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### **Research Note**

# Automatic detection of oestrus cows via breath sampling with an electronic nose: A pilot study



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#### ARTICLE INFO

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Keywords: Diagnosis Electronic nose (eNose) Oestrus Exhaled breath To explore the possibility of automating the process of detecting oestrus cows via electronic olfaction, we designed and built an integrated measurement system consisting of a breath sample device, electronic nose, and a diagnosis model. The diagnosis performance, using a data set of 71 measurements on 52 cows in total, was a receiver operating characteristic curve with an area under the curve of 0.86, which had 83% sensitivity and 86% specificity for a specifically chosen threshold value. These results indicate a potential for automated detection based on breath sampling.

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#### 1. Introduction

Over the past fifteen years, the focus in dairy cow management shifted from the individual animal towards herd level (LeBlanc, Lissemore, Kelton, Duffield, & Leslie, 2006). For farmers, it has become more difficult to monitor fertility, disease, and health in cows on an individual level due to the increasing size of herds. In addition, industrialisation has led to selective breeding of high-producing cows. Consequently, there is a higher susceptibility to diseases, such as claw problems and mastitis (Alban, Agger, & Lawson, 1996; Waage, Sviland, & Odegaard, 1998). Health problems may cause economic losses due to suppressed milk production, deterioration in milk quality, increased veterinary care, medicine costs, shortened longevity, and increased culling rate. Automated diagnosis of the fertility and health status of cows could help improve farm management efficiency.

This study focuses on oestrus detection. Traditionally, this is done by visual observation. This is time consuming, labour intensive, and with varying detection rates (90% to less than 50%) (Roelofs, López-Gatius, Hunter, van Eerdenburg, & Hanzen, 2010). There are many devices on the market for (semi-) automatic detection (Roelofs et al., 2010). One such device is an activity meter, which automatically records the

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activity of a cow. Disadvantages of activity-based oestrus detection, besides high false positive detections, are inability to detect silent heat, high investment costs, and high replacement costs for lost devices, since every cow needs a device.

There is an emerging interest in electronic nose (eNose) devices, which potentially can be used as a new technique in automatic fertility status and disease detection. An eNose device is able to detect different volatile organic compounds (VOCs) originating from normal and disease-associated metabolic processes throughout the body, such as: acute puerperal metritis (Burfeind et al., 2014) and bovine tuberculosis (Ellis et al., 2014). Several studies have been performed for oestrus detection (Lane & Wathes, 1998; Mohamed, Maher, Shaban, & Hussein, 2009; Mottram et al., 2000; Wiegerinck et al., 2011). However, these studies differed from ours, since they were done in the perineal area of the cow. All samples were transported to the analysing device before analysis. For daily measurements, and with a larger number of animals, an automated sampling and detection procedure is desired.

Traditional eNoses are known for their nonreproducibility, as they suffer from drift and fouling (Wyse et al., 2004). Furthermore, standard metal-oxide sensors suffer from large temperature disturbances up to about 50 °C (AppliedSensor, Reutlingen). Temperature disturbances can have a dramatic effect in terms of reproducibility (Bruins, Gerritsen, van De Sande, van Belkum, & Bos, 2013). The eNose device used in this study uses an accurate temperature stabilisation, resulting in temperature disturbances of 3–5 °C. Together with the data-processing methods designed to deal with fouling, drift, and sensor-to-sensor differences, this resulted in reproducible performance in time and other circumstances (Bruins et al., 2012).

The objective of this pilot study was to test automatic differentiation between oestrus and non-oestrus cows via an electronic nose.

#### 2. Materials and methods

#### 2.1. Electronic nose device

All measurements were performed with one electronic nose (eNose) device, made available by The eNose Company, Zutphen, the Netherlands. Three different metal-oxide sensors (with different surface material) in quadruplicate were mounted inside the eNose device (in total 12 sensors). Sensor signals were generated from reactions of the VOCs with the surface material of the sensors, thereby changing the conductivity. The sensors (MLC, MLN, and MLX [Pt]) were supplied by AMS AG, Premstättin, Germany. The sensors were mounted on a hotplate, which went through a sinusoidal temperature profile (from 260  $^\circ\text{C}$  to 320  $^\circ\text{C}$ ). The temperature was controlled with high accuracy (within a few degrees Celsius). In the course of the sinusoid, the conductivity was recorded 32 times. One measurement cycle contained two consecutive sinusoids, with a total duration of approximately 20 s. The eNose device contains a pump that sucks in air, resulting in a constant airflow across the sensors. Each measurement consisted of two phases, the exposure phase and the recovery

phase. In the exposure phase, the sensors were exposed to the sampled exhaled breath from cows and the equilibrium of the sensors changed due to the introduction of new substances. After the exposure phase, the recovery phase allowed the sensors to return to the baseline equilibrium by releasing the substances from the sensor surface. This was done by leading filtered air over the sensors. The air from inside the barn was filtered from environmental influences with an active carbon filter (ABEK1 gas filter, R2157, Respirátor Rt., Budapest, Hungary).

#### 2.2. Breath-sampling device

To sample and analyse the exhaled breath of every cow on the spot, the cow had to breathe into a handheld bucket-like sampling device (Fig. 1). The device consisted of a modified 31 L, polypropylene bucket. Another polypropylene bucket of 6 L capacity was placed inside it. Self-made one-way valves (with a diameter of 2.3 cm each) were placed in the bottoms of the buckets. As the cow inhaled, air from outside the bucket passed through the one-way valves and entered the larger bucket. As the animal exhaled, the air from inside the device exited through the one-way valves in the smaller bucket. Air was sucked from the bucket and transported into the eNose by a pump (50 mL min<sup>-1</sup>) which was positioned inside the eNose. The air was transported to the eNose through polyethylene tubes (inner diameter 4.35 mm, length 60 cm). Before the air entered the eNose, it was filtered twice. First, through a rough disposable filter (FT 4874, Coopers Fiaam, Guyancourt Cedex, France), to filter out the major polluting particles, and second, two Teflon filters (50 mm diameter, Savillex, Eden Prairie, US) with a filter membrane of  $1-2 \mu m$  (PTFE, Savillex, Eden Prairie, US) in order to remove the remaining dust, dirt, and moisture (Fig. 1). For comfort, a polyester nylon sheet was positioned between two lids, having a hole in the centre of between 10 and 19.5 cm. In this way, it was suitable for animals with varying head sizes.

#### 2.3. Measurement protocol

Measurements were performed over 3 weeks, from mid-August until the beginning of September 2015. 52 cows were measured on five different farms located in the east of the Netherlands.

Every farmer monitored all cows for oestrus behaviour. In some cases, this was done using an activity sensor and/or a heat-expectancy chart. Before each insemination, the inseminator checked by rectal palpation whether the cow was in heat. Breath measurements were then performed, and repeated after 6 days, when the cow was non-oestrus. The complete dataset contained 71 measurements from 36 oestrus and 35 non-oestrus cows. During di-oestrus (6th to 17th day of cycle) or when she was pregnant, the cow was classified as non-oestrus.

Each measurement lasted 10 min (5 min breath sampling, and 5 min filtering with air from inside the barn). The cow breathed into the bucket during 40 s, after which a closing lid was put on top while the measurement continued for 9 min and 20 s. A timeframe of 40 s was chosen because cows have a tidal volume of  $\pm 127$  L min<sup>-1</sup> (Gallivan, Viel, Baird, & McDonell,

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