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## Methods for Diagnosing Ketosis in Dairy Animals and Their Relative Effectiveness

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### Abstract:

Ketosis is a major metabolic disorder affecting dairy animals during early lactation, particularly high-yielding cows and buffaloes experiencing negative energy balance. Increased energy demands for milk production combined with insufficient feed intake led to excessive fat mobilization and formation of ketone bodies such as  $\beta$ -hydroxybutyrate, acetoacetate and acetone. Subclinical ketosis is especially challenging because affected animals often do not exhibit visible clinical signs, yet they suffer from reduced milk yield, impaired reproductive efficiency, weakened immunity and increased susceptibility to secondary diseases. Early detection is therefore essential to minimize production losses and improve herd health management. Conventional diagnostic approaches include blood, milk and urine testing. Blood  $\beta$ -hydroxybutyrate estimation remains the most reliable method, although it is invasive, expensive and less suitable for routine herd screening. Milk and urine-based tests are more practical under field conditions but have limitations in sensitivity and accuracy. Recent advances in precision dairy farming have encouraged the development of non-invasive diagnostic technologies based on animal behaviour monitoring and breath analysis. Among these, breath acetone measurement has emerged as a promising tool for rapid detection of ketosis because acetone produced during fat metabolism is exhaled through respiration. Modern gas sensor technologies, including semiconductor, electrochemical and infrared-based sensors, allow sensitive detection of breath acetone under farm conditions. The use of Tedlar bags for breath sample collection further improves sample handling and enables portable as well as laboratory-based analysis. Breath acetone testing offers several advantages such as minimal animal stress, rapid results, repeatability and suitability for large-scale screening. Despite challenges related to environmental influences and sensor calibration, this technology has strong potential for integration into precision dairy farming systems. Wider adoption of affordable and field-adapted breath analysis tools could significantly improve early diagnosis and management of subclinical ketosis in dairy animals, particularly in developing dairy sectors like India.

**Keywords:** Acetone breath analysis, Dairy animals, Ketosis, Precision dairy farming, Tedlar bag

### Introduction:

Ketosis is one of the most common metabolic disorders affecting dairy animals, particularly high-

yielding cows during early lactation usually within four to six weeks after calving (Duffield, 2000; Oetzel, 2004). During this period the animal experiences a sharp rise in energy demand to support milk production, while feed intake often remains insufficient (Grummer, 1995). This imbalance forces the body to mobilize stored fat reserves, leading to the formation of ketone bodies that accumulate in blood and are excreted through milk, urine and expired air (Baird, 1982). A major challenge in managing ketosis is that a large proportion of affected animals suffer from subclinical ketosis, which does not present clinical signs (Duffield, 2000). Although such animals appear normal, they experience reduced milk yield, impaired reproductive performance and compromised immunity (McArt *et al.*, 2012). These hidden effects result in considerable economic losses to dairy farmers. Therefore, early and accurate diagnosis of ketosis plays a vital role in maintaining animal health, sustaining milk production and improving overall farm profitability. Several diagnostic techniques have been developed over the years to detect ketosis. These methods differ widely in terms of accuracy, cost, ease of use and suitability under field conditions. The advantages and limitations of each method help farmers and veterinarians choose the most appropriate diagnostic approach for their production system.

### Importance of Early Detection:

Delayed detection of ketosis can lead to a series of negative consequences. Milk production may decline by five to fifteen percent, directly affecting farm income (Rajala-Schultz *et al.*, 1999). Animals with undetected ketosis are more susceptible to disorders such as mastitis, metritis and displaced abomasum, which further increase treatment costs (Duffield *et al.*, 2009). Fertility is also adversely affected, resulting in delayed conception and prolonged calving intervals (Walsh *et al.*, 2007). Since subclinical ketosis does not show visible symptoms, intervention is often delayed, allowing the condition to worsen. Hence, early and efficient detection forms the foundation of effective ketosis management.

### Blood Testing: The Most Reliable Diagnostic Tool

Blood-based diagnosis focuses on measuring the concentration of  $\beta$ -hydroxybutyrate (BHBA), the principal ketone body produced during excessive fat mobilization (Oetzel, 2004). Animals with blood BHBA levels below 1.2 mmol/L are considered metabolically normal. Levels equal to or above 1.2 mmol/L indicate subclinical ketosis, while concentrations of 3.0 mmol/L or higher are associated with clinical ketosis (Duffield, 2000). Blood BHBA estimation offers very high diagnostic accuracy and allows detection of metabolic disturbances even before visible clinical signs appear. Because of its precision and reliability, this method is widely regarded as the reference or “gold standard” for diagnosing ketosis (Iwersen *et al.*, 2009). However, blood sampling is invasive and requires trained personnel and laboratory facilities. The procedure is also relatively costly and time-consuming, making it less practical for routine herd-level screening. As a result, blood testing is mainly recommended for confirmatory diagnosis, research purposes and valuable animals.

### **Milk-Based Diagnostic Methods: Practical Options for Farmers**

Milk-based diagnostic techniques provide a convenient and non-invasive alternative for monitoring ketosis. Ketone bodies present in blood pass into milk and can be detected using milk analyzers or laboratory tests (Enjalbert *et al.*, 2001). Although milk ketone estimation is less precise than blood BHBA testing, it is useful for screening animals at the herd level. Another commonly used milk-based indicator is the milk fat-to-protein ratio, which reflects the animal's energy balance. A fat-to-protein ratio exceeding 1.4 is generally associated with an increased risk of ketosis (Heuer *et al.*, 2000). These milk-based methods are easy to integrate into routine milk recording systems and are particularly suitable for large herds. However, results can be influenced by feeding practices, breed differences and stage of lactation, and therefore milk indicators cannot be used alone to confirm ketosis.

### **Urine Ketone Testing: A Simple Field-Level Method**

Urine ketone testing is a widely used field technique in which ketone bodies are detected using color-changing dipstick strips (Geishauser *et al.*, 1998). This method is inexpensive, easy to perform and does not require specialized equipment. Results are obtained immediately making it especially useful under village and field conditions. Despite these advantages, urine ketone testing provides only semi-quantitative results and may fail to detect early cases of subclinical ketosis (Carrier *et al.*, 2004). Variations in urine concentration can also affect test accuracy. Nevertheless, urine testing remains a practical option for quick screening, particularly on small-scale dairy farms where access to laboratory facilities is limited.

### **Behaviour-Based and Sensor-Assisted Monitoring**

Advances in precision dairy farming have enabled continuous monitoring of animal behaviour using sensor-based technologies (Rutten *et al.*, 2013). These systems track parameters such as rumination time, feeding behaviour, physical activity and lying or standing patterns. Animals developing ketosis typically show reduced feed intake, decreased rumination and lower activity levels (Soriani *et al.*, 2012). Sensor-based monitoring provides real-time information and can generate early alerts before clinical signs become evident, making it particularly useful for large and organized dairy farms. However, this approach offers indirect evidence of ketosis and requires confirmation using biochemical tests. High initial investment costs also limit its adoption in smallholder farming systems.

### **Breath Acetone Measurement: A Modern Non-Invasive Technique**

Ketone bodies are also eliminated through respiration in the form of acetone, which can be measured using specialized breath sensors (Andersson, 1988). Subclinical ketosis generally corresponds to approximately two parts per million of acetone in exhaled air (Münster *et al.*, 2014). Breath acetone analysis is completely non-invasive, stress-free for animals and allows rapid, repeatable measurements. However, this technique requires specific equipment that is not yet widely available under rural farming conditions. Despite this limitation, breath analysis holds strong potential as a future on-farm diagnostic

tool due to its simplicity and animal-friendly nature.

### Scientific Basis of Breath Acetone in Dairy Animals:

Acetone is a volatile ketone body that readily passes from the bloodstream into the lungs and is exhaled through breath (Andersson, 1988). When a dairy animal experiences negative energy balance, fat breakdown increases, leading to elevated acetone production. A strong correlation between blood ketone levels and breath acetone concentration in cattle has been reported (Münster *et al.*, 2014). This means that measuring acetone in exhaled air can serve as an indirect indicator of ketosis without the need for needles or laboratory analysis. Because dairy animals breathe continuously, breath sampling offers real-time insight into metabolic status, making it especially useful for monitoring animals during the critical transition period.

### Technology Behind Breath Acetone Measurement:

Modern breath acetone measurement systems use high-sensitivity gas sensors capable of detecting very low acetone concentrations (Smith *et al.*, 2011). The main technologies include:

- **Semiconductor Gas Sensors:**

These sensors detect changes in electrical conductivity when acetone molecules interact with the sensor surface. They are affordable, compact and suitable for portable field devices (Righettoni *et al.*, 2015).

- **Infrared-Based Sensors:**

Acetone absorbs infrared radiation at specific wavelengths. Infrared sensors use this property to measure acetone accurately and consistently, even in variable environmental conditions (Miekisch *et al.*, 2004).

- **Electrochemical Sensors:**

These sensors generate an electrical signal when acetone reacts at an electrode. They offer good selectivity and low power consumption, making them suitable for handheld instruments (Turner *et al.*, 2009). With advancements in sensor miniaturization, these technologies can be integrated into portable, battery-operated devices suitable for farm use.

### How Breath Sampling Works in Dairy Animals:

Breath acetone measurement in dairy animals involves capturing exhaled air near the nostrils or muzzle using a sampling chamber or handheld device (Münster *et al.*, 2014). The process is:

- Quick and stress-free
- Safe for both animals and handlers
- Suitable for repeated measurements

Animals do not require restraint or sedation, which is particularly important in Indian farm conditions where labour availability and animal temperament vary.

## Tedlar Bags: An Effective Tool for Breath Sample Collection

Tedlar bags are widely used in breath research due to their chemical inertness, durability, and ability to preserve volatile compounds without contamination (Groves and Zellers, 1996). Made from polyvinyl fluoride (PVF), these bags are particularly suitable for collecting and storing breath samples containing acetone.

### Why Tedlar Bags Are Ideal:

- Non-reactive with acetone and other VOCs
- Lightweight and portable
- Reusable after proper cleaning
- Compatible with laboratory and portable analyzers
- Suitable for field and farm conditions

These properties make Tedlar bags a practical solution for breath sample collection in dairy animals, especially in rural and semi-urban Indian settings.

### Methodology: Breath Collection Using Tedlar Bags

Breath sampling using Tedlar bags is a simple and stress-free method for collecting exhaled air from animals (Miekisch *et al.*, 2004). The procedure involves positioning a sampling tube near the animal's nostrils, which is connected to the Tedlar bag, ensuring that the sample is free from contamination by ambient air or feed particles. This technique does not require restraint or sedation, takes only a few minutes, and can be repeated regularly, causing minimal disturbance to the animal. Once collected, the breath samples can either be analyzed immediately using portable sensors or transported to laboratories for more detailed examination with gas chromatography or sensor-based systems.

### Advantages:

Using Tedlar bags for breath acetone measurement offers several advantages over direct sampling methods:

- Non-invasive and animal-friendly
- Reduced handling stress
- High sample integrity
- Flexible analysis timing
- Suitable for large-scale screening
- Cost-effective for repeated monitoring

### Advantages Over Conventional Ketosis Detection Methods:

Breath acetone measurement offers several advantages (Andersson, 1988; Turner *et al.*, 2009):

- Non-invasive and animal-friendly
- No need for blood or milk sampling

- Rapid results within seconds
- Reduced dependence on laboratory infrastructure
- Lower long-term monitoring cost
- Suitable for on-farm and field conditions

These benefits allow veterinarians, extension workers, and farmers to identify subclinical ketosis early and take corrective nutritional or management actions.

### **Role in Precision Dairy Farming:**

Precision dairy farming focuses on using technology to monitor individual animals and optimize productivity (Rutten *et al.*, 2013). Breath acetone measurement aligns well with this approach by enabling:

- Individual metabolic profiling
- Early disease detection
- Data-driven feeding strategies
- Improved herd health planning

In the future, breath acetone sensors could be integrated with smart collars, mobile apps, or herd management systems, providing real-time alerts and recommendations.

### **Implications for Animal Welfare:**

Animal welfare is gaining increasing importance in modern dairy farming. Non-invasive diagnostic techniques reduce stress, pain and risk of infection (Rushen *et al.*, 2008). Breath acetone measurement supports ethical farming practices by minimizing physical intervention while still ensuring effective health monitoring. For dairy animals in early lactation already under physiological stress this gentle approach is particularly valuable.

### **Challenges and Practical Considerations:**

Despite its promise, breath acetone measurement faces certain challenges. Environmental factors such as temperature, humidity and barn air quality can influence readings (Miekisch *et al.*, 2004). Standardizing sampling protocols and calibration methods is essential for consistent results. Additionally, farmers and field workers require basic training to interpret results correctly and take timely action. However, ongoing research and field trials are steadily refining these aspects, making the technology more robust and user-friendly.

### **Future Prospects in the Indian Dairy Sector:**

India's emphasis on digital agriculture, livestock health and affordable innovation creates strong opportunities for adopting breath acetone technology. Indigenous development of low-cost sensors tailored to Indian conditions can accelerate large-scale implementation (NITI Aayog, 2021). With support from veterinary universities, dairy cooperatives and government schemes, breath acetone measurement could become a routine screening tool much like milk testing or body condition scoring.

### Selection of Diagnostic Methods at Farm Level:

The choice of diagnostic method should be based on farm size, resources and management practices (Oetzel, 2004). Small-scale farms can effectively use urine ketone strips combined with milk indicators. Medium-sized farms benefit from regular milk testing supplemented with periodic blood analysis. Large and organized farms are better suited to sensor-based monitoring systems supported by confirmatory blood or breath testing.

### Conclusion:

Early and accurate detection of ketosis is essential for maintaining dairy animal health, sustaining milk production and reducing economic losses. While conventional methods like blood, milk and urine testing remain valuable, modern non-invasive techniques such as breath acetone measurement offer rapid, stress-free and practical alternatives, especially for large herds and precision dairy farming. By selecting diagnostic methods suited to farm size and resources, farmers can identify subclinical cases early, implement timely interventions and improve overall herd productivity and welfare.

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