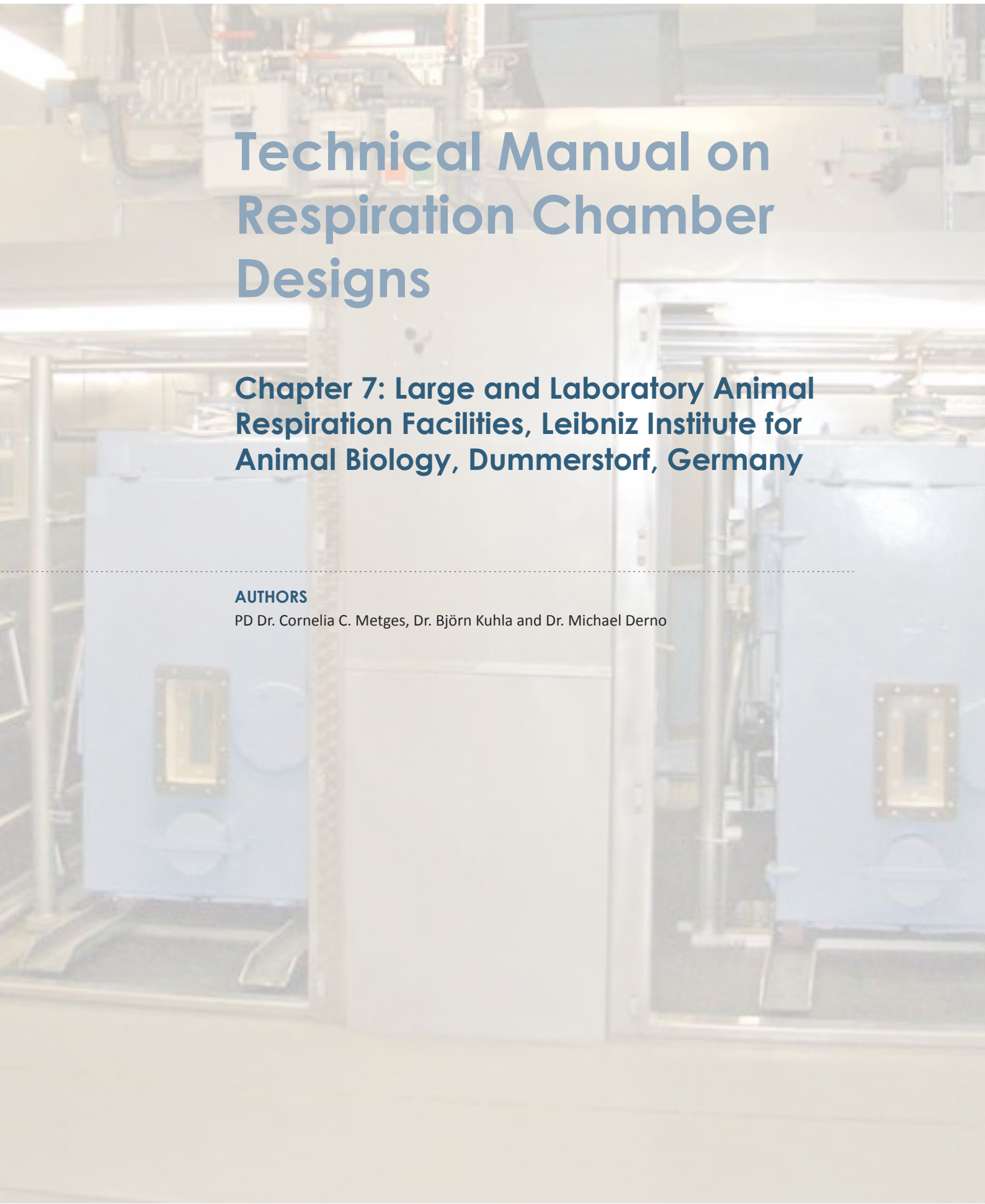

Technical Manual on Respiration Chamber Designs

Chapter 7: Large and Laboratory Animal Respiration Facilities, Leibniz Institute for Animal Biology, Dummerstorf, Germany

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7.1 Summary

The open-circuit indirect calorimetry system at the Leibniz Institute for Farm Animal Biology consists of 4 respiration chambers for cattle or sheep, 4 chambers for pigs and 6 chambers for mice. All chambers as well as the gas analyzing and data acquisition system are located in a dedicated facility. Chambers can be light-cycle and climate controlled in the temperature and relative humidity range from 0°C to 35°C and 50% to 70%, respectively. The air is sucked through the chambers by rotary vane vacuum pumps having a capacity of 40 m³/h. The airflow through the chambers can be controlled by means of a bypass. CH_4, CO_2 and O_2 concentrations are measured using specific gas analysers. Continuous monitoring CO_2 production and O_2 consumption, CH_4 emission, feed and water intake, and physical activity is possible. The facility has been designed to achieve a high standard of animal welfare.

7.2 Location of the facility

The facility is located at the campus of the Leibniz Institute for Farm Animal Biology in Dummerstorf, a village near the city of Rostock in the North of Germany at the Baltic Sea (2 hours drive to Berlin and Hamburg). The Institute is a premier European Institute for interdisciplinary basic as well as applied research on farm animals relating to resource efficient, sustainable animal production and the health and welfare of animals. The Institute employs over 300 members of staff and is a member of the Leibniz Scientific Community in Germany.

The physical address of the facility is:

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The facility houses all cattle and pig chambers in one room, and rodent chambers in another room. In front of the cattle chambers a balance is located. Instrumentation for calibration, gas analysis, data acquisition and data handling is located in a separate dedicated room. Adjacent rooms, equipped with temperature and humidity control, provide tie-stall housing for a limited number of cattle as well as pens and metabolic cages for pigs and sheep so that animals have short-way access to the chamber area. A video monitored surgery room with treatment stanchions for endoscopy and ultrasound, an anaesthesia box, as well as rooms for feed storage, feed preparation, milling and drying are also nearby.

7.3. Description of the chambers structure

Cattle chamber

The general outline of the system is shown in Plate 1. The open-circuit indirect calorimetry system consists of four chambers built in a pair-wise manner. Each chamber pair is constructed of stainless steel (frame, 3 side walls and roof) and separated by a divider wall constructed of acrylic glass enabling visual contact between animals in adjacent chambers (Plate 2). The entire chamber dimension is 4 m x 2 m x 2 m with a chamber volume of approximately 15.5 m^3. Inside the chamber a 2.5 m x 1.5 m stanchion is fixed which allows the individual animal to stand or lie down. The stanchion is designed for keeping animals up to 850 kg of body mass in tie stall (Plate 3). Space between the chamber walls and the stanchion allows the staff to walk around the stanchion. The chamber floor is completely covered with a rubber mat that can easily be cleaned. Feces, urine and water used for cleaning can leave the stand through openings in the back of the stanchion and pass into airtight tanks located in a cellar underneath the floor of the chambers. Each chamber is equipped with a feed bin (1 m wide, 1 m high and 0.5 m deep) that can hold 40 kg of feed (Plate 3). Feed disappearance is assessed automatically by a scale connected to an electronic registration device (PAARI, Erfurt, Germany). Water intake is registered by water flow meters equipped with electromechanical registration (Elster Messtechnik, Lampertheim, Germany). The chamber has a front door through which the animal or the smaller pig chamber (see below) can be brought in. On the rear site, the chamber can be entered through an air lock permanently rinsed with chamber air (Plate 2). To minimize disturbances of the air composition by staff entering the chamber in cases of longer-lasting feeding, milking, or sampling procedures, a facemask connected to the ambient air via flexible tubing can be put on (Plate 6). The milking device can be connected to two vacuum and one milk tubing from inside of the chamber to which a milking machine from outside can be connected (Plate 6).

Plate 1: Outline of the calorimetric system for large animals.

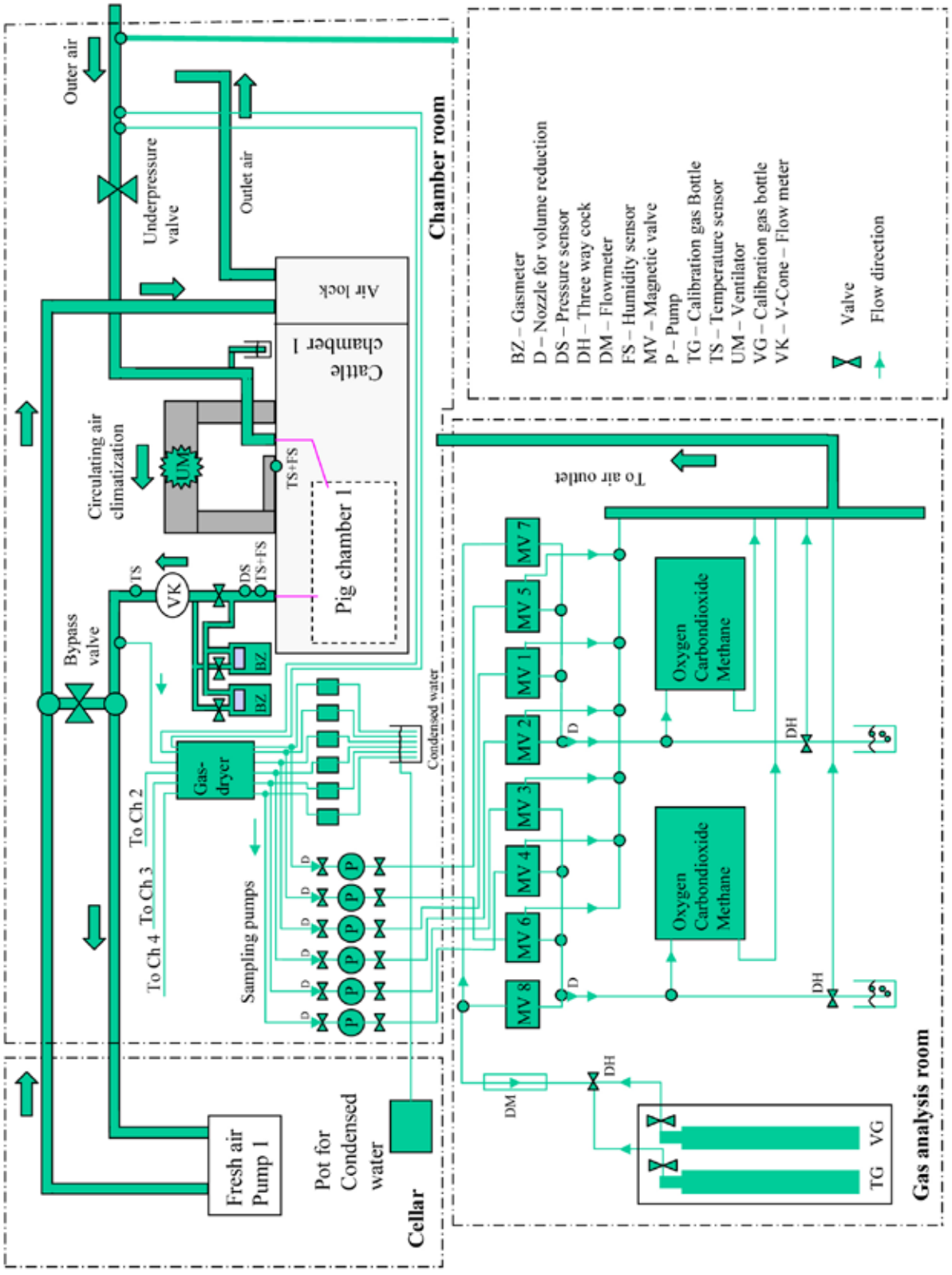


Plate 2: Respiration chamber for cattle with opened door to the airlock.

Plate 3: View into the chamber with stanchion and feed bin.

Moreover, blood can be withdrawn from catheterized animals, via a special catheter extension (4 m, inner diameter 2 mm, Perfusor, BRAUN, Melsungen, Germany) that is guided by rolls and ends outside the chamber in the air lock (Plate 4).

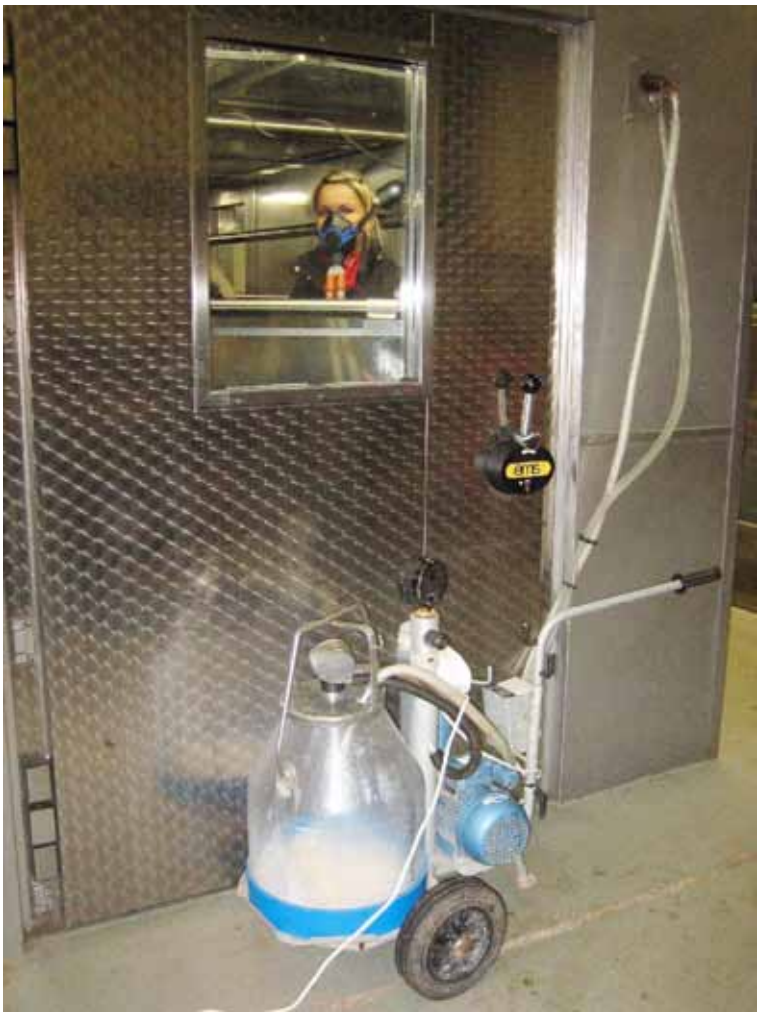
A communication set consisting of a microphone and a speaker inside the chamber and a monitor and a speaker outside the chamber (Orchid MD-502, Orchid Electronics, Langnau am Albis, Switzerland) allows for communication between persons in and outside the chamber. Standing and lying of the animals is registered by a photoelectric switch (SA1E, idec Elektrotechnik GmbH, Hamburg, Germany). Other physical activity is detected by a modified infrared-based motion detector (IS 120, STEINEL, Herzebrock – Klarholz, Germany) converting movements of the animal to impulses. In order to monitor the behavior of the animals, each chamber is equipped with an infrared reflector and a camera plugged into a video computer. The latter is connected via a virtual private network (VPN) to home computers of staff members allowing the animals to be observed from outside the experimental station.

The chambers are light and climate-controlled and designed to regulate the circadian ambient temperature and relative humidity in a range from 0°C to 35°C and 50% to 70%, respectively. The airflow through the cattle chamber can be set from 0–30 m^3/h. The chambers and the operating system were designed by staff members of the FBN and assembled by a contractor (LANTEC, Steinhagen, Germany). The description of the chambers is published (Derno *et al.,* Technical note: a new facility for continuous respiration measurements in lactating cows. *J Dairy Sci*. 2009; 92:2804-8).

Plate 4: Cow lying in the chamber with jugular catheter extension.

Plate 5: Chambers with attached mobile milking devices.

Plate 6: Chambers with attached mobile milking devices.



Pig chambers

For investigation on pigs, smaller steel chambers on wheels (height x width x length: 1.2 x 0.7 x 1.7 m; 1.5 m³) can be introduced into the larger cattle chambers (Plates 7 and 8). They can be easily connected by tubes to the airflow system (airflow for pigs: 6 – 12 m³/h) of the cattle chamber. The pig chambers are equipped with an infrared motion detector. The animals are fed through an opening at one end of the chamber, which can be closed airtight by a cover placed in a water groove. Temperature and light control is performed by the climatization system of the cattle chambers.

Plate 7: Pig chambers standing on rails in cattle chambers.

Plate 8: Opened pig chamber with ramp for driving the animals into the chamber.

Chambers for rodents

Six chambers for mice are available, which consist of transparent plastic cylinders (Ø 10 cm, 10 or 13 cm; ~ 0.8 L or 1 L) with wire mesh bottoms. Each chamber is equipped with a water flask and a hanging basket for feed pellets (Plate 9). An infrared based motion sensor mounted on the chamber cover registers physical activity of the mice. Airflow through the chamber can be regulated up to 60 l/h. Gas exchange is measured continuously in 6 to 21 min intervals, by infrared absorption based CO_2 and paramagnetic based O_2 gas analyzers (Maihak AG, Hamburg, Germany), respectively. The respiration chambers are placed inside a temperature controlled closet where temperature can be regulated from 0 to 40 °C and the light-dark cycle can be programmed (Plate 10).

Plate 9: Mice chambers equipped with water flasks and urine collecting bottles.

Plate 10: Mice chambers in the climatization closet (left) and gas analyzers (middle) and airflow meters (right).

7.4. Animal holding, feeding and cleaning

Before measurement, animals are adapted to the cambers at least 3 times for 4 h each. The criteria for successful adaptation are the voluntary entrance of an animal into the chamber, calm behavior, feed and water intake, and the lying down of an animal. Animals are moved into the chambers prior to the evening feeding and remain resident within the chambers over night before the measurement starts the next morning. This allows for priming the chamber air and the adaptation of the animal to the default ambient temperature. Depending on the specific experiment, animals remain in the chamber for two to three 24-h periods.

Feeding of cattle is performed by a staff member entering the chamber via the air-lock. Feed is normally offered as mixed ration to prevent feed selection. Lactating cows are milked at times of feeding to reduce disturbances. For daily cleaning, feed residues are removed, mats and grills are scrapped and the tanks located in a cellar beneath the chambers are emptied every morning. Once an animal has been removed, the chamber is intensively cleaned with water which drains away to the tanks located in the cellar.

7.5. Chamber airflow piping and measurement

The air is sucked through the chambers from outside of the building by rotary vane vacuum pumps having a capacity of 40 m³/h (VT 4.40, Fuergut, Aichstetten, Germany). By means of a bypass, the airflow through the cattle chamber can be set from 0 up to 30 m³/h, through the pig chamber from 0 to 24 m³/h and the mice chamber from 0 to 60 L/h. A differential pressure type V-cone flow meter (McCrometer, Hemet, CA, USA) is used to measure airflow rate in cattle and pig chambers. The airflow through the mice chambers is measured by mass flow meters (Hastings Instruments, Hampton, Virginia, USA). In all cases the airflow is measured after passing the chamber. All types of gas meters are calibrated by means of wet gas meters.

7.6. Sampling, sample conditioning and analysis

The air sample for the analysis of gas composition is drawn by membrane pumps (80 l/h) (KNF Neuburger Laboport, Freiburg, Germany) located 10 cm behind the flow meters. It is then passed through infrared absorption-based analyzers for the determination of the CO_2 and CH_4 content, respectively, and through a paramagnetic analyzer for measurement of the O_2 content (SIDOR, SICK MAIHAK, Reute, Germany).

Because two sets of analyzers are available, it is possible to switch the gas sampling between two chambers. The controlling software allows the free selection of the time between switching in accordance with the flushing time of the sampling system. In each cycle, the measurement of the gas concentration of the outer air is included in order to detect any drift of the analyzers. In common experiments with large animals (cattle and pigs) the time of measuring intervals is 6 min (2 min flushing time for each unit). For special purposes, it is possible to run only one chamber with cycle times as short as 10 s. With all four cattle chambers working in parallel, data sets for airflow and gas concentrations are measured and stored every 6 minutes. It is also possible to operate chambers for cattle and mice in parallel with a maximum of 10 chambers at the same time. Barometric pressure, air temperature and relative humidity of each chamber and of the exhaust line are measured 3 times per second, averaged over 6-min intervals, and stored for further calculations.

The measured variables (gas concentrations of O_2, CO_2, and CH_4, air flow rate, feed disappearance from the feed bin, water consumption, air temperature and relative humidity in and behind the chamber, standing time, standing or lying position, activity counts, barometric pressure) are transferred to an acquisition system (Simatic, Siemens, München, Germany)

and collected by purpose-adapted software (WinCC, Version 5.1, SP 2, Siemens, München, Germany). A DELPHI-based (Delphi 2007, San Francisco, CA, USA) software (Copyright H. Scholze, FBN Dummerstorf, Germany)specifically adapted for the automatic calculation of heat production (HP) according to BROUWER (1965) [HP (kJ) = 16.18 O_2 (L) + 5.02 CO_2 (L) – 2.17 CH_4 (L) – 5.99 N (g)] collects all continuously measured data in EXCEL (Microsoft Office) files.

Collection of milk, feed, feces, and urine and subsequent analysis for carbon, nitrogen and gross energy, energy and nitrogen balance of individual animals can be calculated. Upon analyses of plasma samples, interrelations between plasma metabolites and hormone concentrations and variables obtained from gas exchange measurement or activity indices can be calculated.

7.7. Gas recovery test

The whole system can be calibrated by introduction of defined volumes of chemically pure CO_2 from a gas cylinder. The mean recovery rate is 99.9%. The analyzers are calibrated by calibration gases of known composition (zero: pure N_2, endpoint: CO_2 – 1.0 Vol%, O_2: 19.9 Vol%, CH_4: 0.1 Vol%).

7.8. Emissions calculation

The measured airflow is corrected to standard conditions and water vapour pressure. The CH_4 emission is calculated by multiplying the corrected airflow with the mean CH_4 concentration in the measuring interval.

7.9. Animal welfare and operators' safety

An alarm system that is activated by the failure of the pumps delivering fresh air to the animals is installed (comline 2016, TELENOT ELECTRONIC, Aalen, Germany) in the chambers. It responds to an increase of pressure in the chambers normally working under low pressure conditions and operates a telephone that automatically calls staff members responsible for the experiment. In case of emergency, doors of the chambers have to be opened within one hour time.

7.10. Weaknesses of the system

One weakness of the system - as compared to others - is the lack of an automatic door opening system activated in case of emergency. Thus, rapid action of the staff is required. However, animal welfare has never been compromised in case of serious risk.

Currently, it is not possible to regulate humidity in the chamber. The cooling device in the air conditioning system removes water vapour from the chamber air establishing a mean level of 50 to 70 % relative humidity in the chamber. A humidity control system will be implemented allowing the adjustment to a desired humidity value.

7.11. Description of components and equipment suppliers

- The sandwich panels and doors: stainless steel; Windows: acrylic glass; LANTEC, Steinhagen, Germany
- Stanchion: LANTEC, Steinhagen, Germany
- Feeding bin and electronic registration device: PAARI, Erfurt, Germany

- Drinking trough with electromechanical registration: Elster Messtechnik, Lampertheim, Germany
- Communication set: Orchid MD-502, Orchid Electronics, Langnau am Albis, Switzerland
- Photoelectric switch: SA1E, idec Elektrotechnik GmbH, Hamburg, Germany
- Infrared-based motion detector: IS 120, STEINEL, Herzebrock – Klarholz, Germany
- Air conditioning: provided by Hildebrandt und Kindt, Rostock, Germany
- Temperature Sensor: E + E Elektronik, Engerwizdorf, Austriaw
- O_2 gas analyzer: SIDOR, SICK MAIHAK, Reute, Germany
- CO_2 gas analyzer: SIDOR, SICK MAIHAK, Reute, Germany
- CH_4 gas analyzer: SIDOR, SICK MAIHAK, Reute, Germany
- Rotary vane vacuum pumps: Fuergut, Aichstetten, Germany
- Membrane pumps: KNF Neuburger Laboport, Freiburg, Germany
- Flow meter: McCrometer, Hemet, CA, USA
- Data acquisition: Standard PC, Software; Simatic, Siemens, München, Germany (WinCC, Version 5.1, SP 2, Siemens, München, Germany)
- Data handling: DELPHI-based (Delphi 2007, San Francisco, CA, USA) tailored software (Copyright H. Scholze, Leibniz Institute for Farm Animal Biology, Dummerstorf, Germany)

7.12. Costs of the facility

Carcass, stanchion, feed bins (including labour)	200,000 €
Flow meters, Temperature - humidity sensors, sample pumps, tubes, wires	60,000 €
Climatization	50,000 €
Feed bin balances, electronic registration	7,000 €
Milking system	6000€
Gas analyzers	24,000 €
Data acquisition and control unit	15,000 €

Design and part of construction was made by staff members of the Leibniz Institute.