



**Fig 1.** Effects due to luminal infusion of different fractions of CMP on CCK release. Results are in percent increase above basal level. Asterisks represent the significant differences from basal level (\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ). (S Beucher *et al*)

Only the fraction containing the slightly glycosylated forms of variant A (GMP2A) strongly stimulated CCK release (fig 1). The carbohydrates, and especially sialic acid, played a major role in activity, because the carbohydrate-free fraction of CMP, variant A and B (CMP(A + B)) was inactive and desialylation of the active fraction suppressed its effect. However, sialic acid solution induced only a very short and low response, consequently the peptide chain also appears to be involved in the peptide activity. The carbohydrate content and the amino-acid replacement of genetic variant A are also supposed to

play a major role. Indeed, highly glycosylated forms of variants A and B (GMP1) and slightly glycosylated forms of variant B (GMP2B) were not potent CCK secretagogues.

Therefore, the peptide activity is due to a definite structure involving sialic acid, which is always located at the end of sugar chains, and replaced amino-acid residues of variant A. The threonine 31 residue of this variant may be particularly involved, because it is a glycosylation site.

These results show that this casein peptide is able to stimulate the release of a hormone involved in the regulation of gastrointestinal functions, probably through binding to intestinal receptors.

**Effects of sorghum tannins on the activity of peptidases in the small intestine of the weaned piglet.** R Lizardo <sup>1</sup>, J Peinau <sup>1</sup>, JM Rouanet <sup>2</sup>, P Besançon <sup>2</sup>, A Aumaitre <sup>1</sup> (<sup>1</sup> INRA, Station de Recherches Porcines, 35590 Saint-Gilles; <sup>2</sup> Université de Montpellier II, 34095 Montpellier Cedex 05, France)

The presence of peptidases, *N*-aminopeptidase (NAP), dipeptidyl peptidase IV (DPP IV) and  $\gamma$ -glutamyl transferase ( $\gamma$ GT), in the membrane of the brush border enterocytes of the pig small intestine has been demonstrated [Kenny and Maroux (1982) *Physiol Rev* 62, 91-128]. The deleterious effect of sorghum tannins on the intestinal

**Table I.** Intestinal peptidases, alkaline phosphatase and maltase specific activities <sup>1</sup> in the weaned piglet: effects of site and dietary tannins (R Lizardo *et al*).

Peptidase	Site of measurement S <sup>2</sup>			Diet D <sup>3</sup>			Statistical analysis <sup>4</sup>		
	Duodenum	Jejunum	Ileum	Maize	Sorghum 0.03%	Sorghum 1.36% <sup>5</sup>	S	D	RSD
<i>N</i> -Aminopeptidase	24.0 <sup>b</sup>	38.7 <sup>a</sup>	44.2 <sup>a</sup>	31.2	37.5	36.9	**	ns	19.5
Dipeptidyl peptidase IV	43.7 <sup>b</sup>	61.5 <sup>a</sup>	66.8 <sup>a</sup>	66.8	48.8	53.2	*	ns	33.1
$\gamma$ -Glutamyl transferase	16.8 <sup>b</sup>	22.6 <sup>ab</sup>	28.6 <sup>a</sup>	29.7 <sup>b</sup>	15.6 <sup>a</sup>	14.5 <sup>a</sup>	*	**	13.7
Alkaline phosphatase	365.2 <sup>b</sup>	403.6 <sup>ab</sup>	481.0 <sup>a</sup>	458.7	413.4	370.9	*	ns	182.9

<sup>1</sup> International units per mg protein; <sup>2</sup> average value for the 3 sites of measurements; <sup>3</sup> average value for the 3 diets; <sup>4</sup> \*, \*\*  $P < 0.05$  and  $0.01$  respectively; RSD: residual standard deviation; <sup>a, b</sup> are significantly different; <sup>5</sup> tannins as % of catechin in the diet.

hydrolysis of dietary proteins has also been demonstrated [Butler *et al* (1984) *IAOCS* 61, 916-920].

The activity of NAP, DDP IV and  $\gamma$ GT was measured in 3 sites of the small intestine (duodenum, jejunum and ileum) of 24 piglets weaned at 21 d of age and slaughtered 5 weeks later fed 1 of the 3 experimental diets based on maize or sorghum containing 0.03 or 1.36% of tannin expressed as catechin (table I). It was found that the specific activity of all peptidases increased from the duodenum to the ileum ( $P < 0.05$ ). Peptidase activity was affected in piglets fed the sorghum-based diets, but only a significant decrease ( $P < 0.01$ ) of  $\gamma$ GT was observed. The activity of alkaline phosphatase decreased linearly but not significantly. We suggest that tannin, which induced a decrease in the apparent digestibility of dietary nitrogen, has an effect on the enterocyte of the small intestine of the piglet, disturbing the process of protein hydrolysis and absorption through the membrane.

**Involvement of L-arginine/nitric oxide pathway in the absorption of calcium in the rat small intestine.** R Schleiffer, M Galluser, F Raul (*INSERM U61, Biologie Cellulaire et Physiopathologie Digestives, 3, avenue Molière, 67200 Strasbourg, France*)

Nitric oxide (NO) is produced enzymatically *in vivo* from L-arginine. It was originally identified as the main endothelium-derived relaxant factor and is now recognized as a second messenger mediating a variety of biological processes, including platelet aggregation, neurotransmission and immunological reactions [Moncada *et al* (1991) *Pharmacol Rev* 43, 109-142]. Recent evidence indicates that enterocytes also produce NO [Blachier *et al* (1991) *Biochem Biophys Acta* 1092, 304-310]. The aim of this study was to determine the involvement of the L-arginine/NO pathway in intestinal calcium absorption.

An inhibitor of NO production ( $N^G$ -nitro-L-arginine methyl ester, L-NAME) and sodium nitroprusside (SNP), a donor of NO, were perfused in adult Wistar rats anesthetized with ethyl carbamate. An intestinal segment (duodenum + proximal jejunum) was perfused by both intraluminal and vascular routes [Scheiffer *et al* (1993) *Biomed Pharmacother* 47, 19-23]. The intraluminal solution (NaCl 155 mM + CaCl<sub>2</sub> 1.25 mM)

contained <sup>45</sup>Ca and was perfused at a flow rate of 0.2 ml•min<sup>-1</sup>. Calcium absorption was quantitated by measuring <sup>45</sup>Ca appearing in the superior mesenteric vein. The drugs were intravenously perfused at a flow rate of 0.1 ml•min<sup>-1</sup>. Mesenteric blood flow (MBF) and mean arterial pressure (MAP) were also measured throughout the experiment. Perfusion of L-NAME (0.123  $\mu$ mol•min<sup>-1</sup>) resulted in an increase in MAP (+17 mmHg) and a decrease in MBF (30%). Calcium absorption was decreased from basal  $0.21 \pm 0.02$  to  $0.15 \pm 0.01$   $\mu$ mol•min<sup>-1</sup>•kg<sup>-1</sup> body weight within 20 min of L-NAME perfusion. Inversely, SNP (0.032  $\mu$ mol•min<sup>-1</sup>) decreased MAP and increased MBF. An increase (30%) in calcium absorption was observed within 20 min of SNP perfusion.

These experiments suggest that the level of a product of the L-arginine/NO pathway may be involved in the regulation of calcium absorption in the rat small intestine.

**Is gut permeability affected by age and soyabean antigenicity in the preruminant calf?** P Branco Pardal <sup>1</sup>, JP Lalles <sup>1</sup>, F André <sup>2</sup>, E Delval <sup>3</sup>, R Toullec <sup>1</sup> (<sup>1</sup> *INRA, Laboratoire du Jeune Ruminant, 65, rue de Saint-Brieuc, 35042 Rennes, Cedex;* <sup>2</sup> *INSERM, Centre Hospitalier Lyon-Sud, Pierre-Bénite;* <sup>3</sup> *INRA, Station de Recherche sur la Nutrition des Herbivores, 63122 Saint-Genès-Champagnelle, France*)

Various small marker probes such as xylose, Cr-EDTA and mixtures of oligosaccharides are used to assess the permeability of the gut in health and disease, but the actual mechanisms of their uptake are still unclear. Dual marker tests like lactulose/mannitol [André *et al* (1990) *Hepato-Gastroenterol* 37 (suppl II), 113-117] rather than single probes are to be preferred to overcome extraneous influences, including gastric emptying rate or intestinal transit on marker absorption. Here, we assessed intestinal permeability using different markers in veal calves, which were fed milk replacers differing in protein source and antigenicity.

Twenty-eight male Holstein calves were placed in cages and fed various diets from 6 weeks of age until slaughter. Protein milk replacers was provided by either skim-milk pow-