METHODOLOGICAL APPROACHES IN RUMINANT METABOLIC RESEARCH[1]

PENDEKATAN METODOLOGI DALAM PENELITIAN METABOLISME PADA RUMINANSIA

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ABSTRACT

Climatic factors of high temperatures and humidities of humid tropical countries, alongside with the occurrence of diseases and parasites have created a complex situation in terms of the effect on animal production. Studies on quantitative relationships for better understanding of digestion, metabolism and related areas are lacking. There are however a number of appropriate methodologies available for conducting onfarm metabolic research aiming at giving better understanding of animal production and health in the humid tropics. The paper discusses methodologies and experimental approaches developed in the authors's laboratory on growing, draft, pregnant and lactating ruminants. Several examples of the results are presented.

ABSTRAK

Faktor-faktor suhu tinggi dan kelembaban lingkungan tropika lembab, digabungkan dengan tingginya kejadian penyakit dan parasit menciptakan situasi yang kompleks yang berpengaruh negatif terhadap upaya produksi ternak. Penelitian-penelitian mengenai aspek kwantitatif fungsi pencernaan, metabolisme hewan dan bidang-bidang terkait boleh dikatakan sangat minim. Meskipun demikian, sebenarnya cukup tersedia metodologi yang sesuai untuk melaksanakan *"onfarm metabolic research"* yang bertujuan memperoleh pemahaman yang mendalam mengenai produksi dan kesehatan ternak di daerah tropika lembab. Makalah ini membahas beberapa metodologi dan pendekatan percobaan yang dikembangkan di laboratorium penulis pada hewan ruminansia yang sedang tumbuh, bunting, laktasi dan temak kerja. Beberapa contoh hasil penelitian disajikan untuk ilustrasi.

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INTRODUCTION

Food is necessary to built up tissue and to act as a source of energy. Food has to be digested into absorbable units in the gastrointestinal tract. Metabolism is the process refered to all the chemical and energy transformations that occur in the body.

The humid tropical countries have special and often unique problems associated with animal production. Both imported and indigenous animals used to build the livestock industry shows variation in adaptability to the existing **agro-ecological** setting of the region. The countries have dietary materials available, particularly for ruminants, which in many instances have not been evaluated for their ability to support the desired production. Humid tropical countries have adverse climatic factors of high temperatures and humidities and, in addition, there are a wide variety of diseases and parasites which reduce production. Direct effects of these factors **and** interactions between the factors have created a complex situation in terms of the effect on production.

As qualitative knowledge increased, detailed consideration is given to develop quantitative relationships to increase further understanding and integrate various aspects, **e.g.** to bring together quantitative approaches concerned with elucidating mechanisms, used in the ***study*** of digestion (monogastric and ruminant), metabolism and related areas. In his address to the 11th Symposium on Energy Metabolism of **Farm** Animals on "Past Achievements and Future Perspective in Energy Metabolism", the late Sir Kenneth L. **Blaxter** (1989) made the following **remarks:** *"Current world literature did not contain much information which relates to climatic and seasonal or exogenous hormonal effects on metabolism, pregnancy in mammals, egg secretion in birds and effects **of muscular** work have not been considered. Nor has the comparative aspect of metabolism been considered On the practical side we need these are items **of comerce**. We also need, more accurate and rapid **methods** for estimating the energy values of fee& **sice;** to take into account breed and strain **differences** in' animal requirements. Better estimates of the composition of body gains (retentions) are required. New **demands** will arise as a **result** of modern techniques of genetic manipulation of livestock and the use of **exogenous** hormones derived through, biotechnology, to increase reproductive **performance** and the rate and composition of growth. On the **fundamental** side, we must explore aspects of adaptation of metabolism to undernutrition, season and climate at the cell, organ and whole animal levels and unravel the complexity **of the** endocrinee and neural control mechanisms involved focussing on* *the roles of the sympathetic nervous system and growth factors. New techniques must be explored since they may well enable studies to be made in natural environments rather than the laboratory. Biochemical methods of assessing the energy status of animals demands attention*". These hold true for the humid tropical situation as well.

Brody (1945) stated that growth is the basis of and closely related to many animal productive processes, which includes egg, milk, fat and other production. He defined growth as the constructive and assimilatory synthesis of one substance at the expense of another, i.e. nutrient which undergoes dissimilation. Growth, and hence animal production, is biological synthesis, production of new biochemical units and it is the aspect of development concerned with the increase in living substance or protoplasm and includes one or all of the following processes, e.g. cell multiplication, cell enlargement, and incorporation of material taken from the environment. From the practical point of view of quantitative measurement of growth of the organism as a whole, nonprotoplasmic inclusions in the body must be considered as parts of the growth process.

The aforesaid processes are more dramatic during periods following starvation and injury. Seasonal variations in animal feed supplies often cause interrupted patterns of growth in young animals due to feed restriction resulting from seasonal variations or other causes. A subsequent restoration of feed supplies causes the animals to exhibit enhanced gain in weight called compensatory growth. The underlying physiological processes of growth are complex involving feed utilization, visceral tissue mass and carcase protein and fat deposition, variation in energy requirements according to a particular condition and also in other nutrients, to meet maintenance and body performance, partition of nutrients, endocrine control, etc. So defined, growth (which includes production) is inseparable from metabolism.

Our studies on the responses of ruminant (and other livestock) animals to different rations or related treatments rely basically on traditional balance methods alongwith proximate analysis of foodstuffs and waste. To measure the transfer of substances within the intact animal and their partition to organs and tissues, tracer isotope methodologies are used in combination with experimental approaches such as the application of the Fick principle and dilution techniques. The data range over a wide field area and include important considerations as the energy cost of maintaining animals, of transforming feed into body tissue and the desired animal products. Data on body composition enable assessment of growth responses to changes in nutrition or in the environment. The studies are reviewed in this presentation highlighting on experience in the use of methodologies appropriate to the existing conditions.

The methodologies and experimental approaches presented are based on the work on growing animals. As the discussions proceed to touch on other animal productive states, the appropriate methodologies will be inserted.

GROWING RUMINANTS

Animal Balance Trial

The balance trial provides information related to the requirements of nutrients for growth, maintenance and production and the availability of nutrients from feedstuffs. It is used to show changes in percentage absorption of nutrients as a function of the level of nutrients in the diet. For energy balance, the energy categories of feed, i.e. gross energy (GE), faecal energy (FE), digestable energy (DE), urinary energy (UE), Energy loss with digestive gas production (En-CH_4), metabolizable energy (ME), energy expenditure (EE) = health production (HP) and retained energy (RE), should be calculated. Energies in feed, faeces and urine are determined by bomb calorimetry or calculated from organic ingredients multiplied by their respective caloric equivalents, e.g. UE may be found from g urinary-N.d^{-1} x 34.0 kJ, but energy in gases and CH_4 is rather difficult" to measure. It could be reasonably well approximated from gas test results or simply taken as 8-10 % of GE, or using Blaxter's equation En.CH_4 = 4.28 + 0.059 D Kcal/100Kcal GE (D = digestibility of energy, %). For the energy balance, data on HP and RE are needed. When physical exercise is absent, it is safe to use the relationship ME = HP + RE. Since ME is found from GE substracted by all energy found in waste, either HP or RE should be determined while the other could then be calculated by difference from the ME. If HP should be measured, a suitable technique of animal calorimetry is required. On the other hand if RE is choosen to be determined, the slaughter technique or a suitable *in vivo* body composition technique should be used. Alongside energy balance, protein (or N) balance could be simultaneously measured.

Whole Animal Calorimetry and Body Composition

All major components of the body are in a continuous state of flux: intakes, excretions, degradations and resynthesis. All endproducts of digestion not only act as substrates for the accretion of body tissue (anabolism), but also serve as precursors for the production of the high-energy compounds needed (catabolism) for maintenance and to provide the energy for the synthesis of the macromolecules of the body. In addition, there is an extensive interconversion of metabolites and the rate of productive processes in the body is governed by the complex interplay between the intermediary metabolism of the various nutrients and the rate of synthesis and degradation of tissue lipid, protein, nucleic acid and carbohydrate stores.

1, *Measurement of Whole Body HP by Carbondioxide Entry Rate'Technique*

Due to unavailability of respiration chamber calorimetry for large animals, but on the other hand we have in our laboratory at our disposal radioisotope detection equipment, Carbondioxide Entry Rate Technique (CERT) using tracer ^{14}C-bicarbonate is the method of choice to measure whole body energy expenditure of small ruminants (Sastradipradja, 1992). The method involves primed continuous infusion of the label solution into a blood vein and after reaching steady state condition of isotope concentration in body fluid bicarbonate, serial blood samples are withdrawn. The CO_2 production rate (rCO_2) is calculated by dividing the rate of label infused by the plateau specific activity of blood bicarbonate. At an accepted RQ value, the rCO_2 value is converted into its energy equivalent which is HP. Double polyethylene catheters are implanted in the jugular veins for minimal disturbance to the animal, easy delivery of isotope and convenient serial blood sampling. It is important to measure the specific activity of CO_2 over a sufficiently long period of time (e.g. 12h or longer) to ensure a more representative mean value of specific activity. For such long trials, instead of blood, samples of any body fluid *can* be taken (e.g. saliva instead of blood). To get faster attainment of stable specific activity we apply the primed-continuous infusion technique and our experience shows that the ratio between primer dose and infusion rate per minute is 80 to 1 giving satisfactory results. Primer dose is 1 ml of $NaH^{14}CO_3$ = 40 µCi delivered within 1 min. followed by continuous infusion at 0.5 µCi/min. The CO_2 production *can* be estimated from the plateau specific activity according to the equation (Corbett *et* al., 1971) :

$$CO_2 = \frac{\text{rate of tracer bicarbonate infused}}{\text{plateau specific activity of } CO_2}$$

The advantage of CERT is that the animal is free to move around without restraint. The use of CERT also enables measurement of gluconeogenesis involving $^{14}CO_2$ fixation. Another advantage of CERT is the ability of measuring glucose kinetics during the same trial by administering ^{3}H-glucose at the same time with the bicarbonate label.

2. ***Measurement* of HP *as the Difference Between ME and RE***

In our work with swamp buffaloes during the last four years, we have calculated HP as the difference between ME and RE included physical work (Mahardika *et al.*, 1997) with satisfactory results (see exercise metabolism below).

3. ***Heart Rate* as *A Predictor of HP***

We are still developing this technique on small ruminants and cattle following the success of longterm heart rate measurement in swamp buffaloes using the Polar Sport Tester (Finland).

4. ***Estimation* of *Body Composition* in vivo**

4.a ***Urea Space Technique*** (Rule *et al.,* 1986)

Urea is used as a marker for body water. A measured amount of urea in saline is injected into the jugular vein within one minute. Exactly 12 minutes after the injection, a sample of blood is withdrawn from the jugular vein and the concentration of urea determined. Urea space is found by dividing the dose (mg) of urea infused by the increment of blood urea concentration following the infusion from the preinfusion value times body weight times 10. The empty body water (EBW, %) = 59.1 + 0.22 US (%) - 0.04 BW, while empty body fat (%) = 19.5 - 0.31 US (%) + 0.05 BW. The choice of this technique is necessary considering the hazards involved in the use of radioisotope labelled water on large ruminants and the costly slaughter technique with ruminant livestock animals. We have introduced this technique in our work with goats and sheep. Validation of the *in vivo* body composition method using US was separately done on four goats by doing slaughter technique (ST) analysis in addition to the *in vivo* calculations on the same animals in question (Arta Putra *et al.,* 1997). EBW estimated according to Rule *et al.* (1986) resulted in an underestimation of 4.2% from the EBW value by ST. Therefore, the use of US in goats required a correction factor of 1.044. For use with small ruminants, Panaretto's equation for body protein (Panaretto, 1963) and for body fat (Panaretto and Till, 1963) are based on EBW. The use of our corrected values in aforesaid equations resulted in good agreement with our ST results. The results are presented in Table 1.

Table 1. Body Composition (% BW) According to Slaughter Technique Analysis and in *vivo* Equations Estimated from Four Female Growing Goats

	Body water		Body protein		Body fat	
	ST	Eq. 1	ST	Eq. 2	ST	Eq.3
	--------- %BW---------		--------- %BW---------		--------- %BW---------	
Mean ± SD	60.9±5.94	60.9±0.06	20.2±0.39	20.2±0.02	16.2±5.74	17.6±0.08

ST = slaughter technique analysis
Eq. 1 = EBW according to Rule *et* al. (1986) US equation multiplied by 1.044,
Eq. 2 = Panaretto (1963) body protein equation using EBW value from Eq. 1,
Eq. 3 = Panaretto and Till (1963) body fat equation using EBW value from Eq. 1.

Validation results with the Javanese thin tail (JTT) sheep (Saka and Sastradipradja, unpublished data) revealed that only the equation which relate EWB with US was significantly different from the regression equation according to Rule et *al.* (1986), as follows

$$\text{EBW (\%)} = 64.1 - 21.1\text{US\%} - 0.378\text{BW}$$
$$(P<0.05;\ S_{y,x} = 2.185;\ R^2 = 0.425)$$

Consequently, only this equation can be used for sheep with correction factors respectively for the intercept, US and BW to be 1.0846, -95.9091 and 9.45. From our studies with female swamp buffaloes, the equation of Rule et *al.* (1986) if applied for working buffaloes should be modified to:

$$\text{Fat} = 13.69 - 0.21\,\text{US} + 0.03\,\text{BW}; \quad \text{Protein} = 19.7 + 0.08\,\text{US} + 0.11\,\text{BW}.$$

4.b Body Density Method (Kleiber, 1961)

Application of this technique in small ruminants and cattle is being conducted after it has been succesfully practiced in the swamp buffalo. Contrary to the situation in swamp buffaloes who love wading in water, other livestock animals will resist to immersion in water. Therefore, a solid frame should be constructed where the animal can be immobilized and the whole device including the animal can be subsequently lowered in the water tank leaving the head above water level.

Supporting Measurements on Metabolism and Digestion

Glucose is an important nutrient for all tissues, especially it is indispensable as energy source for nerve cells and for foetus in pregnant animals, for the synthesis of lactose, fat and providing carbon skeleton for others. An important component of growth is fat synthesis. Glucose is required during growth, in particular for the supply of the NADPH via the pentosephosphate pathway for fat synthesis. A lower amount is required for protein synthesis. The major portion of glucose available to the ruminant is supplied by gluconeogenesis. Additional demands for glucogenic precursors occur during lactation, namely for the lactose secreted in the milk and for the reducing power needed to provide energy for milk synthesis. Impaired gluconeogenesis is believed to contribute to various metabolic disorders frequently seen in ruminants, such as acetonaemia in dairy cows and pregnancy toxaemia or twin lamb disease in sheep. In ruminant studies, glucose metabolism warrants attention.

Successful operation of whole animal metabolism requires low levels to function properly. Many metabolic peculiarities of ruminants stem from the proper functioning of the splanchnic region. It is understandable that special attention should be given to metabolism in this region.

A great deal of research has been carried out on the digestive system of the ruminant giving understanding of the characteristic metabolism which cope with the peculiar products of microbial digestion. We attempted to measure products of ruminal digestion and integrate it for our overall understanding of animal performance.

1. *Gluconeogenesis Involving CO_2 Fixation*

Tracer labeling of the bicarbonate pool (CERT) may enable one to estimate gluconeogenesis (GNG) from carbon transfer involving CO_2 fixation into the glucose pool. The rationale involves using the transfer quotient (TQ) between CO_2 and glucose to assess the extent of CO_2 fixation in GNG from precursors like propionate and lactate. The TQ in question is calculated as the ratio between the plateau specific activities (per at. C) of ^{14}C in glucose (product) and in bicarbonate (precursor). The rate of GNG involving CO_2 fixation is found by multiplying the value of the glucose flux by the TQ times 6. Measurement of the glucose flux and pool size is done simultaneously with the CERT infusion in the same animal by pulse injection with tritiated glucose (Sastradipradja, 1992).

2. Metabolism of the Portal Drained Viscera

Many metabolic peculiarities of ruminants stem from the viscera, that is a group of organs whose blood supply drains into the portal vein. Quantification of nutrients in the splanchnic region is useful to understand the metabolism in this region. We have experience to study this aspect in small ruminants by the arteriovenous difference technique (Katz and Bergman, 1969) involving surgical procedures to obtain samples from the mesenteric, portal and hepatic veins and of any artery (Astuti, 1995; Sastradipradja *et al.,* 1997). Portal blood flow were measured on unaesthetized animals (i.v. injection of xylazine 0.05 ml/kg BW, followed by i.v. injection of ketamine 0.11 mlkg BW). Lidocaine (1 ml/ animal) was applied locally on the site of incision. The primer dose of PAH-^{3}H was 5.75 µCi delivered in one minute via the jugular vein followed by continuous infusion at 0.6 µCi/min into the right mesenteric vein. After around two hours of infusion, blood samples were collected for analysis of blood gasses (CO_2, O_2, VFA) and other relevant constituents. Metabolism of the region and absorption rates of nutrients can be measured. The technique is invasive however, which need appropriate skills to perform. So far we haven't been successful in placing permanent catheters in aforesaid blood vessels. Feasible techniques to measure ruminal fermentation production rates would be an alternative approach to measure the nutrients' supply to the animal.

3. In vitro *Techniques for Ruminal Fermentation*

The techniques closely simulate *in vivo* conditions especially for rumen fermentation studies: rate of formation of endproducts (VFA), rate of release of NH_3, production rate of microbial protein and gas production.

3.a' *Microbial Protein Synthesis* (Suwandyastuti *et al.,* 1985)

A shortterm incubation of rumen contents at 39 °C in an artificial rumen: test tubes, glass syringes or flasks. The anaerobiosis is maintained by introducing N_2 gas replacing the air in the artificial rumen above the incubation mixture. ^{32}P is used as tracer for measuring microbial protein synthesis (MPS).

3.b *Incubation of Rumen Contents in Glass Syringes (gas test)* (Menke and Steingass, 1988)

The amount of gas which is released when feedstuffs are incubated *in vitro* with rumen fluid measures CO_2 and CH_4 production. It is closely related to digestibility and consequently to the energetic feed value of feedstuffs for ruminants. The incubation mixture consists of feed sample, main and trace element solution, buffer solution, rezazurin solution (indicator), reduction solution and rumen fluid. Hundred ml *syringes* are used preheated at 39 °C. The syringes plus contents free of air bubbles are incubated in a (39 °C) preheated incubator. The volume of gas in the piston is read off eight hours after the start of the incubation (V_8). Incubation is continued and the final reading is done after 24 hours (V_{24}). Regression equations will be derived relating ME with gas production, feed nutrient components and digestibilities.

3.c ***VFA Production*** Rates: ***Zerotime*** in vitro ***Method*** (Whitelaw ***et al.,*** 1970)

A sample of rumen contents is taken and subsamples incubated ***in vitro*** under anaerobic conditions. The rate of production of individual and total VFA is calculated from the increments in acid concentration obtained by incubating the subsamples for different periods and extrapolating back to zero time incubation to give the rate of VFA production per unit volume at the time the sample was removed. Equations for performing the calculations are given by Whitelaw ***et al.*** (1970). The rumen volume should be known in order to calculate total ruminal production.

3.d ***Use of Nylon Bags Incubated in The Rumen*** (IAEA, 1985)

The digestibility test requires animals fitted with permanent rumen fistulae. Nylon bags should have a pore size 20 - 40 mm, dimensions 15 x 8 cm, sample size 3-5 g of air-dry feed, ground through screen 2-5 mm, incubation times up to 24h for protein concentrates, up to 72h for roughage feeds. The technique can describe both the rate and the extent of degradation, affected by the rumen environment such as ammonia level, pH, type of feeds, trace minerals, etc.

4. In vivo ***Estimation of Digestion***

Evaluation on the intake and digestion characteristics of feedstuffs, needs quantitative data to describe the movement of digesta along the gastrointestinal tract.

4.a ***Estimation of Rumen Volume*** (IAEA, 1985)

The volume of rumen iiquid is estimated from the dilution of ^{51}Cr-EDTA tracer introduced intraruminally by way of a syringe and needle in a period between rumen contractions. After 1 hour and then every hour up to eight hours, sample/withdraw representative rumen contents by way of a stomach tube. The animal should receive a (nearly) continuous feeding regime. Plot