Automatic blood sampling in dairy cows

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Abstract

Loose housing systems for dairy cows are becoming increasingly common, raising new questions in the area of animal health and welfare. Some of these questions can be addressed by studying the variation in blood parameters, such as glucocortioids. However, the traditional manual blood sampling procedure can in itself affect the responses of the animal. To address this issue we have developed a device for automated collection of multiple blood samples. The device is placed on the back of the animal and allows the animal to be kept in all types of environments, either alone or in social groups. The animal can move freely: no restraints and no handling of the animal are necessary during blood sampling.

Three experiments were conducted to study the performance of the system and how the blood sampling procedure affected the cortisol responses in dairy cows. In the first experiment the accuracy of sample size and timing were investigated.

In the second experiment, automatic samples were collected from six cows kept in tie-stalls, with samples taken at 3-min intervals for 38 min. At 12, 24, 36 and 38 min, a manual sample was taken by vein puncture. None of the cows showed a consistently increased cortisol response to automatic sampling, while two cows showed increased cortisol concentration after vein puncture.

In the third experiment automatic blood sampling was conducted with 12 cows kept in a loose housing system. In the morning, over a period of 1.5 h, 14 blood samples were taken at varying time intervals from each cow. In the afternoon, over a period of 2.5 h, a further 14 samples were taken. Cortisol concentrations in the blood samples suggest that the cows were not affected by the blood sampling procedure.

On some occasions during these experiments, it was not possible to get a sample. For the manual vein puncture 3 out of 24 samples (12.5%) were not successfully taken within a time limit of 2 min. During the automatic blood sampling process, the catheter occasionally became twisted and blocked by the movement of the cow’s head. This occurred in 8 out of 84 samples (9.5%). In four of the manual samples, considerable hemolysis had occurred during the sampling procedure. No hemolysis was observed in the automatic blood samples.

Automated blood sampling offers the opportunity to collect a series of samples without disturbing the animal. Data suggest that automatic blood sampling is a superior alternative to manual blood sampling, even in a tie barn.

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1. Introduction

Loose housing systems are becoming increasingly common as they facilitate the automation of daily routines such as milking and feeding, thereby allowing the farmer to produce more milk per work hour. However, from a health, welfare and nutritional perspective, this creates a wide range of new questions that need to be addressed.

Blood sampling is a powerful tool to address the physiological responses of an animal and can give important information on its health, welfare and nutritional state. From a blood sample many parameters can be accessed, such as hormonal and immune status and metabolic levels. However, many of these parameters are correlated with the method by which one chooses to take the blood sample (Hopster et al., 1999; Goddard et al., 1998). In most cases manual blood samples are taken by restraining and eventually separating the animal during the blood sampling process, but catching and restraining can in itself induce stress responses. For example, in repeated blood sampling of cows in a loose housing system using a head lock, Hopster et al. (1999) found increased plasma cortisol concentration, even in animals accustomed to the procedure. In reindeer the cortisol level increased up to five times when the animals were caught and restrained during blood sampling compared to automatic blood sampling (Cook et al., 2000; Sikkinen et al., 2004). Increases in the cortisol level indicate that the hypothalamus–pituitary–adrenal-axis (HPA-axis) is activated during manual blood sampling. The HPA-axis is one of the primary adaptive mechanisms in response to stressors (e.g. review by Sapolsky et al., 2000). In various animal species including ruminants, increased HPA-axis activity acts to mobilise energy during stress and therefore energy metabolism can be affected (Elässer et al., 2000). The corticotrophin-releasing factor (CRF) is also active at the central nervous system level and is involved in the regulation of feed intake as well as behavioural responses to stress (Matteri et al., 2000; Dunn and Berridge, 1990; Koob, 1999). Obviously it is important to minimise the restraining and handling of animals when carrying out behaviour, health, nutrition and stress research, as the effect of restraining the animal may alter the responses and induce larger individual variation. A system that can collect blood samples automatically and be placed on the animal would be more appropriate than manual blood sampling, especially when a series of blood samples is required.

Two other groups have reported the use of an automated blood-sampling device on large animals: the ABSE described by the Goddard et al. (1998) and the Dracpac described by Ingram et al. (1994). The Dracpac system is based on a single lumen jugular vein catheter, from one lumen a heparin solution is pumped to the tip of the catheter where it mixes with jugular blood. From the second lumen the mixed jugular blood is drawn continuously into the second line. The ABSE system works through a single lumen catheter. Once a sample has been taken, the ABSE flushes the catheter line with heparin. Before the ABSE takes a new sample, the heparin in the sampling line is deposited in a waste container, a motion that at the same time draws fresh blood into the sampling line. Both systems use a multiport valve to distribute the blood into vacuum tubes. However it appears that none of the systems are available today. Inspired by the work of Ingram et al. (1994) and Goddard et al. (1998) we started developing a system for automatic blood sampling.

The objective of this project was to develop a system for automatic blood sampling of freely moving cows. The system should be lightweight and compact in order to be carried by the animal. A special backpack was designed that allows the animal to move around in its normal environment during tests. After the prototype blood sampling system was created, the company IceRobotics entered into an agreement with DIAS to develop a production version. The resulting device was used in this study and is now commercially available as the IceSampler.

2. Materials and methods

2.1. Technical description of the IceSampler

The IceSampler consists of a small peristaltic pump for pumping anticoagulant solution and drawing blood, a distribution unit that can switch between evacuated sample tubes, a waste container, and anticoagulant solution. It connects to a catheter inserted into the jugular vein of the animal.

The catheter uses a 900-mm length of Tygon® Micro-Bore Tubing S-54-HL (Saint-Gobain, France) (1.02 mm × 1.78 mm: I.D. × O.D.). It is inserted using a 2.5-mm × 110-mm special needle (Mediplast ref: 62600259, Sweden). This needle is inserted into the jugular vein, and the catheter is inserted into it, and then further into the jugular vein. The needle is then removed leaving 150–200 mm of the catheter in the vein. The catheter is connected to a sampling line made from an additional 900-mm length of catheter tubing and connected to the peristaltic pump. The pump is fitted with 85 mm of SaniTech® STHT-C-125-2 (Saint-Gobain, France) (32 mm × 64 mm: I.D. × O.D.). It has a maximum flow of 0.52 ml/s. From the pump a modified Safety-Lok™ blood Collection Set (Becton Dickinson, USA) is attached, the vein needle is removed and the silicone tubing is connected to the pump. At the other end of the modified collection set, the needle is attached to a carrier mechanism for moving the needle in and out of tubes and containers. The carrier mechanism is actuated by a stepper motor and its initial position is located by a hall sensor. The sample tubes, anticoagulant solution and waste container are mounted in a carousel. The carousel holds 16 sample tubes and two bags, one for the anticoagulation solution and the other for the waste. The sample tubes are 4.5 ml S-Monovette® (ref 05.1106.100 Sarstedt, Germany). The two bags are 50 ml Viaflo 9 mg/ml infusion bags (Baxter, USA). Both infusion bags’ penetrable membrane is positioned at the same arc as the sample tubes and the rest of the bags are fitted within the centre of the carousel. The waste container is an empty infusion bag. The anticoagulation bag is filled with an additional 30 ml of saline and added heparin to a final concentration of 25 IU/ml (Leo Heparin 25,000 IU/ml, ref 585464, Leo Pharma Nordic, Denmark).

The motion of the carousel is actuated by a DC motor and controlled by an encoder and a hall sensor for resetting the position. The IceSampler is powered by a 12-V recharge-
able battery pack (NiMH 2000 mAh). It is housed in an IP66 fibre-reinforced impact-resistant plastic casing and measures 280 mm × 190 mm × 130 mm with a total weight of 2.9 kg. The IceSampler is illustrated in Fig. 1.

The system is controlled by a custom-built circuit board that is further mounted in protective casing. The unit is programmed by connecting a computer (Windows XP) to the circuit board via a USB port and using software supplied with the IceSampler. The software gives full control over the timing of samples, both in terms of the time delay before sampling commences and the time intervals between each sample. The pump can be programmed for speed as well as pumping duration for each operation (filling, sampling and flushing).

2.2. The IceSampler’s operations

When the system is started or on standby, the sampling line is filled with a heparin solution and the needle is inserted in the heparin bag. The sampling operations are described in Table 1.

2.3. The backpack

A backpack for the IceSampler has been designed for cattle (Fig. 2). The backpack positions the IceSampler on the left side of the thoracic vertebrae and is kept in place by two 8-cm wide elastic straps. A lycra chest cover is used to prevent the backpack from skating to either side. This goes all way up to the atlas joint and covers up the sampling line and the catheter entrance. The backpack is then sealed with a strong zipper and secured by cable ties. Inside the backpack, the IceSampler is further protected by a 5–10-cm foam surround.

2.4. Experiment using the IceSampler

Three experiments were conducted to evaluate the IceSampler performance compared to manual sampling, as well as efficiency of sampling in a loose housing system. Performance was evaluated in terms of stability, precision, diluting levels and cortisol concentration.

2.4.1. Experiment 1

Six Holstein Friesian dairy cows kept in tie-stalls were used for experiments 1 and 2. All cows were catheterised 2 days prior to blood sampling.

The IceSampler was used to collect blood samples at varying time intervals. Samples were taken at intervals of 2, 4, 8, 16, 30 and 60 min for each cow in a randomly assigned order. The weight of the sample tube before and after blood sampling was recorded. For each sample, the exact time when the IceSampler started filling the sample tube was recorded by visual observation of the catheter.

2.4.2. Experiment 2

Dilution and cortisol response were evaluated by comparing manual and automatic blood samples. Before use the IceSampler was cooled down to 2–5°C. Once mounted in the backpack, an additional five icepacks were inserted into the backpack, surrounding the IceSampler. Inside the IceSampler a temperature logger was installed, and data was collected every 10 min, before and during the experiment. The IceSampler was connected to the cow’s catheter, and then the cow

<table>
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<th>Table 1 – The basic operations the IceSampler performs during sampling</th>
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<td>1. Standby</td>
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<td>2. Filling</td>
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<td>3. Sampling</td>
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<td>4. Flushing</td>
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was left undisturbed for 20 min before any sampling occurred. Automatic blood samples were taken at time 0, 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, 36 and 38 min. At time 12, 24, 36 and 38 min, a manual sample was taken by vein puncture. The manual samples were taken from the jugular vein on the opposite side from that attached to the catheter being used with the IceSampler. Manual samples were taken by two persons. One person held the cow by pulling the cow’s head to the side using a rope. The second person collected the blood sample using a 20G × 1.5 in. needle Vacuette®, Greiner bio-one. Manually collected blood was extracted into the same vacuum tubes as used in the IceSampler and stored on ice. After each manual sample the cow was left undisturbed until the next sample. If the manual sample was not taken within 2 min after approaching the cow, it was considered failed. After all blood samples had been collected, the samples were centrifuged for 20 min 2000 × g and aliquots of plasma were stored at −20 °C until analysis for cortisol and calcium concentration.

2.4.3. Experiment 3
The IceSampler was tested for its performance in a loose housing system.

Twelve cows, 7 Holstein Friesian and 5 Danish Red, kept in a group of 50 animals were used for automatic blood sampling. Two cows were tested at a time. At the first day, the backpacks were fitted to the two selected cows to enable them to become accustomed to them. On the second day both cows were catheterised. On the third and fourth days blood samples were taken. In the morning 14 samples were taken with intervals of 10, 10, 10, 3, 2, 2, 2, 3, 3, 5, 5, 5, 15 and 15 min. At midday 14 samples were taken at 15, 15, 5, 5, 5, 5, 5, 5, 20, 20, 20 and 20 min. Before the automated blood sampling was initiated, the cow had its catheter tested and flushed, the IceSampler installed and then the cow was left for 20 min before the first sample was taken. The IceSampler was kept cool as described in experiment 2. After the 14 samples had been collected, the IceSampler was removed, the blood samples were centrifuged, stored and analysed for cortisol concentration as described below.

2.5. Assay
Plasma cortisol concentration was determined using time resolved florescence TR-FIA (Wallec OY, Turku, Finland), modified for use in cattle by dissolving calibrators in charcoal stripped cattle plasma. Intra-assay variation (CV = 15.8%), inter-assay variation (CV = 17.8%). Because of this high variation, all series of samples or sample pairs were analysed in neighbouring wells where the assay variation was (CV = 1.65%) between wells.

Blood plasma calcium was determined by a traditional colorimetric method. The analyses were performed using an autoanalyser, ADVIA 1650® Chemistry System (Bayer Corporation, Tarrytown, NY 10591, USA). Intra-assay variation (CV < 0.6%), inter-assay variation (CV < 1.2%) and relative bias (2.7% deviation from ideal value).

2.6. Statistics
The weight of blood samples, calcium concentration and cortisol concentration are presented as mean ± 95% confidence interval. Differences in cortisol and calcium concentrations between manual and automatic samples from the same individual and taken at the same time were analysed using paired t-test. Differences in cortisol concentration between samples were analysed using the mixed procedure of SAS (Littell et al., 1996). The model included sample number as fixed factor and sample within animal as repeated measure. The best fit according to Akaike’s Information Criterion or Schwarz’ Bayesian Criterion was chosen. Due to the distribution of data, a logarithmic transformation of the dependent variable was implemented to meet the assumptions of normality and homogeneous variances.

3. Results and discussion

3.1. Performance and success rate of the IceSampler
Numbers of full samples were recorded for experiments 1–3. In experiment 1, 77% of the samples were successfully collected (65 out of 84). The main cause of failure to collect samples was attributed to a mechanical error in the positioning of the carousel which resulted in 10 missing samples. The remaining nine missing samples were due to a blocked catheter, since occasionally a cow managed to block the catheter by moving its head to the same side as the catheter. The catheter opened up again once the cow moved its head away from this position. In experiment 2, 89% of the automatic samples were collected successfully (75 of 84). The nine missing samples were again caused by a twisted catheter. Out of the total number of manual blood samples, three samples (12.5%) were not taken within 2 min and were regarded as missing samples. In experiment 3, more mechanical errors occurred during the blood sampling, see Fig. 3.

The total success rate was 75%. However, many of the mechanical errors occurred at the outset of the experiment. The mechanical errors included a problem with the needle holder and the carousel’s position mechanism (worm gear backlash). As these problems occurred, the IceSampler mechanical parts were modified and improved, and from Fig. 3 it can be noted that on week 6 the IceSampler was working at a 97% success rate.

In the loose housing system mechanical failure due to interactions between cows was to some extent expected. However, none of the system failures were caused by rough treatment of the backpack either by the carrier or by the other cows in the group. On one occasion a cow managed to pull the catheter out during the mounting of the equipment, this resulted in the loss of 4% of the total number of samples. The catheter was occasionally blocked by the position of the cow’s neck, causing failure in 6% of all samples.

Furthermore, a human error caused the loss of 2% of the samples owing to incorrect connection of the pump.
3.2. Volumes and timing

The volumes of the blood samples in experiment 1 were fairly consistent (3.04 ± 0.1 g). The data does not indicate that the sample weights were affected by whether the sample was taken after long intervals (60 min; 3.10 ± 0.31 g) or short intervals (2 min; 3.13 ± 0.37 g). The IceSampler internal clock lost 4.6 ± 0.35 s/h. A higher accuracy can be obtained by calibrating the internal clock at 4–7 °C, which was the operating temperature in this experiment.

3.3. Cooling

The average temperature inside the IceSampler was 6.3 ± 2.08 °C at the beginning of experiment 2. Over a period of 70 min the temperature inside the IceSampler increased slightly to 6.9 ± 1.75 °C. During the experiment average and maximum outside temperature were in the range of [−7.8 to 16.4 °C] and [−4.5 to 23.0 °C], respectively (DMI, Denmark). The ice packs were able to keep the internal temperature low, but they were not able to lower the temperature. Hence, it is important to keep the starting temperature low during programming and transport.

3.4. Dilution

The calcium in an animal’s blood is involved in the control of muscle contraction and nerve function, and therefore the calcium level is strictly regulated and maintained at a constant level (Ganong, 1975). Furthermore, calcium is not degraded in a blood sample, thus the calcium concentration can be used as a marker of dilution of a blood sample. In this experiment the calcium concentration in the automatic blood samples was compared to that of the manual samples to investigate if the IceSampler’s flushing and drawing mechanisms were diluting the calcium concentration. The results showed that the manual samples had a significantly higher calcium concentration than the automatic samples (P < 0.0001) (Table 2).

The blood samples from the IceSampler had a calcium concentration that was 3.44 ± 1.14% lower than the manual samples, thus the IceSampler was diluting the samples slightly. This dilution is probably caused by the changes in tubing diameter when the sampling line connects to the pump tubing. This creates a pressure drop where mixing of blood and heparin solution is predominant. However, both the diluting percentage and the variation between samples were fairly low. If diluting is considered to be a problem, a solution could be to lengthen the time the IceSampler pumps to waste before taking an actual sample. This will remove more of the mixed fraction in the pump, but the capacity of the waste bag would be the limiting factor.

In the same samples as used for measuring the calcium concentration, the cortisol concentration showed a high correlation (r = 0.84) between manual and automatic samples. There were no differences in the cortisol concentration between the two sampling methods (P = 0.85, Table 3).

### Table 2 - The calcium concentration (mmol/l) in automatic and manual blood samples

<table>
<thead>
<tr>
<th>Method for blood sampling</th>
<th>Mean (mmol/l)</th>
<th>95% confidence interval</th>
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<tbody>
<tr>
<td>Manual</td>
<td>2.44</td>
<td>0.066</td>
</tr>
<tr>
<td>Automatic</td>
<td>2.36</td>
<td>0.081</td>
</tr>
</tbody>
</table>

### Table 3 - The cortisol concentration (ng/ml) in automatic and manual blood samples

<table>
<thead>
<tr>
<th>Method for blood sampling</th>
<th>Mean (ng/ml)</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manual</td>
<td>4.88</td>
<td>1.92</td>
</tr>
<tr>
<td>Automatic</td>
<td>4.78</td>
<td>1.80</td>
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</table>
3.5. **Response to manual blood sampling**

Experiment 2 was designed to reveal if the cows were affected by the handling sustained during manual sampling. The average cortisol concentration in the samples taken before manual blood samples was 3.04 ± 0.71 ng/ml (Fig. 4), which is in agreement with baseline levels found in other experiments (Hopster et al., 1999).

The results suggest that two of the six cows were affected by the handling during manual blood sampling. The animals were normally tethered and accustomed to handling during milking, feeding and bed cleaning thus they were used to humans and being handled. Hopster et al. (1999) found that most cows responded with increased cortisol concentrations during repeated blood sampling by jugular puncture in cows kept in a loose housing system. In a loose housing system animals usually have less experience of being close to humans, while the cows in our experiment were very accustomed to being close to humans. Other authors have reported that cow’s responses to handling or being close to humans can easily be changed by their previous experience of handling (Rushen et al., 1999; Munksgaard et al., 1997).

3.6. **Response to automatic blood sampling in loose housing system**

The experiment was conducted to investigate how cows in a loose housing system reacted to the automated blood sampling system. Concentrations of cortisol both in the morning (mean 4.24 ± 0.53 ng/ml) and around midday (4.93 ± 0.62 ng/ml) were within the normal range of baseline levels (Fig. 5). During these two periods the cows were lying, eating, grooming, and sometimes milked in the robotic milking machine or brushed by automatic mechanical brushes.

In the morning the cortisol concentration was higher in sample 1 compared to samples 4–14 ($P < 0.05$) and in sample 2 compared to samples 5, 8, 9 and 13 ($P < 0.05$). However, around midday no differences were found in the samples ($P = 0.78$). High cortisol values in the first two morning samples could indicate that the animal was affected by the handling and the mounting of the IceSampler. An alternative explanation could be that the animal was affected by morning routines in the stall, e.g. feeding or cleaning. Further experiments are needed to clarify this.

4. **Conclusion**

The automatic blood sampling system is a robust technique for repeated blood sampling that can be applied in a loose housing system. The system can withstand the animal’s daily routine and interaction with other animals. Technical problems have been corrected and now the primary cause of missing samples is believed to be caused by the twisting of catheters when the cows move their head. Our data indicate that even in a tie-stall automatic blood sampling is less stressful to the animal.

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**References**


