Methods of Detecting Ketosis in Jersey and Holstein Herds

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Table of Contents

List of Tables

Abstract

The purpose of this study was to compare different methods of detecting ketosis in dairy herds. Data from two separate studies were used; one study included a commercial Holstein herd and the other a Jersey herd owned by the University. Methods used to detect ketosis at 7 to 14 days in milk were Ketostix (Bayer Corporation, Leverkusen, Germany) for urine, milk fat from right fore-quarter strippings measured using a Lacticheck (Page & Pedersen, International, Ltd., Hopkinton, MA) instrument, and blood beta-hydroxybutyrate (BHBA) concentrations. Data from 30 Jersey and 55 Holstein cows were analyzed using SAS. Average values for the Jersey herd were 11.4 mg/dL, 17.6 mg/dL, and 1.85% for urine ketones, blood BHBA, and milk fat, respectively. Urine, BHBA, and milk fat for Holstein cows were 3.36 mg/dL, 11.6 mg/dL, and 2.57%, respectively. For both herds, the BHBA and urine tests had the highest correlations; 0.44 with $P < 0.05$ for Jersey and 0.38 with $P < 0.01$ for Holstein. Jersey had the least correlation (0.07) between milk fat and BHBA with $P = 0.74$, while Holstein milk fat and BHBA had a correlation of 0.13 with $P = 0.39$. Milk fat concentrations in our study were not correlated to urine and blood ketones as has been reported in some other studies, possibly because fewer cows were used in our study. Both blood and urine ketones are effective measures for detection of sub-clinical and clinical ketosis.

Introduction

Ketosis is a metabolic disorder that occurs when cows enter into a negative energy balance. According to the Merck Veterinary Manual, ketosis occurs in times of severe negative energy balance with a high demand for glucose, causing mobilization of adipose tissue. Mobilizing body fat causes the concentration of non-esterified fatty acids (NEFA) in blood to increase. A large portion of serum NEFA are directed to the liver to be catabolized into ketone bodies. Ketone bodies in serum are acetone, acetoacetate, and ß-hydroxybutyrate (BHB). In ketotic cows, serum concentrations of NEFA and ketone bodies are high, while glucose concentrations are very low.

Because ketosis is associated with increased risk of many other diseases, it is very important to be able to diagnose it and treat animals quickly. Suthar et al. (2013) found that BHB concentrations greater than 1.1 mmol/L increased risk of lameness in herds 1.8 times. Cows with subclinical ketosis (SCK), defined by Suthar et al. (2013) as blood BHBA concentrations greater than 1.2 mmol/L, had 1.5, 9.5, and 5.0 times greater risk of developing metritis, clinical ketosis, and displaced abomasum, respectively. In addition to being a risk factor for other diseases, ketosis is associated with a decrease in milk production. McArt et al. (2012) found that cows testing positive (>1.2 mmol/L BHBA) for SCK within the first week of lactation produced 2.2 kg/day less milk for the first 30 days of lactation. A decrease in milk

production of 0.5 kg/day was associated with every increase of 0.1 mmol/L in BHBA at the first test positive for SCK. Subclinical ketosis defined between 1.2 and 1.4 mmol/L serum BHBA was found to be a risk factor for displaced abomasum, clinical ketosis, and metritis (Duffield et al., 2009).

There are several methods available to test for ketosis. The method with the highest sensitivity and specificity is BHBA concentration in the blood. Test strips and powders have been developed to detect BHBA in milk and acetoacetate in urine. Monitoring the fat-to-protein ratio (FPR) also has been found to give some indication of ketosis. Blood BHBA concentrations are often coined the "gold standard" because of the high specificity and sensitivity of the tests.

Using blood BHBA as a standard comparison, the Ketolac BHBA strip (Hoescht, Unterschleißheim, Germany) detects BHBA in milk and was found to have a sensitivity of 92% at 50 μmol/L and 72% at 100 μmol/L BHBA (Geishauser et al., 1998). These results were found to be very highly correlated with the BHBA concentration in blood at 0.84 and 0.92, respectively. Geishauser et al. (1998) also tested four Rothera tests, which are based on the reaction of acetoacetate with sodium nitroprusside. Rothera tests included Ketostix (Bayer, Etobicoke, ON, Canada), Bioketone powder (Societé d' Analyses Biopharmaceutiques, Lava, QC, Canada), Ketocheck powder (Great States, St. Joseph, MO), and Utrecht powder (University of Utrecht, Utrecht, The Netherlands). While all of the Rothera tests were highly specific, the sensitivities of each when used with milk were 0.43, 0.33, 0.28, and 0.05 for the Utrecht powder, Bioketone powder, Ketocheck powder, and Ketostix, respectively (Geishauser et al., 1998).

Carrier et al. (2004) found that, at 15 mg/dL, Ketostix had a sensitivity of 0.78 with a specificity of 0.96. This study also compared Ketocheck powder and the KetoTest milk strip (Sanwa Kagaku Kenkyusjo Co. Ltd., Nagoya, Japan) against blood BHBA concentrations.

While the Ketocheck powder was a more subjective measure of BHBA and was highly specific (0.99), it was very poorly sensitive (0.41). Using a cut off of 100 μmol/L of BHBA, the KetoTest milk strip had a sensitivity of 0.73 and specificity of 0.96.

The FPR is determined using the milk fat and protein percentages. Krogh et al. (2011) used a FPR > 1.5 to diagnose ketosis. When compared to the Ketolac BHBA test strips and Ketostix, FPR was the least specific at 0.79. FPR sensitivity ranked in the middLe of the three methods at 0.63 (Krogh et al., 2011). Duffield et al. (1997) found similar sensitivity (0.58) and specificity (0.69) and determined that a high milk fat percentage and low protein percentage was associated with an increased risk of SCK. However, the study did not suggest using FPR as a method of discriminating between cows with or without ketosis but suggested using it more as a tool of monitoring the herd.

Materials and Methods

Two farms were used in the study: a commercial Holstein operation and a Jersey research operation owned by the University. Holstein cows were part of a study determining the effect of a probiotic on lactation and were tested for ketosis between 7 and 14 days in milk. Jersey cows were on a separate trial and were also tested for ketosis between 7 and 14 days in milk.

Urine acetoacetate concentrations were measured using Ketostix (Bayer Corporation, Leverkusen, Germany) after stimulating the cows to urinate. The dipstick was inserted into the stream of urine, and after several seconds, was compared to the color chart on the container. Milk samples were stripped from the right fore-quarter and analyzed with a LactiCheck (Page & Pedersen, International, Ltd., Hopkinton, MA) instrument to determine milk fat concentration. Blood samples were taken from the tail vein and ß-hydroxybutyrate (BHBA) concentrations

were determined using an enzymatic assay kit (Sigma-Aldrich Co. LLC, St. Louis, MO). Concentrations were calculated using absorbance values from the microplate spectrophotometer (Molecular Devices Corp., Sunnyvale, CA).

Results from all of these analyses were collected and run through SAS to calculate correlation values between milk fat, urine acetoacetate, and blood BHBA. Cows missing information were not included, resulting in 55 Holstein and 30 Jersey cows being included in the study. Ketosis was defined as BHBA levels greater than 14 mg/dL or urine ketone levels greater than 40 mg/dL.

Results and Discussion

Holstein cows averaged 3.36 mg/dL urine ketones, 11.6 mg/dL BHBA, and 2.57% milk fat. Incidence of ketosis based on BHBA > 14 mg/dL was 20% and 1.8% based on urine ketones > 40 mg/dL. Urine ketones and milk fat in cows displaying clinical ketosis based on BHBA averaged 8.6 mg/dL and 3.27%, respectively (Table 1). The clinical ketosis cases based on urine ketones had 15.2 mg/dL BHBA and 2.15% fat (Table 1). Blood BHBA and urine ketones showed the highest correlation of 0.38 with $P < 0.01$, while fat and urine showed the least correlation with 0.06 and $P = 0.64$ (Table 2). Milk fat and BHBA had a correlation of 0.13 with $P = 0.39$.

Jersey cows displayed a higher incidence of ketosis. Average urine, blood BHBA, and milk fat values were 11.4 mg/dL, 17.6 mg/dL, and 1.85%, respectively. Incidence of clinical ketosis based on blood BHBA values was 87% and 13% based on urine ketones. Cows displaying clinical ketosis based on BHBA values averaged 18.4 mg/dL, 12.6 mg/dL, and 1.87% for BHBA, urine ketones, and milk fat, respectively (Table 1). Based on urine ketones, cows

with ketosis averaged 21.8 mg/dL, 60.0 mg/dL, and 2.18 % for BHBA, urine ketones, and milk fat, respectively (Table 1). Similarly to Holstein, Jersey cows data on BHBA and urine had the highest correlation of 0.44 with $P = 0.02$ (Table 3). Milk fat and BHBA had the smallest correlation of 0.07 with $P = 0.74$, while urine and fat showed a correlation of 0.17 with $P = 0.44$.

Despite having lower correlations than previous studies, the study did find a positive, significant correlation between BHBA and urine ketones. Studies have found that blood BHBA assays are more sensitive and specific, but that Ketostix also are a viable indicator of ketosis. Incidence of ketosis was extremely high in the Jersey cows due to the ration formulation. Once this was addressed, cows returned to normal, but this is not seen in the study as only the second week of lactation served as a collection time for data.

Some of the disparity between the BHBA levels and the urine ketone levels in the Jersey cows may be due to differences in milk fat. In a study done by Enjalbert et al. (2001), it was found that acetoacetate and BHBA concentrations were lower in milk than in blood. This may be due to the mammary gland using BHBA to synthesize fatty acids and the conversion of acetoacetate to butyrate. White et al. (2001) found that Jersey milk contains more short-chain fatty acids than Holstein milk. Because Jersey cows naturally have a higher fat content to their milk, some of the discrepancy seen in the incidence of ketosis based on blood BHBA or urine ketones may be explained by this. Excess BHBA in the blood may be flowing to the mammary gland to form the short-chain fatty acids.

Conclusion

While the most accurate method for ketosis detection is the blood BHBA concentration, it can be very costly and time-consuming. The urine ketone analysis with Ketostix is an applicable

on-farm test. Milk fat levels by themselves should be used as a management tool, rather than a diagnostic tool for ketosis.

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Table 1. Average blood ß-hydroxybutyrate (BHBA), urine ketones, and milk fat (right fore-quarter stripping) values for cows with ketosis.

	Cows with Ketosis (%)	Number of Cows with Ketosis	BHBA (mg/dL)	Urine Ketone (mg/dL)	Fat (%)
Holstein, based on BHBA[1]	20.0	11	15.5	8.6	3.27
Holstein, based on urine ketones [2]	1.8	1	15.2	40.0	2.15
Jersey, based on BHBA[1]	86.7	26	18.4	12.6	1.87
Jersey, based on urine ketones[2]	13.3	4	21.8	60.0	2.18

[1] Cows with BHBA $\geq$ 14 mg/dL were considered ketotic.
[2] Cows with urine ketones $\geq$ 40 mg/dL were considered ketotic.

Table 2. Correlation between urine ketones, blood ß-hydroxybutyrate (BHBA), and milk fat from fore-quarter strippings for Holstein cows

	Urine	Fat	BHBA
Urine	1.00	0.06	0.38
		0.64	<0.01
Fat		1.00	0.13
			0.39
BHBA			1.00

Table 3. Correlation between urine ketones, blood ß-hydroxybutyrate (BHBA) and milk fat from fore-quarter strippings for Jersey cows

	Urine	Fat	BHBA
Urine	1.00	0.17	0.44
		0.44	0.02
Fat		1.00	0.07
			0.74
BHBA			1.00