PORTAL BLOOD FLOW OF FEMALE GROWING AND LACTATING GOATS ON DIFFERENT FOOD INTAKES[1]

LAJU ALIR DARAH VENA PORTA KAMBING BETINA TUMBUH DAN LAKTASI PADA BERBAGAI TINGKAT PEMBERIAN PAKAN

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ABSTRACT

Net production or uptake rates of the portal drained viscera (PDV) were estimated by measuring portal venous blood flow (PBF) according to the Fick principle in association with measurement of arterio-venous blood concentration differences. ^{3}H-*para*-aminohippuric acid was constantly infused into a mesenteric vein to measure PBF on female growing and lactating PE goats fed different levels of feed intakes. Cardiac output (CO) and whole body energy expenditure were measured by the ^{14}C-carbondioxide entry rate technique (CERT). PBF (n=2 for each group) of growing goats were 1032, 624, 652, 486 and 394 ml/min for 100, 90, 80, 70 and 60 % of *ad libitum* feeding which corresponded to 32.7, 17.4, 19.8, 14.8 and 13.7 % of CO. For lactating goats, PBF (n=2 each) were 500, 371 and 223 ml/min for 100, 90 and 80 % of *ad libitum* feeding, corresponding to 13.6, 13.7 and 7.6 % of CO. The calorigenic effect associated with feed intake of growing goats varied in descending order from 45 to 20 % of total body energy expenditure, while that of lactating goats was around 7 %. Absorption of volatile fatty acids was proportional to level of dietary intake.

Key words: portal blood flow, cardiac output, calorigenic effect of feed.

ABSTRAK

Besarnya laju-laju produksi atau absorbsi jeroan yang bermuara ke vena porta ditentukan dengan melakukan pengukuran laju aliran darah (LAD) *V. porta* dengan kaedah berdasarkan azas Fick serta pengukuran beda kadar nutrien/metabolitnya di darah arteri dan vena. Asam *para*-aminohipurat-^{3}H diberikan sebagai infusi kontinyu ke dalam suatu cabang *V. mesenterica* untuk mengukur laju alir darah porta pada kambing betina yang sedang tumbuh dan yang laktasi yang dikenai perlakuan perbedaan pemberian tingkat pakan. Curah jantung dan pemakaian energi tubuh diukur dengan kaedah "^{14}*C-carbondioxide entry rate technique (CERT)*". Nilai LAD *V.*

[1] The subject has been presented at the 6th Asia and Oceania Congress of Nuclear Medicine and Biology, Kyoto, Japan 1996. Abstract published *in Ann. Nucl. Med.* Vol. 10 (supplement), 1996 S144.

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porta (n=2) pada yang tumbuh adalah 1032, 624, 652, 486 and 394 ml/min untuk yang memperoleh 100, 90, 80, 70 dan 60 % dari catuan *ad libitum* yang setara dengan 32,7, 17,4, 19,8, 14,8 dan 13,7 % dari curah jantung. Untuk yang laktasi, LAD *V. porta* (n=2) adalah 500, 371 and 223 ml/min untuk 100, 90 dan 80 % dari catuan *ad libitum*, setara dengan 13,6, 13,7 dan 7,6 % dari curah jantung. Dampak kalorigenik yang menyertai masukan pakan pada kambing yang tumbuh berkisar menurun mengikuti masukan pakan dengan nilai 45 hingga 20 % dari pengeluaran energi tubuh total, tetapi pada kambing yang laktasi besarnya hanya sekitar 7 %. Absorbsi asam-asam lemak atsiri total hasil rumen sebanding dengan tingkat masukan pakan.

Kata kunci: aliran darah porta, curah jantung, dampak kalorigenik pakan.

INTRODUCTION

Variation in nutrient absorption is the main reason for rythmic variation in the concentrations of nutrients in blood plasma and other compartments of the extracellular nutrient pools. Dietary changes would cause metabolic adaptations to maintain homeostasis. A change in the plasma concentration of nutrients will immediately affect secretion rates of hormones which regulate the turnover of tissue pools to the change in nutrient availability. The large capacity of the ruminant stomach may maintain a relatively constant flow of digesta through the intestine producing only small variations in rate of nutrient absorption, and relatively stable concentrations of nutrients in plasma is then maintained.

Animal production demands adequate supply of nutrients through absorption from the gastrointestinal tract, the magnitude of which depends on the physiological state of the animal. Rates of absorption of nutrients into the blood would depend on blood flow of the splanchnic circulation. The rate of blood flow together with the difference in concentration of the nutrient in the blood across the splanchnic bed will yield knowledge on the production or utilization of nutrients by that organ. Adjustment of the splanchnic circulation is expected to occur in response to elevated work of digestion as indicated by the occurrence of the calorigenic effect of feeding. It is therefore obvious that the tissues of the splanchnic bed play a central role in the quantitative supply of nutrients of the animal. A 500 kg lactating cow on a 60:40 hay-to-grain ratio demonstrated typical portal VFA absorption rates of 31, 17 and 3 mol/day for acetate, propionate and butyrate, respectively, accounting for 16, 16 and 3.5 % of the cow's total digestible energy intake (Reynolds and Huttington, 1988). Values between 35-50 % of the cow's total digestible intake have been reported for total VFA absorption rates in lactating and nonlactating dairy and beef cattle.

The indicator dilution technique (*para*-aminohippuric acid = PAH) based on the Fick principle satisfactorily would measure PBF (Katz and Bergman, 1969a). Studies on the effects of diets on blood flow and metabolism in PDV under different physiological conditions could then be undertaken (Katz and Bergman, 1969b). This group of authors developed a method for simultaneous chronic cannulation of the major splanchnic blood vessels allowing collection of blood in unesthetized sheep (Katz *et al.*, 1969). They found on dogs that the hepatic flow was about the same in both the conscious and anesthetized state, although the portal flow seemed reduced and the hepatic arterial share of liver circulation increased. This observation has also been reported by others (Fisher *et al.*, 1956; Gilmore, 1958; Evringham *et al.*, 1959; Fisher *et al.*, 1963). No such studies have ever been made on tropical indigenous ruminants. The small PE goat would be a suitable experimental ruminant to study absorption rates of nutrients, but its relatively small size would make the animal less suitable for catheterization of the splanchnic vessels. The present investigation was carried out to measure the effects of dietary intakes on blood flow and nutrient uptake by the PDV in anesthetized growing and lactating female PE goats. PAH-^{3}H was used instead of common chemical PAH for the PBF measurements.

MATERIALS AND METHODS

Animals, Dietary Treatments, and Experimental Protocol

Two experiments with a completely randomized design were conducted that had the following protocols:

Experiment A : used 10 female growing goats (14 $\pm$ 2.0 kg BW) which were randomly assigned to five dietary treatment groups (n=2 per group): R1 daily *ad libitum* feeding 490 g DM (dry matter) + 106 g CP (crude protein), and R2, R3, R4 and R5 were respectively 90 %, 80 %, 70 % and 60 % of daily *ad libitum* feeding.

Experiment B : used 6 lactating does (25 $\pm$ 5 kg BW) which were divided into 3 dietary treatment groups (n=2 per group): R-I daily *ad libitum* feeding 925 g DM + 142 g CP, R-II and R-III were respectively 90 % and 80 % of daily *ad libitum* feeding.

All animals were kept in individual metabolic cages and were adapted to the experimental and dietary treatments for one month. After adaptation, the animals underwent a two-week balance trial involving total collection of faeces, urine and unconsumed feed. The week after termination of the balance trial, all animals - still kept in their metabolic cages - underwent CERT measurement to get data on whole body energy expenditure and cardiac output (CO). Double polyethylene catheters were implanted in both jugular veins for easy delivery of isotope and serial blood sampling from the cardiac right atrium with minimal disturbance to the animals. Portal blood flow were measured one day after the CERT trial.

CERT for Measuring Whole Body Energy Expenditure and CO

The CERT is an isotopic dilution technique involving either ^{14}C- or ^{13}C-carbondioxide. The tracer for CERT in the present study was $NaH^{14}CO_3$ (Amersham, U.K.) solution in saline administered into the body via the jugular vein at a constant infusion rate preceded by a primer dose, which underwent dilution with endogenous CO_2 produced by the tissues (Sastradipradja, 1992). The primer dose of 1 ml $NaH^{14}CO_3$ of 40 μCi was delivered in the first minute followed immediately by continuous infusion at 0.5 μCi/min. After some time, approximately during the second hour of infusion, the tracer as well as the tracee within the body will be in equilibrium and a plateau specific activity of body $^{14}CO_2$ will be reached. Blood samples were collected every 30 minutes from the beginning, until around 4 hours later, taken from the right atrium. Body CO_2 production was estimated from the plateau specific activity according to the equation (Corbett *et al.,* 1971)

rCO_2 (mass/min) = (rate of tracer bicarbonate infused, μCi/min)/(plateau specific activity of CO_2, μCi/mass).

A potential error in the calculation of energy expenditure based on CO_2 production concerns the precise estimation of the respiratory quotient (RQ). The energy equivalence per liter of CO_2 depends on the substrate mixture being oxidized. For humans, Black *et al.* (1986) proposed the use of food quotients (FQ) calculated from diet records to approximate RQ. Sastradipradja *et al.* (1991) proposed the calculation of the FQ for ruminant animals.

The CERT enables one to calculate CO minute volume also, by dividing CO_2 production rate by the arterio"atrial mixed venous" blood difference in CO_2 content:

$$\text{CO (ml/min)} = r\ CO_2\ \text{(mass/min)}/\ \Delta AV_{CO_2}\text{(mass/ml)}.$$

Portal Blood Flow Method and Surgical Preparation

PBF was measured one day after CERT to enable estimation of heat production of ($HP_{portal\ drained\ viscera}$) and VFA absorption by the PDV ($VFA_{absorption}$) at peak digestive activity 2-3h postfeeding, measured by acute surgical method on anaesthetized animals (i.v. injection of xylazine 0.05 ml/kg BW, followed by i.v. injection of ketamin 0.11 ml/kg BW). Lidocain (1 ml/animal) was applied locally on the site of incision. The surgical technique applied was basically that of Katz *et al.* (1969) with the goat placed in left lateral recumbency. Incision of the skin was made aseptically 15 to 20 cm long parallel to and behind the last rib on the right side of the goat at a position halfway between the last rib and the tuber coxae. A branch of the mesenteric vein, the right ruminal vein, was prepared and cannulated with Intramedic® polyethylene tubing I.D.0.015"-O.D.0.043" for the PAH-^{3}H infusion. To cannulate the portal vein, a Vygon intravenous catheter (France) of diam. 1.5-2.0 mm-G.14 was introduced via the cranial mesenteric vein and pushed forward until it reached the place where the vein entered the liver. Figure 1 shows a schematic diagram illustrating the positions of the sampling and infusion cannulas, while Figure 2 shows the position of the right ruminal vein.The theoretical aspects of the PAH blood flow method have been presented by Katz and Bergman (1969a). The method requires that PAH should not be excreted or chemically altered in its passage through the portal bed or liver. PAH is rapidly excreted by the kidneys and therefore, provided that the infusion rate is less than the maximal ability of the kidneys to excrete PAH, the PAH concentrations will remain constant and their actual values will depend upon the infusion rates. A chemical alteration, i.e. acetylation of part of the PAH has been shown to occur in the liver. Thus, the method requires deacetylation of sample blood filtrates prior to measuring PAH concentration. No other chemical alterations by the liver have been reported. We modified to use PAH-^{3}H (Amersham, U.K.) instead of PAH and used whole plasma for radioactivity measurement without separation of the chemical and would measure both ^{3}H attached to PAH as well as to acetylated PAH. This would then avoid the deacetylation step of the original method. Thus, a primer dose 5.75 μCi of PAH-^{3}H, was injected into a jugular vein within one minute at the beginning of the infusion period, followed by continuous infusion at a constant rate of 0.6 μCi/min through the right ruminal vein. A Siropump (Everest Electronics, Seaford, S. Australia) was used for the infusion. During the second hour of infusion, arterial and portal venous heparinized blood samples were collected for analysis of blood gasses CO_2, O_2, pH, Hb (Corning blood gas analyzer), VFA and some other analysis of blood gasses CO_2, O_2, pH, Hb (Corning blood gas analyzer), VFA and some other nutrients. VFA was analysed using gas chromatography. An Aloka LSC 753 double channel liquid scintillation spectrometer was used for ^{14}C and ^{3}H counting.

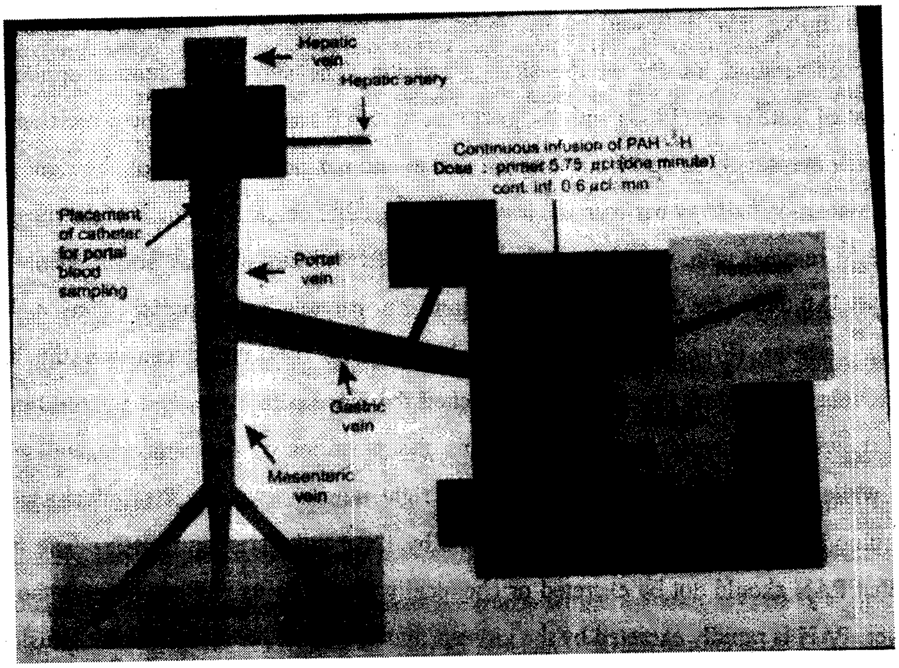


Fig. 1. Schematic Diagram Illustrating the Positions of the Sampling and Infusion Cannulas for the Measurement of Portal Venous Blood Flow by the PAH-Method

Statistical Analyses

The significance of difference between means was compared using Duncan multiple range test after ANOVA for one way classified data (Steel and Torrie, 1986).

Fig. 2. Position of the Right Ruminal Vein where the Infusion Cannula is Placed for Portal Blood Flow Measurement by the PAH-Method

RESULTS AND DISCUSSION

The results of the experiment on growing goats are presented in Table 1. Table 2 summarizes results with the lactating does. The small number of animal replications may have caused most of the differences between treatments nonsignificant.

Although not significant, the PBF in growing goats tended to decrease as DM intake decreased, the values ranged from 1032 ml/min (with *ad libitum* intake) to 394 ml/min (with restricted DM intake). For the lactating does the values significantly decreased from 500 ml/min to 223 ml/min proportional with feed intake. On a per kg body weight basis, the values ranged from 30-70 ml/min for the growing and 9-20 ml/min for the lactating animals. These values corresponded to 32.7 % of CO with *ad libitum* reduced to 13.7 % with 60 % of *ad libitum* Feeding (Fig. 3). Similar observations were obtained for the lactating does, 13.6 (R-I), 13.7 (R-II) and 7.6 % (R-III) of CO, the values being lower in the lactating as compared to the growing goats. Even when calculated per $kg^{0.75}$ (metabolic body size, $Wkg^{0.75}$) basis, the values for the lactating does were lower (17.2-38.5 ml/min, compared to 60-133 ml/min for growing goats).

Table 1. Portal Blood Flow of, and Energy Expenditure by the Portal Drained Viscera in Anesthetized Growing Female Goats on Different Dietary Intake Levels. The data are average values of 2 animals

Parameter	Level of feeding					P level of significance
	R1	R2	R3	R4	R5	
PBF (ml/min)	1032	624	652	486	394	NS
ΔAv_{porta} CO_2 (ml/dl)	4.84	5.09	3.82	4.32	4.32	NS
$HP_{portal drained viscera}$ (MJ/d)	1.64	1.0	0.79	0.68	0.53	NS
ΔAV_{cava} CO_2 (ml/dl)	5.85	4.33	4.20	3.72	4.32	NS
CO (ml/min)	3160	3590	3160	3290	2880	NS
$VFA_{absorption}$ (mM/min)	22.8^{a}	13.6^{ab}	15.4^{ab}	9.9^{ab}	8.6^{b}	P<0.05

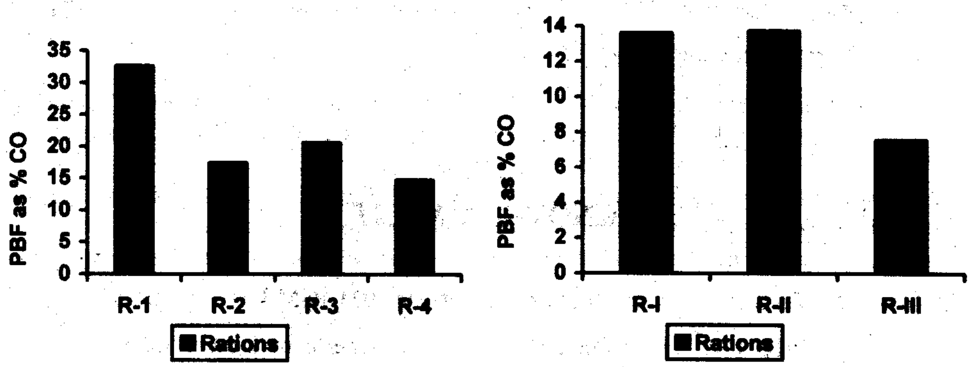
Values in a row with different superscripts differ significantly at theP level indicated.

Table 2. PBF, and O_2 and VFA Uptakes by the Portal Drained Viscera in Anesthetized Lactating Does on Different Dietary Intake Levels

Parameter	Dietary treatment groups			P level of significancy
	R-I	R-II	R-III	
PBF (ml/min)	500^{a}	371^{b}	223^{c}	P<0.05
ΔAV_{porta} O_2 (ml/dl)	3.83	4.84	4.18	NS
$O_{2\,absorption}$ (l/d)	19.19	18.09	9.44	NS
ΔAV_{cava} CO_2 (ml/dl)	4.99	5.95	5.16	NS
CO (ml/min)	3670	2700	2940	NS
$VFA_{absorption}$ (mM/min)	16.7^{a}	12.8^{ab}	7.7^{b}	P<0.05

Values in a row with different superscripts differ significantly at the P level indicated.

The results indicated that gastrointestinal function was more active in the growing goats than in the lactating does. Katz and Bergman (1969a) reported mean values of PBF of 34 ml/ kgBW/min in the fed and 31 ml/kgBW/min in fasted nonpregnant sheep. They also found that fasting significantly decreased the mean rates of blood flow in pregnant and nonpregnant sheep. Reduction in gastrointestinal fill causes lowered gastrointestinal function and PBF seems to be reduced proportionally. Our results were in agreement with this notion.



Exp. A : Growing Female Goats Exp. B : Lactating Goats

Figure 3. Portal blood flow as percentage of cardiac minute volume (PBF/CO) of growing female goats (Exp. A) and lactating does (Exp. B) as affected by level of feed intake.

A similar tendency due to lowered feed intake was also observed for energy expenditure of the PDV. Calculation showed that $HP_{portaldrained\ viscera}$ of growing goats fed *ad libitum* accounted for 45 % of whole body HP, the value reduced to 28, 24, 21 and 16 % of HP for those fed respectively 90, 80, 70 and 60 % of *ad libitum* intakes. The calorigenic effect associated with feed intake expressed as percentage values of whole body HP for lactating does were much lower, around 7 %.

VFA absorption by the PDV of both growing and lactating goats showed significant positive relationships with level of feed intake ($P<0.05$). These instant peak digestive activity values may not necessarily represent total daily absorption rates. Bergman (1990) reported values around 2.79 mol/day total VFA for temperate sheep.

CONCLUSIONS

Portal blood flows of growing female goats and lactating does are affected by level of feeding, the more remote from *ad libitum* dietary intake the lesser the flow. Absorption of volatile fatty acids which are the major energy-source nutrients of ruminants is proportional to level of dietary intake. Heat production of the portal drained viscera of growing female goats is reduced by decreasing level of dietary intake, with the highest value of 45 % of whole body energy expenditure on goats fed *ad libitum* diets. The gastrointestinal function of lactating does is less active than that of growing goats.

ACKNOWLEGMENT

This research was supported by Research Grants no. 021/P4M/DPPM/PHB I/2/1993 and 061/P4M/DPPM/94/PHB I/3/ 1994 from the Directorate General of Higher Education of the Department of Education and Culture of the Republic of Indonesia.

REFERENCES

Bergman, E.N. 1990. Energy Contribution of Volatile Fatty Acids from the Gastro-Intestinal Tract of Various Species. *Physiol. Rev.*, 70:567-590.

Black, A.E., A.M. Prentice and W.A. Coward. 1986. Use of Food Quotients to Predict Respiratory Quotients for the Doubly-Labelled Water Method of Measuring Energy Expenditure : *Clin. Nutr.*, 40C:381-391.

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

Evringham, A., E.M. Berenneman and S.M. Horvath. 1959. Influence of Sodium Pentobarbital on Splanchnic Blood Flow. *Am. J. Physiol.*, 197:624-630.

Fisher, A. 1963. *In*: The Liver, edited by C. Roullier. New York: Academic, p329-378.

Fisher, B., C. Russ, R.G. Selker and E.J. Fedor. 1956. Observations on Liver Blood Flow. A. M. A. Arch. Surg. 72:600-611.

Gilmore, J.P. 1958. Effect of Anesthesia and Hepatic Sampling Site Upon Hepatic Blood Flow. *Am. J. Physiol.*, 195:465-468.

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

Katz, M.L. and E.N. Bergman. 1969b. Hepatic and Portal Metabolism of Glucose, Free Fatty Acids, and Ketone Bodies in the Sheep. Am. *J. Physiol.*, 216:953-960.

Katz, M.L. and E.N. Bergman. 1969. A Method for Simultaneous Cannulation of the Major Splanchnic Blood Vessels of Sheep. Am. *J. Vet. Res.*, 30:655-661.

Reynolds, P.J. and G.B. Huttington. 1988. Net Portal Absorption of Volatile Fatty Acids and L-lactate by Lactating Holstein Cows. *J. Dairy Sci.*, 71:124-133.

Sastradipradja, D., N.G.F. Katipana, D.A. 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*: Energy Metabolism of Farm Animals (C. Wenk and M. Boessinger, eds) EAAP Publ. No. 58, Inst. f. Nutztierwiss. *Gruppe Ernaehrung*, ETH-Zentrum, Zürich, p.276-279.

Sastradipradja, D. 1992. Tracer Techniques for Measuring Energy Expenditure and Related Metabolic Parameters. *Proceedings* 5th AOCNMB vol. 2, PKBNI/ BATAN Jakarta p.451-457.

Steel, R.G.D. and J.H. Torrie. 1986. Principles and Procedures of Statistics. McGraw-Hill Book Co. Inc., Singapore-New York.