Buletin Peternakan, Vol. 20, 1996. *ISSN 0126-4400*

EFFECT OF EXOGENOUS ACETATE AND GLUCOSE LOAD ON RATE OF RECYCLING BETWEEN ACETATE AND ACETYL-CoA AND PORTAL DRAINED VISCERA OF SHEEP

Bugi Rustamadji[1]

ABSTRACT

The study was undertaken to measure the rate of acetate-acetyl-CoA recycling in portal drained viscera (PDV) for estimating its contribution to total heat production of the body and to investigate whether by increasing exogenous acetate the rate of acetate-acetyl-CoA recycling increases sufficiently to account for a significant part of heat increment of feeding (HIF). Three Merino wethers were fed a diet chosen to provide intake above maintenance with low propionate. Polyvinyl chloride catheters were surgically implanted into rumen, an iliac artery, portal and jugular veins. All catheters were kept patent by flushing 2 times a week with sterile heparinized saline (10^6 IU heparin and 9 g sodium per litre of distilled water). In this experiment sheep were administered with saline as control (T_1), 1 moles/d acetate infusion (T_2), 2 moles/d acetate infusion (T_3) and 2 moler/d acetate plus 0.3 mole glucose infusion (T_4). Sheep were measured for calorimetry measurement and [1-^{14}C] sodium acetate was infused through the jugular vein. The rate of substrate cycling between acetate and acetyl-CoA in PDV increased in response to increased rumen acetate load. The heat produced in PDV as a result of recycling between acetate and acetyl-CoA equivalent to 2-3% of the total heat production of the sheep. This heat producted in PDV was 2-4% of the extra energy supplied by the infused 1 mole and 2 moles/d of acetate diet.

(Key Words : Acetate, Acetyl-CoA, PDV, Heat Production, Sheep)

Buletin Peternakan 20: 44-51, 1996.

[1] Fakultas Peternakan UGM. Yogyakarta 55281

Buletin Peternakan, Vol. 20, 1996. *ISSN 0126-4400*

PENGARUH INFUSI ASETAT EKSOGEN DAN GLUKOSA PADA LAJU SIKLUS ANTARA ASETAT DAN ASETIL-CoA DI DALAM "PORTAL DRAINED VISCERA" DOMBA

INTISARI

Penelitian dilaksanakan untuk mengukur laju siklus Asetat-Asetil-CoA di dalam *portal drained viscera* (PDV) dalam rangka mengestimasi produksi energi untuk keperluan tubuh domba dan menyelidiki apakah dengan perubahan asetat dan glukosa laju siklus Asetat-asetil-CoA dapat meningkat cukup sebagai tambahan energi karena adanya infusi asetat dan glukosa. Tiga domba Merino dikebiri diberi pakan terpilih dengan kandungan propionat rendah di atas hidup pokok. Kateter *polyvinyl chloride* dengan pembedahan dimasukkan ke dalam rumen, arteri iliaka, vena-vena portal dan jugular. Semua kateter dirawat dengan baik menggunakan cairan 10^6 IU heparin dan 9 g NaCl setiap liter air distilasi. Pada penelitian ini domba-domba diberi 4 perlakuan yaitu infusi cairan NaCl sebagai kontrol (T1), infusi 1 mole/hari asetat (T2), infusi asetat 2 mole/hari (T3) dan infusi asetat 2 mole/hari ditambah infusi glukosa 0,3 mole/hari (T4). Domba-domba diukur kebutuhan energinya dengan kalorimeter dan domba-domba mendapat infusi [1-^{14}C] Na-asetat melalui vena jugularis. Laju siklus substrat antara asetat dan Asetil-CoA di dalam PDV meningkat karena adanya infusi asetat melalui rumen. Energi yang dihasilkan di dalam PDV sebagai akibat adanya siklus antara asetat dan asetil-CoA adalah setara dengan 2-3% dari total energi dalam tubuh domba. Panas yang diproduksi di dalam PDV adalah 2-4% dari ekstra energi karena infusi asetat sebanyak 1 dan 2 mole/hari yang dimasukkan ke dalam rumen.

(Kata Kunci : Asetat, Asetil-CoA, PDV, Energi, Domba)

Introduction

The efficiency with which energy is utilized by an animal depends upon the quantities of individual nutrients absorbed in relation to the kinetics of computing biochemical reactions. Gill *et al.* (1984) developed a computer program that simulates these biochemical reactions and Black *et al.* (1987) used that computer program to evaluate factors that may influence the efficiency of acetate utilization in ruminants. Direct comparison between productions from the computer program and experimental results can't be made because there are a few or rare published data relating the absorption of all nutrients to either metabolism of individual nutrients of energy utilization in sheep.

Therefore this experiment was undertaken (1) to measure the rate of acetate-acetyl-CoA cycling in portal drained viscera (PDV) for estimating its contribution to total heat production of the body; (2) to investigate whether by increasing exogenous acetate the rate of acetate-acetyl-CoA cycling increases sufficiently to account for a significant part of heat increment of feeding (HIF).

Materials and Methods

Sheep and ration

Three Merino wethers (approximately 12 months of ages) were brought indoors. The wethers were fed chopped phalaris hay (90%), chopped lucerne (10%) and urea (2%). The diet was chosen to provide an energy intake above maintenance but with relatively low proportions of propionate in the rumen VFA. Sheep were fed continously with an automatic belt feeder. Amounts of fed offered were monitored every day and water was provided at all times. Sheep were adapted to the experimental conditions and fed at least 2 weeks prior to the experiment began. Acetate and glucose infused were included in the energy utilization calculation as a diet component contributing energy. This experiment was done in Dairy Research Unit at Sydney University at Canden, New South Wales (NSW) Australia for 4 (four) months.

Surgical and experimental procedures

Polyvinyl chloride catheters (Dural Plastics, Sydney) were surgically implanted into rumen, an iliac artery, portal and jugular veins as mentioned by Katz and Bergman (1969). Correct final placement of these catheters were confirmed at post-partum examination. All catheters were kept patent by flushing 2 times a week with sterile heparinized saline (10^6 IU heparin and 9 g sodium chloride per litre of distilled water).

In this experiment sheep were administered with saline infusion as control (T1), 1 mole/d acetate infusion (T2), 2 moles/d acetate infusion (T3) and 2 moles/d acetate plus 0.3 mole/d glucose infusion (T4).

This experiment was designed as an incomplete Latin square with 4 treatments and 3 sheep. The sheep had 8 days between treatments to return to basal state. Each treatment period consisted of infusion of either saline (9 g of sodium chloride plus 1 litre of distilled water) or acetate solution (potassium acetate : sodium acetate = 2 : 1, Max Chemical, Sydney) into the rumen for 5 days. In treatment 4 glucose solution (0.3 mole/d, 50% w : v, Abbott Australia Pty Ltd) was also infused via jugular vein for 5 days. On the day 4 of infusion, sheep was put in a ventilated hood (modified by Nutrition and Feeds Evaluation Unit Department of Agriculture Glenfield, NSW) as calorimetry measurement. A transit time blood flow meter (Transonics Inc. Cornell University, USA) was implanted around the portal vein. On the last day of each rumen/or jugular infusion [1-^{14}C] sodium acetate (4.63×10^4 Bq/ml, Amersham International, plc.) was infused through the jugular vein at the rate of 0.4 ml/min for 4 hours and 10 paired samples of blood from the artery (A) and the portal vein (PV) were taken hourly for measurements of acetate and CO_2 radioactivities (SRA'S), O_2 saturation concentrations of haemoglobin, VFA, FFA, glucose, insulin and urea. Portal blood flow was monitored continously throughout day 5.

Statistical analysis

This experiment was designed as an incomplete Lasquare and Tukey's studentized range test (TSRT) was used to evaluate the significance of

Calculations

The calculation used to estimate production and utilization of acetate were based on Bergman (1975), while acetate entry rate and proportion of CO_2 derived from acetate was calculated by a method of Pathick *et al.* (1981).

(a) Acetate entry rate $= \frac{I}{SA_a}$

Where I is labeled acetate infused (Bq/h)

SA_a is the SRA of acetate arterial blood (Bq/m mole)

(b) CO_2 derived from acetate = $\frac{\text{arterial } CO_2 \text{ SRA at plateau}}{\text{arterial acetate SRA at plateau}} \times 100\%$

(c) Net portal appearance

(NPA) = Fp (Cp = Ca)

Where

Fp = portal blood flow (L/h)

Cp = portal acetate concentration (mM)

Ca = arterial acetate concentration (mM)

(d) Net hepatic production (NHP) :

Fp (Ch - Cp) + Fa (Ch - Ca)

Where Fa = arterial blood flow (L/h)

Ch = hepatic acetate concentration (mM)

(e) Portal utilization (Pu) =

$$\frac{Sa - Sp}{Sa} \times (Fp \,.\, Ca)$$

Where Sa = arterial concentration of labeled acetate (Bq/m mole)

Sp = portal concentration of labeled acetate(Bq/m mole)

(f) Hepatic utilization (HU) :

$$\frac{(Fa \,.\, Sa) + (Fp \,.\, Sp) - (Fh \,.\, Sh)}{(Fa \,.\, Sa) + (Fp \,.\, Sp)} \times (Fa \,.\, Ca) + (Fp \,.\, Sp)$$

Where Fh = hepatic blood flow (L/h)

Sh = hepatic concentration of labelled acetate (Bq/m mole)

Calculations of the rate of acetate-acetyl-CoA (Crabtree *et al.*, 1987) were as follows :

(g) $K = \frac{(U + O)}{3} \mu$ mole/min

Where K = tricarboxylic (Krebs) cycle

U = NPA of acetate = PBF x [PV - A]

O = O_2 use in RDF = PBF x [A - PV]

(h) $C = \frac{B \,.\, K \,.\, Ca\,(r - 1)}{K + B[Cpv - (a \,.\, r)]}$

Where C = the rate of acetate-acetyl-CoA cycling

B = portal blood flow (PBF)

Ca, Cpv = arterial and portal concentra-tion of acetate

r = SRA of arterial acetate over SRA of portal acetate

(i) $\frac{\text{SRA of acetyl-CoA}}{\text{SRA of acetate}} = \frac{(C + U)}{(C + K)}$

HP due to acetate cycling = C x 2 x 80 since 2 moles of ATP are hydrolyzed by the cycle and 1 mole of ATP produced approximately. 80 KJ of heat from acetate and 70 KJ from glucose (Crabtree *et al.* 1987).

Results

The entry rate of acetate, CO_2 derived from acetate, acetate oxidized,

blood concentrations of VFA and portal blood flow are showed in Table 1. Acetate entry rate tended to increase with acetate infusion, but the increase was not significantly different. The percentage of CO_2 derived from oxidation of acetate and the amount of acetate oxidized tended (P>.05) to increase with infusion of acetate or acetate plus glucose. There were no effect of acetae or acetate plus glucose infusion on portal blood flow. Intra ruminal acetate infusion did not significantly affect blood VFA concentration, both in artery or portal vein, although there was a tendency for higher PV concentrations.

The net portal appearance (NPA) of acetate and other VFA and concentrations of FFA, urea, glucose and insulin are shown in Table 2. There were no differences (P>.05) in NPA of propionate, concentrations of blood FFA, and plasma glucose. NPA of acetate infused from 1 mole to 2 moles/d but declined (P<.05) when glucose was infused. More butyrate appeared in portal blood of T2, T3 compared to T1 and T4 (P<.01). Blood urea concentrations declined with increasing acetate load and was even lower during acetate plus glucose infusion.

Table 1. Measurement of acetate entry rate, proportion of CO_2 from acetate, acetate oxidized, blood concentrations of VFA and portal blood flow

Parameter	Treatment			
	1	2	3	4
Acetate entry rate (μ mole/min)	2261±270	2461±230	3046±230	2575±290
Proportion CO_2 derived from acetate (%)	21.2±2.98	28.0±2.47	24.8±2.75	29.0±5.1
Acetate oxidized* (μ mole/min)	507±120	655±77	727±38	778±204
Blood concentrations of (μM)				
-Acetate A**	2330±130	2671±150	2277±100	2682±230
PV**	3919±350	3630±310	4164±560	4014±480
-Propionate A	42±10	76±10	55±10	44±10
PV	322±50	304±20	234±20	164±50
-Butyrate A	24±10	30±10	41±18	11±1
PV	55±23	94±29	81±35	23±1
Portal blood flow (ml/min)	981±80	1017±60	1087±20	1110±20

*) Acetate oxidized = acetate entry rate x CO2 derived from acetate
**) A = artery; PV = portal vein.

Table 2. Net portal appearance of VFA and concentrations of nutrients in blood

Parameter	Treatment 1	2	3	4
NPA (μ mole/min) of				
-Acetate (U)	1517±290^{ab}	979±140^{a}	2064±490^{b}	1448±280^{ac}
-Propionate	277±60	230±30	188±40	137±50
-Butyrate	36±15^{qr}	65±19^{p}	54±20^{pr}	16±2^{q}
Concentrations (μM)of				
- Blood FFA A	294±18	300±30	373±71	245±71
PV	424±25	443±47	575±188	284±79
- Blood urea A	5730±440^{a}	5110±1000^{ab}	4260±600^{ab}	1970±580^{c}
PV	5730±420^{a}	5080±1000^{ab}	3360±580^{bc}	2270±570^{c}
- Plasma glucose A	3104±150	3113±100	3140±330	3622±90
PV	3190±160	2910±100	3210±220	3620±150
- Plasma insulin A	1044±90^{a}	1010±120^{a}	1011±150^{a}	1945±610^{b}
(ng/L) PV	1388±110^{a}	1315±110^{a}	1338±150^{a}	2082±240^{b}

Value with different superscript differed significantly :
a, b, c, ($P<.05$)
p, q, r, ($P<.01$)

There were no significant differences in insulin concentration due to acetate infused alone but insulin tended to be higher ($P<.05$) during acetate plus glucose infusion. Interestingly there was a consistent appearance of insulin in portal blood in all treatments, indicating pancreatic secretion of insulin directly into portal blood.

The transactions of acetate metabolism in PDV likely contribution to heat of both the whole body (WB) and portal drained viscera (PDV) are shown in Table 3. No statistically significant differences were found in parameters measured except in the proportional contribution of NPA of acetate entry rate.

Discussion

There are variations in measurement of acetate concentration in A and PV, portal blood flow, SRA of acetate and CO2 contributed to wide variation in estimates of parameters of acetate kinetics, both in the whole body and particularly in the PDV. The range of errors (expressed as CV %) arising at each step outcome are shown in Table 4. Because of the variations, it has been difficult to demonstrate statistically significant differences between treatments, nonetheles it is useful to compare some results as means.

There was no apparent increase in (PV - A) recovery of acetate in NPA when acetate was infused into rumen at 1 mole/d, and at 2 moles/d plus 0.3 mole/d glucose infused. This variation illustrates the subs- tantial errors in measuring NPA, the major component of which is variation in (PV - A) acetate concentration (Table 4). However, NPA of acetate accounted for 41% to 66% of

the entry rate in the whole body is consistent with the other results suggesting portal drained viscera (PDV) was the major site of acetate release in sheep (Annison and White, 1962; Bergman and Wolff, 1971; Pethick *et al.*, 1981). Blood urea concentration was substantially decreased in sheep receiving acetate infusion and decreased further when glucose was also infused.

Table 3. Krebs cycle, acetate cycling, the heat produced by portal drained viscera and oxygen in PDV and in whole body

Parameter	Treatment 1	2	3	4
Heat production (HP)	4.3±0.22^{ac}	5.5±0.64^{b}	4.8±0.28^{ab}	3.9±0.47^{c}
Krebs cycle (µmole/min)	938±160	770±50	1066±140	866±90
Acetate cycling (µmole/min)	434±80	451±50	588±130	528±70
$\frac{\text{NPA acetate}}{\text{Acetate entry rate}}$ (%)	65±9^{p}	41±4^{r}	66±8^{p}	56±5^{q}
$\frac{\text{Acetate SRA (A)}}{\text{Acetate SRA (PV)}}$	1.45±0.05	1.39±0.02	1.51±0.04	1.34±0.03
$\frac{\text{Acetate CoA SRA}}{\text{Acetate SRA}}$	1.39±0.04	1.19±0.07	1.60±0.17	1.41±0.10
$\frac{CO_2 \text{ SRA (A)}}{CO_2 \text{ SRA (PV)}}$	0.98±0.08	1.05±0.03	1.11±0.10	0.95±0.04
O_2 in PDV (µmole/min)	1196±190	1345±100	1148±50	1125±60
O2 in whole body/WB (µmole/min)	6666±490	7847±1480	7360±550	5903±660
$\frac{O_2 \text{ use in PDV}}{O_2 \text{ use in WB}}$	20±4	19±6	16±2	20±2
HP due to PDV acetate cycling (KJ/d)	99.7±18.68	115.7±11.64	131.3±31.90	112.3±17.09
Contribution PDV acetate cycling to total HP (%)	2.4±0.40	2.1±0.12	2.9±0.86	2.8±0.26
Contribution of acetyl-CoA to HP in PDV (%)	12.3±2.34	12.7±2.85	18.0±3.22	15.7±2.03

Value with different superscripts differed significantly : a, b, c, (P<.05), p, q, r, (P<.01)

Table 4. Some errors in parameter measured based on coefficient of variation

Parameter	Coefficient of Variation (%)	
	Average	Range
Heat production measurement	15	9 - 20
Portal blood flow measurement	8	4 - 14
Arterial acetate concentration	13	8 - 24
Portal acetate concentration	19	14 - 24
(PV - A) acetate concentration	33	25 - 43
Pump flow measurement	4	2 - 7
Acetate infusion rate	7	4 - 9
Acetate SRA concentration in artery	24	10 - 41
Acetate SRA counts in artery	16	7 - 24

The rate of substrate cycling between acetate and acetyl-CoA in PDV increased from 434 to 580 µmole/min in response to increased rumen acetate load. The heat produced in PDV as a result of cycling between acetate and acetyl-CoA was approximately 100 KJ/d in the control group and increased to 131 KJ/d during acetate infusion, or this is equivalent to 2-3% of the total heat production of the sheep. However, this heat produced in PDV would represent an extra heat of approximately 31 KJ/d in which equivalent to 2-4% of the extra energy supplied by the infused 1 and 2 moles/d of acetate diet. This suggests the acetyl-CoA acetate cycle in PDV could contribute to heat production in PDV, although the effect is small when related to HP in the whole body. These results are to some extent similar to those of Crabtree *et al.* (1987) who indicated that the acetate-acetyl-CoA cycle in muscle contributed only approximately 0.5% of the total heat produced by the animal and would probably make only a relatively small contribution to any heat increment of feeding associ- ated with diets producing large amounts of acetate. Unfortunately, these researchers did not measure total heat production in the body, so it was uncertain from their work that the failure to see an increase in HP due to acetate-acetyl-CoA cycling was due the small change in acetate cycling or lack of increment. Howe- ver, given the results obtained here. It is now clear that they may not have seen an increases in HP with diets plus acetate infused used.

The idea that inefficiency of acetate use was derivat from a futile cycle was introduced in the model of Gill *et al.* (1984) and Black *et al.* (1987). They did not, however, allow interrelationship between acetate and glucose. It has been known for some time free fatty acid (FFA) have direct effect on glucose metabolism. In ruminants, Pethick and Vernau (1984) have shown that acetate infusion resulted an increase of acetate uptake by muscle accompanied by

maintenance of glucose uptake, but increased output of lactate. The energetics of conversion of glucose to lactate and subsequent cycling rather than oxidation of glucose to CO_2 indicated that a considerable energy saving may result from this interaction. Gill *et al.* (1984) did not consider such an interaction. To account for their results they invented an ATP degradation pathway. It now seems clear that such a separate pathway does not exist, but it is simply a consequence of the simplification used in generating the model.

Conclusion

Acetate and glucose infusions showed increases in acetyl-CoA acetate cycle, heat production (HP) due to acetyl-CoA acetate cycle in PDV, contributions PDV acetyl-CoA acetate cycle to total HP and acetyl-CoA to HP in PDV.

Literature Cited

Annison, E.F. and R.R. White. 1962. Further studies on the entry rate of acetate and glucose in sheep, with special reference to endogenous production of acetate. Biochem. J. 84 : 546.

Bergman, E.N. and J.E. Wolf. 1971. Metabolism of volatile fatty acids by liver and portal drained viscera in sheep. Am. J. Physiology. 221 : 586-592.

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.

Black, J.L., M. Gill, D.E. Beever, J.H.M. Thornley and J.D. Oldham. 1987. Simulation of the metabolism of absorbed energy yielding nutrients in young sheep : efficiency of utilization of acetate. J. Nutrition, 117 : 105-115.

Crabtree, B., S.A. Maur, S.E. Anderson and J.C. MacRae. 1987. Measurement of the rate of substrate cycling between acetate and acetyl-CoA in sheep muscle in vivo. Biochem. J. 243 : 821-827.

Gill, M., J.H.M. Thornley, J.L. Block, J.D. Oldham and D.E. Beever. 1984. Simulation of the metabolism of absorbed energy yielding nutrients in young sheep. Br. J. Nutr. 54 : 621-649.

John, J.H. and M.H. Quenoville. 1977. Experiments : Design and Analysis. Charles Griffin and Co. Ltd. London and High Wycombe.

Katz, M.L. and E.N. Bergman. 1969. A Method for simultaneous cannulation of the major splanchnic blood vessels of the sheep. Am. J. Vet. 30 : 655-661.

Pethick, D.W., D.B. Lindsay, P.J. Barker and A.J. Northrop. 1981. Acetate supply and utilization by the tissues of sheep in vivo. Br. J. Nutr. 46 : 97- 110.

Pethick, D.W. and B. Vernau. 1984. The effect of acetate on utilization of glucose by skeletal muscle of the sheep. Can. J. Anim. Sci. 64 (Suppl.) : 291-292.