

MONITORING THE INCIDENCE OF KETOSIS IN FRESH COWS USING MILK
COMPOSITION, URINE KETONES, AND MILK KETONES

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INTRODUCTION

Fresh cows have a high risk for ketosis within the first 30 days in milk (DIM) due to low dry matter (DM) intake and the rapid mobilization of fat after parturition. In addition, ketosis in fresh cows is most commonly associated with a negative energy balance postpartum (Oetzel, 2004). In a study by Carrier et al. (2004), subclinical ketosis was more prevalent in multiparous cows than single parity cows and highest in the first two weeks of lactation based on beta-hydroxybutyrate (BHBA) concentrations in serum of $\geq 1400 \mu\text{mol/L}$ (14.4 mg/dl). Cows with serum BHBA levels of $\geq 1400 \mu\text{mol/L}$ are three times more at risk for displaced abomasums and clinical ketosis (Oetzel, 2004). Ketosis in dairy cattle is defined as the increase in concentrations of ketones such as BHBA, acetoacetate (AcAc), and acetone (Duffield et al., 1997). This increase in ketone concentrations in the serum of fresh cows has a negative impact on the health of the cow and is associated with a loss in milk production (Duffield et al., 1997). Fresh cows with serum BHBA levels of $\geq 1800 \mu\text{mol/L}$ have an estimated milk production loss of 300 kg for that lactation (Duffield et al., 2009). Early detection of ketosis, including subclinical ketosis, can allow for earlier intervention and minimize the loss of milk production.

The use of cow-side test strips to measure BHBA in milk and AcAc in urine on a semiquantitative scale is widely practiced in the dairy industry to detect subclinical ketosis (Krogh et al., 2011). However, according to Oetzel (2004), cow-side tests for milk and urine are lower than blood BHBA in both sensitivity and specificity. In addition to the cow-side test strips, previous studies have shown the milk fat to protein ratio (F/P) to be a useful indicator of subclinical ketosis (Duffield et al., 1997). In a study by Čejna and Chladek (2005), during the first third of lactation the F/P ranged between 1.45 and 1.91 and were higher compared to the rest

of the lactation. The recommended F/P used in this study was 1.05 to 1.18 for Holstein cows (Čejna and Chladek, 2005). Additionally, a F/P of >1.5 increased the risk of ketosis along with other metabolic conditions (Heuer et al., 1999). Toni et al. (2011) found the incidences of retained placenta, left displaced abomasum, metritis, and endometritis increased with $F/P \geq 2.00$. The F/P for the herd is readily available from the Dairy Herd Improvement (DHI) test days, but individual cow F/P at given DIM when cows are most at risk for ketosis require on-farm milk composition analytical tools.

Milk fat is a highly variable milk component as compared with other less variable components, such as lactose and protein. Milk fat will be higher during the first third of the lactation due to the rapid mobilization of fat post-parturition (Čejna and Chladek, 2005). Therefore, elevated milk fat could be used as an indicator of clinical ketosis in fresh cows. On-farm milk component analysis tools are readily becoming more common and less expensive. The availability to analyze milk components from individual cows on the farm may allow farmers the opportunity to early detect metabolic diseases, such as ketosis. In addition, the use of an on-farm milk component analysis tool may be a better economical choice for large dairy herds, eg. > 1000 cows, as cow-side strip tests can be costly.

The objective of this study was to determine the accuracy of an on-farm milk component analyzer and identify a correlation between milk fat composition and the incidence of sub-clinical and clinical ketosis in fresh cows.

MATERIAL AND METHODS

Animal selection and housing

A 700-cow Holstein farm (Twin Oak Dairy, LLC.) in South Solon, Ohio was used for collecting samples at 7 and 14 DIM. A total of 204 fresh cows were sampled between August and November 2011. Cows were selected based on their calving date. The majority of cows were housed together in a fresh pen in a free-stall barn; however, sick cows were housed in an adjacent hospital pen in the same free-stall barn. Composite milk samples and right-front (RF) quarter strip samples were collected from individual cows at a single milking at 7 and 14 DIM. Two cow-side test strips were used to measure ketones in milk and urine on the same day as milk was sampled for component analysis.

Milk collection and analyses

Composite milk samples were collected using an in-line BouMatic (Madison, WI) sampler. The RF quarter strip samples were collected after udder preparation and just prior to attaching the milking unit. Both samples (composite and RF) were analyzed for milk components at the farm using a LactiCheck (LIC) (Page & Pedersen International, Ltd., Hopkinton, MA). The LactiCheck was calibrated weekly with raw milk samples from Eastern Lab Standards, Ltd (Medina, OH). Composite milk samples were also sent to DHI Cooperative, Inc (Columbus, OH) for analysis of fat, protein, lactose, and other solids using infrared spectroscopy (B2000 Infrared Analyzer, Bentley Instruments, Chaska, MN). Keto-Test[®] strips (Elanco[®] Animal Health, Greenfield, IN) were used to measure ketones (β -hydroxybutyrate) in milk stripped from the RF quarter (same sample used for component analysis). The increments of BHBA

measurement were based on a color scale at 0, 0.5, 1, 2, 5, and 10 mg/dl. The Keto-Test[®] strips were dipped into the milk samples when the samples reached room temperature.

Urine analyses

Urine ketones (acetoacetate) were measured with Ketostix[®] (Bayer Corporation, Leverkusen, Germany). The urine test strips were wetted in the urine stream and read after 15 seconds. The Ketostix[®] also uses a block color scheme, with the increments of concentrations of AcAc at negative (< 5 mg/dl), trace (5 mg/dl), small (15 mg/dl), moderate (40 mg/dl), large (80 mg/dl), and largest (160 mg/dl).

Statistical Analyses

The Proc Corr procedure of SAS (Version 9.1, SAS Institute Inc., Cary, NC) was used for data analysis. Significant differences were declared at $P \leq 0.05$ and a trend at $P \leq 0.10$.

RESULTS

Milk fat concentration

The average milk fat concentration from LIC composite samples was $5.36 \pm 2.05\%$ and $5.14 \pm 1.90\%$ from DHI, with the RF strippings having a lower milk fat ($3.18 \pm 1.88\%$) (Table 1). The correlation coefficient for LIC and DHI composites was 0.69 ($P < 0.01$), and the correlations between the composites and the RF samples were similar for both methods of analysis ($r=0.30$; $P < 0.01$) (Table 2). The milk fat concentrations for all three methods of measurement (LIC, DHI, and RF) decreased from 7 to 14 DIM, -0.63, -0.88, and -0.77, respectively (Figure 1). The decrease in fat percentage from 7 to 14 DIM indicates the negative energy balance is being corrected and the mobilization of fat is decreasing. Overall

the herd had a very low incidence of ketosis, 3.4% based on urine ketones ≥ 40 mg/dl or 6.9% based on milk ketones ≥ 2.0 mg/dl. In order to determine the fat percentage values in Table 1, an average of the fat percentages were determined for cows with ≥ 2.0 mg/dl based on milk ketones. The same method was also used for cows with urine ketones of ≥ 40 mg/dl. Based on cows with ≥ 2.0 mg/dl milk ketones, the corresponding mean fat percentages are 7.42, 6.40, and 4.74% (LIC, DHI, and RF (LIC) respectively). Based on cows with ≥ 40 mg/dl urine ketones, the corresponding mean fat percentages are 6.54, 6.98, and 4.20% (LIC, DHI, and RF (LIC) respectively).

Milk and urine ketones

Average milk ketone concentration was 0.55 ± 0.98 mg/dl, and urine ketone concentration was 4.41 ± 15.4 mg/dl; however, the incidence of clinical ketosis was relatively low in the herd (3.4% based on urine ketones ≥ 40 mg/dl or 6.9% based on milk ketones ≥ 2.0 mg/dl). The correlation of milk ketones and urine ketones was 0.48 ($P < 0.01$). The correlation of the RF milk fat percentage and the milk and urine ketones was similar ($r=0.22$; $P < 0.01$).

Table 1. Milk fat percentages relative to the incidence of ketosis among the fresh cows (n=204).

	Composite (LIC)⁴	Composite (DHI)⁴	RF (LIC)⁴
Overall Fat % ± SD	5.36 ± 2.05	5.14 ± 1.90	3.18 ± 1.88
Fat, %, Ketotic cows²	6.54	6.98	4.20
Fat, %, Ketotic cows³	7.42	6.40	4.74

¹Mean fat percentages for all cows sampled at 7 and 14 days in milk.

²Clinical ketosis based on urine ketones.

³Clinical ketosis based on milk ketones.

⁴LIC=LactiCheck instrument, DHI= Dairy Herd Improvement, and RF= right front quarter.

Table 2. Correlation coefficients of composite milk fat (LIC & DHI), right front (RF) milk fat, milk ketones, and urine ketones.¹

	Fat (DHI)¹	Fat (LIC)¹	RF (LIC)¹	Milk Ketones	Urine Ketones
Fat (DHI)	1.00	--	--	--	--
Fat (LIC)	0.69 P < 0.01	1.00	--	--	--
RF (LIC)	0.31 P < 0.01	0.30 P < 0.01	1.00	--	--
Milk Ketones	0.20 P < 0.01	0.28 P < 0.01	0.22 P < 0.01	1.00	--
Urine Ketones	0.28 P < 0.01	0.16 P < 0.01	0.23 P < 0.01	0.48 P < 0.01	1.00

¹LIC= LactiCheck instrument, DHI= Dairy Herd Improvement, and RF= right front quarter.

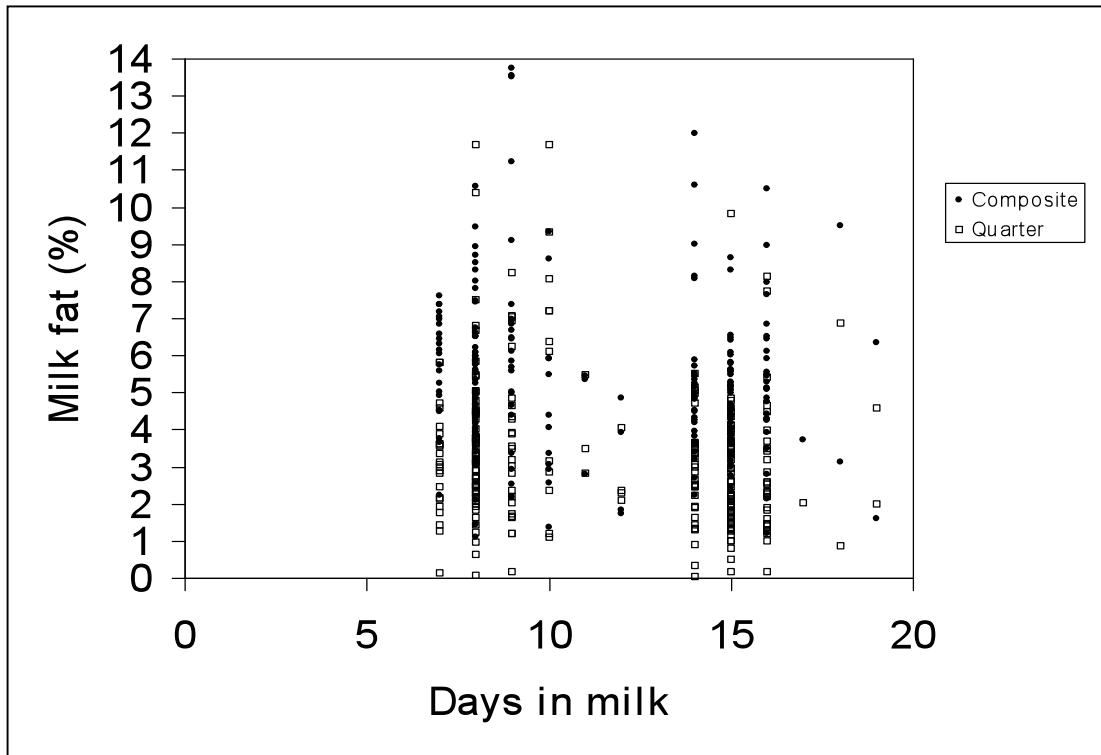


Figure 1. Right front quarter and composite fat percentages in milk from cows sampled at about 7 and 14 days in milk.

DISCUSSION

The purpose of this study was to determine if individual cow milk fat percentages could be used as a baseline for early detection of subclinical or clinical ketosis risk cows. Previous research in this area investigated using DHI test day milk fat and protein percentages as an early indicator of subclinical ketosis (Duffield et al., 1997). In the Duffield et al. (1997) study, $\geq 4.1\%$ fat was used as a cutoff point and had 54% sensitivity (Se) and 72% (Sp) specificity. The sensitivity and specificity for milk fat indicates its usefulness as a tool for detecting ketosis risk cows. The $\geq 4.1\%$ cutoff point was derived from the constructed receiver operator characteristic curve for DHI test-day milk fat (Duffield et al., 1997). In our

study, the milk fat percentage cutoff point for detecting cows with subclinical ketosis was $\geq 6.98\%$ and $\geq 6.40\%$ (DHI composite) based on urine ketones of ≥ 40 mg/dl and milk ketones ≥ 2.0 mg/dl respectively. These milk fat percentage cutoffs are likely higher due to only testing on day 7 and 14 versus the Duffield et al. (1997) study which used DHI test date data resulting in milk sampling occurring on average 7 days prior to blood sampling. In the study by Carrier et al. (2004), urine test and milk test strips were first used to identify at risk cows before the milk fat percentages because of these test's higher sensitivity and specificity (Se=0.49, Sp=0.99 and Se=0.27 Sp=0.99, KetoStix[®] and KetoLac[®], respectively; KetoLac[®] is a similar milk BHBA test to the Keto-Test[®] used in our study).

Compared to the typical Holstein fat percentage of 3.79%, the mean milk fat percentages for cows in the current study are well above the typical breed percentage (Table 1) (Bremmer, accessed June 2012). According to Duffield et al., 1997, a 1% increase in milk fat doubles the risk for subclinical ketosis. Based on this, the 2% to $\geq 3\%$ milk fat percentages (Table 1) above breed average in the current study are more than 2 times as likely to have subclinical ketosis.

CONCLUSIONS

Based on this study, the use of on-farm milk component analysis tools to measure milk fat composition is beneficial for early detection of subclinical and clinical ketosis in individual fresh cows, in addition to cow-side test strips. Both RF stripping and composite milk fat percentages can be used as an indication of the ketosis risk for cows, allowing for herd managers to monitor these cows sooner and thus reduce milk loss from disease.

IMPLICATIONS

Subclinical ketosis costs are estimated at \$78 per case; therefore, based on 40% prevalence in a 100-cow herd, the economic loss is approximately \$3,120 per year (Geishauser et al., 2001). Based on these numbers and 92 million dairy cows, the yearly projected loss nationwide due to ketosis is about \$2.8 billion per year. With earlier detection of subclinical and clinical ketosis, the cost associated with the treatment and loss of milk production associated with ketosis can be decreased.

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