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Technical Note: Measuring Portal Blood Flow in Sheep Using an Ultrasonic Transit Time Flow Probe¹

D. Rémond, I. Ortigues-Marty, A. Isserty, and J. Lefaivre

Département Elevage et Nutrition des Animaux, INRA de Clermont-Ferrand Theix,
63 122 St Genès-Champagnelle, France

ABSTRACT: Our objective was to validate the use of an ultrasonic transit-time flowmeter for the measurement of portal blood flow (PBF) in sheep. Data recorded with this technique were compared with those obtained with an indicator dilution method, and probes were calibrated *in vivo*. Wethers were fitted with catheters in the portal, jejunal, and ruminal veins and in a mesenteric artery. Ultrasonic flow probes were implanted around the portal vein: S-series probes in three wethers, and A-series probes in

four wethers. The PBF measured with A-series probes was within 10% of that measured by indicator dilution, but PBF measured with S-series probes were 52 to 77% of that determined by indicator dilution. *In vivo* calibration indicated that A-series probes provided accurate measurement of PBF (absolute accuracy: 5% ± zero flow error). In conclusion, an ultrasonic transit-time flowmeter, with an A-series probe, can be used to reliably measure PBF in sheep.

Key Words: Sheep, Blood Flow, Portal Vein

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Introduction

Portal blood flow measurement is a key parameter for the determination of nutrient net fluxes across the splanchnic bed. Since the work conducted by Katz and Bergman (1969), most data reported in the literature have been obtained using an indicator dilution technique, and direct measurements with electronic devices are scarce. In ruminants, conflicting observations have been reported concerning portal blood flow (PBF) measurements with ultrasonic transit-time flowmeters. Huntington et al. (1990) observed in cattle an underestimation of PBF with the ultrasonic technique. However, Neutze et al. (1989) reported that the ultrasonic flow probes provided accurate estimate of PBF in sheep. Recently, developments in ultrasonic technology have led to the availability of a new type of flow probe (A-series), which feature worthwhile characteristics for PBF measurement. Our objective was to evaluate the effectiveness of ultrasonic flow probes (standard S-series and new A-series) for measuring PBF in sheep.

Materials and Methods

Animals and Diet

Texel wethers (52.3 ± 5.4 kg BW) were surgically prepared with indwelling catheters in portal, jejunal, and right ruminal veins and in a mesenteric artery. A blood flow probe was fitted around the portal vein immediately caudal to the liver. The catheter materials and the procedures for surgery and implantation of catheters have been described in detail (Rémond et al., 1993; Ortigues et al., 1994).

Two types of ultrasonic transit-time flow probes were used: 14-mm S-series (three animals) and 14-mm A-series probes (four animals). The A-series probes are less bulky (vessel length required for flow measurement is 9 mm instead of 26.2 mm for the S-series), they are easier to position on the portal vein (20 to 30 mm length in sheep), and they place less constraint on this vessel. Furthermore, the technique used by this type of probe is more adapted to measure turbulent flow, as could be the case in the portal vein because of the junctions with open angles of the mesenteric vein, the gastrosplenic vein, and the right gastroduodenal vein, just upstream from the probe. For probe implantation, the portal vein was isolated over a length of 2 cm by blunt dissection. The S-series probes were wrapped in silicone strips to ensure a good probe/vessel alignment and to prevent an excessive curve of the portal vein across the reflector plate;

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this was done to provide good operating conditions for the probes.

The sheep were housed in individual cages and were fed at maintenance with orchardgrass hay (970 g of DM/d, 18.3 g of N/kg DM, 7.5 MJ of ME/kg DM). The daily ration was provided in eight equal meals distributed every 3 h, and the animals had continuous access to water and salt block. Sheep had a period of recovery of at least 2 wk before measurements. The experiment was conducted in a manner compatible with national legislation on the care and use of laboratory animals (statutory order no. 87-848, October 19, 1987, *Journal Officiel*, France).

Experimental Procedures

Infusion of para-aminohippuric acid (PAH) was achieved via mesenteric and ruminal routes as described by Ortigues et al. (1994). Portal and arterial blood samples were simultaneously collected into 4-mL syringes, containing EDTA-K as anticoagulant, every 20 min over two feeding cycles (2×3 h), providing 20 sets of samples. Samples were frozen immediately after collection for chemical analysis later. Portal blood flow was measured continuously with an ultrasonic transit-time flowmeter (Transonic Systems, Ithaca, NY) interfaced with a computer for data acquisition. In each sheep fitted with an A-series probe, measurements were carried out on 2 d separated by an interval of at least 1 d.

After in vivo comparison of PBF obtained with the probes and the PAH, A-series probes were calibrated in vivo according to the time-syringe method (Gorewit et al., 1989; Rémond et al., 1993). Animals were anesthetized. A catheter was inserted in the carotid artery for blood collection. After a small left paracostal incision, a catheter (i.d. 4 mm, o.d. 5.6 mm) was introduced into the splenic vein and pushed into the vessel toward the portal vein. Another catheter (i.d. 4 mm, o.d. 5.6 mm) was inserted into the cranial mesenteric vein after a right paracostal incision. Both catheters were secured so that their tips were located approximately 3 to 4 cm upstream from the probe. Due to the difficulty in accessing the vessel, it was not possible to occlude other tributaries of the portal vein. Both catheters were linked together and connected to a large cylinder equipped with a piston. Animals were then injected i.v. with 10,000 IU of heparin. Calibration was achieved by collecting blood from the carotid artery into the cylinder and then infusing it simultaneously into the splenic and cranial mesenteric veins pushing down the piston. The cylinder was equipped with two sensors to measure the transit time of the piston between the positions of the two sensors. Blood volume in the cylinder between the two sensors was accurately determined before the calibration procedure. Acquisition of data from sensors and flowmeter were done on the same computer. For each

probe, a calibration curve was obtained from 10 comparisons between true flow and measured flow (ranging from 0 to 5 L/min). The whole calibration procedure lasted about 1 h; the animals were then slaughtered.

Analysis

Blood concentrations of PAH and hemoglobin (cyanmethemoglobin method) were simultaneously determined with an autoanalyzer (Isserty et al., 1998).

Calculations and Statistics

Portal blood flow was calculated from PAH dilution as proposed by Katz and Bergman (1969) using hemoglobin concentration for correcting the water transfer in the portal drained viscera. Portal blood flow recorded by the flowmeter was averaged on the sampling time, which ranged from 40 s to 2 min (mean: 70 ± 25 s). The paired *t*-test (SAS, 1988) was used to compare the different techniques of blood flow measurement.

Results and Discussion

The ruminal vein catheter of sheep G and the mesenteric vein catheter of sheep C lost patency during the experimentation. For these two sheep, PAH infusions were achieved by only one infusion site. Necropsies showed correct placement of portal catheters; their tips were located at least 3 to 4 cm downstream from the porta hepatis. All probes were correctly placed on the portal vein. They were covered by fibrotic tissue, and the portal veins did not display inflammation or important wall thickening.

The PBF observed in this experiment with a PAH dilution technique were compared with published data obtained in sheep with the same technique and for which ME intake was given or easy to estimate. When considering PBF values in relation to the ME intake, our data obtained with a PAH method compared well with those of the literature (Figure 1), as did PBF measured with A-series probes. However, S-series probes gave consistently lower PBF. In the present experiment, PBF recorded with S-series probes amounted to 77, 52, and 67% of that determined by indicator dilution (Table 1). This observation agrees with that of Huntington et al. (1990) who reported that PBF in cattle fitted with S-series probe was 40 to 60% of that determined with PAH (one probe gave 14%, but it was incorrectly placed). Kristensen et al. (1996) also reported that PBF measured in sheep with an S-series probe was 60 to 100% of PBF measured by PAH dilution, and Figure 1 shows that their data obtained with probes are lower than those generally reported in the literature. However, using

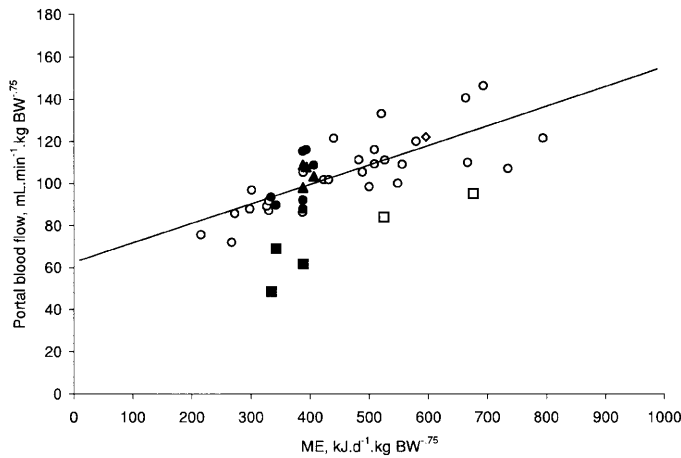


Figure 1. Relationship between portal blood flow and metabolizable energy intake in sheep. The line shown ($y = 61.4 + .093 x$, $SEy = 11.4$, $r^2 = .78$) represents the regression that was obtained for sheep with para-aminohippuric acid dilution, and published by Bergman et al. (1970), Bergman and Wolf (1971), Heitmann et al. (1986), Gross et al. (1990), Burin et al. (1991), Goetsch et al. (1994), Freetly et al. (1995), Goetsch and Ferrell (1995), and Patil et al. (1995). These data are denoted by \circ . Data from the present experiment are figured by \bullet for PAH, \blacksquare for S-series probes, and \blacktriangle for A-series probes. Data from Kristensen et al. (1996) \square and Neutze et al. (1989) \diamond were obtained with S-series probes in sheep.

an in situ calibration method, Neutze et al. (1989) demonstrated that S-series probes can provide an accurate estimate of true PBF in sheep. Furthermore, their data compare well with those of the literature (Figure 1).

In cattle the portal vein is very short, and Huntington et al. (1990) attributed the poor correlation observed in steers between PAH and probe measurement to the fact that the probe was implanted not on a tubular but rather on a T-vessel with the junctions of the gastrosplenic and mesenteric veins nearly within the probe. In sheep the portal vein is proportionally longer than in cattle, which provides a tubular section long enough for a correct implantation of the S-series probes. Furthermore, in Neutze et al. (1989), Kristensen et al. (1996), and the present work, the reflectors of S-series probes were equipped with a silicone sheet to ensure a good probe positioning on the portal vein. Despite this precaution, our data and those of Kristensen et al. (1996) show that the use of S-series probes on the portal vein can lead to an extensive underestimation of PBF. The presence of a turbulent flow within the probes (not compatible with accurate measurements with S-series probes) could be assumed to explain this underestimation. However, the data obtained by Neutze et al. (1989) do not support such a conclusion. In any case, it seemed to us that S-series probes, because of their dimensions and technology, are not well adapted to the measurement of PBF in ruminants.

This reason prompted us to test the newly designed A-series flow probes. These cuff-type probes need, to be operational, a portal vein section one-third the size of that necessary for S-series probes. Mean PBF obtained with A-series probes in the four sheep compared well with those measured by PAH dilution. Averaging the 2 d of sampling, differences between these two techniques were only +10, -5, -3, and -6% relative to PAH measurement (Table 1). During each sampling period, the variability of PBF measurement

Table 1. Comparisons of portal blood flow measured using ultrasonic transit-time flowmeter with portal blood flow determined by para-aminohippuric acid (PAH) dilution technique

Sheep	BW, kg	Portal blood flow, L/min ^a				Statistics ^b		
		Probe		PAH		Probe-PAH		
		Mean	SD	Mean	SD	Mean	SE	P
S-series probes								
A	59	1.431	.084	1.908	.267	-.477	.054	.001
B	61	1.064	.145	2.035	.258	-.971	.047	.001
C	50	1.103	.276	1.730	.267	-.626	.086	.001
A-series probes ^c								
E	50	1.830	.114	1.592	.174	.237	.030	.001
		1.810	.141	1.723	.329	.087	.081	.295
F	50	1.976	.077	2.216	.093	-.240	.040	.001
		2.051	.139	2.039	.354	.012	.079	.879
G	49	2.137	.106	2.107	.348	.030	.082	.717
		2.033	.095	2.186	.443	-.153	.098	.134
H	48	1.881	.106	1.985	.309	-.095	.070	.191
		1.791	.107	1.915	.158	-.123	.027	.001

^aMean of 20 sampling times evenly distributed over 6 h.

^bPaired comparison of instantaneous portal blood flow (n = 20).

^cTwo days of sampling for A-series probes.

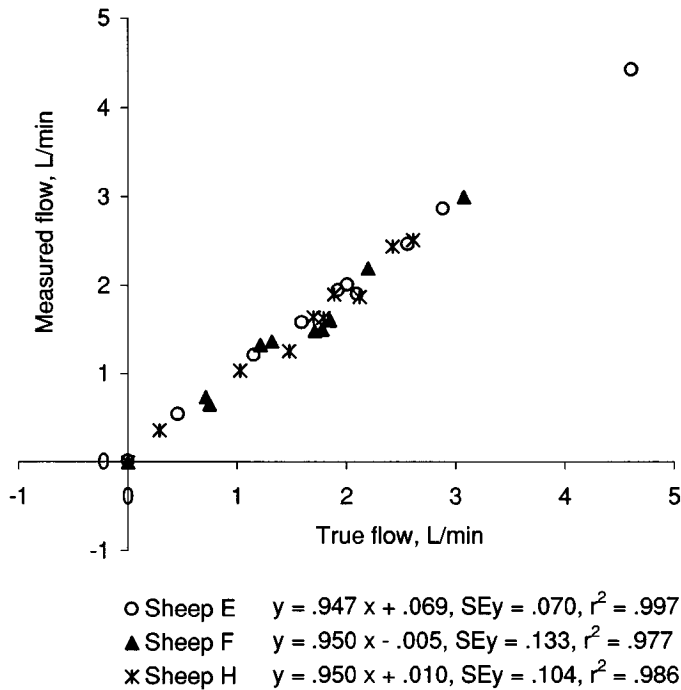


Figure 2. In vivo calibration of three A-series probes implanted on the portal vein of sheep.

was higher with PAH than with the probes. On some sampling days, instantaneous PBF recorded with the two methods were different ($P < .01$). This method effect appeared when the variability of PBF measurement with PAH was low. When instantaneous PBF are considered, differences up to 40% were observed between the two methods, but, within a sampling day, the mean difference observed for each of the animals never exceeded 15%. In conclusion, a good agreement between mean PBF measured with A-series probes and PAH dilution was observed.

In vivo calibration of probes was difficult to achieve because of the high blood flow required. One sheep died during the catheter implantation procedure, and the probes were calibrated on only three sheep. Probes were calibrated 1 to 3 mo after implantation. Blood was infused via the two major tributaries of the portal vein to approach as closely as possible the natural velocity profile of the flow. For generating blood flow up to 5 L/min, the use of a large volume of blood (800 mL) was necessary. Withdrawing such a quantity of blood from the animal inevitably produced a drop in PBF (already limited because of the splenic and mesenteric vein ligation). However, after blood infusion, the increase in blood volume generated an increase in PBF. Basal blood flow during infusion was, therefore, calculated on the basis of PBF measured immediately before and after infusion (when returned to a stable level), assuming linear evolution. This basal blood flow was subtracted from recorded blood flow between sensor signals. Calibration results are shown in Figure 2. For each animal, a linear

relationship was observed between true flow and measured flow. Zero offset of the implanted probes determined by regression was within or close to manufacturer specifications (maximum zero flow error of 60 mL/min). For blood flow ranging from 0 to 5 L/min, the slope of the calibration curves was correct (deviating by about 5% from linearity). This study demonstrated that A-series ultrasonic probes provide an accurate estimate of true PBF and can, therefore, be reliably used for PBF measurement in sheep. The data obtained with these probes compared well with those using a PAH dilution technique. As previously discussed by Eisemann et al. (1987), the choice between these two techniques mainly depends on the experimental design. When average PBF is of interest, either technique can be used. However, considering the inherently high variability of PBF estimated with PAH dilution, direct and continuous measurement of PBF with ultrasonic probes is the method of choice to record acute changes in blood flow and to record PBF in sheep that are not submitted to continuous feeding (non-steady state conditions).

Implications

The development of a reliable method for direct and continuous measurement of portal blood flow is of great interest for nutrition studies. When combined with measurements of arteriovenous differences, it will allow one to determine the changes in portal-drained viscera net fluxes of nutrients with the rhythm of feed distribution or with the degradation rate of the feeds. This would provide a better understanding of nutrient interactions during absorption and portal-drained viscera metabolism in meal-fed animals.

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