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EFFECT OF EXOGENOUS ACETATE LOAD ON HEPATIC ACETATE METABOLISM IN LACTATING AND NON-LACTATING SHEEP

Bugi Rustamadji[1]

ABSTRACT

The experiments were undertaken to quantify the production and utilization of hepatic acetate by increasing exogenous acetate load in non-and lactating sheep. Two lactating and 3 non-lactating sheep were used in the experiments. In each experiment [1-^{14}C] sodium acetate was administered to the mesenteric vein and unlabelled acetate was also infused via another mesenteric vein. Results indicated in lactating sheep with large infusion of acetate might make significant utilization of acetate in liver and in non-lactating sheep, portal utilization (PU) and net hepatic production (NHP) of acetate were affected by increasing load into liver.

(Key Words: Acetate, Liver, Sheep.)

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PENGARUH INFUSI ASETAT TERHADAP METABOLISME ASETAT DI DALAM HATI DOMBA YANG SEDANG DAN TIDAK LAKTASI

INTISARI

Tujuan penelitian ini adalah untuk mengetahui besarnya produksi dan penggunaan asetat dalam *liver* domba yang sedang dan tidak laktasi dengan menambahkan asetat masuk dalam tubuh domba. Dua domba yang sedang laktasi dan 3 domba yang tidak laktasi digunakan dalam penelitian. Pada masing-masing penelitian "[1-^{14}C] sodium acetate" diinfusikan melalui vena mesentrik dan *unlabelled acetate* juga dimasukkan melalui vena mesenterik yang lain. Hasil-hasil penelitian menunjukkan bahwa pada domba-domba yang sedang laktasi infusi asetat yang banyak ini kemungkinan dapat memperbesar penggunaan asetat di dalam *liver*, sedangkan pada domba-domba yang tidak laktasi *portal utilization* dan *net hepatic production* dari asetat sangat dipengaruhi adanya infusi asetat yang dimasukkan ke dalam *liver*.

(Kata Kunci: Asetat, Hati, Domba.)

[1] Faculty of Animal Science, GMU Yogyakarta 55281

Introduction

There were various reports of entry rate of acetate in lactating and non-lactating sheep (King *et al.*, 1985 and Hough *et al.*, 1986). Pethick and Lindsay (1982) have established relationships between rate into blood and feed intake (fed or fasted) and arterial concentration of blood acetate.

Acetate which accounts for 60-70% molar percentage of total Volatile Fatty Acids (VFA) production entered the circulation largely unchanged and becomes mixed with acetate from endogenous sources. Most of these were used for energy purposes and for lipogenesis. Endogenous acetate was produced by many tissues including the liver, skeletal muscle and the mammary gland (Giesecke *et al.*, 1985).

Therefore, the experiments were undertaken to quantify the production and utilization of liver acetate by increasing exogenous acetate load in lactating and non-lactating sheep.

Materials and Methods

Sheep and rations

Experiment 1. Two lactating crossbred sheep (Border Leicester >< Merino) ewes, approximately 40 kg of liveweight were used in the experiment. They were kept indoors in metabolism cages and a good quality diet 40% lucerne chaff and 60% rolled barley grain (10.6 MJ metabolizable energy and 158 g crude protein per kg dry matter) was fed continously using a belt feeder, to avoid post-prandial changes in metabolites and hormones. Amounts of feed offered were sufficient to satisfy requirements for metabolizable energy for maintenance plus milk production (MAFF, 1975). Water and salt licks were provided *ad libitum*.

Experiment 2. Three non-lactating crossbred sheep (Border Leicester >< Merino) ewes, with live weight around 50 kgs were put in the metabolism cages indoors. The sheep were fed with 1000 g/d of lucerne chaff (approximately 1.25 times of maintenance requirement). Water was given *ad libitum*. Feed were given continously with an automatic belt feeder as for in experiment 1 above. Those experiments were done in Dairy Research Unit at Sydney University, Camden, New South Wales (NSW) Australia.

Surgical and experimental procedures

Surgical preparation for experiment 1 and 2 were conducted to facilitate blood sampling and infusions. Catheters were surgically inserted into a femoral artery (A), hepatic vein (HV), portal vein (PV) and mesenteric vein (MV). Catheters were kept patent by flushing with a solution of heparin (2500 IU/ml of saline) once a week. During experiment, they were flushed with sterile heparinized saline (10^5 IU heparin and 9 g sodium chloride per litre distilled water).

Experiment 1 consisted of a control period, followed by [1-^{14}C] sodium acetate (3.7 x 10^4 Bq/ml, Amersham International, plc) was infused through the mesenteric vein at the rate of 1 ml/min for 150 minutes, while unlabelled acetate (acetic acid glacial, Ajax, Chemical, Australia) at the rate of 1 mmole/min was infused via another mesenteric vein during the last 75 min of the whole experiment. Three sets of blood samples from A, HV and PV were taken at 15 minutes intervals before and during

infusion of unlabelled acetate. Blood was sampled simultaneously using heparinized syringes and immediately placed into tubes chilled on ice.

The experimental procedures of experiment 2 were as experiment 1, but duration of labelled acetate infusion was 180 minutes, whereas unlabelled acetate infusion was 90 minutes. At the same time, para-amino hippuric acid (PAH, Merck, West Germany) was infused for 180 minutes via mesenteric vein as well for simultaneous measurements of portal and hepatic blood flows. Unlabelled acetate was prepared by mixing acetic acid glacial (Ajax, Chemicals, Australia) with sodium hydroxide (40%) and saline, then it was adjusted to pH 7. All those were done under sterilized conditions. All animals remained healthy and consumed to eat well throughout the experiment. Five sets of blood samples of A, HV and PV were taken at 15 minutes intervals before and during infusion of unlabelled acetate.

Calculation and statistical analysis

The calculation and to estimate production and utilization of acetate were based on Bergman (1975), while acetate specific radioactivity was calculated by a method of Pethick *et al.* (1981).

The paired t-test (Steel and Torie, 1980) was used to evaluate the significances of differences between the mean values.

Results and Discussion

Experiment 1. Blood acetate concentration of A, HV and PV were increased by 56, 28 and 53% between control and acetate treated groups, respectively (Table 1). NPA of acetate was 4.1 mmoles/min (control) and 4.5 mmoles/min (treatment), whereas PV of acetate was 1.5 mmoles/min versus 2.4 mmoles/min, respectively for control and treated groups. NHP and HV of acetate were increased by 20% in NHP and decreased by 18% in HV. Blood flows were assumed based on previous experiment. Acetate flow of the experiment can be seen in Figure 1.

The results of the present study indicated that large amounts of acetate were absorbed into the portal blood of ruminants. The time course of spesific radioactivity (SRA) in portal and hepatic veins is shown in Figure 3. The course showed that equilibrium was relatively reached in PV and HV after 2 hours of [1-^{14}C] acetate infusion eventhough from the graph was clear, but SRA was ranging approximately from 14 to 19 μCi/g C both in PV or HV.

Blood acetate concentrations of A, HV, PV, NPA of acetate, PU acetate, NHP of acetate, HU of acetate and blood flows of control group have higher values compared to another experiment. These may probably due to individual variation and number of animals were used in the experiment, but it should be emphasized that large experimental errors are possible in calculation of the experiment. Seven sets of blood samples were analyzed in the experiment, but the errors would still be greater for portal-arterial differences of acetate concentration and also due to high measurement of blood flows.

However, results in lactating ewes showed that acetate was more utilized in portal drained viscera (PDV) and liver during acetate infusion (Figure 1), indicated exogenous acetate or additional energy was more needed in lactating ewes. Furthermore, acetate depressed utilization of acetate in the liver during

Table 1. Acetate production and utilization across Portal-Drained Viscera (PDV) and Liver, Blood concentration, Blood flows in lactating sheep treated with acetate infusion via mesenteric vein (Experiment 1)

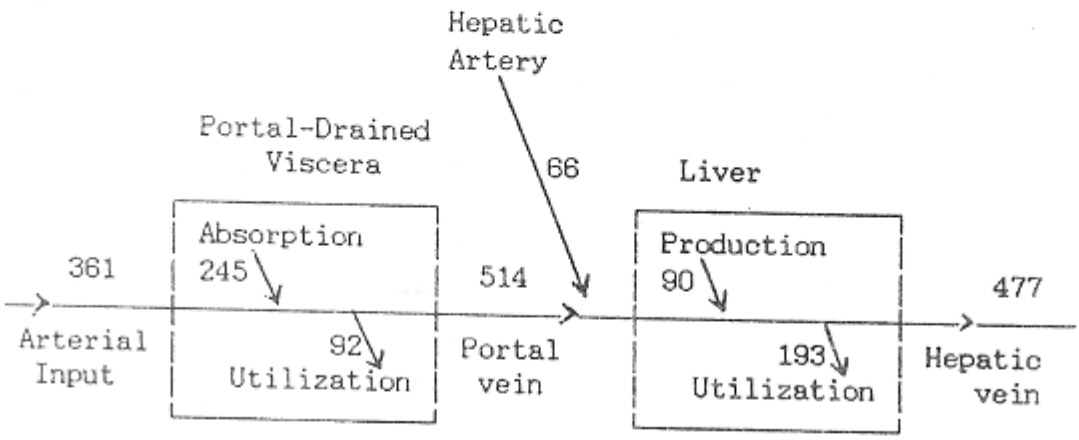
Parameter		control	Acetate infusion	Change (%)	P
Blood acetate concentration (μM)	A	1600 ± 360	2500 ± 430	+56	NS
	PV	2800 ± 300	4300 ± 400	+54	NS
	HV	2100 ± 230	2700 ± 340	+54	NS
Blood flow (PAH*) (ml/min)					
	A	800 ± 180	800 ± 180	0	NS
	PV	3100 ± 610	3100 ± 610	0	NS
	HV	3900 ± 720	3900 ± 720	0	NS
Net portal appearance (NPA) (μmole/min)		4100 ± 990	4500 ± 860	+10	NS
Portal utilization (PU) (μmole/min)		1500 ± 280	2400 ± 480	+60	NS
Net hepatic production (NPA) (μmole/min)		1500 ± 290	1800 ± 330	+20	NS
Hepatic utilization (HU) (μmole/min)		3200 ± 800	2600 ± 650	+19	NS

* Based on previous experiment
Experiment 1, n = 2
A = artery; PV = portal vein; HV = hepatic vein
P= significant level (P>.05)
NS = Not significant

acetate infusion suggested that acetate activation by ruminant liver was very low, while uptakes of acetate by adipose, muscle and mamary tissue were relatively high (Pethick and Lindsay, 1982; King *et al.*, 1985).

Experiment 2. Blood acetate concentrations of A, HV and PV were 1.3 μM, 2.0 μM and 2.0 μM in the control group compared to 2.0 μM, 3.0 μM and 3.0 μM in the treatment group respectively. NPA of acetate was increased by 38%, whereas PU of acetate was decreased by 20%. NHP and HU of acetate were 0.5 and 0.7 mmole/min in the control group compared to 0.4 and 0.8 mmole/min in the treatment group, respectively. Blood flows of A, HV and PV are 0.7; 2.7 and 2.0 L/min (control) compared to 0.8; 3.1 and 2.3 L/min (treatment). In all parameters measured were found no statistically differences (P>.05) between control and acetate treated groups. Figure 4 shows the flow of acetate in non-

Control :



Acetate Infusion (60 mmole/h) :

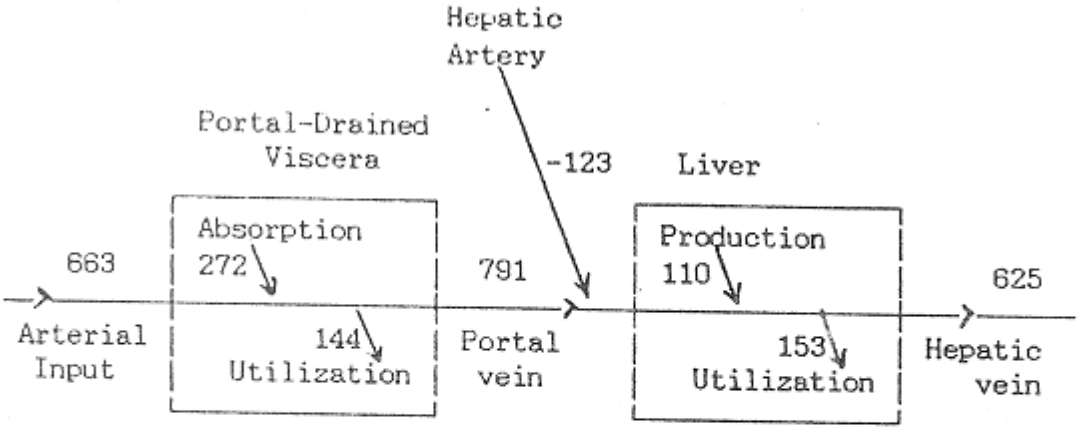


Figure 1. Diagram of flow of acetate (mmole/h) in portal drained viscera and liver of lactating sheep treated with acetate (experiment 1)

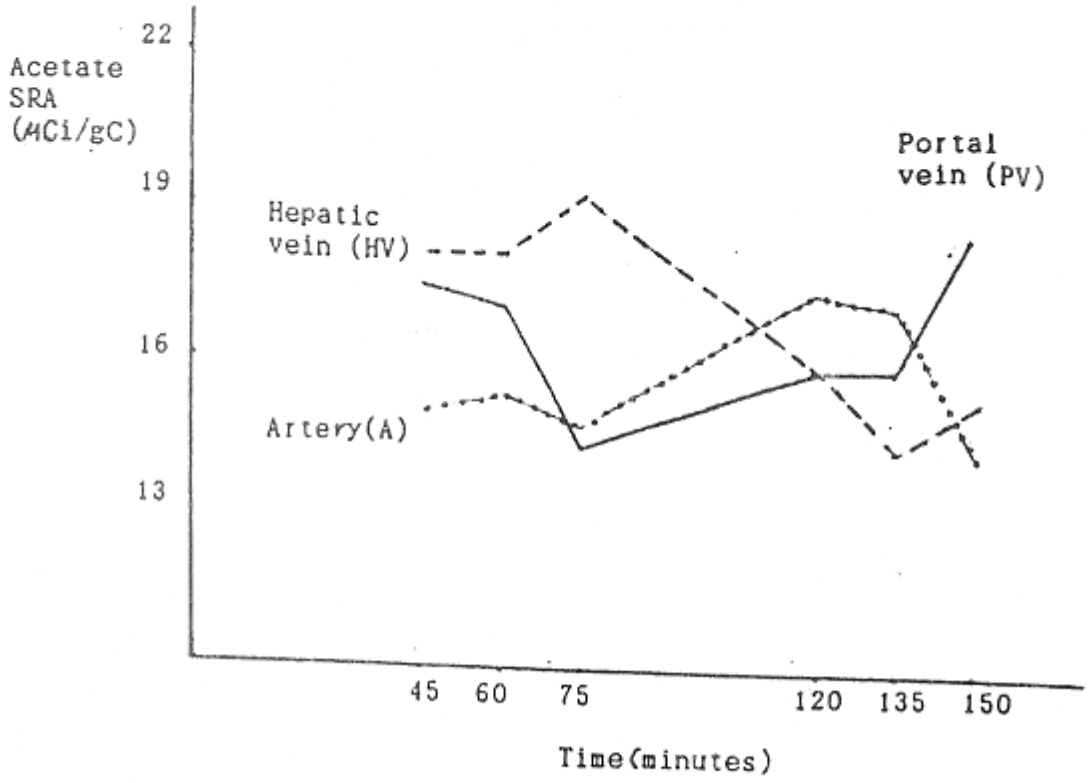


Figure 2. Acetate SRA (μCi/gC) in A (.....), HV (----), and PV (————) of lactating sheep (Experiment 1)

lactating sheep.

In experiment 2, infusion of acetate at 60 mmoles/h for 1.5 hours via mesenteric vein and infusion of [1-^{14}C] acetate (3.7 x 10^4 Bq/ml) at the rate 1 ml/min for 3 hours through the mesenteric vein as well, leave increased blood acetate concentrations of A, PV, HV, NPA of acetate. HU of acetate and blood flows of A, PV and HV. On the other hand infused of acetate has decreased PU and NHP of acetate (Table 2).

The time course of SRA in PV and HV reached equilibrium clearly in non-lactating ewes after 2 hours of [1-^{14}C] acetate infusion (Figure 4).

Acetate utilization in liver increased by 14% was detected in the experiment (Figure 4), suggesting acetate activation by ruminant liver is relatively high. The results are also indicating an average of 78 mmoles/h of acetate coming

Table 2. Acetate production and utilization across Portal-Drained Viscera (PDV) and Liver; Blood acetate concentrations and blood flows in Non-lactating sheep treated with acetate infusion via mesenteric vein (Experiment 2)

Parameter		Control	Acetate	Change	P
Blood acetate concentration (μM)	A	1300 ± 340	2000 ± 500	+54	NS
	PV	2000 ± 500	3000 ± 530	+50	NS
	HV	2000 ± 610	3000 ± 630	+50	NS
Blood flow (PAH*) (ml/min)					
	A	700 ± 200	800 ± 180	+14	NS
	PV	2000 ± 170	2300 ± 220	+15	NS
	HV	2700 ± 330	3100 ± 340	+15	NS
Net portal appearance (NPA) (μmole/min)		1300 ± 220	1800 ± 170	+39	NS
Portal utilization (PU) (μmole/min)		500 ± 110	400 ± 100	-20	NS
Net hepatic production (NPA) (μmole/min)		500 ± 170	400 ± 180	-20	NS
Hepatic utilization (HU) (μ mole/min)		700 ± 110	800 ± 210	+14	NS

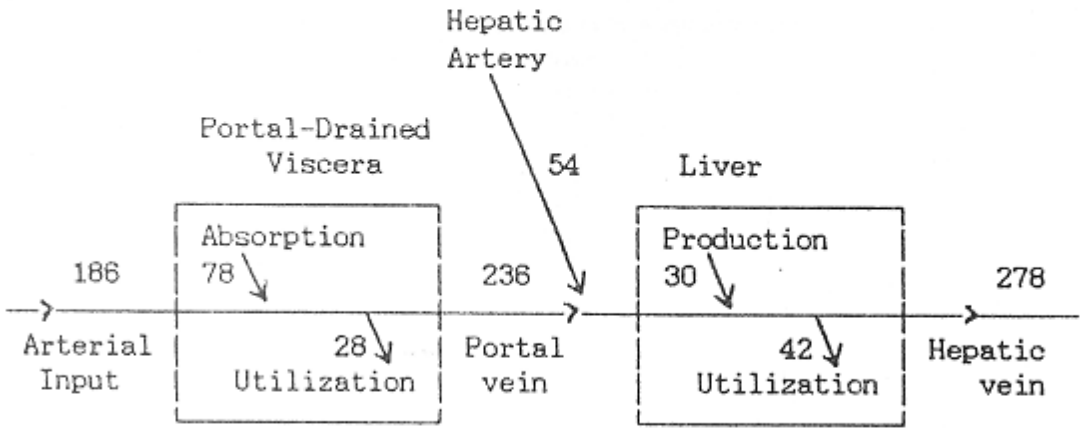
* Based on previous experiment
Experiment 2, n = 3
A = artery; PV = portal vein; HV = hepatic vein
P = significant level (P > .05)
NS = not significant

into the blood of fed sheep, if could be extrapolated to daily rates, since the animals were constantly fed throughout the entire 24 hours period. This gives values of 1872 moles/d and 2592 moles/d of acetate, respectively for control and treated groups. Unlike in lactating sheep, in non-lactating sheep exogenous acetate load via the mesenteric vein decreased PU and NHP of acetate. This may be due to large quantities of acetate apparently in the liver enter non-oxidative pathways (Pethick *et al.*, 1981).

Conclusion

In lactating ewes, that only with large infusion of acetate seem to be any significant hepatic utilization of acetate, while in non-lactating sheep portal utilization and net hepatic production of acetate were affected by increasing acetate load into liver.

Control :



Acetate Infusion (60 mmole/h) :

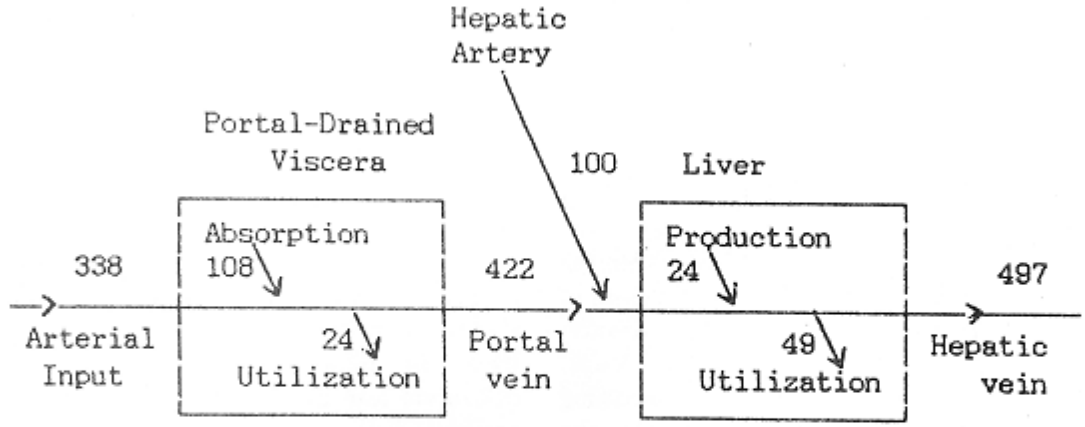


Figure 3. Diagram of flow of acetate (mmole/h) in portal drained viscera and liver of non-lactating sheep treated with acetate (experiment 2)

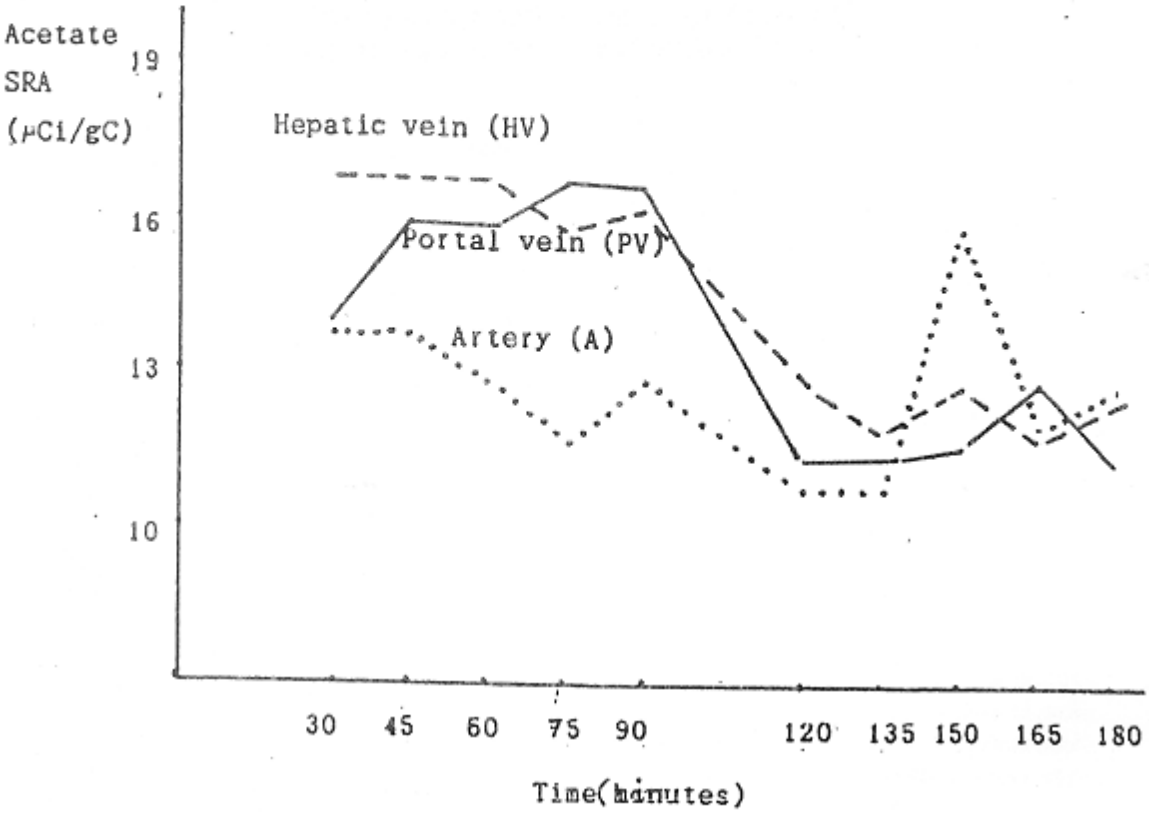


Figure 4. Acetate SRA (μCi/gC) in A (.....), HV (----), and PV (————) of non-lactating sheep (Experiment 2).

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