RESPIRATION CHAMBER FOR STUDY OF ENERGY EXPENDITURE OF MOOSE

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<u>Abstract</u>: The respiration chamber and associated equipment used at the Kenai Moose Research Center to measure energy expenditure of moose is described. Methods used to construct the chamber and to measure respired gas volume and composition are discussed.

Partitioning the flow of energy through a ruminant animal requires a measurement of energy lost in feces, urine, respiratory gases and as heat increment (HI). Fecal and urinary energy loss can be sampled and measured with standard digestion cages and routine laboratory analysis. Determination of energy lost as methane and HI requires a means of measuring the exchange of respiratory gases or production of heat. Direct measurement of heat flux is difficult and requires close confinement of the animal. Indirect calorimetry is the method used most often with large bodied animals. This technique estimates metabolic heat production from the amount of oxygen consumed and carbon dioxide produced (Kleiber

1961, Blaxter 1967).

A respiration chamber or face mask can be used to collect respired gases. Systems involving a chamber can be closed-circuit, in which air is recirculated through the system, or open-circuit in which fresh air is continuously circulated through the system. The open-circuit indirect calorimetry method has great versatility. Animals can be confined in the chamber for long periods, allowing a wide variety of experimental procedures. We describe the open-circuit respiration chamber and gas analysis equipment used at the Kenai Moose Research Center in Alaska. Our system is similar to that used at the Ritzman Laboratory, University of New Hampshire (Haven Hayes, pers. comm.). Several alterations have been made to adapt it to moose and low temperatures in Alaska.

## THE CHAMBER

The respiration chamber measures 2.4 X 2.3 X 2.2 m in size with a 0.9 X 0.9 X 2.2 m addition in one corner to accommodate a refrigeration unit and feed bunk (Fig. 1). The chamber was constructed of 5 X 20 cm floor joists and 5 X 10 cm wall and ceiling joists covered with high quality 1.9 cm plywood fastened with screws. A subfloor of plywood slopes to the center and one end to aid urine flow out of the chamber. The moose stand or lie on a floor of expanded sheet metal suspended 5 cm above the sloping subfloor. The expanded metal has holes of sufficient size to allow feces and urine to pass through thus maintaining a clean, dry floor. Seven plexiglass windows (30 X 76 cm) were placed in the chamber walls. The entry door is 1 X 2.1 m; it fits tightly against rubber material to prevent air leaks. All joints and screw holes were sealed with silicone sealer and all interior walls were painted with

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several coats of epoxy paint to prevent air leakage.

Humidity in the chamber is controlled by a refrigeration unit<sup>1</sup> suspended from the ceiling (Fig. 2c). This unit maintains relative humidity at about 30% and temperature between 2 and 4 C. It has a fan that continuously mixes the chamber air. Air is moved at less than 1 m/sec. This velocity does not increase heat loss (Moen 1973). Water vapor removed by the refrigeration unit is drained outside the chamber. A thermostatically-controlled electric heater<sup>2</sup> (Fig. 2b) warms the air during winter so the refrigeration unit will function. Walls, floor and ceiling are insulated with fiberglass. A feeding stall with a remote control access door (Fig. 2d) is located below the refrigeration unit. Food can be added or removed without entering the chamber.

Air volume of the chamber is 13,200 liters. Volume can be reduced to accommodate smaller animals by displacing air with large air mattresses. Chamber volume should be as small as possible without distressing the experimental animal. This allows  $CO_2$  level in the chamber to increase to about 1% rapidly and provides a faster response to changes in respiratory gases due to animal activity.

Outside air enters the chamber through a 4.5 cm valve (Fig. 2a). The entry valve is partially closed to keep the chamber at a slight negative pressure. This insures that any air leaks will be into the chamber and that no gas expired by the moose can escape.

<sup>1</sup>Model M100, Nor-Lake Inc., Hudson W.I. 54016

<sup>2</sup>Glassheat, K&L Contruction, Soldotna, AK



Fig. 2. Schematic drawing of the respiration chamber and gas handling system at the Kenai Moose Research Center, Alaska. a, air inlet valve; b, heater; c, refrigeration unit; d, feed bunk; e, expanded metal floor; f, sloping subfloor; g, urine drain; h, vacuum cleaner; i, manometer; j, line thermometer; k, pressure valve; l, spirometers; m, gas meter; n, wet and dry thermometers.

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Gas is pumped out of the chamber at a constant rate by a reversed vacuum cleaner motor<sup>3</sup>. The flow rate is regulated by a rheostatic control of the vacuum cleaner motor. Flow rate for an adult moose is 280 1/min. This rate maintains the  $CO_2$  level inside the chamber between 0.5 and 1.0%. Values within this range can be measured accurately; animals can tolerate a CO $_{
m 2}$  level as high as 2.0% without any respiratory stress. The gas is pumped into a 5.1 cm plastic line and through a gas meter<sup>4</sup> that measures total volume to the nearest liter (Fig. 2m). Pressure in the gas line is kept slightly positive by a valve placed in front of the gas meter. The positive pressure permits aliquot subsamples to be collected continuously in three 9 liter spirometers  $^5$  (Fig 2 ). Needle valves in the flow line to each spirometer enable the collection of the aliquot samples over 2- to 24-hour periods. Gas is dried by passing it through  $CaCl_2$  and filtered through glass filter paper prior to entering the spirometers. A stopcock valve in the main flow line (Fig. 2k) permits continuous analysis of the gas throughout the trial. This line bypasses the spirometers and flows directly to the gas analysis equipment after the gas has been dried and filtered.

Temperature and moisture content of the gas is monitored by wet and dry bulb thermometers. Barometric pressure is measured with a standard

<sup>&</sup>lt;sup>3</sup> Model L, Electrolux Co., Stanford, CT

<sup>&</sup>lt;sup>4</sup> Model AL1400, American Meter Co., Philadelphia, PA

<sup>&</sup>lt;sup>5</sup> Warren E. Collins, CO., Braintree, MA

mercury barometer. All gas volume measurements are converted to standard temperature and pressure before any calculations are made. Air pressures inside the chamber (negative) and in the main flow line (positive) are monitored by simple home-made manometers.

Composition of the gas is determined by passing it through three instruments that measure the content of oxygen, carbon dioxide and methane  $(CH_4)$ . 0xygen is measured by a paramagnetic analyzer<sup>6</sup> to the nearest 0.01%.  $CO_2^{-7}$  and  $CH_4^{-8}$  by non-dispersive infrared analyzers-- $CO_2$  to the nearest 0.01\% and  $CH_4$  to the nearest 0.0001\%. The instruments are connected so that the same gas sample flows through each one. Gas from the spirometers or directly from the main flow line passes through each machine at a constant rate of 500 ml/min.

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The instruments are calibrated every hour during a trial using gases of known composition. Three gas mixtures are used for calibration, one being outside air and the other two provided by a chemical supply company<sup>9</sup> in compressed gas cyclinders. The calibration gasses are pumped out of the spirometers at the same rate of flow as the resiratory gas.

All instrument readings are made manually. Automatic recording devices are available for all instruments, but they are expensive.

<sup>6</sup> Model OM14, Beckman Instruments, Inc., Schiller Park, IL <sup>7</sup> Model LB2, Beckman Instruments, Inc., Schiller Park, IL <sup>8</sup> Model 865, Beckman Instruments, Inc, Schiller Park, IL <sup>9</sup> Scientific Gas Co., Denver, CO Heat production is calculated by multiplying the volume of  $0_2$  consumed during the trial by the thermal equivalent (caloric value) of the  $0_2$  at the extant respiratory quotient (Brody, 1968). Energy expenditure is expressed in terms of heat production. Standard units of measure are either Kcal/24 hr or Kcal/Kg BW<sup>.75</sup> (Kg BW<sup>.75</sup> = Body weight of animal in Kg raised to the .75 power). The recent trend in Europe has been to express energy expenditure as kilo joules/24 hr. (1 kJ - 0.239 Kcal).

## DISCUSSION

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The first chamber we built was 2.4 X 1.2 X 2.4 m and had only one small window, at one end. Adult moose had great difficulty in turning around and refused to lie down. They became agitated after a few hours of confinement. It was important that the moose remain calm in a recumbent position to enable accurate measurement of resting metabolic rates. We enlarged this chamber to its present size and added 5 more windows. The new dimensions provided adequate space for the moose to lie down and turn around but minimized movement. The windows helped keep the moose calm, especially if they could see other moose outside the chamber. The windows also allowed us to observe the moose and record their activity.

Because the expanded metal floor had a rough surface which we felt might injure the feet of the moose, we placed a  $1.3 \text{ m}^2$  plywood board in the center of the chamber floor. The moose stand and lie on this board nearly all the time they are in the chamber.

We have conducted 48 energy expenditure trials in this chamber using six moose during the past 18 months. The age of the moose varied from 6 to 30 months. They were either in a fasted condition (no food for

48 hours) or on ad libitum food intake. Length of trials varied from 2 to 24 hours. The trials have been used to measure  $CH_4$  production in relation to food intake, energy costs of standing and diurnal variation in energy expenditure. Seasonal changes in energy requirements have been examined. The measurements have a high degree to repeatability indicating the system is capable of producing precise results.

The respiration chamber has been operated at outside temperatures ranging from -35 to 20 C without problems. The electric heater warms the air sufficiently, even at extremely low temperatures, to make the refrigeration (dehumidifying) system operate. The cooling system easily lowers high air temperatures. The system does not have the capability to reduce chamber temperature or increase wind velocity to critical levels for moose.

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The entire system cost \$17,000 excluding labor at 1979 prices. Of this total, the gas handling and gas analysis equipment cost \$14,000. The chamber with attachments cost \$3,000; about half of which was accounted for by the refrigeration system.

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