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 résearch 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 ternak 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 feeds 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:

Fat = 13.69 - 0.21 US + 0.03 BW; Protein = 19.7 + 0.08 US + 0.11 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 ml/kg 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 liquid 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 log concentration against time and find the intercept to give the ^{51}Cr concentration at zero time. Rumen volume is calculated as dose devided by zero time concentration. Flow of rumen content is obtained from the kinetics' data. $^{51}CrCl_3$, obtained from Amersham UK, mixed with Na-EDTA will easily form ^{51}Cr-EDTA. Alternatively, the volume of rumen can also be estimated from *per oral* administration of an aliquot of labeled water (^2H_2O or D_2O) and follow the tracer disappearance with time in rumen water (Mac Farlane *et al.*, 1974).

4.b *Determination of Rate of Passage*

A practical method to determine the rate of passage of digesta is to use an appropriate marker introduced with the feed offered and follow its appearance in the faeces. The resulting marker concentrations are plotted against time of collection, and the appropriate curve derived can be mathematically analyzed to determine the outflow rate constants. A suitable external marker is the PA6 (polyamide granules) (Becker *et al.*, 1992).

EXERCISE METABOLISM

Draft animals have been an integral part of agricultural development throughout Indonesia for centuries but mechanisation is now being introduced in some areas as a consequence of the scarcity of human labour and animal power at peak periods of land preparation. There is a great demand for additional power which could be met by providing more draft animals, especially in areas where tractors cannot operate and in transmigration areas. The large number of animals used for draft power and their importance to agricultural development in Indonesia makes it imperative that they are used efficiently by providing adequate diet. To provide the required amount of energy for optimum performance and production, it is necessary to know the energy expenditure of draft animals under the conditions in which they typically work. The actual expenditure is proportional to the type of work and work load. To estimate requirements, expenditure must be measured over a range of workloads and the predicted requirement related to work output. Expenditure for any given workload will vary with breed and will be influenced by the prevailing environmental conditions. There are presently no reliable figures from which to predict the energy requirements of draft animals in Indonesia. We used the following techniques on female swamp buffaloes in addition to balance trials.

Appropriate Methodologies on Working Animals.

1. *Relation Between HR and EE of (Growing) Working Female Swamp Buffaloes*

Using Polar Sport Tester, continuous long term (up to 36 hours) HR monitoring has been used successfully on swamp buffaloes in Indonesia (Mahardika *et al.*, 1995). Simultaneously calculation of energy expenditure was carried out by the factorial method of measuring work output on exercising animals. Increase in energy expenditure was related to increase in HR following the equation: EE = 17.22 + 0.23HR (r = 0.95). Results also reveal that it is not advisable to impose a work load exceeding 15 % of liveweight on female swamp buffaloes.

The heart rate monitoring is sturdy and relatively inexpensive and our experience has shown that the necessary equipment can be used reliably in field studies. But, although the method shows great promise, it has yet to be validated in the field. For this purpose, an appropriate method would be measuring EE as the difference between ME and RE. The *in vivo* body density method was used to estimate body composition and RE.

2. *Body Density (Water Displacement) Method*

The swamp buffalo prooved to be a suitable animal for the water displacement method. It attempted to estimate longterm EE of working buffaloes from energy balance within a fortnight experimental period and benefiting from the relationship EE equals to ME minus RE. Measuring *in vivo* body composition with this body density technique showed that the fat content of buffaloes ranged from 16.8 to 18.7 %, and the protein ranged from 17.4 to 18.7 %. Treatment with 3-hours work for 14 days did not have a significant effect on body composition, although there was a tendency that the fat content decreased in the working buffaloes. Non-working buffaloes had fat retention of 0.07 kg/day, whereas working buffaloes showed negative retentions (Table 2) (Mahardika *et al.*, 1997).

Similar results also happened to protein retention. However, negative retention of protein only occured in buffalo with 3-hours work/day. The decreased content of fat and protein resulted from the use of both substances as energy sources for work. Fat degradation would occur earlier than protein's. Both decrements of body constituents are exponential in nature. For the calculation of HP, RE should be corrected by dividing it by the efficiency coefficient for the formation of body tissue. This alternative method of HP measurement was used for the validation of the heart rate monitoring technique and will be routinely applied with ruminants in our laboratory. Equations for swamp buffaloes relating components of lean (water, bone, protein and meat) and fat was made by Mahardika *et. al.* (1997) who used the data found in Natasasmita's PhD dissertation (Univ. of Melbourne, 1978). Although the method is rather laborious, facilities are easy to build and is not too costly. The method is attractive to be developed for other ruminant species.

Table 2 . Retentions of Fat, Protein and Energy for Working Buffaloes Subjected to Different Working Duration

Variables	Treatment			
	No work	1-hour work	2-hour work	3-hour work
Fat Retention (kg/day)	0.07[a]	-0.04[b]	-0.08[bc]	-0.09[c]
Protein Retention (kg/day)	0.11[a]	0.08[a]	0.01[b]	-0.10[c]
Energy Retention (MJ/day)	4.96[a]	0.03[b]	-2.75[c]	-4.92

Note: The values with different letters on the same line are significantly different ($P<0.05$) (Source: Mahardika *et al.*, 1997)

PREGNANT AND LACTATING RUMINANTS

The indigenous small ruminant breeds of Indonesia are adapted to the humid tropical environment. They show independence of photoperiodicity for breeding and are generally believed to be prolific. In the free living state, they feed on grasses or poor roughage without being given any feed supplement. ME supply with such diet is insufficient to support energy retention and ADG, the more for pregnant animals. The conceptus depends largely on glucose for its energy supply and demands high maternal glucose production rates (Sastradipradja *et al.*, 1994). The rapid increase in foetal growth in late pregnancy imposes a progressive limitation on the use of poor quality roughage as the sole feed. Concentrate supplementation improves ME and protein supplies (Katipana and Sastradipradja, 1994), however, such feeding needs long training for the animal's acceptance.

ME use for lactation is considered in general more efficient than for fattening and tissue gain. Total amounts of specific nutrients available within a lactating animal that are utilized for milk production, and other productive processes in the body, are not equal to amounts absorbed from the digestive tract. Therefore, a balance model of milk synthesis of the indigenous female is needed to evaluate carbon and nitrogen flows into and out of the gland, which generates sufficient energy and reducing power to meet synthetic requirements. Calculated uptakes of metabolites, energy requirements of the gland for synthesis of milk components and other related quantitative data are needed to evaluate effects of changing nutrient availability and metabolic control mechanisms.

Our studies with pregnant and lactating small ruminants requires measurements of the same metabolic parameters as required for the growing animal, e.g. balance trials, whole animal calorimetry, glucose kinetics and activity of the splanchnic bed. For the studies on the lactating animal, additional data are needed on milk production and composition, and the metabolic activity of the mammary gland.

1. *Milk Production and Composition*

Prior to weaning, the development and growth performance of the young is dependent upon the milk supply from the mother animal, hence the amount of milk produced is an important parameter to be looked at for judging milk performance of the mother animal. Production can be measured after handmilking, usually oxytocin is injected prior to milking. Samples of milk can be analyzed for milk components, e.g. lactose, fat, crude protein and citrate. Direct measurement of milk yield may fail to provide an accurate estimate of milk production, especially for non-dairy animals where production is very limited. Therefore, an appropriate dilution method (deuterium oxide) for the determination of milk yield of such animals would be useful. The principle steps of this procedure includes injection of the D_2O into the body of the infant, collection of blood samples, separation of the water moiety, determination of the D_2O content, calculation of water turnover and calculation of water intake (Prawirodigdo and Sastradipradja, 1992).

2. *Mammary Gland Metabolism*

2.a *Mammary Blood Flow*

This physiological parameter is essential in basic trials for the calculation of nutrient utilization in lactating animals. Mammary blood flow (MBF) is estimated by a variety of techniques. For our purposes we choose an indirect technique employing the Fick principle and initially used total N in milk, arterial and venous blood for the calculations (Astuti, 1995). Later measurements applied a technique according to Cant *et al.* (1993) using A-V difference of phenylalanine and tyrosine, and their contents in milk protein. Thus,

$$MBF \text{ (liters per hour)} = \{(FY_B \times .965) + FY_F\}/\{FY_{A\text{-}V}\},$$

where:

FY_B = Phe + Tyr output in milk protein (moles per hour),
FY_F = free milk Phe + Tyr (moles per hour), and
$FY_{A\text{-}V}$ = Phe + Tyr A-V difference (moles per hour).

2.b *Metabolite Uptake, Oxygen Uptake*

Data on blood and plasma metabolite concentrations is needed and using the Fick principle, extraction percentages and uptake by the mammary gland can be calculated. Metabolites of interest are oxygen, glucose, acetate, betahydroxybutyrate (BHBA), lactate, glycerol, triglycerides (TG), other lipids, aminoacids. Oxygen uptake and CO_2 production provide the basis for estimating substrate oxidation.

2.c *Biochemical Analysis*

Conventional chemical methods for the determination of concentration levels of metabolites in body fluids can provide adequate information to the understanding of how feed nutrients are converted into animal products and biochemical status of the animal. Such laboratory work includes determination of total and individual Volatile Fatty Acid (VFA), ruminal ammonia-N, blood glucose, FFA, TG, alpha-keto butyric acid, urinary allantoin, blood plasma aminoacids and minerals.

3. *Lactating Dairy Cows*

Onfarm research with dairy cattle owned by commercial enterprises suffers from limitations imposed by the owners by not allowing major manipulations to be given to the animals or to the existing system. Under these cicumstrances one has to fully make use of the limited data collected. From such limited data, Sigit (1995) managed to construct material balance of lactating FH cows at the Baruajak Farm, Bandung.

With minimal disturbance to the animal, data were collected on body weight by measuring the chest circumference according to Schoorl's equation:

$$BW \text{ (kg)} = 0.01[\text{ chest circ. (cm)} + 22]^2,$$

feed intake as the difference between feed offered and refused, total collection of wastes, daily milk production and milk analysis, while data on digestibilities were obtained from *in vitro* estimations.

HP was calculated as follows:

N retention (g/d) was calculated as amount of N digested - urinary N - milk N

C retention (g/d) was calculated as digested C - milk C - urinary N

The value still contains the amount of C which would escape as respiratory CO_2. HP was calculated from CO_2 production (Mcal/day) = [retained C - C of meat and fat which disappeared due to loss of body weight] x 94/12/1000 (Brody, 1945).

1 mole C = 12g, produces CO_2 with 94 Kcal. (1 Kcal = 4.185 KJ)

EXAMPLES OF RESULTS

Data of relevant metabolic parameters of growing female goats (av. BW 14 kg) and lactating does (av. BW 25 kg) fed *ad libitum* and restricted diets are presented in Table 3. Calculations demonstrated that energy maintenance requirement for growing goats was 0.47 MJ/kg$^{0.75}$/d while ME_m for the lactating doe was 0.71 MJ/kg$^{0.75}$/d. Portal blood flow ranged from 400 - 1000 ml/min which means about 30 % of cardiac output in growing goats. The data for lactating does were about 15 %. We found evidence that the glucogenic capacity of the growing goat is adequate even with restricted feeding and similar conclusion could be drawn for the lactating doe. Manik and Sastradipradja (1989) found that gluconeogenesis in the lactating goat is high persumably from ruminal propionate. Metabolism of the splanchnic area in growing goats seemed to be more active than is the case with lactating does.

With JTT growing lambs, the effect of multiple clenbuterol injection (CB) was investigated. It was found that there were,significant quadratic responses (P<.05) to dose of CB (X) for average weights of 5 individual muscles (I-Y) and 5 cut yields (II-Y) of which the equations were respectively :

I-Y = 196.3292 + 8.5304X - 0.3164X^2 ($P<.05$; R^2 = 0.8687; $S_{y.x}$ = 10.4522).
max. 253.8 g for CB 13.48 : g/kg BW

II-Y = 0.8194 + 0.0393X - 0.0015 X^2 ($P<.05$; R^2 = 0.7003; $S_{y.x}$ = 0.0573).
max. 1.069 kg for CB 12.70 : g/kg BW. (Saka *et al.*, 1997).

Table 3. Digestibility and Metabolism of Nutrients of Growing Female and Lactating Etawah Cross-Breed Goats

Parameter		*ad libitum*	medium	low	P level of significancy
Energy/nutrient balances:					
GE (MJ/d)	G	8.53	6.27	4.71	
	L	15.98	14.07	11.38	
DE (MJ/d)	G	5.22[a]	3.71[bc]	2.83[c]	< 0.01
	L	10.70[a]	10.12[a]	8.18[b]	< 0.01
CP (g/d)	G	102	75	56	
	L	158	152	135	
ME (MJ/d)	G	4.47[a]	3.17[bc]	2.40[c]	< 0.01
	L	9.26[a]	8.85[a]	7.10[b]	< 0.01
HP (MJ/d)	G	3.62	3.25	3.31	NS
	L	6.28	5.46	5.21	NS
HP/GE (%)	G	43	51	70	
	L	39.3	38.8	45.8	
RE (MJ/d)	G	0.85[a]	-0.085[ab]	-0.91[b]	< 0.05
	L	3.16	3.09	1.68	NS
MBS ($kg^{0.75}$)	G	7.75	7.12	6.48	NS
	L	10.98	11.34	10.45	NS
Catprot(UNx6.25g/d)	G	12.68[a]	7.55[b]	11.72[a]	< 0.01
	L	39.6	29.9	33.2	NS
MPS (g/d)	G	3.14[a]	2.65[ab]	2.29[ab]	< 0.05
	L	6.28[a]	4.50[b]	4.14[b]	< 0.05
RProt (g/d)	G	67.14[a]	48.50[b]	31.01[c]	< 0.01
	L	59.95[a]	57.81[ab]	37.35[b]	< 0.05
Glu. Flux (mg/min)	G	21.02[a]	12.20[b]	4.63[d]	< 0.01
	L	29.43	24.20	14.46	NS
Cardiac min.vol.(ml/min)	G	3160	3160	2880	NS
	L	3670	2700	2940	NS
Organ metabolism:					
$BF_{splanch.bed}$ (ml/min)	G	1032	625	394	NS
	L	500[a]	371[b]	223[c]	< 0.05
$HP_{splanchnic}$/HP (%)	G	45	24	16	
	L	6.3	6.8	3.7	
VFA absorp. (mM/min)	G	22.8[a]	15.4[ab]	8.6[b]	< 0.05
	L	16.7[a]	12.8[ab]	7.7[b]	< 0.05
Mammary BF (ml/min)	L	229[a]	193[b]	128[c]	< 0.05

G = growing goats, source :Astuti *et al.* (1997)
L = lactating does, source: Astuti (1995).

Experimental data on pregnant ewes fed grass as the sole feed revealed that the diet was insufficient and the straineous effecis of pregnancy would cause maternal fat may had been mobilized to get access to fat glycerol for endogenous glucose production, eventhough no signs of acetonemia were observed (Table 4). RP, RE and ADG were improved by concentrate supplementation (Sastradipradja *et al.*, 1991). The beneficial effect of adequate feeding has been demonstrated also on pregnant does (Katipana and Sastradipradja, 1994).

Table 4. Metabolic Responses of Pregnant Ewes and Does to Adequate Feeding

	R0			R1			R2		
Parameter	P0	P1	P2	P0	P1	P2	P0	P1	P2
EWES*:									
ME (MJ/d)	3.11	3.12	2.88	5.34	4.83	5.05			
HP (MJ/d)	4.22	5.85	6.08	3.75	6.00	5.53			
RE (MJ/d)	-1.12	-2.73	-3.20	1.58	-1.17	-0.48			
RP (g/d)	6.38	-3.83	-7.65	35.02	21.29	30.61			
DOES**									
ME (MJ/d)				3.09	5.99	6.06	5.51	11.98	12.18
HP (MJ/d)				2.92	4.36	4.75	2.65	6.51	9.48
RE (MJ/d)				0.17	1.64	1.31	2.86	5.47	2.6611
RP (g/d)				19.43	47.19	48.37	36.06	110.19	112.8

P0 = non-pregnant, P1 = single and P2 = twin pregnancy.
* for ewes: R0 = sole grass feed; R1 = grass + concentrate; Significant difference due to ration.
** for does: R2 = low dietary level; R3 = medium dietary level.

Table 5. Metabolic Responses of Lactating FH Cows Fed a Ration Supplemented with Hydroxymethionine Analogue (Sigit, 1995)

Variable	A0	A1	A3
Urinary-N (g/d)	50.52	65.68	51.44
N retention (g/d)	55.7	41.88	37.05
C retention (g/d)	2488	2474	2280
ADG (g/d)	-554.7	-404.7	-781.3*
HP (MJ/d)	63.98	67.56	73.17
HP (MJ/kg$^{0.75}$)	0.790	0.797	0.852

A0, A1 and A3 were respectively 0, 0.1 and 0.2%. *supplementation with ammoniated zeolite at 3.0% level improved ADG (+83.33 g/d).

Table 5 contains data on metabolic parameters in lactating FH cows fed a diet supplemented with hydroxy-methionine analogue (A0, A1 and A3 were respectively 0, 0.1 and 0.2 %) (Sigit, 1995). Addition of ammoniated zeolite at 3.0 % improves ADG +83.33 g/d.

CONCLUSION

There are a number of appropriate methodologies available for conducting onfarm metabolic research aiming at gaining better understanding of animal production and health in the humid tropics.

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REFERENCES

Arta Putra, I G. , D. Sastradipradja, D.A. Astuti, B. Kiranadi and L.A. Sofyan. 1997. Nutrient Utilization of Growing Female Etawah Crossbreed Goats Fed Diets Containing Different Protein/ Energy Ratios. *In:* Energy Metabolism of Farm Animals, Proc. 14th Symp., Belfast UK 14-20 September 1997.

Astuti, D.A., D. Sastradipradja, T. Sutardi and B. Haryanto. 1997. Energy and Protein Requirements of Growing Female Etawah Crossbreed Goats Determined From Metabolic Parameters. *In:* Energy Metabolism of Farm Animals, Proc. 14th Symp., Belfast UK 14-20 September 1997.

Astuti, D.A. 1995. Evaluasi Pemanfaatan Nutrien Berdasarkan Curahan Melalui Sistem Vena Porta dan Organ Terkait pada Kambing Tumbuh dan Laktasi. Disertasi Doktor. Institut Pertanian Bogor.

Becker, K., F. Mahler, E. Schlecht and A. Susenbeth. 1992. Plant/Animal Interaction on Savanna Shrubland in Sahelian Mali. Proceedings 5th Asia and Oceania Congr. Nucl. Med. and Biology, vol. 2, PKBNI Jakarta. p413-434.

Blaxter, K. 1989. Energy Metabolism in Farm Animals: Past, Present and Future. *In:* Y. van der Honing and W.H. Close (eds). Energy Metabolism of Farm Animals, EAAP Publ. No. 43, Centre for Agric. Publ. And Documentation, Pudoc Wageningen, Netherlands. p379-384.

Brody, S. 1945. Bioenergetics and Growth. Reinhold Publ. Corp., New York, USA.

Corbett, J.L., D.J. Farrell, R.A. Leng, G.L. McClymont and B.A. Young. 1971. Determination of the Energy Expenditure of Penned and Grazing Sheep from Estimates of Carbondioxide Entry Rate. *Brit. J. Nutr.*, 26:277-291.

Cant, J.P, E.J. DePeters and R.L. Baldwin. 1993. Mammary Aminoacid Utilization in Dairy Cows Fed Fat and its Relationship to Milk Protein Depression. *J. Dairy Sci.*, 76:762-774.

IAEA. 1985. Laboratory Training Manual on the Use of Nuclear Techniques in Animal Nutrition, Vienna. p191-192.

Katipana, N.G.F. and D. Sastradipradja. 1994. Energy and Protein Requirements of Etawah Cross Goats During Late Pregnancy. *In:* J.F. Aguilera (Comp.). Energy Metabolism of Farm Animals, EAAP Publ. No. 76, CSIC Publ. Serv., Madrid, Spain. P.63-66.

Katz, M.L. and E.N. Bergman. 1969. Simultaneous Measurements of Hepatic and Portal Venous Blood Flow in the Sheep and Dog. *Am. J. Physiol.,* 216:946-952.

Kleiber, M. 1962. The Fire of Life. An Introduction to Animal Energetics. John Willey and Sons, Inc., New York- London. p44-59.

Macfarlane, W.V., B. Howard and B.F. Good. 1974. Tracer in Field Measurements of Water, Milk and Thyroxine Metabolism of Tropical Ruminants. *In:* Tracer Techniques in Tropical Animal Production. IAEA,Vienna. p.1-23.

Mahardika, I G., D. Sastradipradja, I K. Sumadi, T. Sutardi and Kartiarso. 1995. Energy Expenditure of Water Buffaloes for Draft Power in the Humid Tropics. *In:* K. Becker, P. Lawrence and B. θrskov (Ed.). Sustainable Small-Scale Ruminant Production in Semi-Arid and Sub-Humid Tropical Areas. Inst. An. Prod. Tropics and Subtropics, University of Hohenheim, Stuttgart, Germany. p127-130.

Mahardika, I G., D. Sastradipradja and I K. Sumadi. 1997. Daily Energy Expenditure and Protein Requirement of Working Female Swamp Buffaloes Estimated from Energy Balance and

Body Composition. *In:* Energy Metabolism of Farm Animals, Proc. 14th Symp., Belfast UK. 14-20 September.

Manik, I G. and D. Sastradipradja. 1989. Effect of Heat-Moisture Treated Cassava in Urea Containing Feed Supplement on Carbohydrate, Protein and Energy Metabolism of Goats. *In:* Y. van der Honing (Comp.). Energy Metabolism of Farm Animals, EA/ P Publ. No. 43, Pudoc-Wageningen, Netherlands. p73-76.

Menke, K.H. and H. Steingass. 1988. Estmation of the Energetic Feed Value Obtained from Chemical Analysis and *in vitro* gas production. *Anim. Res. and Dev.,* 28:7-55.

Natasasmita, A. 1978. Body Composition of Swamp Buffalo. A Study of Developmental Growth and of Sex Differences. PhD dissertation, University of Melbourne, Australia.

Panaretto, B.A. and A.R. Till. 1963. Body Composition *In Vivo*. II. The Composition of Mature Goats and its Relationship to the Antipyrine, Tritiated Water and N-acetyl-4-aminoanti-pyrine Spaces *Austr. J. Agric. Res.*, 14:926-943.

Panaretto, B.A. 1963. Body composition *in vivo*. III. The Composition of Living Ruminants and its Relation to the Tritiated Water Spaces. *Austr. J. Agric. Res.*, 14:944-952.

Prawirodigdo, S. and D. Sastradipradja. 1992. Application of the Deuterium Oxide Dilution Technique to Estimate Milk Yield of Sows. Proceedings 5th Asia and Oceania Congr. Nucl. Med. And Biology, vol. 2, PKBNI Jakarta. p435-449.

Rule, D.C., R.N. Arnold, E.J. Hentges and D.C. Beitz. 1986. Evaluation of Urea Dilution as a Technique for Estimating Body Composition of Beef Steers *In Vivo*: Validation of Published Equations and Comparison with Chemical Composition. *J. Anim. Sci.*, 63:1935-1948.

Saka, I K., D. Sastradipradja, and B. Kiranadi. 1997. Nutrient Metabolism and Carcase Characteristics of Male Thin Tail Sheep Receiving Clenbuterol. *In:* Energy Metabolism of Farm Animals, Proc. 14th Symp., Belfast UK. 14-20 September.

Sastradipradja, D. 1992. Tracer Techniques for Measuring Energy Expenditure and Related Metabolic Parameters. Proceedings 5th Asia and Oceania Congr. Nucl. Med. And Biology, vol. 2, PKBNI Jakarta. p451-457.

Sastradipradja, D., N.G.F. Katipana, D.R Astuti, D.R. Ekastuti and H. Maheshwari. 1991. The Effect of Concentrate Supplementation on Glucose, Energy and Protein Metabolism of Single and Twin Pregnant Ewes. *In:* C. Wenk and M. Boessinger (*Ed.*). Energy Metabolism of Farm Animals, EAAP Publ. No. 58, Inst. Nutztierwiss. *Gruppe Ernaehrung*, ETH-Zentrum, Zurich, Switzerland. P. 276-279.

Sastradipradja, D., D.R Astuti, H. Permadi and P. Paridjo. 1997 Portal Blood Flow of Female Growing and Lactating Goats on Different Food Intakes. *Media Veteriner*, 4(2): 23-33

Sigit, N.A. 1995. Penggunaan Zeolit Beramonium dan Analog Hidroksi Metionin dalam Ransum Sapi Perah Laktasi. Disertasi Doktor. Institut Pertanian Bogor.

Suwandyastuti, S.N.O., 1986. Peningkatan Mutu Jerami Padi Ditinjau dari Neraca Mineral Esensial pada Sapi Perah. Disertasi Doktor. Institut Pertanian Bogor.

Whitelaw, F.G., J. Hyldgaard-Jensen, R.S. Seid and M.G. Kay. 1970. Volatile Fatty Acid Production in the Rumen of Cattle Given an all Concentrate Diet. *Brit. J. Nutr.*, 24:179-195.