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APPENDIX

APPENDIX A
Comparative Slaughter procedure

The comparative slaughter technique is currently method being used at the California Agricultural Experiment Station and to discuss its application in determining energy retention of beef cattle. The details of this method are describes according to Lofgreen (1968) follow as;

1. Animals to be used in the study are selected in uniformity and prior the experiment, they are vaccination and deworming.
2. The animal are placed on the same ration, allowed to adjust to it and fed foer a minimum of 10 days on an *ad libitum* intake.
3. An initial liveweight measurement is made 12 h after standing without feed and water.
4. A randomly selected group is slaughtered to present the initial body composition. At the conclusion of experimental feeding trial, all animals are slaughtered and determined the final body composition.
5. The energy retention in body is calculated from body fat and body protein in final group deducting by body fat and body protein in initial group.
6. The energy retained is determined by assuming the caloric values of fat and protein to be 9367 kcal / kg of fat (Blaxter and Rook, 1953) and 5686 kcal / kg of protein (Garrett, 1958).

Slaughter procedure and body composition analyses

Before slaughter, Animals are standing without feed and water at least for 12 h according to Lofgreen (1968). Shrunk body weight is measured as the body weight. At the slaughter, animals are stunned using wood knocker and killed by exsanguinations. Body composition components are split into three fractions (Figure appendix A1).

1. Non carcass (hide, head, feet and tail)
2. Carcass

3. The organs (gastrointestinal and visceral organ)In organ part especially digestive tract was cleaned by emptying and flushing with water.

Weighing and record all organ parts, Non carcass were separated in bone, hide and soft tissue. Carcass was separate in bone and soft tissue. Each part was cut into small piece, homogenized, and following ground into two or three times of grinder with sieve plate with holes 2.0 mm in diameter before proportionally sampling approximately 10% of total samples. All homogenized ground materials, placed in a sealed plastic bag and store at -18 °c before analyzed the chemical composition.

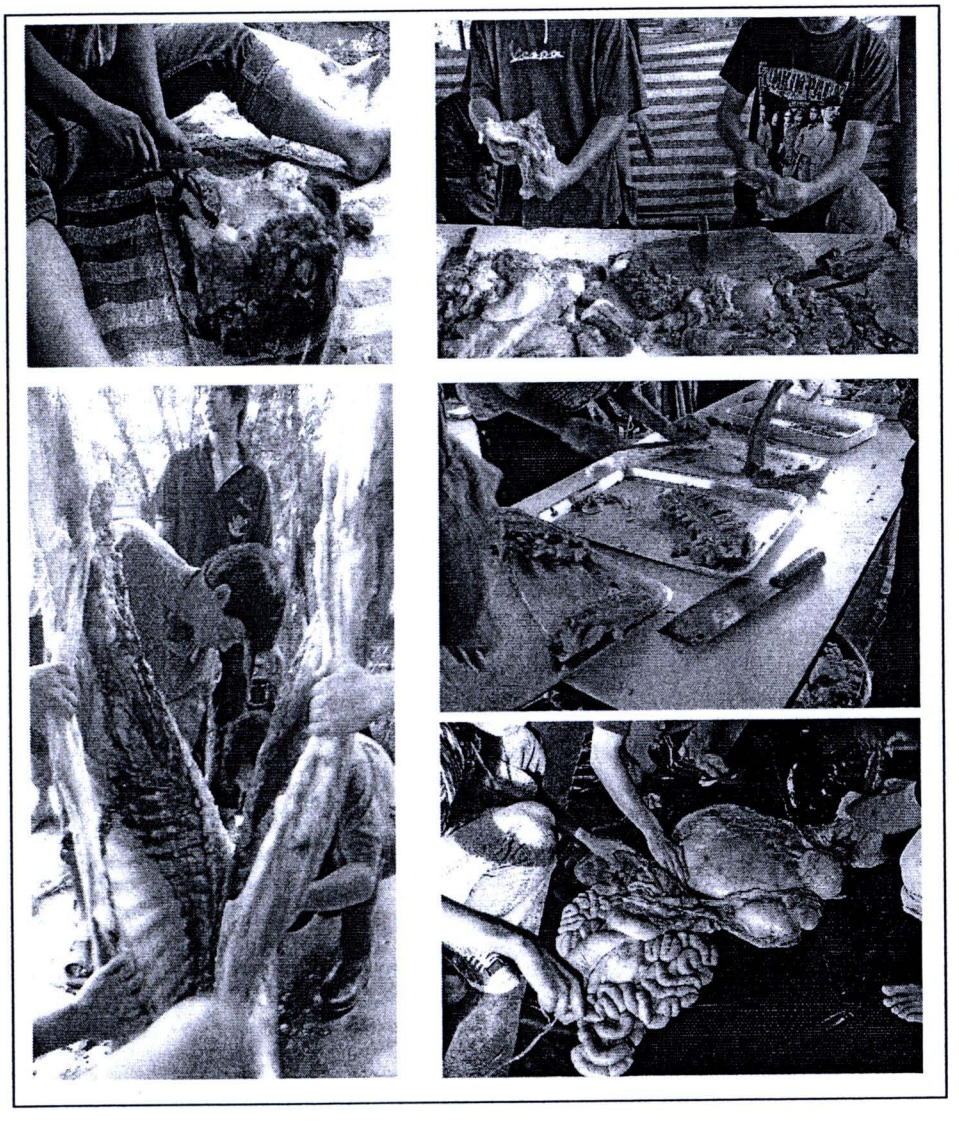

Figure appendix A1. The slaughtered procedure for energy retained determination

Dry matter was determined by the difference from wet weight after sample was placed in an oven at 105 °c for at least 80 h. according to Brown et al. (2005), Chizzotti et al. (2007) and Galvani et al. (2008). Crude protein was determined by Kjeldahl method, and ether extract was determined by Soxhlet apparatus for 18 h. Ash was analyzed until complete combustion in a muffle furnance at 600 °c according to AOAC (1990).

APPENDIX B

Respiration Calorimeters System

A respiration trial system using a ventilated hood-type indirect respiration calorimetry method was constructed at Khon Kaen Animal Nutrition Research and Development Center (KKANRDC), Khon Kaen, Thailand. It comprises the following four components, including

(a) The digestion trial pen

(b) Head cage or head box

(c) Gas sampling and analysis unit, and

(d) Data acquisition and processing unit.

A detailed description of these components follows. A schematic diagram of the ventilated hood-type calorimeter used in this current study was shown in Appendix B (The figure 1 and figure 2). The animal calorimeter using a ventilated hood was conducted a trial to estimate the energy balance in the cattle (Suzuki et al., 2007; Suzuki et al., 2008).

Calorimeter design and its constituents

1. The digestion trial pen

Digestion trial pen is designed to house animals to allow correct measurement of feed intake and excreta output to be made. The experimental equipment is typical of other apparatus used in many animal nutrition laboratories around the world where classical feed digestibility studies are conducted. The pen also purposes to position the animals head inside the head cage.

2. Head cage or head box

The head cage is installed in front of the digestion trial pen. It is designed to be sealed tight, with the exception of an air inlet that has an adjustable "loose fitting" collar. The position of the head cage and yoke are modifiable in order to permit animals of various sizes to be housed in it.

3. **Gas analysis**

Flow meters: Flow meters are the simplest type of flow meter available; they are factory calibrated to measure the output flow rate under conditions of standard temperature and pressure (0°C and 760 mm Hg).

Blower: The blower is to pull the main air stream through the calorimetric system, i.e., from the inlet point at the loose-fitting collar of the ventilated hood through to the exhaust point.

Air filter and dryers: Air filters and dryers are set up in the system to eliminate any dust particles and moisture prior to the transfer of the gas samples to the gas analysis system.

Gas analyzers: The gas sample was analyzed at three places (i.e., background air and two different head boxes) by using a set of gas analyzers. In this system, the gas sampling point changes at 90-s intervals among the three places. The first 60 s are used to allow stabilization of the gas concentrations earlier to measurement, while the final 30 s are for data achievement. The gas analyzer consists of three parts: oxygen analyzer, carbon dioxide analyzer and methane analyzer.

4. **Data acquisition and processing**

It is necessary to have a system for collecting accurate data for correct calculations. A make routine system should be established to allow 24-h data collection. All data of respiration gas sampling and collections, flow rate and concentration of carbon dioxide (CO_2) production, methane (CH_4) production and oxygen (O_2) consumption. Calculate the data set to compute energy partition of feed and also energy requirement of animals.

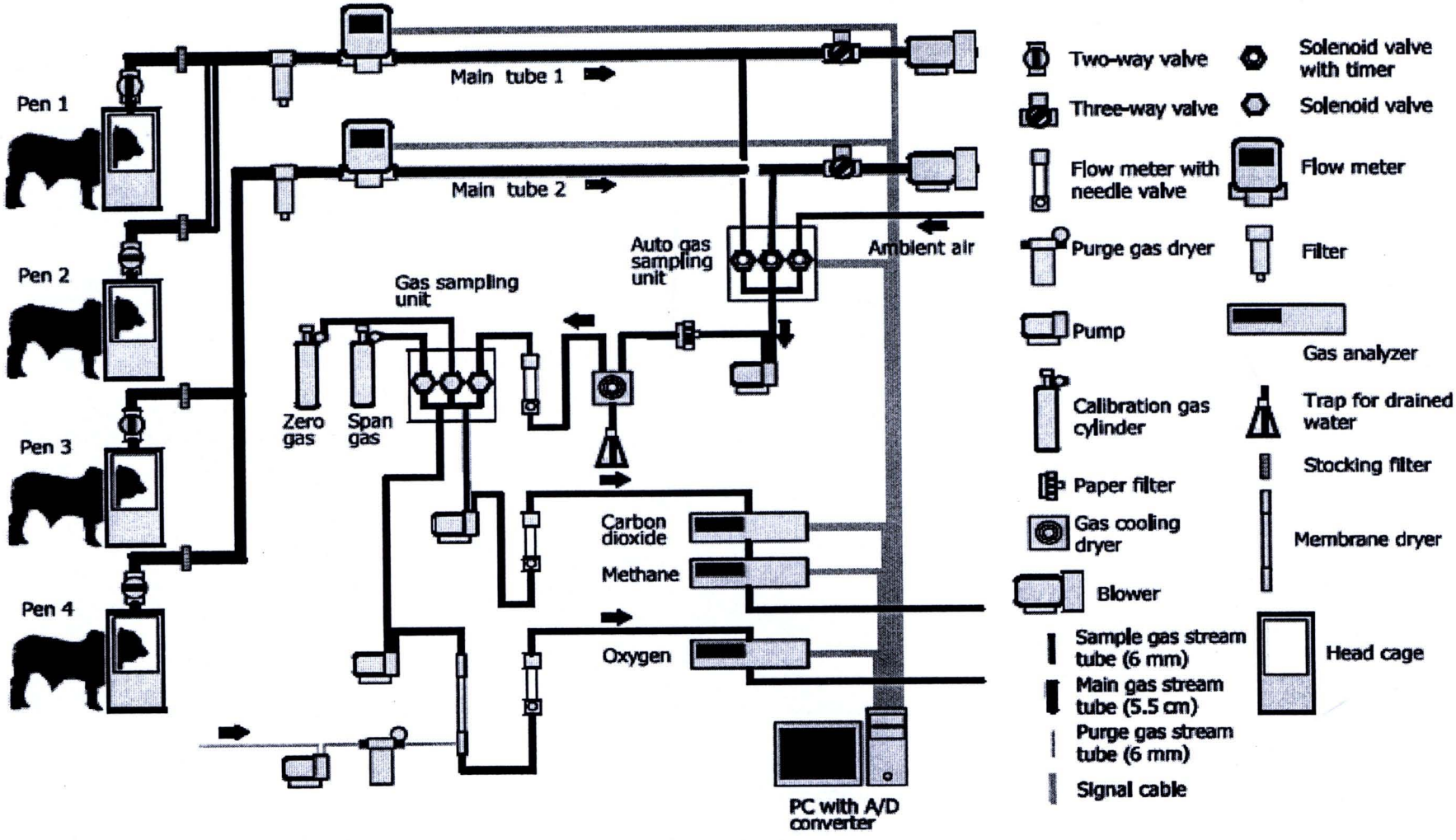

Figure appendix B1. A schematic diagram of the ventilated hood-type respiration chamber system (Suzuki et al., 2007, 2008b).

Figure appendix B2. The ventilated hood-type respiration chamber system consisted of the digestion trial pen, head cage (head box), gas sampling and analysis unit and data acquisition processing unit

Operation of the system

1. Instrumentation

The accuracy of the calorimeter system is only as accurate as the section instruments being used. It is therefore sensible to match the accuracy of the parts at the time of obtain.

2. Recovery tests

Recovery tests were carrying out for the final corroboration of the system. The procedures are described in full detail by McLean and Tobin (1987). Pure carbon dioxide was introduced into the head cage from a gas cylinder that was positioned on a gravimetric balance. A newly constructed calorimeter system should achieve 95-105% recovery values before any measurements are made with the system.

3. Measurement of net volume of the system

It is essential to regard as the effect of differences in gas concentration in the chamber throughout periods of gas measurement. To calculate this effect, it is important to know the real volume of the respiration calorimeter system, is described in detail by Suzuki et al. (2007, 2008b). Discrepancy of CO_2 gas volume after infusion can be calculated. When the concentration difference of CO_2 becomes 1%, we defined that time as the effective volume and 99% CO2 recovery time. The measurement of net volume of the system is used to regard as the accuracy of the whole measurement system.

4. Calibration of gas analyzers with certified standard gas

Calibrating the gas analyzers with certified standard gases is an important part of quality control for the measurement system. At the beginning of each measurement day, the gas analyzers were standardized against certified gases (Takachiho Chemical Industrial Co., Ltd., Tokyo, Japan) with well-known gas concentrations.

5. Training animals for measurement and experimental design

It is important to train the animals to be used in the experiment and consider the experimental design carefully before making measurements with a respiration calorimeter.

APPENDIX C

Lab Procedure

C1: Determination of Dry matter and Ash

Instruments

1. Porcelain crucible
2. Desiccators
3. Forced air oven
4. Muffle furnace
5. Digital analytical balance

Procedure

1. Dry porcelain crucible in oven at 100 °C for 2 h.
2. Cool pan in desiccators, weighed and record weight.
3. Add 2 to 3 g of samples and record weight of crucible contained sample.
4. Dry crucible contained sample in oven at 100 °C for 12 h or overnight.
5. Cool in desiccators, re-weighed and record weight.
6. Place crucible contained sample in muffle furnace and ash at 550-600 °C for at least 2 h.
7. Cool in muffle for at least 12 h, then in desiccators, reweigh and record weight.

Calculations

Crucible + sample (before drying) – Crucible weight = Sample wet weight

Crucible + sample weight (after drying) – Crucible weight = Sample dry weight

%DM = (Dry weight ÷ Wet weight) x 100

Crucible + sample weight (after ashing) – Crucible weight = Sample ash weight

%Ash = (Ash weight ÷ Dry weight) x 100

C2: Determination of Crude Protein by Kjeldahl Method

Instruments

1. Digestion equipment
2. Distillation equipment
3. Burette
4. Erlenmeyer flask (250 ml)
5. Digital analytical balance

Reagents

1. Catalyst tablets, (5g tablets consisting of potassium sulphate (K_2SO_4): copper sulphate ($CuSO_4$): selenium (Se) at 100:6:1 ratio)
2. 98 %(w/v) of sulfuric acid (H_2SO_4)
3. 45% of sodium hydroxide (NaOH) (prepared from 45 g of NaOH added to 100 ml of distilled water)
4. Boric acid 4 % (prepared from 4 g of boric acid added to 100 ml of distilled water)
5. Indicator (0.3125 g of methyl red and 0.2062 g of methylene blue dissolved in 95% ethanol 250 ml)
6. 0.1 N of hydrochloric acid (HCl)

Procedures

Digestion

1. Weigh in duplicate about 0.5-1.0 g of sample and transfer into KJeldahl tube follow with 25 ml of H_2SO_4 and Kjeldahl catalyst tablets.
2. Tubes are heat up on digestion units until the digestion was completed oxidation when the sample color turned a clear color.

Distillation

1. After cooling the digested sample was place into the stream distilling unit of distillation equipment.
2. Added boric acid in to 250 ml Erlenmeyer flask and place the delivery tube from the condenser into the boric acid.
3. Collect 150 ml of solution from condensed.
4. Titrate distillate with 0.1 N HCl, recording quantity of acid of used.

Calculations

Nitrogen (N), % = (A x B x 0.014 x 100)/C

Crude protein (CP), % = %Nitrogen x 6.25

where A = HCl (ml), B = Normality of HCl, C = Dry matter sample weight

C3: Determination of Ether Extract (EE)

Instruments

1. Petroleum ether
2. Soxhlet apparatus
3. Thimble filter
4. Florence flask
5. Permeable cotton
6. Desiccators
7. Digital analytical balance

Procedure

1. Dry the florence flask in oven at 100 °C for at least 2 h.
2. Cool Florence flask in desiccators for 30 min., weight and record weight.
3. Add 2 g of sample into thimble filter, covered with permeable cotton.
4. The thimble filter containing the sample is placed in the siphon of the Soxhlet apparatus.
5. Ether in excess of that required for refluxing is added into the fat flask of known constant weight, and extraction is carried out for 18 h.
6. After extraction, the thimble filter is removed, the ether solution is heat again, and collected until a small quantity of ether remains.
7. The florence flask is removed and the remaining ether is driven off over a water bath.
8. The exterior of the florence flask is wipe up with gauze, dried at 100 °C for at least 24 h., allowed to cool in a desiccators for 30 min., weighed and record.

Calculations

Ether extract content (%) = (A-B)/C x 100

A = Florence flask weight + sample weight after dried extract (g)

B = Florence flask weight (g)

C = Sample weight (g)

C4: Determination of Neutral Detergent Fiber (NDF)

Instruments

1. Filtering flasks and suction pump
2. Refluxing apparatus or plate heater
3. Crucible
4. 600 ml Berzelius beaker
5. Filter paper NO. 541
6. Digital analytical balance (Four figure)

Reagents and preparation

1. NDF solution to 1 liter of H_2O added:

   30 g sodium lauryl sulfate

   18.61 g disodium dihydrogen ethylene diamine tetraacetic dihydrate

   6.18 g sodium borate decahydrate

   4.56 g disodium hydrogen phosphate

   10 ml 2-ethoxy- ethanol

2. Amylase solution, Dissolve 2 g α-amylase, type IIIA (Sigma No. A6505) in 90 ml H_2O filter through 541 paper. Add 10 ml Ethoxy-ethanol store at 4 °C.

3. Acetone

Procedure

1. Place 0.5-1.0 g sample in 600 ml Berzelius beaker.
2. Add 100 ml NDF solution.
3. Heat to boiling (~ 10 min). Reduce heat as boiling begins. Boil for 60 min.
4. After 60 min, filter contents into crucible under vacuum at first, increasing only as more force is needed.

5. Rinse contents with hot water, filter, and repeat twice.
6. Wash twice with acetone.
7. Dry overnight in 100 °C oven.
8. Cool in desiccators at least 30 min, and weighed.

Calculations

%NDF = (A-B)/C x100

A= Crucible weight and sample weight after dry in drying oven

B= Crucible weight

C= Sample weight

C5: Determination of Acid Detergent Fiber (ADF)

Instruments

1. 600 ml Berzelius beakers
2. Fiber digestion apparatus
3. Sintered glass crucibles, 40 to 50 ml course porosity
4. Filtering flasks and suction pump
5. Digital analytical balance (Four figure)

Reagents and preparation

1. ADF solution: add 27.84 ml H_2SO_4 and 20 mg CH_3 $(CH_2)15N$ $(CH_3)3$ Br and bring to 1 liter volume with H_2SO_4.

** Add some H_2O before acid

2. Acetone

Procedure

1. Place 0.5-1.0 g sample in 600 ml Berzelius beaker.
2. Add 100 ml ADF solution.
3. Heat to boil (~ 10 min) and boil closely 60 min.
4. Filter with light suction into previously tarred filtering crucibles.
5. Wash with hot water 3-4 times.
6. Wash thoroughly with acetone until no further color is eliminated and suck dry.
7. Dry in oven at 100 °C for at least 24 h.
8. Cool in desiccators for at least 30 min, weighed and record weight.

Calculations

%ADF = (A-B)/C x100

A= Crucible weight + sample weight after dry in drying oven

B= Crucible weight

C= Sample weight

C6: Determination of Gross Energy by Adiabatic Bomb Calorimeter

Instruments

1. Automatic adiabatic bomb calorimeter (CA-4PJ; Shimadzu, Kyoto, Japan)
2. The jacket bomb
3. Plastic bag (accurately constant energy content measured)

Reagents

1. Corn starch (Standard to sample to standardize sample analysis, calorific value of con starch equivalent to 15246.19 J/g of corn starch)
2. Benzoic acid (Standard sample to standardize bomb calorimeter, calorific value of benzoic acid equivalent to 26.44 J/g of Benzoic acid)

Procedure

1. Weigh out a 1 g of sample and place (paper or plastic bag) into a clean combustion crucible (the urine samples were oven-dried (60°C, 48 h) prior to use). Turn on heater box on instrument.
2. Attach a 10 cm combine wire between the electrodes of the bomb.
3. Place 1 ml of water into the bomb cylinder and swirl the water to wet the sides.
4. Assemble the bomb. Fill with oxygen to 20 atmospheres gauge pressure. Place the oval bucket in the calorimeter, set the bomb in the bucket and attach the clip terminal.
5. Weight out 2,000 g deionized-water and pour the water into the calorimeter bucket. Make sure the water temperature within the range of the calorimeter thermometer.
6. Slide the cover and lower the thermometers and turn on the water circulating motor, the instrument will automatically equilibrate.
7. Check the calorimeter temperature at one-minute intervals for 3 min.

8. After the entire calorimeter has equilibrated, read and record the initial temperature. Ignite the sample.

9. After ignition has occurred and temperature of the outer jacket and the inner bucket are equal, read and record print output of the final temperatures (FIN) at 1-min intervals for 3 min.

Calculations

Gross energy (GE, J/g) = FIN – (CHC x Cwt) / S

FIN = Final temperatures read and record

CHC = Calorific value of place (46488 of paper or 16050 of plastic bag)

Crucible weight

Cwt = Place weight (paper or plastic bag)

GE of standard sample (15246.19 J/g of con starch or 26.44 J/g of benzoic acid)

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