

## Effects of Hyperketonemia within the First Six Weeks of Lactation on Milk Production and Reproductive Performance

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### Abstract

Hyperketonemia is a common disease in early lactating dairy cows and diagnosed by measurement of blood  $\beta$ -hydroxybutyric acid. The objectives of our study were to describe the occurrence of hyperketonemia within the first six weeks of lactation and to evaluate the effects of hyperketonemia on milk production (1st test day milk yield and 100 DIM milk yield), reproductive performance (time to first service, first service conception risk, and time to pregnancy within 200 DIM) and early lactation culling risk.

A total of 655 Holstein dairy cows from 6 commercial dairy farms in Germany were enrolled between 1 and 4 DIM. Cows were tested twice weekly using an electronic handheld meter for  $\beta$ -hydroxybutyric acid for an examination period of 42 days resulting in 12 test results per cow. Hyperketonemia was defined as a  $\beta$ -hydroxybutyric acid concentration  $\geq 1.2$  mmol/l. The onset of hyperketonemia was described as early onset (first hyperketonemia event within the first 2 weeks postpartum) and late onset (first hyperketonemia event in week 3 to 6 postpartum). Prevalence and incidence of hyperketonemia were assessed based on the 12 examinations.

Cumulative incidence of hyperketonemia was 48% and 72% for primiparous and multiparous cows, respectively. Mean prevalence was 17.5%. Early onset ketotic cows had a higher 1st test day milk yield (+3.0 kg/d,  $P < 0.001$ ) and 100 DIM milk production (+301.6 kg;  $P < 0.001$ ) compared to non-ketotic cows. There was no effect of late onset of ketosis on milk production. There were no effects of hyperketonemia on reproductive performance and culling risk, irrespective of onset of ketosis.

**Keywords:** Hyperketonemia; Transition; Dairy cow; Adaptation; Milk production; Reproduction

**Abbreviations:** AI: Artificial Insemination; BHBA:  $\beta$ -hydroxybutyric Acid; DHIA: Dairy Herd Improvement Association; DIM: Days in Milk; ECM: Energy-Corrected Milk Yield; FSCR: First Service Conception Risk; HYK: Hyperketonemia; NEB: Negative Energy Balance; TMR: Total Mixed Ration; VWP: Voluntary Waiting Period

### Introduction

The transition period of dairy cows is often accompanied by hyperketonemia (HYK) caused by a poor adaptation to negative energy balance [1]. Negative energy balance (NEB) is a result of an imbalance between energy input and output because the energy requirements for milk production and maintenance exceed the available energy from feed intake [2]. Some authors speculated that there are two different types of HYK differing in their onset and pathophysiology [3,4]. Type I ketosis was described as spontaneous or underfeeding ketosis. It occurs 3 to 6 weeks postpartum when milk secretion is so extensive that the demand for glucose exceeds the capacity for gluconeogenesis in the liver. The plasma levels of glucose and insulin are low; the levels of ketone bodies are high. Type II generally occurs earlier in lactation as a result of insulin resistance and excessive body fat mobilization prior to or at calving and is also known as "fat cow syndrome". Blood insulin and glucose concentrations are

high, whereas blood ketone concentrations are lower in type II ketosis than in type I [3,5].

Several studies evaluated the effects of HYK on milk yield [6-10] and reproductive performance [7,9,11] within 2 wk after parturition. Most studies showed that milk production in cows with HYK in early lactation is decreased compared to cows with lower  $\beta$ -hydroxybutyric acid (BHBA) values [1]. It was also shown, however, that in the long-term HYK was not associated with a milk loss but that cows with HYK within the first 2 wk postpartum produced more milk across four Dairy Herd Improvement Association (DHIA) tests [8] and that cows with HYK in the second week postpartum produced more milk at the third DHIA test [6].

No consistent results have been found for the evaluation of associations between elevated postpartum BHBA and reproductive measures [1]. An odds ratio (OR) of 0.7 was found for the outcome of pregnancy to 1st service for a BHBA cut-point  $\geq 1.0$  mmol/L ( $P = 0.04$ ) [11]. The results of other studies investigating the association between BHBA (for BHBA  $\geq 1.0$  or 1.2 mmol/L) and pregnancy to 1st service [8-10] and the time to pregnancy [7,9] were not significant ( $P > 0.05$ ).

The first 14 to 16 days after calving have been described as the main risk period for hyperketonemia [9,12]. It was shown, however, that the period in which early lactation dairy cows are at risk for hyperketonemia lasts at least until 42 days in milk (DIM) [13]. To the author's knowledge, there is limited evidence for the effect of HYK from week 3 to 6 on milk production, reproduction, and health.

Therefore, the objectives of our study were (1) to describe the occurrence of hyperketonemia within the first six weeks of lactation, (2) to evaluate the effect of onset of hyperketonemia within the first six weeks of lactation on milk production and reproduction and (3) to evaluate the effect of onset of hyperketonemia within the first six weeks of lactation on early lactation culling risk.

## Materials and Methods

The experimental procedures reported herein were conducted with the approval of the Institutional Animal Care and Use Committee of Freie Universität Berlin. Cows were managed according to the guidelines set by the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medical Products [14].

### Study population

This study was conducted between June 2013 and July 2015. A total of 655 Holstein dairy cows from 6 different commercial dairy farms in Brandenburg, Sachsen and Sachsen-Anhalt, Germany, were included in the trial. All farms kept at least 600 dairy cows in freestall barns, fed

a total mixed ration (TMR) and recorded medical treatments using computer based farm management programs (HerdeW, version 5.8, dsp-Agrosoft Ltd., Ketzin, Germany). The energy-corrected milk yield (ECM) was calculated using the following formula:  $ECM (kg) = (0.38 \times fat\% + 0.21 \times protein\% + 1.05) / 3.28 \times milk\ yield (kg)$  [15].

Data of farms 1 to 3 were reported previously [13]. Farm 4 kept approximately 1,600 cows in a freestall barn with slatted floors and cubicles equipped with rubber mats. Feed was delivered once daily and pushed up 3 times a day. Cows were milked 2 times a day in a rotary parlor. The average annual milk yield was 8,465 kg (ECM). Farm 5 kept approximately 1,300 cows in a freestall barn with concrete solid floors and cubicles equipped with rubber mats. Feed was delivered once daily and pushed up 5 times a day. Cows were milked 3 times a day in a side-by-side parlor, the average annual milk yield was 9,509 kg (ECM). Farm 6 kept approximately 1,400 cows in a freestall barn with slatted floors and cubicles equipped with rubber mats. Feed was delivered over a conveyer belt system 6 to 8 times per day. Cows were milked 3 times a day in a rotary milking parlor, the average annual milk yield was 9,785 kg (ECM). Some cows received a prophylactic treatment against ketosis (Table 1).

Parameter	Herd					
	1	2	3	4	5	6
Enrolled cows, n	124	121	60	136	104	110
Herd size, n	1,200	1,800	600	1,600	1,300	1,400
Annual milk production <sup>1</sup> , kg	10,733	10,900	11,127	8,465	9,509	9,785
Ketosis prophylaxis	All cows: TMR including 300 g glycerol per cow/d and oral 300 g dextrose at 0 DIM. 3 <sup>rd</sup> lactation and higher: 0.04 mg/kg dexamethasone at 0 DIM	All cows: TMR including 300 g glycerol per cow/d. 3 <sup>rd</sup> lactation and higher: 160 g dextrose per cow intravenous at 0 DIM	All cows: TMR including 300 g propylene glycol per cow/d during the first 21 DIM	2 <sup>nd</sup> lactation and higher: oral 35 g calcium propionate and 21 g sodium propionate at 0 DIM	2 <sup>nd</sup> lactation and higher: 500 ml dextrose (40%) intravenous on 1 <sup>st</sup> DIM	All cows: TMR including 100 g propylene glycol and 150 g glycerol per cow/d for cows between 7-42 DIM

**Table 1:** Description of the 6 study herds. <sup>1</sup>Energy corrected milk (4.0% fat; 3.4% protein).

### Milk recording

All participating herds were enrolled in a DHIA milk recording program in which milk production and other data were recorded on-farm at intervals of approximately 35 to 40 d. We used test day milk yield 1 to 4 and a projection of milk yield in the first 100 DIM based on test day milk yield 1 to 3.

### Reproductive management

The management of reproduction of farms 1 to 6 is shown in Table 2. Pregnancy diagnosis was performed by the herd veterinarian using transrectal ultrasonography between day 28 and 42 after breeding. A positive pregnancy diagnosis was based on visualization of an embryo with a heartbeat.

Breeding dates and results of pregnancy diagnoses were obtained from the on-farm computer system.

### Experimental design

Cows were enrolled between 1 and 4 DIM and were tested for BHBA twice weekly at intervals of 3 to 4 days for an examination period of 42 days, resulting in 12 test results per cow. The term lactation week was used to describe the time of sample collection relative to calving (e.g., lactation week 0.5 for the first measurement on DIM 1 to 4, lactation week 1 for the second measurement on DIM 4 to 7, lactation week 1.5 for the third measurement on DIM 8 to 11). Hyperketonemia was defined as a BHBA concentration  $\geq 1.2$  mmol/l. The onset of HYK was categorized as early (first HYK event within lactation week 0.5 to 2.0) and late onset (first HYK event within lactation week 2.5 to 6.0).

The collection of blood samples and the measurement of BHBA by an electronic BHBA meter (NovaVet, Nova Biomedical, Waltham, USA) were described in the study by Mahrt et al. [13] for farm 1 to 3. The same methods were used for farm 4 to 6.

A total of 32 cows were excluded from analysis because they received one of the following treatments that have been shown to have

effects on blood BHBA: (1) intravenous application of butaphosphan-cyanocobalamin combination (Catosal, Bayer Animal Health, Leverkusen, Germany) because of its role in increasing gluconeogenesis by increasing the activity of methylmalonyl-coenzyme A (CoA) mutase [16], (2) intravenous application of dextrose which seems physiologically sound, because the requirement for glucose for milk production drives fat metabolism and hypoglycemia [16], (3) intravenous application of dexamethasone (Dexatad ad us.vet., aniMedica, Senden-Bösensell, Germany) due to glucocorticoids ability to produce hyperglycemia as a result of changes in glucose use and to increase catabolism of fat and protein stores [16], (4) oral administration of propylene glycol which stimulates gluconeogenesis and helps to decrease fat breakdown and hepatic ketone body production by entering the Krebs cycle or being converted to propionate [16], (5) monensin (Kexxtone, Elanco Animal Health, Hampshire, UK) which was shown to alter the rumens microbial population and significantly reduces the prevalence and incidence of subclinical ketosis [12].

Parameter	Herd					
	1	2	3	4	5	6
VWP <sup>1</sup>	50	50	42	40	42	42
TAI (DIM) <sup>2</sup>	Presynch (70)	No	No	No	Ovsynch (80)	No
21d pregnancy rate	13	15	21	17	13	24
Time of PD <sup>3</sup>	35	30	28	38	39	42

**Table 2:** Description of reproductive management. <sup>1</sup>VWP: Voluntary Waiting Period. <sup>2</sup>TAI (DIM): Synchronization protocol for the first AI and responding DIM of fixed AI. <sup>3</sup>Time of pregnancy diagnosis in days after insemination.

Animals that received a blanket prophylaxis with dextrose or dexamethasone against disorders in energy metabolism on DIM 0 or 1 (n=115) were not excluded because a one-time intravenous bolus administration of 50% dextrose in postpartum dairy cows is unlikely to prevent or resolve hyperketonemia [16] and a single treatment with glucocorticoids is considered equivocal [16]. However, an effect of a second treatment or a treatment in combination with other therapies is possible and therefore cows that received a second treatment or a combination were excluded from the trial as described above.

Farms monitored during this study implemented different feeding and disease prevention strategies. This is inevitable when conducting a study on multiple commercial dairy farms, as has been discussed previously [17] and is comparable to the situation in the field. Furthermore, two cows were excluded due to an abortion. After exclusion, 621 cows (195 primiparous; 426 multiparous) were used for the final analyses.

For the analysis of milk production and reproductive performance some cows had to be excluded due to incomplete data recording on farm, culling, or the decision to not breed anymore. In total, 593 and 533 cows were analyzed for 1st test day milk yield and 100 DIM milk production, respectively. For the evaluation of reproduction 614, 558 and 544 cows were analyzed for insemination within 100 DIM, first service conception risk and pregnancy within 200 DIM, respectively.

For the evaluation of incidence, number of HYK events per cow and time from first positive HYK test to one blood BHBA concentration of

<1.2 mmol/L only cows with a complete set of test results (i.e. 12 tests) were considered. In total, 458 cows had 12 test results and were considered for the analysis of incidence and number of HYK events. Of those cows, 294 cows were ketotic and were considered in the analysis for time from first positive HYK test to one blood BHBA concentration of <1.2 mmol/L.

### Statistical analysis

Data from the cowside evaluation of BHBA and data from the on farm computer system were exported to Excel spreadsheets and analyzed using SPSS for Windows (version 22.0, SPSS Inc., IBM, Ehningen, Germany).

To evaluate the effect of ketosis status on milk yield in early lactation linear regression analysis was performed using the GENLIMMIXED procedure of SPSS. The outcome variable was either 1<sup>st</sup> test day milk yield (kg/d) or a projection of cumulative 100 d milk yield (kg). Cow was the experimental unit and herd was considered as a random effect. According to the model-building strategies described by Dohoo et al. [18] each parameter considered for the mixed model should be separately analyzed in a univariate model, including the parameter as a fixed factor (i.e., categorical parameter) or covariate (i.e., continuous parameter). Only parameters resulting in univariate models with  $P \leq 0.2$  should be included in the final mixed model. The initial model contained the following explanatory variables as fixed effects: ketosis status (non-ketotic, early onset, late onset), parity (primiparous vs. multiparous), DIM at test day and calving season (spring, summer, autumn, winter). Selection of the model that best fit the data was performed by testing each effect separately in a univariate model and finding the model with the lowest value for the Akaike information criterion using a backward elimination procedure that removed all variables with  $P > 0.10$  from the model. Regardless of the significance level ketosis status was forced to remain in the model.

The analysis of pregnancy per artificial insemination at first artificial insemination (AI) was performed by logistic regression using the GENLIMMIXED procedure of SPSS. Cow was the experimental unit and herd was considered as a random effect. The initial model contained the following explanatory variables as fixed effects: ketosis status (non-ketotic, early onset, late onset), parity (primiparous vs. multiparous) and calving season (spring, summer, autumn, winter). Model building and selection of the model that best fit the data was performed as described earlier. Regardless of the significance level ketosis status was forced to remain in the model.

Survival analysis was conducted to evaluate the effect of ketosis status on the hazard of insemination within 100 DIM and pregnancy within 200 DIM by creating Cox semiparametric proportional hazard model using SPSS. The outcome variable for the Cox model for insemination within 100 DIM was DIM at first insemination and for pregnancy within 200 DIM was the DIM at which cows conceived. The models included ketosis status, herd, parity and calving season (spring, summer, autumn, winter) as categorical explanatory variables. Selection of the model that best fit the data was performed by using a backward elimination procedure that removed all variables with  $P > 0.10$  from the model. Regardless of the significance level, ketosis status was forced to remain in the model.

Survival analysis was conducted to evaluate the effect of ketosis status on the hazard of culling (live or dead) within the study period (42 DIM) by creating Cox semiparametric proportional hazard model using SPSS. The outcome variable was DIM at culling within 42 DIM.

The models included ketosis status, herd, and parity as categorical explanatory variables. Selection of the model that best fit the data was performed as described earlier. Regardless of the significance level, ketosis status was forced to remain in the model.

Kaplan-Meier survival analysis curves were constructed to illustrate the rate at which cows resolved HYK, received first AI, conceived until 200 DIM, and were culled within 42 DIM by using the Kaplan-Meier survival analysis option of MedCalc (version 12.5.0.0, MedCalc Software, Mariakerke, Belgium).

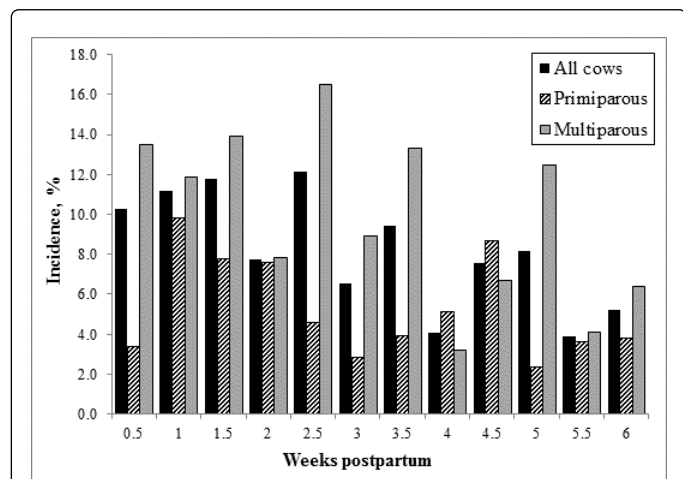
A significant difference between the levels of a classification variable was declared when  $P < 0.05$ , whereas differences between  $P \geq 0.05$  and  $P \leq 0.10$  were considered a statistical tendency.

## Results

Of 655 cows enrolled in the trial, 34 (5.2%) were excluded from analysis as described above; 621 cows remained for final analysis. Of these cows, 195 (31.4%) were in first lactation, 173 (27.9%) were in second lactation, and 253 (40.7%) were in the third or greater lactation. Overall, 235 cows were non-ketotic, 224 cows were early onset-ketotic, and 162 cows were late onset-ketotic.

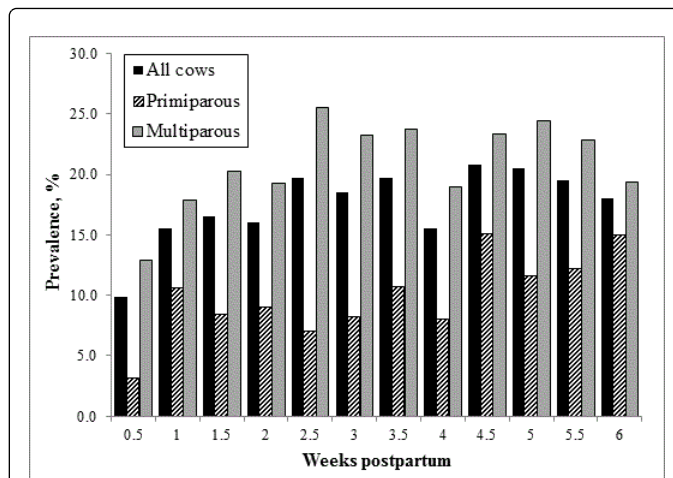
### Occurrence of HYK within the first six weeks of lactation

Only cows with 12 test results were considered in the analysis for incidence (n=458). Cumulative incidence of HYK was 48% and 72% for primiparous and multiparous cows, respectively. The peak incidence of HYK for primiparous cows occurred in wk 1.0 of lactation whereas for multiparous cows it was found in wk 2.5 (Figure 1).



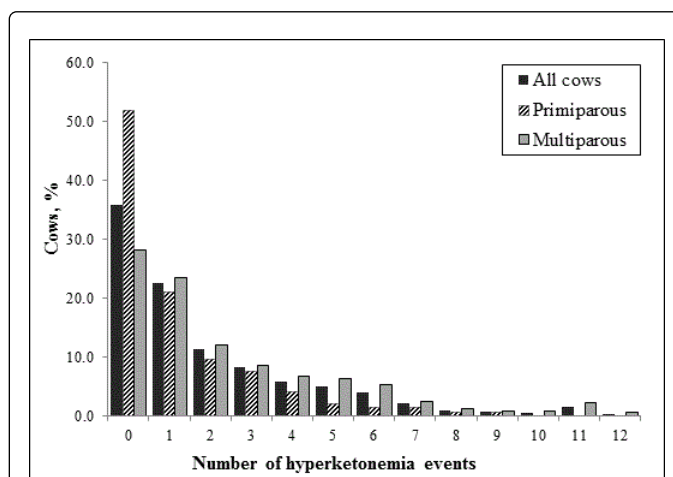
**Figure 1:** Incidence of hyperketonemia (BHBA concentration  $\geq 1.2$  mmol/L) in primiparous, multiparous, and overall Holstein dairy cows during the first 6 wk of lactation (n=458).

Mean prevalence was 17.5%, ranging from 9.9% in lactation wk 0.5 and to 20.8% in lactation wk 4.5. Overall prevalence stayed on the same level within the first six weeks of lactation (Figure 2). Peak prevalence occurred in week 4.5 and 2.5 for primiparous (15.1%) and multiparous cows (25.5%), respectively. Multiparous cows had a higher mean prevalence of HYK (21%) within the first six weeks of lactation than primiparous cows (10%).

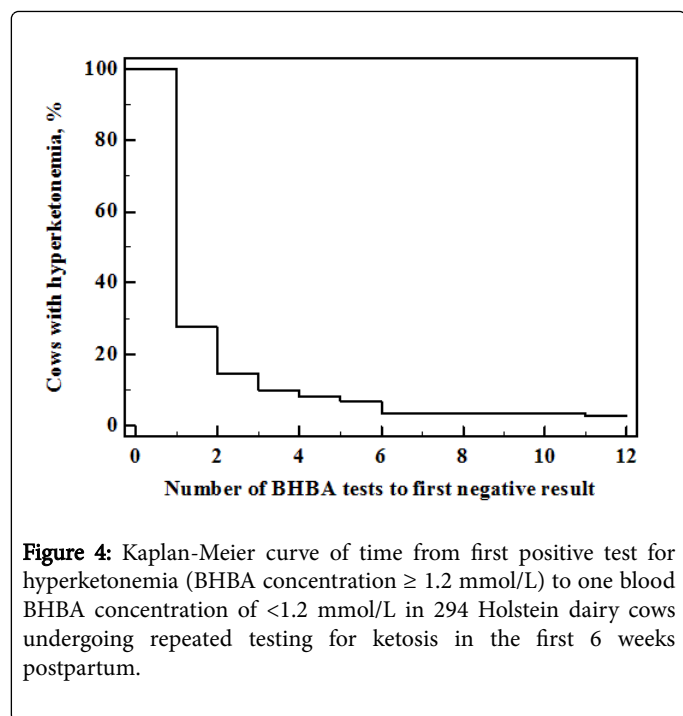


**Figure 2:** Prevalence of hyperketonemia (BHBA concentration  $\geq 1.2$  mmol/L) in primiparous, multiparous, and overall Holstein dairy cows during the first 6 wk of lactation (n=621).

The number of HYK events within the first six weeks of lactation is shown in Figure 3. Only cows with 12 test results (n=458) were considered. Of those, 164 cows (35.8%) had no positive BHBA test result within the first six weeks of lactation, 104 cows (22.7%) had one event of HYK only. A total of 50 cows (10.9%) had 6 or more HYK events. Figure 4 shows the time from the first positive test for HYK (i.e., BHBA concentration  $\geq 1.2$  mmol/L) to one blood BHBA concentration of  $< 1.2$  mmol/L in 294 cows with HYK. Only ketotic cows with 12 test events were included. Because of the testing scheme (i.e., Tuesday and Friday or Monday and Thursday, respectively) time between two test events were either 3 or 4 days. In total, 72% of HYK positive cows were test negative 3 to 4 days after the initial diagnosis of HYK.



**Figure 3:** Number of hyperketonemia (BHBA concentration  $\geq 1.2$  mmol/L) events per cow within the first 6 weeks of lactation (n=458).



**Figure 4:** Kaplan-Meier curve of time from first positive test for hyperketonemia (BHBA concentration  $\geq 1.2$  mmol/L) to one blood BHBA concentration of  $<1.2$  mmol/L in 294 Holstein dairy cows undergoing repeated testing for ketosis in the first 6 weeks postpartum.

### Effect of onset of HYK within the first six weeks of lactation on milk production

In total, 593 and 533 cows were used for the analysis for the 1<sup>st</sup> test day milk production and the 100 DIM milk productions, respectively.

The effects of onset of HYK on 1<sup>st</sup> test day and 100 DIM milk productions are shown in Tables 3 and 4, respectively. Early onset ketotic cows produced 3.0 kg more milk on the 1<sup>st</sup> test day ( $P<0.001$ ) and 301.6 kg more milk in the first 100 d of lactation ( $P<0.001$ ) than non-ketotic cows. There was no significant effect of late onset of ketosis on 1<sup>st</sup> test day milk yield (+1.2 kg/d;  $P=0.1$ ). Cows with late onset of ketosis showed a trend to a higher 100 DIM milk yield (+118.6 kg;  $P=0.075$ ) than non-ketotic cows.

Variable	Estimate <sup>1</sup>	SE <sup>2</sup>	95% Confidence Interval		P
			Lower CI	Upper CI	
	Kg/d				
Parity					
Primiparous	Referent				
Multiparous	9.4	0.6	8.1	10.6	<0.001
Ketosis status <sup>3</sup>					
Non-ketotic	Referent				
Early onset	3	0.7	1.6	4.4	<0.001
Late onset	1.2	0.8	-0.2	2.7	0.1

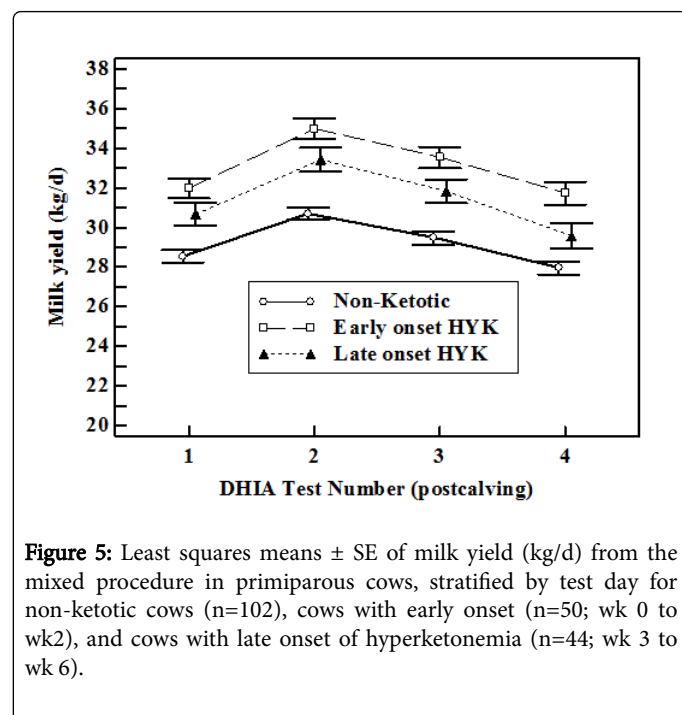
**Table 3:** Effect of ketosis status within the first six weeks of lactation on 1<sup>st</sup> test day milk production in Holstein dairy cows (n=593). <sup>1</sup>Model adjusted for the random effect of herd and the fixed effect of parity. <sup>2</sup>SE=Standard error of the estimate. <sup>3</sup>Ketosis status: Non-ketotic=no HYK event in the first six weeks of lactation; Early onset=first HYK

event within the first two weeks; Late onset=first HYK event in week 3 to 6 postpartum.

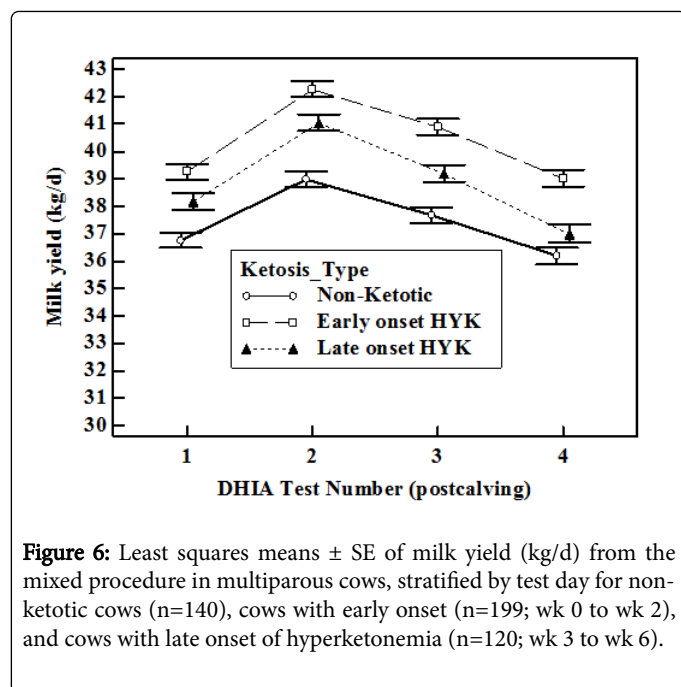
Variable	Estimate <sup>1</sup>	SE <sup>2</sup>	95% Confidence Interval		P
			Lower CI	Upper CI	
	kg				
Parity					
Primiparous	Referent				
Multiparous	853.9	57.3	741.3	966.6	<0.001
Ketosis status <sup>3</sup>					
Non-ketotic	Referent				
Early onset	301.6	64.1	175.7	427.6	<0.001
Late onset	118.6	66.5	-12	249.2	0.075

**Table 4:** Effect of ketosis status within the first six weeks of lactation on 100 DIM milk production in Holstein dairy cows (n=533). <sup>1</sup>Model adjusted for the random effect of herd and the fixed effect of parity. <sup>2</sup>SE: Standard error of the estimate. <sup>3</sup>Ketosis status: Non-ketotic: no HYK event in the first six weeks of lactation; early onset: first HYK event within the first two weeks; late onset: first HYK event in week 3 to 6 postpartum.

Graphs of milk yield by test day for non-ketotic, early onset and late onset ketotic cows were plotted for DHIA test 1 to 4 for primiparous and multiparous cows, respectively (Figures 5 and 6). Both, early and late onset of HYK was associated with an increased milk yield on each DHIA test day. This applied for primiparous as well as for multiparous cows. The increase in milk production was higher for early onset HYK than for late onset HYK.



**Figure 5:** Least squares means  $\pm$  SE of milk yield (kg/d) from the mixed procedure in primiparous cows, stratified by test day for non-ketotic cows (n=102), cows with early onset (n=50; wk 0 to wk2), and cows with late onset of hyperketonemia (n=44; wk 3 to wk 6).



**Figure 6:** Least squares means  $\pm$  SE of milk yield (kg/d) from the mixed procedure in multiparous cows, stratified by test day for non-ketotic cows (n=140), cows with early onset (n=199; wk 0 to wk 2), and cows with late onset of hyperketonemia (n=120; wk 3 to wk 6).

### Effect of onset of HYK within the first six weeks of lactation on reproduction

The association between ketosis status and reproductive performance was evaluated using the time to first service within 100 DIM, first service conception risk (FSCR), and time to pregnancy within 200 DIM.

In total, 614 cows had data concerning insemination within 100 DIM. The risk for insemination within 100 DIM did not differ between early onset ketotic cows (HR=0.9; 95% CI=0.7 to 1.1; P=0.110) and late onset ketotic cows (HR=1.0; 95% CI=0.8 to 1.3; P=0.976) compared to non-ketotic cows.

A total of 558 cows were used in the analysis for the effect of ketosis status on FSCR. An odds ratio <1 indicates a reduced risk for pregnancy at first AI. Multiparous cows had a lower risk for conceiving at first artificial insemination compared to primiparous cows (OR=0.64, 95% CI=0.4 to 1.0; P=0.030). There was no effect of HYK on the risk for conceiving at first artificial insemination, neither for early onset-ketotic cows (OR=0.9; 95% CI=0.6 to 1.4; P=0.604) nor for late onset-ketotic cows (OR=1.0; 95% CI=0.6 to 1.6; P=0.945).

A total of 544 cows were used for analysis of pregnancy risk within 200 DIM. The risk to become pregnant within 200 DIM did not differ between ketotic and non-ketotic cows (Table 5). Risk for pregnancy within 200 DIM was not decreased for early onset ketotic cows (HR=0.8; 95% CI=0.7 to 1.1; P=0.197) nor for late onset ketotic cows (HR=1.0; 95% CI=0.8 to 1.3; P=0.821) compared to non-ketotic cows.

### Effect of onset of HYK within the first six weeks of lactation on early lactation culling risk

The association between ketosis status and culling risk within 42 DIM was evaluated for 621 cows using survival analysis. Culling risk was comparable for early onset ketotic cows (HR=1.5; 95% CI=0.8 to 2.7; P=0.223) compared to non-ketotic cows. Cox proportional hazard analysis for culling risk determined that culling risk for cows with late

onset HYK was numerically lower (HR=0.4; 95% CI=0.1 to 1.0; P=0.062).

Variable	Estimate	SE <sup>1</sup>	P - value	HR <sup>2</sup>	95% CI
Parity					
Primiparous	Referent				
Multiparous	-0.279	0.109	0.011	0.757	0.611 – 0.937
Ketosis status <sup>3</sup>					
Non-Ketotic	Referent				
Early onset	-0.164	0.127	0.197	0.848	0.662 – 1.088
Late onset	-0.03	0.131	0.821	0.971	0.753 – 1.252

**Table 5:** Cox proportional hazard analysis for pregnancy within 200 DIM for 544 Holstein dairy cows. <sup>1</sup>SE: Standard error of the estimate. <sup>2</sup>HR: Hazard ratio for becoming pregnant within 200 DIM. HR <1 indicating decreased risk for pregnancy within 200 DIM. <sup>3</sup>Ketosis status: Non-ketotic=no HYK event in the first six weeks of lactation; early onset: first HYK event within the first two weeks; late onset: first HYK event in week 3 to 6 postpartum.

## Discussion

This study was conducted to describe the occurrence of early and late onset of HYK and to evaluate the effects of early and late onset of HYK on milk production, reproduction and culling risk. To our knowledge this is the first study that evaluates the effects of early and late onset of HYK within the first 6 wk of lactation.

Our results indicated that early onset HYK was associated with higher milk production. Cows were at risk for HYK for at least 6 wk postpartum. There were, however, no effects on reproductive performance and culling, irrespective of the onset of HYK.

### Occurrence of hyperketonemia

In our study, incidence, prevalence and the time from the first positive HYK event to a negative BHBA test (i.e., <1.2 mmol/L) event were evaluated for cows within the first 42 DIM. Incidence describes the percentage of cows with new cases of HYK in a group of cows frequently tested during the risk period, whereas prevalence presents the percentage of all affected cows at a certain moment (i.e., a snapshot; [19]). Time to first negative test event provides information about the self-cure rate of HYK.

The cumulative incidence of HYK within 42 DIM was 48% and 72% for primiparous and multiparous cows, respectively. McArt et al. [20] reported an incidence of approximately 44% when cows were tested 3 times per week for 3-16 DIM. Duffield et al. [12] found a cumulative incidence of 59% when cows were tested once weekly for 1-63 DIM. Incidence depends on the frequency and time of testing. McArt et al. [9] speculated that Duffield et al. [12] most likely underestimated the true incidence as cows were only tested once weekly. The peak incidence in our study was found in wk 1.0 (4-7 DIM) for primiparous cows and in wk 2.5 (15-18 DIM) for multiparous cows. In a study by McArt et al. [1] in which cows was tested 3 times per week between 3 to 16 DIM the peak incidence was found on 5 DIM. Our results confirm that multiparous cows have a higher risk of HYK in early lactation. Also the results indicate that the risk for multiparous cows to

develop new cases of HYK extends further into lactation. It has to be noted that the prophylactic treatment on some farms for multiparous cows could have influenced our results and that the true incidence for multiparous cows on the first testing could be higher than our results indicate. External factors that could influence metabolic conditions such as different feeding and disease prevention strategies on multiple commercial dairy farms have been discussed in detail in a previous study [13].

Different sampling times relative to feeding may also explain some variation among studies. However, it was shown that sampling time of continuously TMR fed dairy cows does not affect the concentration of BHBA [21].

The mean prevalence in our study was 17.5% which is close to the mean prevalence for Germany described by Suthar et al. [22] and in the middle of the prevalence range of 12% to 31% described in a recent review [1]. In our study the peak prevalence was found in wk 4.5 whereas McArt et al. [1] found the peak prevalence to be on 5 DIM. In that study, however, cows were tested until 16 DIM and the period further in lactation was not evaluated. In fact, prevalence increased in our study during the first 2 weeks of lactation and then stayed on a level of approximately 20% until 42 DIM.

Many cows (22.7%) tested positive for HYK had only one single HYK event and 72% of all HYK positive cows were tested negative within 3 to 4 d after the initial diagnose of HYK. These results indicate a high self-cure rate of HYK positive cows which should be considered in future studies when evaluating the effects of a treatment on the cure-risk of HYK. Instead of using only cure (BHBA<1.2 mmol/L) from HYK, studies should also use health and production outcomes with an economic value to the dairy farmer (e.g., reduction of disease or culling risk) to evaluate a treatment effect.

Furthermore, we wanted to investigate the theory of type I and II ketosis by Holtenius et al. [3] as the theory has never been confirmed in a large-scale study using commercial dairy herds. It was proposed that cows with elevated BHBA concentrations within the first 2 wk postpartum would most likely have type II ketosis which generally occurs earlier in lactation as a result of body fat mobilization prior to or at calving especially in obese cows [5]. Cows with elevated BHBA concentrations between 3 to 6 wk postpartum would probably have type I ketosis because milk secretion is so extensive that the demand for glucose exceeds the capacity of gluconeogenesis in the liver [3-5]. Our results indicate that early onset of HYK seems to be primarily an adaptational response to a high metabolic load caused by higher milk production in the beginning of lactation without the negative side effects on health.

## Milk production

Several studies evaluated the effect of HYK on milk production but findings were inconsistent. Some studies reported a decrease in milk yield for cows with BHBA  $\geq 1.2$  or  $1.4$  mmol/L [6,8,9]. Chapinal et al. [8] found, however, that the milk loss concerned only the first DHIA test. Across four DHIA tests cows with HYK were found to produce more milk. A similar result was demonstrated by Duffield et al. [6] who showed that cows with HYK in wk 2 had a higher milk yield on the third DHIA test. McArt et al. [1] suggested that higher producing cows might have impaired production in the short term that might be overcome by mid-lactation. Our findings confirm that early onset HYK was associated with a higher 100 d milk production. In contrast to Chapinal et al. [8] and Duffield et al. [6], however, we found a higher

milk production already on the first DHIA test for early onset ketotic cows. This observation is in accordance with a most recent study [23]. We suppose that the severity of SCK and the individual ability to adapt could be the reason for the inconsistent results. In our study some cows (n=32) were excluded due to individual treatment decisions by the farm personnel. The exclusion of treated animals with clinical symptoms might have masked the negative effects of HYK. It was shown by Duffield et al. [6] that an increasing threshold of BHBA has a more negative impact on 305-d milk yield (i.e. 15.7 kg yield loss for BHBA  $\geq 1.2$  mmol/L vs. 333.7 kg yield loss for BHBA  $\geq 1.8$  mmol/L).

Hyperketonemia is part of a physiological strategy of ruminants of adaptation to NEB in early lactation [4]. In early lactation, an excessive BHBA concentration is considered as a continuous risk measure for maladaptation of energy and fat metabolism to negative energy balance [2]. Our intense testing schedule (i.e., twice a week for 6 wk) and the exclusion of some cows (e.g., treated cows) with more severe HYK might have led to the inclusion of cows that had a disproportionately high metabolic load (i.e., high milk production) but were able to cope with it. We can only speculate that the adaptational response to NEB is animal specific and that some cows with HYK might still be able to undergo a successful adaptation and consequently do not suffer from negative effects whereas other animals with a high metabolic load might not be successful. In the first case the cow would show HYK as a physiological adaptational response to NEB whereas in the second case the HYK is accompanied by negative outcomes such as diseases and reduced milk yield representing maladaptation to NEB.

## Reproduction

Previous studies evaluating the association between HYK and reproductive performance are also inconsistent. A meta-analysis concluded that the precision of results regarding reproductive performance was low and that most relationships between different reproductive indicators were quantified in only 1 or 2 studies [24].

Walsh et al. [11] reported a decreased probability of pregnancy to 1<sup>st</sup> service for a BHBA concentration  $\geq 1.0$  mmol/L in week 1 postpartum (OR=0.7; P=0.04). In other studies, however, pregnancy to 1<sup>st</sup> service did not differ between ketotic and non-ketotic cows [8-10]. In our study we neither detected an effect of early onset HYK nor late onset of HYK on pregnancy to 1<sup>st</sup> service. The statistical power in our study for the effect of HYK on 1<sup>st</sup> service pregnancy risk, however, was limited due to the overall sample size and the different categories of HYK (i.e., early vs. late onset of HYK) and therefore has to be interpreted with caution.

To our knowledge, only one study evaluated time to first service [11] and reported that cows with HYK in either of the first 2 wk after calving were inseminated 8 d later than animals that were never HYK positive (HR=0.85; P=0.04). Our data do not confirm this relationship.

In the study by Walsh et al. [11] the median time to pregnancy for animals never classified as HYK positive was 108 d relative to 124 d for animals above the threshold in either wk 1 (BHBA  $\geq 1.0$  mmol/L) or wk 2 (BHBA  $\geq 1.4$  mmol/L). In other studies time to pregnancy was not different [7,9].

## Culling risk

In contrast to other studies we did not find an effect of HYK on culling risk, neither for early nor for late onset of HYK. Several studies

described that HYK cows were more likely to be removed from the herd [9,25].

A possible explanation for the inconsistent results could be handling of severely sick cows in our study and previous studies. In our study sick cows were treated at the discretion of the farm personnel and consequently excluded from analysis. Those cows were likely to suffer more intensely from HYK as a direct result of a disease. Unfortunately, the reporting of those cows is unspecific in previous research.

It was shown in the past that a high milk yield is protective against culling [26]. In our study HYK was associated with high milk yield. As discussed above we suggest that cows with a high milk production can have HYK as a result of a successful adaptation without necessarily developing short term diseases that might lead to culling.

## Conclusions

Hyperketonemia in the first 2 weeks of lactation was associated with increased milk production. We could not detect a negative impact of HYK on reproductive performance or culling risk in early lactation. As HYK was more pronounced in high producing cows it seems to be part of a physiological adaptational response to negative energy balance in transition dairy cows. Further research is required to determine the effects of occurrence of hyperketonemia considering duration and severity of HYK on subsequent disease risk.

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