Efficacy of different cow side tests for diagnosis of ketosis in lactating cows

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A B S T R A C T

The aim of this work was to compare the efficacy of different cow side tests for diagnosis of ketosis including Precision Xtra ketone method and PortaBHB milk ketone tests in Holstein- Friesian dairy cows. The colorimetric estimation of serum BHBA at a cut-off point ≥1.200 mmol /L was used as a gold standard method. For that purpose, 200 Holstein- Friesian dairy cows up to 6 weeks postpartum were tested. The prevalence of ketosis was 11% by using colorimetric method. The apparent prevalence of ketosis was 12.5% by using of Precision Xtra ketone test at a cut-off point of blood BHBA ≥1.200 mmol /L with 90.9% sensitivity, 97.2% specificity, 80 % positive predictive value (PPV) and 98.9% negative predictive value (NPV). A significant correlation (r = 0.871; *P* <0.01) was recorded between serum BHBA measured by colorimetric method and whole blood BHBA measured by Precision Xtra ketone test. The apparent prevalence of ketosis was 20.5% and 6.5% by using PortaBHB milk ketone test at a cut- off point 100-200 µmol of milk BHBA /L with 86.4% and 45.5% sensitivity, 87.6% and 98.3% specificity, 46.3% and 76.9% PPV and 98.1% and 93.6% NPV, respectively. It was concluded that Precision Xtra ketone test and PortaBHB milk ketone test are simple and rapid tools for diagnosis of ketosis compared with the standard colorimetric method. However, Precision Xtra ketone test is more accurate and sensitive than PortaBHB milk ketone test.

Keywords: cow side tests, Ketosis, PortaBHB milk ketone test, Precision Xtra ketone test

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1. INTRODUCTION

Ketosis is a major metabolic disorder of dairy cows in early lactation, which develops when dairy cows fall into a condition of excessively negative energy balance (NEB) caused by insufficient dietary intake and generous lactation (Xu et al.,2010). Ketosis characterized by relatively high concentrations of the ketone bodies acetoacetate, β-hydroxybutyrate (BHBA) and acetone, and a concurrent low concentration of glucose in the blood (Melendez et al.,2006). The gold standard test for diagnosis of ketosis is measurement of ketone bodies in serum or plasma photometrically in a diagnostic laboratory. BHBA is used most frequently due to its stability in samples (Herdt, 2000). Concentration of BHB>1.200 mmol/L is used as the defining threshold of subclinical ketosis (Oetzel, 2012). There are two cow-side tests that are currently accepted a sufficiently accurate for routine use: PortaBHB milk test strips (PortaCheck Inc., Moorestown, NJ) for measurement of milk BHBA and Precision Xtra ketone meter test (Abbott Laboratories, Abbott Park, IL) for measurement of blood BHB (Iwersen et al.,2009 and Denis-Robichaud et al., 2011). The prevalence of ketosis in Egypt is not well defined although the disease causes great economic losses.

Aim: comparison the efficacy of different cow side tests for diagnosis of ketosis including Precision Xtra ketone method and PortaBHB milk ketone tests in Holstein- Friesian dairy cows

2. MATERIAL AND METHODS

2.1. Animals and design:

This study was implemented on 200 lactating Holstein- Friesian cows of different ages (3- 10 years old) during post parturient period up to 6 weeks postpartum with average of daily milk production (28.47±2.96) kg/day varies from 13 – 50 kg/day, These cows were located in eight dairy farms in four governorates (Kalubia, Dakhalia, Menofia and Ismailia governorates). According to colorimetric enzymatic method diagnosis of ketosis at a cut-off point 1.200 mmol /L (Duffield, 2000 and Zhang et al., 2012a) these cows were classified into 2 groups. Group 1 included control cows (n=178 cows)

negative with colorimetric enzymatic method. Group 2 included (22 cows) which are positive with colorimetric enzymatic method.

All 200 cows enrolled in this study were tested by Precision Xtra ketone test for detection of ketosis in blood at a cut – off point of blood BHBA≥ 1.200 mmol /L and PortaBHB milk ketone test at a cut- off point 100 and 200 µmol of BHBA /L to detect positive and negative cases for ketosis.

2.2. Samples.

2.2.1. Blood and serum Samples.

The blood sample was collected from jugular vein of all cows during postpartum period during the early morning (Kelly, 1984). Two types of blood samples were collected including whole blood for detection of blood BHBA by Precision Xtra ketone test and serum samples for detection of serum BHBA by colorimetric enzymatic method analysis.

2.2.2. Milk samples.

Left fore quarter strip samples were collected after udder preparation (Denis-Robichaud et al., 2011).

2.3. Diagnosis of ketosis by enzymatic laboratory test

Williamson et al. (1962) developed an enzyme catalysis method to test serum BHBA. Based on this method, a test kit was manufactured. The test kit required the use of an ultraviolet spectrophotometer or biochemistry analyzer and could be used to test the serum BHBA levels of humans and animals. Beta- hydroxybutyrate (BHBA) (Randox Laboratories Ltd, UK,)

2.4. cow side tests

2.4.1. Precision Xtra ketone test.

The Precision Xtra meter is a hand-held device that measures BHBA in fresh whole blood samples. Precision Xtra ketone strip contains the enzyme β-hydroxybutyrate dehydrogenase, which oxidizes BHBA to acetoacetate. This reduces nicotinamide adenine dinucleotide (NAD+) to NADH. NADH is then reoxidized to NAD+ by an electron transfer mediator molecule. The electrical current generated by this conversion is measured by the electronic hand-held BHBA meter and is directly proportional to BHBA concentration.

2.4.2. PortaBHB milk ketone test.

The PortaBHB milk ketone test is a semi-quantitative dipstick for the detection of subclinical ketosis (PortaCheck, Inc, Moorestown, NJ). It is used for detection of BHBA in milk it contains color chart provided on the test bottle (0, 50, 100, 200, or 500 µmol/L).

2.5. Statistical analysis.

Correlation coefficient (r) between serum BHBA measured by the colorimetric enzymatic laboratory method and blood BHBA values displayed on Precision Xtra ketone test was calculated by using Spearman's rank correlation analysis by using of the Sigma Stat 3.1, statistical software.

Prevalence of a disease is calculated by Beaglehole et al. (1993) by the following equation: Prevalence= $\frac{\text{Number ofcows with disease or condition at specified time}}{\text{Number ofcows in the population at risk at specified time}}$ (x 100).

True prevalence = $\frac{Apparent\ prevalence+Sp-1}{Se+SP-1}$ (x 100) (Carrier et al., 2004)

For evaluation of cow side tests for field detection of ketosis according to method described by (Lalkhen and McCluskey, 2008) the following terms and equations were used: Sensitivity of Precision Xtra ketone test and PortaBHB test was calculated by following equation:

$$\text{Sensitivity} = \frac{\text{True positive}}{\text{True positive+False negative}} (\text{x } 100)$$

$$\text{Specificity} = \frac{\text{True negative}}{\text{True negative+False positive}} (\text{x } 100)$$

$$\text{PPV} = \frac{\text{True positive}}{\text{True positive+False positive}} (\text{x } 100)$$

$$\text{NPV} = \frac{\text{True negative}}{\text{True negative+False negative}} (\text{x } 100)$$

3. RESULTS

3.1. Prevalence of ketosis by the gold standard colorimetric method

As shown in Table 1, the true prevalence of ketosis was 11% by using colorimetric enzymatic method which is the gold standard method for diagnosis of ketosis (Duffield, 2000 and Oetzel, 2007) as 22 cows suffered from ketosis from 200

Holstein – Friesian dairy cows enrolled in this survey at a cut-off point of serum BHBA ≥1.200 mmol /L.

3.2. Prevalence of ketosis by different cow side tests

The apparent prevalence of ketosis was 12.5% by using of Precision Xtra ketone test at a cut- off point of blood BHBA ≥1,200 mmol /L. Some values of BHBA measured by Precision Xtra ketone test were illustrated in Figure 1 (a-b). The apparent prevalence of ketosis was 20.5% by using PortaBHB milk ketone test at a cut- off point 100 µmol of BHBA /L. The apparent prevalence of ketosis was 6.5% by using PortaBHB milk ketone test at a cut-off point 200 µmol of BHBA /L (Table1). The changes in the color of PortaBHB milk ketone test strips are illustrated in Figure 2

3.3. Evaluation of sensitivity and specificity of Precision Xtra ketone test and PortaBHB milk ketone test.

Precision Xtra ketone test had 90.9% sensitivity, 97.2% specificity, 80 % positive predictive value and 98.9% negative predictive value at a cut-off point ≥1,200 mmol of blood BHBA /L (Table 2).

The correlation between BHBA in serum measured by (colorimetric enzymatic method laboratory method – BHB mmol/L) and BHBA of whole blood measured by (Precision Xtra test – BHB mmol/L) showed a significant positive correlation coefficient r = 0.871; p <0.01; no of cows = 200 (Figure 3).

PortaBHB milk strips had 86.4% sensitivity, 87.6 % specificity, 46.3% positive predictive value and 98.1% negative predictive value at a cut- off point 100 µmol of BHBA /L. (Table *2).* PortaBHB milk ketone test had 45.5% sensitivity, 98.3% specificity, 76.9% positive predictive value and 93.6% negative predictive value at a cut-off 200 µmol of BHBA /L (Table 2)

Figure (1a) Figure(1b)

Figure 1 (a-b) Demonstration of some values of blood BHBA measured by Precision Xtra ketone test using blood samples.

Table 1 The prevalence of ketosis by different diagnostic methods

Parameters	Serum BHBA by Colorimetric enzymatic method at a cut – off ≥1.200 mmol/L	Blood BHBA by Precision Xtra ketone test at a cut- off ≥12.00/mmol/L	Milk BHBA by PortaBHB milk ketone test	
			at a cut – off ≥100 µmol/L	at a cut – off ≥200 µmol /L
No. of examined cows	200	200	200	200
No. of cows Tested positive	22	25	41	13
True or Apparent prevalence%	11%	12.5%	20.5%	6.5%

Figure 2 the change in the color of PortaBHB milk ketone test strips on milk samples.

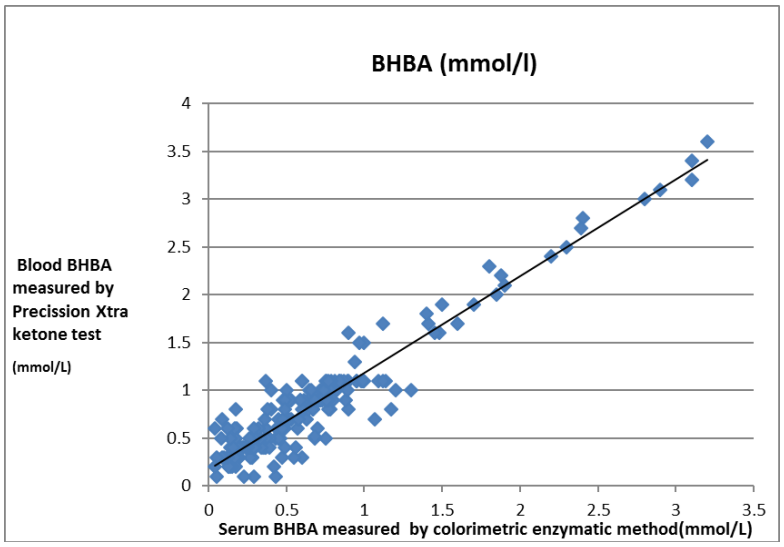

Figure 3 Correlation between BHBA in serum measured by (colorimetric enzymatic method mmol/L) and BHBA of whole blood measured by (Precision Xtra ketone test –mmol/L).

Table 2 Sensitivity, specificity, PPV and NPV of Precision Xtra ketone test at a cut-off point of blood BHBA ≥1.200 mmol//L and PortaBHB milk ketone test at a cut- off point 100-200 µmol of BHBA /L.

Parameters	Blood BHBA by Precision Xtra ketone test at a cut – off point ≥ 1.200 mmol/L	Milk BHBA by PortaBHB milk ketone test at a cut – off	
		≥100 µmol of BHBA/L	≥200 µmol of BHBA /L
Total examined cows	200	200	200
True positive	20	19	10
False positive	5	22	3
True negative	173	156	175
False negative	2	3	12
Sensitivity	90.9%	86.4%	45.5%
PPV	80%	46.3%	76.9%
Specificity	97.2%	87.6%	98.3%
NPV	98.9%	98.1%	93.6%

4. DISCUSSION

The precision Xtra ketone test had 90.9% sensitivity, 97.2% specificity, 80 % positive predictive value and 98.9% negative predictive value at a cut-off point of blood BHBA ≥1.200 mmol /L. This result is in lined with that recorded (Oetzel and McGuirk, 2009) who recorded 91% sensitivity and 94% specificity of Precision Xtra ketone test for the diagnosis of ketosis. Moreover, Iwersen et al. (2009) reported that Precision Xtra ketone test had 88 % sensitivity and 96 % specificity, 82% positive predictive value and 98% negative predictive value at a cut- off point 1.200 mmol of BHBA /L of whole blood.

Correlation between serum BHBA measured by laboratory colorimetric method – BHB mmol/L and blood BHBA measured by Precision Xtra test – BHB mmol/L showed a significant positive correlation (r) = 0.871; $P<0.01$; no of cows = 200. This result complied with those previously recorded (Krempaský et al., 2014) who recorded that r = 0.95; $P < 0.001$ between measurements of whole blood BHBA of dairy cows measured by the Precision Xtra ketone test and serum BHBA concentration measured by photometric method. Additionally, Oetzel, (2013) recorded that correlation coefficient (r) between handheld meter results and laboratory BHBA results were 0.92. These results validating the use of Precision Xtra ketone test for diagnosis of ketosis in cattle. In addition, several advantages have been proposed of using the Precision Xtra ketone test, it is a rapid method i.e. the results are known immediately, accurate method (had a high sensitivity and specificity), the cost of the Precision Xtra ketone test is less than the cost of laboratory testing, only a small blood sample is required and simple method as there is no need to process or mail serum or plasma samples to a laboratory.

The PortaBHB milk ketone test had 86.4% sensitivity, 87.6 % specificity, 46.3% positive predictive value and 98.1% negative predictive value at a cut- off point 100 µmol of BHBA /L. This result is lined with those of Denis-Robichaud et al. (2011) who reported that PortaBHB milk ketone test had 92% sensitivity, 78.1% specificity, 58.5% positive predictive value and 97.7% negative predictive value at a cut- off point 100 µmol of BHBA /L.

On the other hand, the PortaBHB milk ketone test had 45.5% sensitivity, 98.3% specificity, 76.9% positive predictive value and 93.6% negative predictive value at a cut-off point 200 µmol of BHBA /L. This result is comparable to those observed by Denis-Robichaud et al. (2011). Who reported that PortaBHB milk ketone test had 50.7% sensitivity and 99.7% specificity, 92.7% positive predictive value and 85.7% negative predictive value at a cut- off point 200 µmol/L.

There are several advantages of using PortaBHB milk strips, it is rapid and simple method (ease of collection of milk sample), need minimal training and cheap method. Therefore, farm application of PortaBHB milk ketone test is considered a useful tool to identify cows suffered from ketosis.

5. CONCLUSION

Ketosis is a common problem in early lactating Holstein Friesian dairy farms in Egypt. Precision Xtra ketone test is almost simple, accurate and rapid tool compared with diagnostic laboratory colorimetric method for diagnosis of ketosis. PortaBHB milk ketone test can be used for detection of ketosis, but their lower sensitivity and specificity as compared with Precision Xtra ketone test made it less accurate cow side test.

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