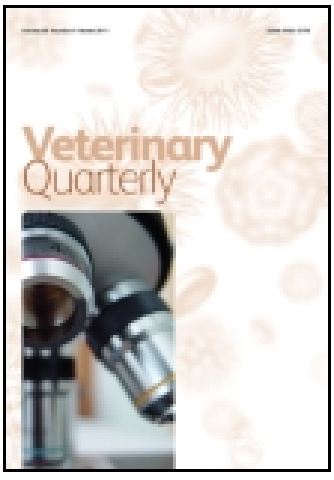

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DETECTION OF KETOSIS IN DAIRY COWS BY ANALYSIS OF EXHALED BREATH

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SUMMARY

In four healthy cows an elevation of ketone bodies was induced by reduction of feed intake. Two cows became clearly ketotic while the other two cows showed only slight increases in ketone body concentrations in serum and milk. Acetone concentrations in exhaled breath were measured by gas chromatography combined with mass spectrometry. These values were correlated with concentrations of serum β-hydroxybutyric acid (r=0.81) and milk acetoacetate+acetone (r=0.70). It is concluded that the ketotic state of dairy cows can be detected by analysis of exhaled breath. This offers a potential non-invasive method of determining the metabolic state of dairy cows.

INTRODUCTION

Ketosis is a metabolic disorder observed in dairy cows and is most likely to occur within the first 6 weeks after calving. Clinical signs include depressed appetite, decreased milk yield, weight loss, dull appearance, and rather dry faeces. There is a distinctive acetone-like odour of the breath and fresh milk. Temperature and heart rate remain normal. Elevation of ketone bodies, decrease in glucose concentration, and increase in free fatty acid concentrations are typical changes in blood composition (10). Observational studies show that ketosis is a risk factor for other diseases such as mastitis, cystic ovarian disease, and abomasal dislocation (1,5,9,11). Kremer *et al.* (6) observed that ketotic cows reacted more severely to experimental *E.coli* mastitis and suggested that components of the immune system may be compromised by ketosis.

Routine detection of subclinical ketosis would enable the farm manager to adopt preventative measures before the onset of the clinical stage and its related disorders. The chemical analysis of blood, urine, and milk enables the detection of subclinical ketosis. However, these methods are either unavailable or difficult to perform on a regular basis by the farmer. Equipment for on-line measurement of milk temperature, conductivity, and yield has been developed (8), but as yet the automatic detection of milk ketone bodies has not been reported. It was proposed that ketosis could be detected by analysing cows' breath with electronic chemical sensors (3). An electronic nose could be deployed in a fixed location such as a feed trough or a drinking device. Such instruments are based on a metal oxide semiconductor and conducting polymer gas sensors and are capable of discriminating between different odours (4). The choice of sensors depends upon the chemical constituents and a calibration should precede implementation. To detect the ketotic state in dairy cows, acetone is an obvious component to consider in exhaled breath. The correlation between the concentration of acetone in exhaled breath with ketone body concentrations in blood and milk of ketonaemic and non-ketonaemic cows are reported.

MATERIALS AND METHODS

Four healthy cows, 10-17 days post partum were given fresh cut grass *ad libitum* and 8 kg of commercial concentrates per day. Individual grass intake was measured over the first 3 days. Hyperketonaemia was induced by reducing the grass intake of each cow by 50% and by reducing the concentrates to 1 kg daily over a period of 12 days. Milking took place at 0.00 and 12.00 h, except during days 8-12 of restricted feed intake when the cows were also milked at 7.30 and 19.00 h. Blood sampling coincided with milking, except at 0.00 h, when no blood samples were taken. Serum β-hydroxybutyric acid (β-HBA) was determined enzymatically using the Randox test kit. In milk, acetoacetate and acetone (AcAc + Ac) were measured using the microdiffusion test with vanillin (2). On day 12 of restricted feed intake, samples of exhaled breath (500 ml) were taken at each milking from two cows showing high serum β-HBA and one cow showing low serum β-HBA prior to day 12. Each sample was drawn into individual poly(terephthalic ester) bags (Nalophan, Hoechst Thin Films Division, Witham, UK) using a purpose built sampling device held in the cow's nostril (7). The sample was discharged onto a multiple bed of adsorbent materials (40-60 mesh silica gel and charcoal) and was refrigerated. Acetone concentration was measured within 48 hours after sampling by gas chromatography combined with mass spectrometry (GC-MS). Samples were removed from the adsorbents by thermal desorption into the GC-MS system, where acetone was identified by mass spectral and chromatographic retention time matching. Quantification was performed by calibration with a known concentration of acetone.

RESULTS AND DISCUSSION

Cows maintained a good appetite and did not show any clinical symptoms. All cows had a mean serum ß-HBA above the normal value of 0.85 mmol/l. The effects of reduced energy intake on conventional chemical parameters were apparently different between cows. Cows A and D became clearly ketonaemic while cows B and C showed only slight increases in ketone body concentrations (Table 1). This is also reflected in the mean glucose concentrations, which were subnormal in cows A and D. The relationship between the conventional measures of ketosis and concentrations of breath acetone

ABBREVATION KEY:

ß-HBA = ß-hydroxybutyric acid
AcAc + Ac = acetoacetate and acetone
GC-MS = gas chromatography combined with mass spectrometry

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Table 1. Means and S.E.M. for serum β-hydroxybutyrate, glucose and acetoacetate + acetone of samples taken from cows on the 8th -12th day of restricted feed intake.

COW	β-HBA mmol/l (serum)		glucose mmol/l (serum)		AcAc +Ac mmol/l (milk)	
A	3.67	(0.11)	1.98	(0.11)	2.28	(0.14)
B	1.43	(0.12)	3.15	(0.09)	0.66	(0.09)
C	1.14	(0.04)	2.78	(0.06)	0.56	(0.06)
D	3.56	(0.18)	1.95	(0.09)	2.36	(0.14)

from GC-MS of cows A, C and D is shown in figure 1. The level of acetone in the breath was positively correlated to β-HBA in serum ($r=0.81$, $p< 0.01$) and to AcAc + Ac in milk ($r=0.70$, $p< 0.05$). Since the data showed two levels of ketone body concentrations, there is a hiatus in ketone body levels, as is demonstrated in figure 1. The relationship between ketone bodies in blood and acetone in breath remains unclear and may be either linear or curvilinear. A curvilinear relationship indicates a different biological mechanism behind the excretion of acetone by the lungs than a linear relationship would. In either situation, our data indicate that ketotic cows can be diagnosed by measuring acetone concentrations in breath. Non-invasive online devices to measure the health status of dairy cows are potentially attractive (8). Early detection of ketosis is of great potential value because the consequences of ketosis are severe while treatment is effective, feasible, and cheap (10).

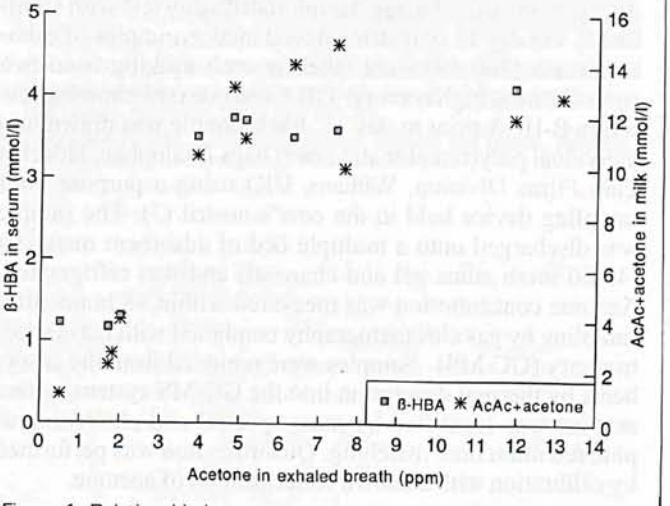

Figure 1. Relationship between acetone concentration in exhaled breath with β-hydroxybutyrate in serum and acetoacetate + acetone in milk of two ketotic and one non-ketotic cow.

Further experiments are planned in order to obtain values over the whole range of concentrations and to investigate the other components of the breath. The next step would be to assess the sensitivity and specificity of the on-line breath measurement. Finally, it has to be determined whether on-line detection of subclinical ketosis in dairy cows is practical and remunerative at farm level. It is clear that the ketotic state of dairy cows can be detected by analysis of exhaled breath by GC-MS.

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