

**EFFECT OF DIFFERENT BLOOD FLOW METERS AND DIETS
ON VOLATILE FATTY ACIDS AND OTHER METABOLITES
INTO PORTAL BLOOD OF SHEEP**

Bugi Rustamadji¹

ABSTRACT

The aim of the study was to observe effect of diets composition and portal blood flow meters on net portal appearance of acetate and other key-nutrients. Three Merino wethers were fed either lucerne chaff : oaten hay (40:60, L/OC); lucerne chaff : rolled barley grain (60 : 40, L/BG), or lucerne chaff : rolled barley grain : fish meal (30:60:10, LBFM) and designed in a Latin Square sequence. Each sheep was fed continuously with an automatic belt feeder in order to avoid post-prandial changes in metabolites. Portal blood flows were measured by probe (blood flow meter) and PAH. Results showed the differences in intakes of crude fiber, crude protein, dry matter and difference for dry matter digestibility and also diets changed the appearance of nutrients. Besides that portal blood flow measured by the probe was more reliable compared to PAH.

(Key Words: Diets, Volatile Fatty Acids, Portal Blood of Sheep.)

Buletin Peternakan 21 (1): 29-36, 1997

¹ Faculty of Animal Science, GMU 55281

PENGARUH PENGUKUR ALIRAN DARAH PORTAL DAN MACAM PAKAN TERHADAP ASAM-ASAM LEMAK TERBANG DAN UNSUR LAINNYA DI DALAM DARAH PORTAL DOMBA

INTISARI

Tujuan dari penelitian adalah dalam rangka melakukan observasi pengaruh macam pakan dan pengukur aliran darah portal terhadap kinerja darah portal dalam hal kandungan asam asetat khususnya dan unsur-unsur lainnya. Tiga domba Merino kebirian diberi 3 macam pakan yaitu *lucerne chaff : oaten hay* (40:60, L/OC); *lucerne chaff : rolled barley grain* (60 : 40, L/BG) dan *lucerne chaff : rolled barley grain : fish meal* (30:60:10, LBFM). Penelitian dirancang dengan menggunakan disain *Latin Square*. Tiap-tiap ekor domba diberi 3 macam pakan tersebut dengan menggunakan alat pemberi pakan otomatis untuk menghindari adanya fluktuasi kandungan unsur-unsur dalam darah. Aliran darah portal diukur dengan *blood flow meter (probe)* dan PAH. Hasil menunjukkan bahwa terjadi perubahan-perubahan pada serat kasar, protein kasar, bahan kering dan pencernaan bahan kering dan juga macam pakan mempengaruhi susunan metabolit dalam darah. Demikian juga pengukuran aliran darah portal menggunakan *probe* lebih meyakinkan dibandingkan dengan metode PAH.

(Kata Kunci : Pakan, Asam Lemak Terbang, Darah Portal Domba.)

Introduction

The universal end-products of fermentation of all diets in the rumen are volatile fatty acids (VFA), carbon dioxide and methane. Some of the potentially fermentable feed will inevitably escape fermentation and will be digested in the intestines. Judson and Leng (1968) demonstrated that a proportion of some feeds invariably escape in tract to the lower tract (i.e. maize grain). Other can be manipulated relatively easily (i.e. protein meal) to avoid the fermentative process in the rumen.

The efficiency of utilization of nutrients for growth in ruminants may be dependent on the rate and pattern of absorption from the gastrointestinal tract. Study by Seal *et al.* (1989) was working using Friesian steers showed that portal absorption rates reflected the pattern of fermentation in the rumen with a lower uptake of acetate and increase in propionate

absorption on the forage concentrate diet.

In the studies reported here, effect of diet composition and portal blood flow on net portal appearance (NPA) of VFA and other key-nutrients were measured in sheep fed with different composition of diets which fed based on energy requirement for maintenance.

Materials and Methods

Sheep and ration

Three Merino wethers, 37-44 kgs liveweight, were kept in metabolism cages. The wethers were fed either *lucerne chaff : oaten chaff* (40:60, L/OC), *lucerne chaff : rolled barley grain* (60:40, L/BG) or *lucerne chaff : rolled barley grain : fish meal* (30:60:10, LBFM) over successive periods of 12 days, and designed in a Latin Square sequence. Each sheep was fed

continuously with an automatic belt feeder in order to avoid post-prandial changes in metabolites. Dry matter intake (DMI) offered for each diet were adjusted so that the wethers were fed to meet calculated requirement for metabolizable energy (ME). Water and salt lick were provided at all times.

This experiment was done in Dairy Research Unit at Sydney University at Camden, New South Wales (NSW) Australia.

Surgical and experimental procedures

The animal had been surgically prepared at least 2 weeks prior to the experiment began, such that polyvinyl chloride catheters (Rural plastics, Sydney) were inserted into the portal vein (PV) and femoral artery (A) as mentioned by Katz and Bergman (1969). A transit time blood flow meter (Transonics Inc. Cornell USA) was implanted around the portal vein. Catheters were kept patent by flushing three times a week with sterile heparinized saline (2,5 x 10⁶ IU) heparin and 9 g sodium chloride per litre of distilled water. Placement of catheter tips confirmed upon *post mortem* examination.

Measurement of portal blood flow used para-amino hippuric acid (PAH) and Probe methods, NPA and arterial plasma concentrations of metabolites were made on day 12 of each period and intakes of nutrients over days 8-12 of each period as well.

Statistical and chemical analyses

A studentized range test (TSRT) was used to evaluate the significance of differences between the mean values (Steel and Torrie, 1980)

Blood concentration of VFA was measured by modification of Pethick *et al.*

(1981). Blood free fatty acid (FFA) was measured with the method of Kelly (1965). Plasma glucose was measured by the method of Bernt and Lachenicht (1974), whereas concentration of urea in blood was measured by method of Marsh *et al.* (1965). The concentration of 3-OHB in blood was determined by the method of Zivan and Snarr (1973) and blood lactate by the method of Gutmann and Wahlefield (1974).

Results and Discussion

Results showed in spite of the marked differences in intakes of crude fiber, crude protein, dry matter and differences for dry matter digestibility, there were no differences in arterial and portal concentrations nor NPA of most metabolites (Table 1 and Table 2). Plasma urea apparently was related to intake of crude protein and it is of interest that concentrations of 3-OHB increases as crude protein intake increased. Plasma FFA was different between treatments in the portal blood and it is evident from arterial plasma concentration of FFA and glucose that the wethers were adequately fed. Portal blood flow was higher when measured by PAH method rather than Probe (Table 2).

Treatments with different crude protein and crude protein intakes in the present experiment resulted variable nutrients come into portal blood of sheep in some parameters measured between treated groups (Table 1).

For high-roughage diets, there was a close relationship between the molar proportions of VFA present in the rumen and the molar proportions in which they were produced (Sutton *et al.*, 1978). However, studies of Leng and Brett (1966) indicated that the same relationship may not apply when diets contained cereal grains.

Table 1. Dietary composition and plasma concentrations of nutrients

Parameter		L/OC	L/BG	LBFM
Intakes of				
dry matter (g/d)		838 ^a	615 ^b	511 ^b
Metabolizable energy (MJ/d)		6.8	5.9	5.7
Crude fibre (g/d)		237 ^a	145 ^b	90 ^b
Crude protein (g/d)		104 ^a	110 ^a	197 ^b
Digestible crude protein (g/d)		64 ^a	76 ^b	84 ^b
Dry matter digestibility (%)		54 ^a	64 ^{ab}	75 ^b
Organic matter digestibility (%)		56	66	77
Digestible organic matter (g/d)		469	406	393
Plasma concentration (μ M) of				
Acetate	A	1130 \pm 80	1040 \pm 90	1040 \pm 90
	PV	1730 \pm 90	1690 \pm 150	1860 \pm 200
Propionate	A	20 \pm 10	20 \pm 10	20 \pm 10
	PV	250 \pm 40	200 \pm 40	210 \pm 20
Butyrate	PV	20	30	80
3-OHB	A	350 \pm 150	400 \pm 50	590 \pm 110
	PV	420 \pm 210 ^a	660 \pm 100 ^{ab}	1010 \pm 130 ^b
FFA	A	197 \pm 22	274 \pm 25	254 \pm 49
	PV	180 \pm 42 ^a	257 \pm 34 ^b	242 \pm 8 ^{ab}
Glucose	A	4050 \pm 250	4300 \pm 280	4070 \pm 560
	PV	3410 \pm 700	4120 \pm 250	3970 \pm 400
Blood lactate	A	970 \pm 60	900 \pm 80	850 \pm 120
	PV	890 \pm 120	880 \pm 80	960 \pm 140
Urea	A	3610 \pm 420 ^a	5480 \pm 650 ^b	6170 \pm 660 ^b
	PV	3320 \pm 610 ^a	5560 \pm 610 ^b	5990 \pm 750 ^b

Value with different superscripts differ significantly (a-b, $P < .05$).

L/OC = lucerne + oaten chaff; L/BG = lucerne chaff + rolled barley grain; LBFM = lucerne chaff + rolled barley grain + fish meal.

Metabolizable energy intake, organic matter digestibility, and digestible organic matter intake are estimated values.

A = femoral artery PV = portal vein

This has been confirmed by studies with sheep (Sutton and Morant, 1978) which showed the relationship between the molar proportion of VFA produced and the molar proportion present in the rumen was much

more variable for diets containing 60 or 90% concentration than for a 100% hay diet. No consistent trend in the relationship was established.

Unfortunately, the present study did

Table 2. Comparative measurements of PAH and Probe methods on portal blood flow and net portal appearance of VFA

Parameter		L/OC	L/BG	LBFM
Portal blood flow (ml/min)				
- PAH		1770 ± 20	1450 ± 200	1690 ± 200
- Probe		1100 ± 190	1010 ± 80	870 ± 240 ^b
Net portal absorption (μmole/min)				
- Acetate	A	820 ± 260 ^a	910 ± 40 ^b	1480 ± 240 ^b
	PV	570 ± 220	660 ± 140	730 ± 180
- Propionate	A	460 ± 10	190 ± 50	350 ± 40
	PV	290 ± 50	120 ± 70	150 ± 60
Butyrate	PV	20	70 ± 40	60 ± 30

Value with different superscripts differ significantly (a-b, $P < .05$)

NPA values were adjusted by Pack Cell Volume (PCV) 30%

PAH = para-amino hippuric acid

Probe = ultrasonic blood flow meter

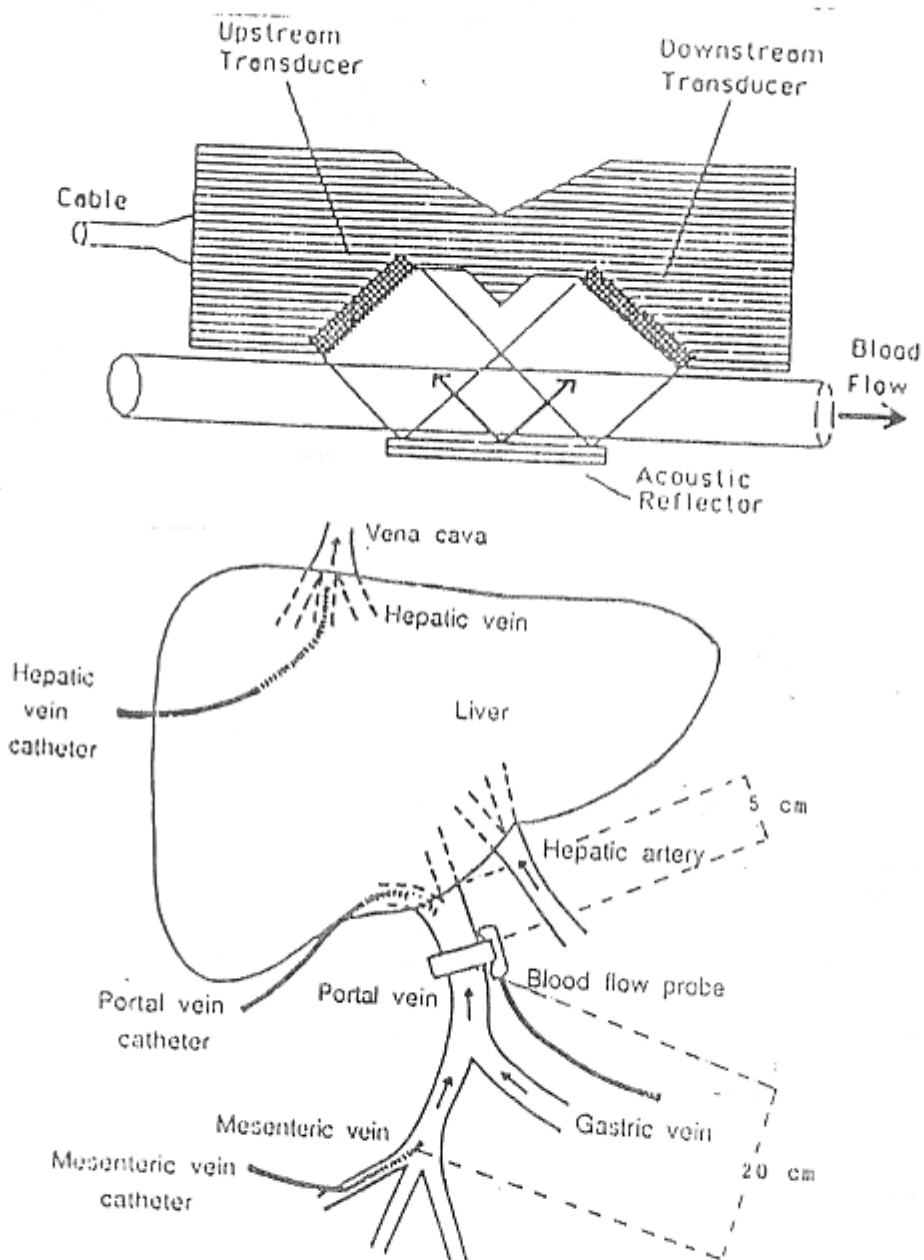
not measure the molar proportions and the rumen production of VFA from each treated diets, so it was difficult to match between production of VFA in the rumen and in portal simultaneously.

Over the 24 hours of the present study average NPA of VFA was ranged from 1.27 to 1.38 moles/d when portal blood flow (PBF) was measured by Probe and from 1.90 to 2.85 moles/d when PBF measured by PAH method. As comparison Bergman and Wolff (1971) was working with sheep fed with 800 g/d of alfalfa pellets, obtained 2.79 moles/d of VFA in the portal blood (PBF was measured by PAH).

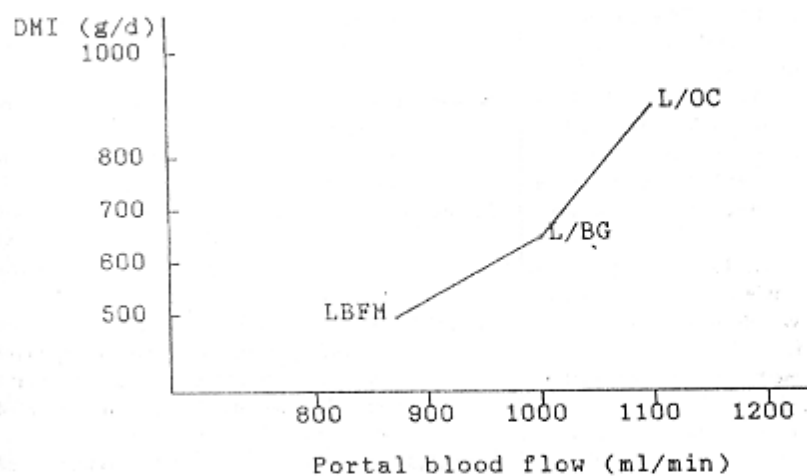
It is not possible to explain the differences of PBF measured with PAH and Probe in the present study. These differences were ranging from 1.4 to 1.9 times higher for PAH. However, the ultrasonic blood flow meter (Probe) values seem close to reality and that PAH are probably overestimates. In this

regard, Neutze *et al.* (1989) showed that there was closed correlation ($r^2 = 0.95$) between portal blood flow measured by Probe and pump in Sheep. Another study Lush *et al.* (1989) concluded no significant difference in the measurement of blood flow in the jugular vein between Probe and the dye-dilution procedure.

The illustration and surgical implantation of Probe into the blood vessel are shown in Figure 1. As informed by the manufacturer, this flow meter utilizes ultrasonic transit-time principle where by volume flow is sensed independent of vessel size. Acoustic contact is required between ultrasonic sensor and blood vessel under test. A flow Probe consist of an epoxy Probe body containing the ultrasonic sensors and stainless steel brackets. The brackets screw into place onto the Probe body, leaving a rectangular acoustic window. The Probe is designed to sense the



Gambar 1. Side section of probe and implantation of probe into portal vein



Gambar 2. Relationship between DMI and PBF of different diets

volume flow of liquid passing through this window, irrespective of where the flow occurs within the window.

Portal blood flows seem to be related to feed intakes. As seen in Table 1 and 2, since dry matter intake (DMI) or digestible organic matter intake (DOMI) increased, portal blood flow increased linearly as well, particularly when portal blood flow was measured by Probe (Figure 2).

The present results were in agreement with study of Lush and Gooden (1988) who demonstrated that increasing feed intake from 370 to 1000 g/d in sheep resulted in a corresponding increase in PBF, which is consistent with Burin *et al.* (1989) who also worked with sheep. They indicated that blood flow and O_2 consumption in both PDV and liver were related to level of nutrient. Unfortunately, the present study did not measure O_2 consumption in PDV.

Plasma urea concentration in the present study was not surprised related to crude protein intake, but plasma concentration of 3-OHB increases as crude protein

increased and the difference of plasma FFA between L/OC and L/BG was remained unclear.

Conclusion

It was concluded that dietary compositions changed the appearances of 3-OHB, FFA and urea into portal blood of sheep.

Literature Cited

- Bergman, E.N. 1975. In "Digestion and Metabolism in the Ruminant". (Eds. I.W. McDonald and A.C.I. Warner). The University of New England Publ. Unit, Armidale, Australia.
- Bergman, E.N. and Wolff, J.E. 1971. Metabolism of volatile fatty acids by liver and portal-derived viscera in sheep. *Am. J. Physiol.* 221: 586.
- Bernt, E. and Lachemicht, R. 1974. In "Methods of enzymsticanalysis". (Ed. H.V. Bergmeyer). Academic Press. New York.

- Burin, D.G., Fenell, C.L., Eiseman, J.H., Britton, R.A. and Nienaber, J.A. 1984. Effect of level of nutrition on splanchnic blood flow and oxygen consumption in sheep. *Br. J. Nutr.* 62: 23.
- Gutmann, I. and Wahlefeld, A.W. 1974. In "Methods of Enzymatic Analysis". (Ed. H.V. Bergmeyer). Academic Press. New York.
- Judson, G.J. and Leng, R.A. 1968. Effect of diet on glucose synthesis in sheep. *Proc. Aust. Soc. Anim. Prod.* 7: 354.
- Katz, M.L. and Bergman, F.N. 1969. A method for simultaneous cannulation of the major splanchnic blood vessels of the sheep. *Am. J. Vet. Res.* 30: 655.
- Kelley, F. 1965. Improved method for microtitration of fatty acids. *Anal. Chem.* 37: 1078.
- Leng, R.A. and Brett, D.B. 1966. Simultaneous measurements of the rates of production of acetic, propionic and butyric acids in the rumen of the sheep on different diets and the correlation between production rates and concentrations of these acids in the rumen. *Br. J. Nutr.* 20: 541.
- Lush, J.M. and Gooden, J.M. 1988. Effect of Feed Intake on Portal Blood Flow in Sheep. *Proc. Nutr. Soc. Austr.* 13: 107.
- Lush, J.M., Gooden, J.M. and Annison, E.F. 1989. Validation of the ultrasonics procedure for the measurement of blood flow. *Proc. Nutr. Soc. Austr.* 14: 147.
- Marsh, W.H., Fingerhut, B. and Miller, H. 1965. A Chemlab autoanalyzer for measurement of concentration of urea in blood. *Clin. Chem.* 11: 624.
- Neutze, S.A., Forbes, W.A., Oddy, O.D. and Gooden, J.M. 1989. Diurnal variation in oxygen uptake by the portal system of the sheep. *Proc. Nutr. Soc. Austr.* 14: 120.
- Pettick, D.W., Lindsay, D.B., Barker, P.J. and Northrop, A.J. 1981. Acetate supply and utilization by the tissues of sheep *in vivo*. *Br. J. Nutr.* 46: 97.
- Seal, C.J., Sarker, A. and Parker, D.S. 1989. Rumen propionate production rate and absorption of fermentation end-products into the portal vein of forage and forage-concentrate fed cattle. *Proc. Nutr. Soc. Austr.*
- Steel, R.G.D. and Torrie, J.H. 1980. Principles and Procedures of Statistics. Second ed. McGraw-Hill Book Co., New York.
- Sutton, J.D. and Morant, S.V. 1978. Measurement of the rate of volatile fatty acids production in the rumen. In : Ruminant Digestion and Feed Evaluation (Osburn, D.F., Beever, D.E. and Thomson, D.J., eds.). London: Agricultural Research Council.
- Zivar, J.A. and Snarr, J.F. 1973. An automatic calorimetric method for the measurement of 3-hydroxybutyrate concentrations. *Biochemistry.* 52: 546.