

## Assessment of gastrointestinal permeability to small marker probes in the preruminant calf

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**Summary** — The intestinal permeability to markers was assessed in preruminant calves fed different milk substitutes containing skimmed milk powder or whey and soyabean products of differing antigenic activity as the protein sources. In *Experiment 1*, the 6 h urinary excretion of lactulose transiently decreased with the antigenic soyabean product but not that of sucrose or D-mannitol. In *Experiment 2*, the 6 h urinary excretion of sucrose and D-mannitol averaged 1–3%, regardless of age and dietary treatment. Cr-EDTA was recovered at rates of 2 and 4% after 6 and 24 h of urinary collection, respectively. The 24 h excretion of Cr-EDTA was lower in the calves fed the antigenic flour than in the controls after 2 weeks of experimental feeding (2.9 vs 6.0%,  $P < 0.05$ ) but not thereafter. This transient decrease was also observed using D-xylose with the antigenic flour and with the non-antigenic concentrate (17 and 22% respectively vs 37%,  $P < 0.05$ ). The present markers, including sucrose, may be suitable for assessing intestinal permeability in the calf even though excretion rates differed from one marker to another. Changes in the intestinal permeability to antigenic soya were of low magnitude and were only transiently detected when measured using lactulose, Cr-EDTA or D-xylose, probes which were therefore more sensitive than sucrose or D-mannitol.

**gut permeability / marker / preruminant calf / soyabean**

**Résumé** — Évaluation de la perméabilité intestinale à l'aide de marqueurs de faible masse molaire chez le veau préruminant. La perméabilité intestinale à divers marqueurs a été évaluée en mesurant leur excretion urinaire chez des veaux préruminants consommant des laits de remplacement qui contenaient de la poudre de lait écrémé ou du lactosérum et des dérivés du soja différant par leur activité antigénique. Dans l'expérience 1, la proportion de lactulose excrétée en 6 h, mais pas celle de saccharose et de D-mannitol, a transitoirement diminué avec le soja antigénique. Dans l'expérience 2,

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*l'excrétion a été voisine de 1 et 3% pour le saccharose et le D-mannitol, indépendamment de l'âge des veaux et du régime alimentaire. Le Cr-EDTA a été excrété à raison de 2 et 4% en 6 et 24 h, respectivement. La proportion de Cr-EDTA excrétée en 24 h a été plus faible avec l'aliment contenant la farine antigénique qu'avec le témoin 2 sem après le début de l'essai (2,9 vs 6,0%, P < 0,05), mais non à 10 sem. Cette diminution transitoire a aussi été observée par le D-xylose avec la farine antigénique et le concentrat non antigénique (excrétion urinaire de 17 et 22% vs 37% pendant 6 h, P < 0,05). Les présents marqueurs, incluant le saccharose, peuvent servir à évaluer la perméabilité intestinale chez le veau, bien que les taux d'excrétion aient différé d'un marqueur à un autre. Les modifications de perméabilité intestinale liées à la consommation de soja antigénique ont été de faible amplitude ; elles ont été transitoirement détectées par le lactulose, le Cr-EDTA ou le D-xylose, qui ont été, de ce fait, des marqueurs plus sensibles que le saccharose ou le D-mannitol.*

**marqueur / perméabilité intestinale / soja / veau préruminant**

**INTRODUCTION**

The intestinal epithelium is permeable to small molecules and is thought to function as a barrier that has a large population of small pores and a smaller population of larger pores (review by Travis and Menzies, 1992). On this basis, the permeation of small probes such as monosaccharides is greater than that of larger probes such as oligosaccharides, chelated metals or low molecular weight polyethylene glycols (PEGs). The monosaccharides are thought to cross the epithelium by a transcellular route, while the oligosaccharides may use a paracellular route, apart from PEGs which are lipid-soluble and diffuse through cell membranes. In addition, D-xylose absorption is similar to glucose absorption since it stimulates sodium ion transport and a short-circuit current in the intestinal epithelium (review by Craig and Atkinson, 1988). Simultaneous administration of 2 markers subject to exactly the same influences except for mucosal permeability have been proposed to circumvent sources of error, including gastric emptying, gut motility and transit time, inherent to all single-marker tests. Changes in gut permeability due to a variety of infectious and non-infectious gastrointestinal diseases are reflected by an increase in urinary excretion of the larger molecules which may be associated with a decrease in that for the smaller probes

(Travis and Menzies, 1992). Thus, the excretion ratio of larger to smaller markers is usually increased. The behaviour of sugar permeability probes is however unknown in the preruminant calf.

Insufficiently processed soyabean products, *eg*, those containing substantial amounts of near-native storage proteins like glycinin and  $\beta$ -conglycinin, are responsible for decreased calf performance and immune-mediated digestive disorders in some predisposed individuals (review by Lallès *et al*, 1993b). A negative relationship between the *in vitro* immunoreactivity of soyabean products and the apparent digestibility of protein from soyabean *in vivo* has been shown (Lallès *et al*, 1993a). The intestinal permeability to macromolecules such as  $\beta$ -lactoglobulin increased after a test meal containing an antigenic heated soyabean flour in calves that had been previously sensitized to the flour during a limited number of liquid meals containing it (Kilshaw and Slade, 1980). This was probably due to an acute inflammation of the gut in response to an immediate hypersensitive reaction to the dietary antigens. In calves chronically fed soyabean-based milk substitutes, the intestinal absorption of small molecules was reduced, as far as xylose was concerned (Seegraber and Morrill, 1986). However, its excretion in urine was also lower when the substitute contained a fish protein concentrate and, to a lesser extent, when casein was present (Seegraber

and Morrill, 1986), suggesting the possible influence of abomasal emptying and protein nature on the plasma concentration of xylose. The effect of soya antigens on xylose absorption was studied by Mir *et al* (1993). They observed that the lowest plasma peak xylose concentration occurred with the most antigenic soyabean product in calves aged 33 d, but not in those aged 21 or 45 d, at the beginning of experiments with soya. They also found a significant but weak negative relationship between peak xylose concentrations in plasma and antisoya antibody titres.

The aim of the present study was to evaluate the use of sucrose as a permeability probe, since sucrose is cheap and also since it is not digested because calves lack intestinal sucrase (Sissons, 1981). The validity of dual-marker tests in calves fed milk substitutes differing in protein source or quality was examined using the disaccharides lactulose and sucrose tested separately, with D-mannitol employed as the monosaccharide. The probes Cr-EDTA and D-xylose were also included in the study for comparison.

## MATERIALS AND METHODS

### Experiment 1

This experiment was set up to assess the suitability of sucrose as the disaccharide, compared to lactulose, in a dual-sugar permeability test involving D-mannitol as the monosaccharide.

### Soyabean protein sources, milk substitute formulas and calf management

Two commercial soya protein concentrates that were either antigenic (ASC, Coopérative de Traitement des Produits de la Pêche, Boulogne-sur-Mer, France) or non-antigenic (NASC1, Oliefabrik, Aarhus, Denmark) were used in *Experiment 1* (table I, Toullec *et al*, 1994). Milk substitute diets were formulated to contain 21–22% crude protein and 20–21% fat on a dry matter basis (Toullec *et al*, 1994). The soyabean products provided approximately two thirds of the total protein of the diets, the remaining portion being supplied by whey powder. Six male Holstein calves per treatment were used to study the nitrogen (N) digestibility, the antisoya antibody production and the gastrointestinal permeability to sugar probes. The

**Table I.** Levels of immunoreactive glycinin and  $\beta$ -conglycinin (% crude protein) in the soyabean protein sources, and soyabean apparent N digestibility and circulating antisoya antibody titres ( $\log_2$  dilution) measured in prerinant calves.

Item	Experiment 1 <sup>a</sup>		Experiment 2 <sup>b</sup>		
	NASC1	ASC	HSPI	NASC2	ASF
Glycinin <sup>c</sup>	< 0.0001	3.29	< 0.0001	< 0.0001	3.94
$\beta$ -Conglycinin <sup>c</sup>	< 0.0001	1.47	< 0.0001	< 0.0001	3.61
Soyabean N digestibility <sup>d</sup>	0.81	0.71	0.90	0.81	0.59
Antisoya antibody titre <sup>d</sup>	0.0	2.3	1.6	3.1	9.1

<sup>a</sup> Non-antigenic (NASC1) and antigenic (ASC) soyabean protein concentrates. <sup>b</sup> Hydrolysed soyabean protein isolate (HSPI), non-antigenic soyabean protein concentrate (NASC2) and antigenic soyabean flour (ASF). <sup>c</sup> Determined in soyabean products by immunometric ELISA (Tukur *et al*, 1993). <sup>d</sup> See references Lallès *et al* (1995) and Toullec *et al* (1994).

calves were purchased and reared on milk substitutes based on skimmed milk powder (SMP) until they reached the age of 1 month. Animals were then switched to the experimental diets over 2 d and remained on these diets for approximately 100 d. Meals were fed at 9.00 and 16.00 h by open pail. As presented in table 1, soyabean N digestibility was found to be lower, and plasma antisoya antibody titres higher with the ASC compared with NASC1 diet (Toullec *et al*, 1994).

### Assessment of the gastrointestinal permeability

Lactulose (0.125 g/kg BW; Sigma, Saint-Louis, MO, USA), sucrose (0.2 g/kg body weight (BW); Béghin-Say, Thumeries, France) and D-mannitol (0.1 g/kg BW; Roquette, Lestrem, France) were dissolved separately in water (15% wt/vol). Lactulose and D-mannitol or sucrose and D-mannitol were fed together with the morning meal on days 16–21 (period P1) and days 58–63 (period P2) after the start of the experimental diets. The calves were randomly dosed with these mixtures of sugars on separate occasions to avoid interference between the disaccharides during their assay. Since the soya protein sources contained sucrose (3–5 mg/g powder), the corresponding amounts ingested were taken into account for the calculations. Urine was collected using a large funnel located under the slatted floor of the crates, for 6 h post-dosing on mercuric chloride (HgCl<sub>2</sub> 5%, 20 ml/l urine). Aliquots were frozen at –20°C until analysis. Urine samples were also collected prior to marker dosing to ensure absence of endogenous interfering substances.

### Experiment 2

This experiment was carried out to study the ability of D-xylose and Cr-EDTA alone, compared to a dual-sugar test using sucrose and D-mannitol, to detect the effect of soyabean antigenicity on the urinary excretion of markers.

### Soyabean protein sources, milk substitute formulas and calf management

Four milk substitute formulas containing either SMP or one of the 3 soyabean products as the

main protein sources were prepared (Toullec *et al*, 1994; Lallès *et al*, 1995). These products were a non-antigenic hydrolysed soya protein isolate (HSPI, Protein Technologies International, Saint-Louis, MO, USA), a non-antigenic concentrate (NASC2, Coopérative de Traitement des Produits de la Pêche) and an antigenic heated soyabean flour (ASF, Société Industrielle des Oléagineux, Bougival, France). Only ASF had noticeable antigenic activities as far as glycinin and  $\beta$ -conglycinin were concerned (table 1). The composition of the milk substitute diets was close to that used in *Experiment 1* (Toullec *et al*, 1994; Lallès *et al*, 1995). Twenty-eight male Holstein calves (7 per treatment) were purchased and reared as described in *Experiment 1*. Permeability measurements were made 1 week before (period P0), and at days 16–21 (period P1) and days 72–77 (period P2) after the start of the experimental diets. As shown in table 1, soyabean N digestibility was the lowest, and anti-soya antibody titres the highest, with the antigenic ASF. Differences between HSPI and NASC2 were also observed, based on these criteria (Toullec *et al*, 1994; Lallès *et al*, 1995).

### Assessment of the gastrointestinal permeability

The calves were deprived of milk the evening before the tests. An aqueous solution mixture of D-mannitol (0.1 g/kg BW), sucrose (0.2 g/kg BW), and Cr-EDTA (5.5 mg Cr/kg BW, prepared according to Binnerts *et al*, 1968) was distributed alone at the usual time of the morning meal. An aqueous solution (10% w/v) of D-xylose (0.5 g/kg BW, Sigma) was similarly given to calves on separate occasions. Warm water and milk substitute were made available 2 and 6 h respectively after the marker administration. All the urine was collected as in *Experiment 1*, but during the intervals 0–6 h and 6–24 h after marker dosing. The aliquots were stored at –20°C.

### Assay of marker probes, calculations and statistics

D-Mannitol, lactulose and sucrose were analysed in urine samples by gas-liquid chromatography (André *et al*, 1987), Cr by atomic absorption spectrometry (Lallès and Poncet, 1990) and D-xylose colorimetrically (Eberts *et al*, 1979).

Results were expressed as marker probe clearance (% oral dose). Additionally, disaccha-

ride to monosaccharide excretion ratios were calculated. The effect of age was analysed by the non-parametric tests of Wilcoxon (*Experiment 1*) and Friedman (*Experiment 2*) while the dietary influences were analysed by the tests of Mann-Whitney (*Experiment 1*) and Kruskal-Wallis (*Experiment 2*) (Hollander and Wolfe, 1973).

## RESULTS

### *Experiment 1*

The mean urinary excretion of lactulose varied between  $1.3 \pm 0.45$  (SEM) and  $3.0 \pm 0.23\%$  of the oral dose (table II). It decreased significantly ( $P < 0.05$ ) with age in the

NASC1 group. Lactulose excretion was significantly ( $P < 0.05$ ) lower with the ASC diet as compared to the NASC1 diet during the first period of measurement but this was not true during the second. In contrast, neither the excretion of sucrose nor that of D-mannitol was significantly affected by age or dietary treatments. Although the excretions of lactulose and sucrose were not significantly different ( $P > 0.05$ ), the data were not significantly ( $P > 0.05$ ) correlated either. Similarly the urinary excretions of D-mannitol obtained on 2 separate occasions were neither significantly ( $P > 0.05$ ) different nor correlated. Only the sucrose to D-mannitol excretion ratio reflected a significant ( $P < 0.05$ ) age effect for the NASC1 diet.

**Table II.** Six-hour urinary excretion of lactulose, sucrose and D-mannitol (means  $\pm$  SEM, % oral dose) and disaccharide to monosaccharide excretion ratio in preruminant calves (*Experiment 1*).

Item	Period <sup>a</sup>	Diet <sup>b</sup>	
		NASC1	ASC
Lactulose	P <sub>1</sub>	3.02 <sup>c</sup> $\pm$ 0.23	1.29 <sup>d</sup> $\pm$ 0.45
	P <sub>2</sub>	2.29 <sup>e</sup> $\pm$ 0.27	2.14 $\pm$ 0.97
Sucrose	P <sub>1</sub>	1.63 $\pm$ 0.56	1.56 $\pm$ 0.69
	P <sub>2</sub>	2.09 $\pm$ 0.41	1.42 $\pm$ 0.42
D-Mannitol-L <sup>f</sup>	P <sub>1</sub>	1.82 $\pm$ 0.50	1.42 $\pm$ 0.45
	P <sub>2</sub>	1.53 $\pm$ 0.31	1.28 $\pm$ 0.55
D-Mannitol-S <sup>f</sup>	P <sub>1</sub>	1.64 $\pm$ 0.38	1.81 $\pm$ 0.77
	P <sub>2</sub>	2.39 $\pm$ 0.47	1.43 $\pm$ 0.53
Lactulose/D-mannitol	P <sub>1</sub>	2.28 $\pm$ 0.48	1.59 $\pm$ 0.61
	P <sub>2</sub>	1.83 $\pm$ 0.27	1.93 $\pm$ 0.30
Sucrose/D-mannitol	P <sub>1</sub>	1.04 $\pm$ 0.25	1.06 $\pm$ 0.25
	P <sub>2</sub>	0.99 <sup>e</sup> $\pm$ 0.21	1.59 $\pm$ 0.51

<sup>a</sup> P1 and P2 during days 16–21 and days 58–63 after the start of the experimental diets, respectively. <sup>b</sup> Milk substitutes containing non-antigenic (NASC1) and antigenic (ASC) soyabean protein concentrates. <sup>c,d</sup> Means with different superscripts in the same row differ significantly ( $P < 0.05$ ). <sup>e</sup> Significant difference between periods for a given diet ( $P < 0.05$ ). <sup>f</sup> D-Mannitol excreted during the lactulose/D-mannitol (D-mannitol-L) and the sucrose/D-mannitol (D-mannitol-S) tests were carried out separately.

### Experiment 2

The 6 h urinary excretion of sucrose varied between 0.9 and 3.7% of the oral dose (table III). The effects of age or dietary treatment were not significant ( $P > 0.05$ ). Similarly the D-mannitol excretion in urine varied between 1.8 and 4.0%, irrespective of the age and dietary treatment. Consequently, the sucrose to D-mannitol excretion ratio ranged between 0.6 and 1.2, irrespective of the factors tested.

In the control calves, the mean Cr-EDTA excretion during the first 6 h of urine collection, the next 18 h and the whole 24 h period ( $2.5 \pm 0.39$ ,  $2.8 \pm 0.63$  and  $5.3 \pm 0.39\%$ , respectively) did not significantly change with age (fig 1). Values were lower with the soyabean diets than with the control diet after 2 weeks of feeding. The differences,

however, were only significant ( $P < 0.05$ ) between the ASF and the control diets when the entire 24 h period was considered. A general trend was no more apparent after 10 weeks of study.

The urinary excretion of D-xylose was the highest among the different marker probes tested; the values observed before the start of the experimental diets averaged 26% of the oral dose (fig 2). In the control calves, this value peaked at 34% 3 weeks later and then decreased to 19% ( $P < 0.05$ ) by the end of experiment. D-Xylose excretion was usually lower in calves fed milk substitutes containing the soyabean products, although significant differences ( $P < 0.05$ ) among the diets were only observed 2 weeks after the start of the experimental diets (fig 2); the values with NASC2 and ASF were lower than with SMP.

**Table III.** Six-hour urinary excretion of sucrose and D-mannitol (means  $\pm$  SEM, % oral dose) and sucrose to D-mannitol excretion ratio in preruminant calves (Experiment 2).

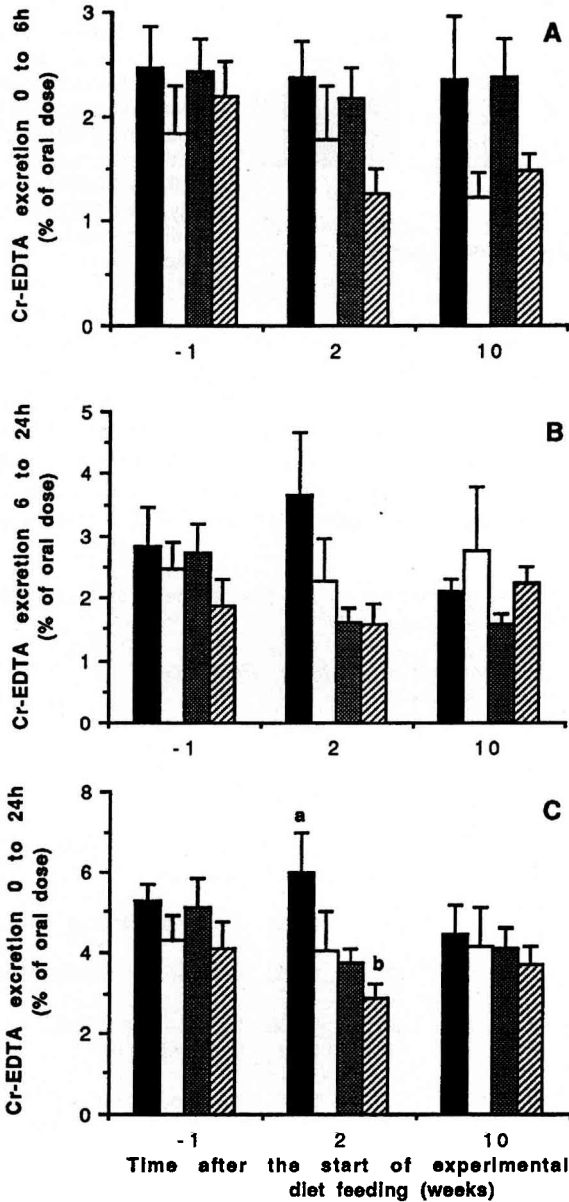
Item	Period <sup>a</sup>	Diet <sup>b</sup>			
		Control	HSPI	NASC2	ASF
Sucrose	P0	3.72 $\pm$ 0.51	2.56 $\pm$ 0.68	3.34 $\pm$ 0.68	2.72 $\pm$ 0.40
	P1	2.35 $\pm$ 0.38	2.15 $\pm$ 0.38	1.98 $\pm$ 0.20	1.29 $\pm$ 0.23
	P2	1.95 $\pm$ 0.59	1.45 $\pm$ 0.30	2.33 $\pm$ 0.35	0.92 $\pm$ 0.28
D-Mannitol	P0	3.15 $\pm$ 0.33	2.37 $\pm$ 0.58	2.72 $\pm$ 0.38	2.93 $\pm$ 0.39
	P1	4.03 $\pm$ 0.95	2.57 $\pm$ 0.59	2.83 $\pm$ 0.39	2.06 $\pm$ 0.29
	P2	2.33 $\pm$ 0.72	1.83 $\pm$ 0.38	2.55 $\pm$ 0.35	1.78 $\pm$ 0.41
Sucrose/D-mannitol	P0	1.23 $\pm$ 0.17	0.98 $\pm$ 0.15	1.19 $\pm$ 0.09	0.93 $\pm$ 0.09
	P1	0.64 $\pm$ 0.09	0.85 $\pm$ 0.14	0.73 $\pm$ 0.06	0.67 $\pm$ 0.12
	P2	0.78 $\pm$ 0.07	0.85 $\pm$ 0.15	0.95 $\pm$ 0.12	0.59 $\pm$ 0.23

<sup>a</sup> P<sub>0</sub>, P<sub>1</sub> and P<sub>2</sub>: one week before, and during days 16–21 and 72–77 respectively, relative to the start of feeding experimental diets. <sup>b</sup> Milk substitutes containing skimmed milk powder (control) non-antigenic hydrolysed soyabean protein isolate (HSPI), non-antigenic soyabean protein concentrate (NASC2) and antigenic heated soyabean flour (ASF).

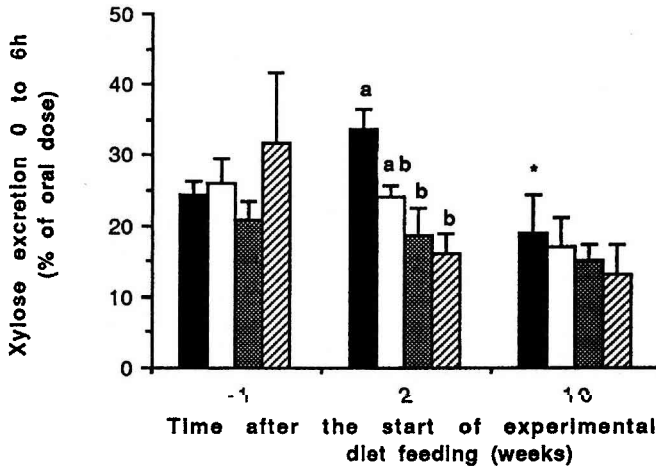
## DISCUSSION

Non-invasive exploration of intestinal permeability using dual sugar tests has become widespread in many species including

humans (André *et al*, 1987; Travis and Menzies, 1992), rats (Turner *et al*, 1988), guinea pigs (Weaver and Coombs, 1988), dogs (Hall and Batt, 1991b; Quigg *et al*, 1993) and cats (Papasouliotis *et al*, 1993). In the



**Fig 1.** Urinary excretion (means  $\pm$  SEM, % of oral dose) of Cr-EDTA 0–6 h (A), 6–24 h (B) and 0–24 h (C) after oral administration to preruminant calves fed milk substitute diets containing skimmed milk powder (■), non-antigenic soyabean protein isolate (□), non-antigenic soyabean protein concentrate (▨) or antigenic soyabean flour (▩). <sup>a,b</sup> Means in the same period with different superscripts differ significantly ( $P < 0.05$ ).



**Fig 2.** Urinary excretion (means  $\pm$  SEM, % of oral dose) of D-xylose 0–6 h after oral administration to preruminant calves fed milk replacer diets containing skimmed milk powder (■), non-antigenic soyabean protein isolate (□), non-antigenic soyabean protein concentrate (▒) or antigenic soyabean flour (▨). <sup>a,b</sup> Means in the same period with different superscripts differ significantly ( $P < 0.05$ ). \* Differs significantly ( $P < 0.05$ ) from control diet group values in weeks –1 and 2.

present study, young calves excreted disaccharides and monosaccharides in similar proportions, *eg*, 1–4% of the oral dose, as indicated by a disaccharide to monosaccharide excretion ratio comprised between 0.6 and 1.9. These values appear to differ from those in other species (table IV). The

calf has a proportionally higher intestinal permeability to disaccharides and a lower permeability to monosaccharides like D-mannitol. The basis for such differences is unknown. Although an influence of methodology cannot be ruled out, these differences for D-mannitol may be linked to those in sol-

**Table IV.** A selection of control values for 5–6 h urinary excretion of sugar probes in various species.

Species	Mean age	Sugars <sup>a</sup>	Urinary excretion (5–6 h)			Reference
			Oligo-saccharide (%)	Mono-saccharide (%)	Ratio	
Human	1 year	L/M	0.04	3.7	0.010	Jalonen <i>et al</i> (1991)
	Adult	L/M	0.26	14.1	0.024	André <i>et al</i> (1987)
Rat	Adult	L/Rh	NI	NI	0.160	Turner <i>et al</i> (1988)
Guinea pig	< 1 week	L/M	NI	NI	0.058	Weaver and Coombs (1988)
Dog	1 year	C/M	2.3	50	0.050	Hall and Batt (1991b)
	4–8 years	L/Rh	1.8	16	0.12	Quigg <i>et al</i> (1993)
Cat	5–12 years	L/M	0.5	2.5	0.27	Papasoulotis <i>et al</i> (1993)

<sup>a</sup> C cellobiose, L lactulose, m mannitol, Rh rhamnose. NI: not indicated.



vent drag which are related to water absorption. Indeed, one third of D-mannitol permeability of perfused rat jejunum was mediated by passive diffusion and two thirds by solvent drag (Krugliak *et al*, 1994); the latter accounted for most (89%) of D-mannitol absorption in the colon. Increasing luminal osmolarity resulted in a decreased net water flux and permeability to D-mannitol (Krugliak *et al*, 1994). In humans, D-mannitol excretion is higher than in the rat, possibly because of a stronger solvent drag linked with a highly hyperosmotic state of their intestinal villi (Bijlsma *et al*, 1993). The differential excretion behaviour of oligosaccharides and monosaccharides has been explained on theoretical grounds by assuming the existence of 2 populations of intestinal pores differing in size (review by Travis and Menzies, 1992) or 2 populations of tight junctions having different dimensions, different resistances to marker passage or different locations in the epithelium (Hollander, 1992). If any of these theories are true then the calf might have 2 different types of pores or tight junctions with limited morphological or functional differences. This speculative view warrants further investigation.

The lack of sucrase activity in calves (Sissons, 1981) is unusual compared with monogastric species, including humans whose gastric, but not intestinal, permeability can be assessed using sucrose (Meddings *et al*, 1993). We found the urinary excretion of sucrose and lactulose to be of the same magnitude, suggesting a similar behaviour for these disaccharides (Meddings *et al*, 1993). Sucrose may therefore be suitable for gastrointestinal permeability studies in the preruminant calf. The lack of correlation between the excretion rates of these disaccharides might result from their generally low excretion, as well as reflecting day-to-day variations, as observed here for D-mannitol. Although expressing the results as excretion ratios tended to reduce individual variations in the present study, this

was not usually sufficient to permit the detection of significant changes in intestinal permeability with the various dietary treatments (see below). This result is different from observations in other species (Travis and Menzies, 1992).

The 6 h urinary excretion of Cr-EDTA in our control calves amounted to 1.7–2% of oral dose. This value is close to that found using the <sup>51</sup>Cr-EDTA radiomarker in rats (2.8%; Ramage *et al*, 1988) but higher than the excretion recorded in humans (0.44–0.85%; Maxton *et al*, 1986; André *et al*, 1990). The 24-h recovery of Cr-EDTA in urine seems to represent marker absorption in both small and large intestines (Maxton *et al*, 1986; Elia *et al*, 1987). It is between 1.2 and 2.6% in healthy humans (Maxton *et al*, 1986; André *et al*, 1990) and close to 12% in dogs (Hall *et al*, 1989; Hall and Batt, 1991a). Here, 2–4% of Cr-EDTA was recovered after a 24 h collection of urine in calves, suggesting that the lower gut would have accounted for half of the marker absorption.

The 5 h urinary excretion of D-xylose is between 18 and 35% in adult humans, depending on age (Haeney *et al*, 1978; Lim *et al*, 1993), 12–16% in preruminant calves (Seegraber and Morrill, 1986; Dawson *et al*, 1988) and 10% in mares (Bolton *et al*, 1976). The values recorded here (19–34%) were substantially higher than those found by others in calves (Seegraber and Morrill, 1986; Dawson *et al*, 1988). Such differences may come from our longer collection period of urine (6 vs 5 h). Secondly, the calves in our experiments grew faster than those in the other studies (1.0–1.2 vs 0.4–0.5 kg per day) which may indicate greater intestinal absorptive capacity. Thirdly, there is a tendency towards increased D-xylose absorption or excretion up to 2 months of age in the calf, which was apparent in the present and other published data (Dawson *et al*, 1988; Mir *et al*, 1993).

Predisposed calves are known to suffer from immune-mediated gut disturbances

when fed insufficiently processed soyabean products (Sissons, 1982; reviews by Lallès *et al*, 1993b). Macromolecule absorption was drastically increased following a test meal containing heated soyabean flour in previously sensitized calves (Kilshaw and Slade, 1980). Absorption of D-xylose appeared to be reduced after the introduction of non-milk protein, including soya or fish protein, in milk substitutes (Seegraber and Morrill, 1986; Dawson *et al*, 1988). This is known to prevent abomasal clotting and to hasten gastric emptying (Guilloteau *et al*, 1979), factors which, in turn, may affect marker absorption (review by Travis and Menzies, 1992). Villous atrophy was demonstrated in experimentally sensitized calves (Kilshaw and Slade, 1982) and in calves chronically fed soyabean products (Seegraber and Morrill, 1986). However, abnormal villi were also observed when calves were fed a fish protein concentrate, and casein but to a lesser extent (Seegraber and Morrill, 1986). In the present study, the duodenal villi observed at slaughter appeared shorter in the calves fed soya than in controls calves (Lallès *et al*, 1995), but a cellular infiltration of the intestinal mucosa was only observed in the calves fed ASF (Dréau *et al*, unpublished data). Only the Cr-EDTA and D-xylose excreted over the 24 and 6 h post-dosing, respectively, indicated dietary effects 2 weeks after the start of soya consumption. A significant decrease in D-xylose absorption (Lallès *et al*, 1995) and excretion in urine (present data) with antigenic soya was expected from previous work, and may be ascribed to alterations of the intestinal epithelium (Kilshaw and Slade, 1982; Seegraber and Morrill, 1986). Curiously, Cr-EDTA excretion was lower in the calves fed antigenic soya compared with the controls, whereas higher values might have been expected from what is known concerning gluten enteropathy in both humans (André *et al*, 1990) and dogs (Hall and Batt, 1990). Decreased Cr-EDTA excretion may be linked to an osmotic effect or

decreased nutrient absorption following mucosal damage.

Changes in intestinal permeability to small marker probes occur in a variety of diseases including infections and gut inflammation which are related or unrelated to food hypersensitivity in humans (André *et al*, 1987, 1990; Lobley *et al*, 1990; Jalonen *et al*, 1991; Lim *et al*, 1993) and animals (Bolton *et al*, 1976; Ramage *et al*, 1988; Turner *et al*, 1988; Hall and Batt, 1991a,b; Quigg *et al*, 1993). The urinary excretion of monosaccharides is usually decreased while that of oligosaccharides or Cr-EDTA is increased (Travis and Menzies, 1992). Thus, the oligosaccharide to monosaccharide ratio is increased. Such variations in marker excretion have been frequently ascribed (but not always, see Bjarnason *et al*, 1994) to intestinal villous flattening in humans and animals (Strobel *et al*, 1984; Hall and Batt, 1991a; Quigg *et al*, 1993). Finally, studies on laboratory animals indicate a correlation (Ramage *et al*, 1988) or a lack of correlation (Turner *et al*, 1988; Weaver and Coombs, 1988) between intestinal absorption of small markers and macromolecules suggesting that different mechanisms of absorption are involved (Jalonen *et al*, 1991). Mir *et al* (1993) reported a significant but weakly negative relationship between plasma peak xylose concentrations and antisoya antibody titres in calves. We have also found such a relationship ( $r = -0.31$ ,  $P < 0.01$ ).

In conclusion, various marker probes including sucrose may be used to assess intestinal permeability in the preruminant calf, although the individual markers behaved slightly differently. The calf appears to substantially differ from other species with regard to urinary excretion of markers. Changes in intestinal permeability to small markers linked with protein source and antigenicity of the soyabean products were only detected using lactulose and D-xylose excreted for 6 h or Cr-EDTA excreted for

24 h; they were of limited magnitude and were only transient in this study. Thus, the pathogenesis of soya sensitivity in calves may not result from underlying abnormalities in intestinal permeability, contrary to those demonstrated in Irish setter dogs sensitive to gluten (Hall and Batt, 1991a).

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