on the development of ketosis can be determined. It must be kept in mind, however, that the causal association between prepartum walking and eating activity and the development of ketosis has not been cleared up yet. Additional research is needed to determine the possible causality between prepartum walking and eating activity and the development of ketosis postpartum.

Goldhawk *et al.* (2009), among others, reported a decline in food intake and feeding behavior in association with a SCK diagnosis[27,28]. Reduced food intake was already a well-recognized sign of CK[9,29]. In contrast to these studies, the results of the present study did not provide a significant association between prepartum eating activity and postpartum ketosis attentions. The difference between the mean eating activity of the ketosis and the no ketosis group of the total prepartum period was only 5%, in contrast to the difference of 11% of the walking activity sample. Nonetheless, a trend between the ketosis and no ketosis group was present from d -28 to d 14 (Figure 3). Moreover, the p-value (0.087) did approach significance and, hence, confirmed the difference between the two groups. Expending the eating activity sample with some extra data could already lead to an even stronger association.

Important discrepancies exist between the present and the foregoing studies. These discrepancies must be considered when evaluating the association between prepartum eating activity and postpartum ketosis attentions. An important discrepancy is the prepartum timeframe in which eating activity was monitored in the present study, in contrast to the mainly postpartum and shorter prepartum timeframe of the foregoing studies. The reported association of these studies, between postpartum eating activity and ketosis, could partly be due to a vicious circle, since cows that do not feel well because of circulating ketone bodies, eat less[12,27,28]. In the present study, the prepartum eating activity preceded the development of ketosis postpartum. Hence, the association that was found between prepartum eating activity and postpartum ketosis attentions is an even more important finding, since it cannot be the result of a vicious circle.

Another important discrepancy refers to the postpartum period in which the ketosis attentions were received in the present study. The ketosis attentions could have been received on any of the days in the period of 60 d postpartum, dependent on the test-days of the MPR, instead of the regular measurement of serum BHB levels in the first 2 weeks postpartum[27,28] or a clinical diagnosis[28,29]. Hence, ketosis could have been missed. Furthermore, the period in which eating activity was monitored, was not corrected for the exact day on which a ketosis attention was received. Correcting for this exact day would lead to the monitored eating activity directly preceding the day on which the ketosis attention was received. For instance, if the ketosis attention was received on d 50 postpartum, the period of 28 d of monitoring eating activity would become from d 32 to 50 postpartum. Not correcting for the exact days on which the ketosis attentions were received could have contributed to the weaker association between prepartum eating activity and postpartum ketosis attentions as presented in this study. The same is true for the walking activity. Hence, correcting for the exact days on which the ketosis attentions were received could lead to stronger associations with both prepartum eating and walking activity.

As for prepartum walking activity, the sensitivity and specificity were calculated from the results in Table 6. The sensitivity and specificity were used to evaluate prepartum eating activity as being useful for detection of cows at risk of a postpartum ketosis attention. The sensitivity and specificity of the mean eating activity in both the total prepartum period (Se = 33%, Sp = 76%) and the first prepartum period (Se = 49%, Sp = 61%) were lower than the values calculated for walking activity. As described for walking activity, both the sensitivity and the specificity would become higher when taking management factors into account, since this will lower the standard deviation of the mean. Ketosis is known to be affected by dietary formulation and can be modified by cow behaviour and feed intake[27-29,37,38]. Hence, especially the management factors that influence cow behaviour and feed intake need to be established.

Incorporating management factors into the calculation can improve the sensitivity and specificity of the early warnings. Also, combining prepartum walking and eating activity into one early warning system could possibly increase the sensitivity and specificity. Hence, additional research is needed in these areas before being able to use them as early indicators of receiving postpartum ketosis attentions. Furthermore, in the present study prepartum walking and eating activity were evaluated as early indicators of ketosis attentions on the MPR. Considering clinical signs and investigate prepartum walking and eating activity as early indicators of both CK and SCK could lead to more practical results. At last, outside the scope of this study, the relative difference between the mean walking and eating activity during lactation and the mean of the transition period could also lead to a useful indication of the development of transition-related diseases such as ketosis. Thus, additional research is needed in several areas before prepartum walking and eating activity can be used to detect cows at risk of developing ketosis postpartum.

Conclusion

Dairy cows that received a ketosis attention on the MPR in the first 60 d postpartum showed less walking activity in steps/d as early four weeks prepartum. Even though the prepartum eating activity did not provide the same significant result, a clear association was established between both prepartum walking and prepartum eating activity and postpartum ketosis attentions. This study does support that an early warning system is feasible. However, because of the large variation among individual cows and the relative small difference of the means between cows that did and did not receive a ketosis attention, it is still difficult to predict the probability of receiving a ketosis attention for individual cows. Moreover, the sensitivity and specificity based on the data of this study are not yet high enough to implement walking and eating activity into an early warning system. Management factors that influence prepartum activity need to be established and considered when evaluating prepartum walking and eating activity as early indicators of postpartum ketosis attentions. This might increase the sensitivity and specificity and help to prevent and control the development of postpartum ketosis.

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