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PROCEEDINGS OF THE NUTRITION SOCIETY

ABSTRACTS OF COMMUNICATIONS

A joint meeting of the Nutrition Society and the Association Française de Nutrition was held at the Ecole Nationale de la Santé Publique, Rennes, on Wednesday–Friday, 9–11 September 1992, when the following original communications were presented.

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Commission des Communautés Economiques Européennes (dans le carde du Programme COMETT).

Ministère de la Recherche et de l'Espace.

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Involvement of sugar composition of dietary fibre in formation of short-chain fatty acids during *in vitro* fermentation with human microflora. By V. SALVADOR, C. CHERBUT, J. L. BARRY, C. BONNET and J. DELORT-LAVAL, *INRA, Laboratory of Nutrition and Applied Technology, BP 527, 44026 Nantes Cédex 03*

Dietary fibre is composed of complex arrangements of sugars whose monomeric forms are known to be specifically utilized *in vitro* by the colonic bacteria to produce short-chain fatty acids (SCFA). However, the role of sugars in the formation of the individual SCFA is still unclear, especially when these sugars are arranged in complex structures in the plant cell wall. The aim of the present study was therefore to analyse the relationship between bacterial breakdown of the fibre sugars and production of the individual SCFA.

Five dietary fibres, obtained from wheat bran, maize, sugar beet, pea and cocoa, were incubated with human faecal inoculum using an *in vitro* fermentation system (Guillon *et al.* 1992), for 6, 12 and 24 h. Neutral and acidic sugar composition was determined in the initial fibres and in the fermentation residues to determine the amounts of sugars which disappeared during fermentation. Concentrations of SCFA were measured simultaneously. Involvement of the fermented sugars in production of acetate, propionate and butyrate were evaluated by a stepwise multiple linear regression. The analysis led to the three following parameters: (1) a multiple correlation coefficient (R), (2) partial correlation coefficients (r), which express the extent of involvement of a sugar in formation of SCFA, (3) partial F values which are predictive of formation of SCFA at the probability level of 5%.

	Acetate		Propionate		Butyrate	
	Partial r	Partial F	Partial r	Partial F	Partial r	Partial F
Arabinose	NS		0·16	7·6	NS	
Xylose	0·33	9·2	0·11	6·8	0·23	42·8
Glucose	0·33	12·1	0·22	16·9	0·08	6·6
Uronic acids	0·72	51·0	NS		0·11	11·1
Multiple R	0·94		0·92		0·88	

NS, not significant.

The results indicate that production of acetate was mainly due to fermentation of uronic acids and, to a lesser extent, of xylose and glucose. These three sugars were also involved in formation of butyrate, but xylose was the best predictor. Finally, breakdown of glucose together with arabinose and xylose significantly accounted for formation of propionate. In conclusion, production of the individual SCFA greatly depends upon the chemical nature of the fibre sugars are utilized during the fermentation.

Guillon, F., Barry, J.-L. & Thibault, J.-F. (1992). *Journal of the Science of Food and Agriculture* (In the Press).

Enumeration of bacteria forming acetate from H_2 and CO_2 and other H_2-utilizing micro-organisms from the digestive tract of animals and man. By J. DORÉ[1], B. MORVAN[1], P. POCHART[2], I. GODEREL[3], F. RIEU-LESME[1], G. FONTY[1], J.-C. RAMBAUD[3] and P. GOUET[1], [1]*Laboratoire de Microbiologie, INRA CR Clermont-Theix, 63122 Saint-Genès-Champanelle,* [2]*CNAM, Chaire de Biologie en vue des Applications, 75003 Paris* and [3]*INSERM U290, Hôpital Saint Lazare, 75010 Paris*

Hydrogen is an important intermediate in the anaerobic breakdown of organic matter in the digestive tract of animals and man. It is produced by major hydrolytic and fermentative micro-organisms *in vitro* and a number of studies have shown that it may be re-utilized *in vivo* by inter-species H_2 transfers. Three microbial groups may contribute to H_2-recycling in the rumen and hindgut: methanogenic archaea (MA), sulphate-reducing bacteria (SR) and acetogenic bacteria (AB). When MA and SR have been estimated simultaneously the importance of acetogenesis has been inferred from labelling techniques which have shown the incorporation of $^{14}CO_2$ into acetate in the presence of H_2 (Breznak & Kane, 1990). As such no comparable technique for the concomitant enumeration of all three microbial groups has been proposed so far.

The aim of the present work was therefore to develop appropriate conditions to obtain comparable estimates of populations of MA, SR as well as AB in digestive ecosystems. We have used minor adaptations of the culture media of Miller & Wolin (1983) and Pfennig *et al.* (1981) for the selective enumeration of H_2-utilizing MA and SR, respectively. Similarly, we have applied a selective enrichment technique to the enumeration of H_2:CO_2-utilizing AB. The method proposed allows the most probable number estimate of the population level of AB based on the difference in acetate production for dilutions incubated under a test atmosphere (4:1) of H_2-CO_2 (2×10^5 Pa) *v.* a control N_2-CO_2 gas phase, in the presence of a specific inhibitor of MA. We give examples of its application to the comparative enumeration of H_2-utilizing MA, SR and AB from the digestive tract of various animals (six sheep, six pigs) and man.

Our population estimates of MA were consistent with previous findings. This included the observation of human faecal MA counts (N) within a very wide range (logN/g from $<2{\cdot}0$ to $>9{\cdot}0$), while MA were dominant in the rumen of adult sheep and the colon of pigs. Wherever comparisons were available, H_2:CO_2-utilizing SR populations were also consistent with previous observations, except for the human colon where population estimates were nonetheless in the range of reported lactate-utilizing SR populations. Where tested, SR were rather stable in population within a given species, including between sites or subjects for which MA populations differed by 2 log units or more.

H_2-utilizing AB could be enumerated using the proposed technique within all the ecosystems tested. Control acetate production under N_2-CO_2 was commonly below 10 mM, while test acetate concentrations under H_2-CO_2 reached 100 mM or more. Using a threshold value the upper limit of the 95% confidence interval of acetate concentrations under N_2-CO_2, test incubations under H_2-CO_2 at the corresponding dilutions could be considered positive or negative. The consistency of observations allowed the estimation of the most probable number for H_2-dependent acetogens. Relatively low levels of AB were observed in the various ecosystems tested (logN/g $<6{\cdot}0$ for sheep rumen and hindgut of pigs), except for faeces from several non-methane-excreting human individuals in which AB were the dominant H_2-utilizing microbial population observed (logN/g as high as 8·5). We feel this technique should prove useful in giving a more complete picture of the relative importance of the three main microbial groups potentially competing for H_2 in the digestive ecosystem. The isolation of AB enriched by the technique described is under way. Finally, this method is being compared to the classical anaerobic technique using pure cultures of AB.

Breznak, J. A. & Kane, M. D. (1990). *FEMS Microbiology Reviews* **87,** 309–314.
Miller, T. L. & Wolin, M. J. (1983). *Applied and Environmental Microbiology* **45,** 317–318.
Pfennig, N., Widdel, F. & Trüper, H. G. (1981). In *The Prokaryotes*, pp. 926–940 [M. P. Stolp, H. G. Trüper, A. Balows and H. G. Schlegel, editors]. Springer-Verlag.

Competition for hydrogen between methanogenesis and hydrogenotrophic acetogenesis in human colonic flora studied by ^{13}C NMR. By A. BERNALIER[1], E. DOISNEAU[2], C. CORDELET[1], P. BEAUMATIN[1], M. DURAND[1] and J. P. GRIVET[2], [1]*Laboratoire de Nutrition et Sécurité Alimentaire, INRA, 78352 Jouy-en-Josas Cédex* and [2]*Département de Physique, Université d'Orléans, 45071 Orléans Cédex*

In the human colon, hydrogen produced by fibre fermentation could be used by methanogenic bacteria (MB), sulphate-reducing bacteria (SRB) or hydrogenotrophic acetogenic bacteria (AcB). In order to study the competition for H_2 between MB and AcB, we investigated the effect of a specific methane inhibitor, 2-bromoethane sulphonic acid (BES), on $^{13}CO_2$ incorporation into short-chain fatty acids (SCFA) by human faecal bacterial suspensions.

Human faeces were collected from two CH_4-producing (A and B) and one non-methanogenic (C) adult volunteers. A continuous culture system was inoculated with flora A and adapted to starch. At the end of the experiment, the flora were collected and incubated in presence of $NaH^{13}CO_2$ under H_2:N_2(4:1) as described by Durand *et al.* (1992). Washed bacterial cell suspensions were obtained directly from faeces B and C by differential centrifugations and mixed with phosphate buffer (pH 5·7) containing trace-elements, vitamins, resazurin, $(NH_4)_2SO_4$ and NaCl. After addition of 100 mM $NaH^{13}CO_2$, the flasks were incubated at 39° for 40 h under 80% H_2 in the absence and presence of 20 mM BES. Reductive acetogenesis activity was measured by ^{13}C NMR.

		Acetate (mM)					
		Single-labelled	Double-labelled	Total	^{13}C acetate (% total)	CH_4 (mM)	H_2 utilized (mM)
A:	− BES	1·3	0·5	41·6	4·3	101·3	381·7
	+ BES	13·7	6·1	66·8	29·6	0·0	208·0
B:	− BES	2·4	2·0	18·7	23·2	58·6	290·0
	+ BES	4·6	3·7	25·0	33·2	0·0	173·0
C:	− BES	21·0	8·4	93·7	31·4	0·0	235·0
	+ BES	19·8	9·2	80·7	35·8	0·0	225·0

The addition of BES to the methanogenic flora increased the proportion of $^{13}CO_2$ incorporated into acetate (Table). In non-methanogenic flora (C) this incorporation was high and similar with or without BES. Some ^{13}C labelling was observed in butyrate but BES had only a small effect.

The present results suggest that competition for H_2 exists between AcB and MB in the human large intestine. The acetogenic activity is related to the intensity of the methanogenic one. When no CH_4 is formed, AcB seem to play an important role in the re-utilization of H_2.

Durand, M., Cordelet, C., Hannequart, G. & Beaumatin, P. (1992). *Proceedings of the Nutrition Society* **51**, 6A.

The intestinal adaptive response of the rat to soluble non-starch polysaccharides is partially dependent on dietary fat. By J. D. PELL, J. M. GEE and I. T. JOHNSON, *AFRC Institute of Food Research, Norwich Laboratory, Norwich Research Park, Colney, Norwich NR4 7UA*

An increased rate of mucosal cell turnover is one manifestation of gastrointestinal adaptation to diet, but the regulatory mechanisms are poorly understood. In previous work we observed that viscous non-starch polysaccharides stimulate cell proliferation throughout the intestinal tract of the rat (Johnson & Gee, 1986). In the present study we questioned whether this effect was dependent on the lipid content of the diet.

In the first experiment, sixty animals were allocated to three groups of twenty and fed on a fibre-free semi-synthetic diet (FF) or diets containing 100 g of either insoluble cellulose (C) or guar gum (GG) kg. These groups were themselves divided into subgroups of ten whose diets contained lipid (maize oil) at either 40 or 80 g/kg. After 28 d the rats were killed and crypt cell production rate (CCPR) was determined by the metaphase-arrest technique (Johnson & Gee, 1986). Ileal CCPR in the FF and C groups was higher in rats receiving maize oil at 80 g/kg, and CCPR was higher still in rats fed on GG. In a second experiment, forty rats were allocated to two groups of twenty receiving either C or GG, and the groups were again subdivided to receive either maize oil (80 g/kg) or linoleic acid (10 g/kg). The trophic effect of GG occurred at the low level of lipid intake. However, the highest CCPR occurred in animals fed on GG and maize oil (80 g/kg). The combined effects led to a three–fourfold higher rate of mucosal cell proliferation in the colon.

Crypt cell production rate (mitoses/crypt per h)

	Ascending colon		Descending colon	
	Mean	SD	Mean	SD
Cellulose (low fat)	3·02[a]	0·29	2·75[a]	0·31
Cellulose (normal fat)	7·14[b]	0·65	5·54[b]	0·60
Guar gum (low fat)	7·68[b]	0·77	3·91[a]	0·55
Guar gum (normal fat)	10·80[c]	0·61	8·40[c]	0·44

[a,b,c] Values with different superscripts differ significantly (*t* test for least-squares regression): $P<0·05$.

These findings suggest that the GG and dietary lipid exert independent trophic effects on intestinal mucosa which may combine synergistically. The relevance of these findings for humans remains to be determined but diets rich in both fat and soluble dietary fibre may have a potentially adverse effect on colonic mucosal cell proliferation.

Johnson, I. T. & Gee, J. M. (1986). *British Journal of Nutrition* **55**, 497–505.

Establishment of *Bifidobacterium bifidum* in the intestine of human neonates: relationship with the bifidus-factors found in the stools. By S. HUDAULT[1], C. BRIDONNEAU[1], P. RAIBAUD[1], C. CHABANET[1] and M. F. VIAL[2], *Ecologie et de Physiologie du Système Digestif,* [1]*Biométrie, INRA, 78350 Jouy-en-Josas* and [2]*Hôpital Béclère, 92150 Clamart*

The aim of the present work was to study the relationship between the establishment of a strain of *Bifidobacterium bifidum (Bb)* requiring human milk bifidus-factors *in vitro* and the presence of bifidus-factors either in the faeces of human neonates or in the diet during the first 2 weeks of life.

A group of thirty-one babies received a single dose of 5×10^8 *Bb* and 5×10^8 spores of transit marker (*Bacillus stearothermophilus*) on 1–8 d after birth. This experimental procedure was agreed by the ethical committee of the hospital. Selective counts of *Bifidobacterium*, *Enterobacteria*, spores of transit marker and *Streptococci* were performed. *Bifidobacterium* strains were isolated and checked for their ability to utilize human bifidus-factors *in vitro*. Bifidus-factor amounts were evaluated in each stool and in the milk received by each baby as described by Neut *et al.* (1981).

Transit marker was quickly eliminated from the digestive tract since about 10^3 spores were detected between 3–6 d post-inoculation. In only six babies out of thirty-one, was *Bb* found in the dominant flora ($>3\times10^7$ cfu/g) on days 3–6 post-inoculation, while it was detected $<10^4$ cfu/g in seventeen out of thirty-one babies. Amounts of bifidus-factors in the faeces of babies, whether harbouring *Bb* or not, were not different. These amounts were significantly higher in breast-fed babies than in formula-fed babies on days 2–6 post-inoculation. However, *Bb* became established in only one out of eighteen breast-fed babies *v.* five out of thirteen formula-fed babies. The populations of *Enterobacteria*, *Streptococci* and other *Bifidobacterium* already present on days 0 and 1, and the rate of elimination of a transit marker, were not different whatever the population size of *Bb*. A correspondence analysis was performed, classifying babies according to microbial and individual variables in a contingency table. No relationship was found between the amounts of bifidus-factor in the faeces and the establishment of *Bb*. Formula-feeding and age on the day of inoculation were two variables correlated to a high population level of *Bb*.

	Bifidus factor V titres† in faeces of babies									
	Harbouring *B. bifidum* ($\log_{10}$cfu/g)						Fed with			
	>7·5 (*n* 6)		7·5–4·0 (*n* 8)		4·0 (*n* 17)		human milk (*n* 18)		formula (*n* 13)	
Days post-inoculation	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
0	1·8	0·3	2·2	0·2	2·0	0·3	1·9	0·2	1·8	0·2
1	2·1	0·2	1·9	0·2	1·8	0·2	1·9	0·2	1·8	0·2
2	1·8	0·2	ND		1·9	0·2	2·2*	0·1	1·4	0·1
3–6‡	1·7	0·3	1·7	0·3	2·1	0·2	2·2*	0·1	1·4	0·2

ND, Not done.

* Significantly different from value for formula-fed babies: $P<0·01$.

† Bifidus factor V titre is the $\log_{10}$ of the inverse of the last positive dilution, i.e. the dilution for which one can measure the diameter of the zone of growth around the wells filled with faecal preparations.

‡ Once between 3 and 6 d post-inoculation.

Hudault, S., Bridonneau, C., Ducluzeau, R. & Raibaud, P. (1991). *Microbial Ecology in Human Health and Disease* **4**, 1–9.

Neut, C., Romond, C., Beerens, H. & Catteau, M. (editors). (1981). *Current Concepts in Microbiology* 67–76 Elsevier.

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Maintaining the patterns of human milk-induced gut microflora after supplementary feeding of beikost. By BRIGITTA KLEEßEN and H.-J. ZUNFT (introduced by Chr. Barth), *Deutsches Institut für Ernährungsforschung Potsdam-Rehbrücke, Germany*

Human milk induces a unique intestinal microflora. Usually, after changing the dietary regimen either by supplementary feeding with infant foods or (beikost) or by feeding non-adapted cow's milk formula, the microflora start to change immediately and develop over a period of 1–2 years towards an adult population. A special casein formula, however, which is low in protein and minerals and high in lactose is able to stimulate the human milk effect. The question is whether beikost, low in protein, phosphate and calcium, may also maintain the micro-ecological action of human milk. We compared the effect of beikost A (<20 g protein/kg, <0·45 g phosphorus/kg, <0·40 g calcium/kg) and B (protein and mineral content above these limits) in three groups of seven infants each. The infants were fed:

up to week 17 on human milk (group 1) or a partially-adapted casein formula (groups 2 and 3),

from week 17 to week 21 additionally on beikost A (groups 1 and 2) or beikost B (group 3).

Group (*n* 7)	Age (weeks)	Milk	SF	Microflora†									
				pH		Bifido-bacteria		Bacter-oides		Entero-bacteria		H_2S-forming bacteria	
				Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	14	Human	—	5·4	0·3	10·2	0·3	8·3	1·2	8·3	0·5	4·4	1·8
	21	Human	A	5·6	0·6	9·9	0·4	8·2	0·6	8·5	0·2	5·7	1·4
2	14	Formula	—	5·7	0·6	9·8	0·5	8·4	1·1	8·4	0·4	5·2	1·9
	21	Formula	A	5·7	0·6	9·8	0·7	8·2	1·0	8·5	0·6	6·4	1·6
3	14	Formula	—	5·7	0·5	9·9	0·3	8·5	1·3	8·5	0·7	5·4	1·6
	21	Formula	B	6·3	0·4	9·2	0·6	9·7	0·8	9·2	0·6	7·3	1·9
				*				**	#	*			

SF, supplementary feeding; A, beikost A; B, beikost B.
Significantly different from group 1 (ANOVA and Student's *t* test): *P<0·05; **P<0·01.
Significantly different from group 2 (ANOVA and Student's *t* test): #P<0·05.
† Mean and SD expressed as $\log_{10}$ of colony-forming units/g of wet faeces.

After the introduction of beikost A in groups 1 and 2 there was no change in pH or marker organisms. But these variables were effected by beikost B (group 3).

The results demonstrate the possibility of preventing the usual response of the intestinal microflora after feeding of beikost and of maintaining the gut ecosystem established by human milk.

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Chemical characteristics of insoluble dietary fibre from sea-lettuce (*Ulva lactuca*). By D. JEGOU and M. LAHAYE, *Institut National de la Recherche Agronomique, Laboratoire de Biochimie et Technologie des Glucides, B.P. 527, 44026 Nantes Cédex 03*

Marine seaweeds are traditionally consumed in Asia and there is a recent interest in sea-vegetables in Europe. The particular nutritional characteristics of these plants are their low energy value, their richness in vitamins oligo-elements and dietary fibre. The marine green seaweeds of the *Ulva* genera are among the algae authorized in France for consumption as vegetables. In recent works, sea lettuce (*Ulva lactuca*) was shown to be particularly rich in dietary fibre (40%) of which 60·5% was insoluble (Lahaye & Jegou, 1993). These insoluble fibres were mainly composed of glucose with small amounts of xylose, rhamnose, uronic acids and sulphate and 27·1% protein. In this presentation we report on a more detailed chemical composition of the insoluble fibre.

Hot water-insoluble residues from *Ulva lactuca* were successively extracted by 1·0 and 4·0 M KOH. By adjusting the pH of the extracts to 4·5, protein-rich precipitates were recovered (P1, P4). Dialysis of the insoluble residues after KOH extraction allowed for the recovery of a protein-rich soluble fraction (SI) in the dialysis bag. All the extracts were analysed for sugar and amino acid composition.

Soluble fractions recovered after the pH adjustment of the alkali extracts (S1, S4) and the alkali-insoluble residues (IN) were rich in polysaccharides. S1 and S4 were made principally of rhamnose, xylose, uronic acids and sulphate whereas IN was essentially composed of glucose and xylose. S1 was richer in glucose and sulphate than S4. The latter fraction contained more uronic acids than S1 and part of these was shown by ^{13}C NMR spectroscopy to come from a linear ß-1,4-glucuronan. Glucose was the main sugar in P1 and P4 whereas uronic acids and sulphates were the major components with some rhamnose and glucose in S1. Starch in all the fractions could not account for all the glucose measured. An attempt to separate the insoluble glucan from all the other sugars in IN by extraction with 4 methyl-morpholine-N-oxide (MMNO) partially succeeded as some xylose remained associated with the MMNO-insoluble glucan fraction.

Proteins were essentially composed of aliphatic and acid amino acids. Hydroxyproline, a typical amino acid associated with cell-wall proteins (extensin), was found at relatively low levels in all but P1 and P4 fractions.

Thus, these extractions demonstrated that *Ulva lactuca* insoluble fibre is composed of several "hemicellulosic" polysaccharide populations closely associated with a glucan. The high content of protein most probably has a cellular origin although the low content of hydroxyproline may indicate the presence of extensin-like cell-wall proteins. The fate in the digestive tract and the metabolic effects of these polysaccharides and of the enzyme-resistant proteins associated with these fibres need to be further investigated.

Lahaye, M. & Jegou, D. (1993). *Journal of Applied Phycology* (In the Press).

Starch hydrolysis in the rabbit intestine and effect on fibre degradation. By T. GIDENNE and J. M. PEREZ, *Laboratoire de Recherche sur l'Elevage du lapin BP 27, 31326 Castanet*

Cell wall degradation in the rabbit caecum is affected by the starch:fibre ratio in the diet (Gidenne, 1992). Furthermore, the starch hydrolysis in the small intestine could vary according to the type of starch, thus affecting the starch quantity entering the caecum and the fermentation activity.

The aim of the present study was, therefore, to investigate variations in the concentration of residual starch at the ileum and the effect on the fibre digestibility, for four diets differing mainly in the source of the starch namely, purified maize starch (PMS), maize, barley and pea. The diets, similar in starch:neutral detergent fibre (NDF) ratio (279–280 g/kg DM) and in crude protein content (224 g/kg DM), were given (100 g/d) to four adult rabbits (3·2–3·6 kg live weight) cannulated at the terminal ileum. Ileal digesta were sampled for 30 min on three consecutive days at 5, 6 and 7 h after feeding (09.00 hours) in order to estimate residual starch level after the enzymic hydrolysis in the small intestine. NDF digestibility was measured from total faecal collection for 6 consecutive days.

Starch origin . . .	PMS	Maize	Barley	Pea	SEM	*P*
Ileal starch (g/kg DM)	3·1[a]	27·2[d]	6·4[b]	11·7[c]	1·6	0·0001
Starch digestibility (%)	0·997[a]	0·990[b]	0·998[a]	0·996[a]	0·001	0·0001
NDF digestibility (%)	0·266[c]	0·385[a]	0·290[bc]	0·332[b]	0·024	0·0001

[a,b,c,d] Means with different superscript letters were significantly different ($P<0·05$).

Faecal digestibility of starch was almost complete whatever the diet; only a slight reduction was observed for maize. Ileal starch concentration differed markedly according to starch origin. The level of ileal residual starch for the maize diet reached almost 30 g/kg DM. Starch from pea was well-degraded in spite of a high dietary inclusion level (600 g/kg DM). For these four diets, of similar fibre content, NDF digestibility was positively correlated to the level of ileal starch ($R^2=0·74$). This suggests a possible control of rabbit caecal fermentation by modifying the starch hydrolysis in the intestine through the nature of dietary starch.

Gidenne, T. (1992). *British Journal of Nutrition* **62**, 133–146.

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The adaptive response of the alimentary tract of the rat to soluble non-starch polysaccharide (NSP) components of oats. By E. K. LUND, S. R. R. MUSK and I. T. JOHNSON, *AFRC Institute of Food Research, Norwich Laboratory, Norwich Research Park, Colney, Norwich NR4 7UA*

Consumption of oats is associated with reduced postprandial glycaemia and a reduction in serum cholesterol levels. Isolated NSP is known to induce gastrointestinal adaptation in experimental animals, but there is very little in the literature relating to the effect on gut morphology of eating oats over an extended period of time. We have previously reported that rats fed on oats for 28 d have markedly higher crypt cell proliferation rates (CCPR) than those fed on a cellulose-based control diet (Lund & Johnson, 1990). This was associated with an increase in small intestinal length from 121 (SEM 1) to 129 (SEM 3) cm ($P<0{\cdot}05$). In the present study we assessed the role of soluble NSP in relation to gastrointestinal adaptation.

Previous studies (Johnson *et al.* 1984) have shown that the addition of guar gum (40 g/kg) to the diet causes an increase in CCPR which is associated with raised plasma enteroglucagon levels. Thus the changes found with oats are likely to be due to the presence of the soluble NSP, ß-glucan. To test this hypothesis we fed rats on isocalorific diets containing 50 g NSP/kg in the form of oat gum, guar gum or solkafloc. The rats fed on either form of soluble NSP showed both an increase in CCPR in the small intestine, and raised levels of enteroglucagon. These effects may arise directly from the delayed absorption of nutrients; alternatively, the rise in enteroglucagon, which is a putative gut growth hormone, may cause the increase in CCPR. The possibility that oats cause a proliferative response via fermentation of soluble fibre and consequent production of volatile fatty acids seems unlikely as there was no increase in CCPR in the caecum.

Non-starch polysaccharide . . .	None		Solkafloc		Guar gum		Oat gum	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Enteroglucagon (nmol/l)	104[a]	17	80[a]	25	170[b]	26	154[b]	24
Distal ileum CCPR (cells/crypt per h)	7·7[a]	0·8	7·5[a]	0·7	12·7[b]	0·5	16·3[b]	1·5
Caecum CCPR (cells/crypt per h)	5·6	1·1	4·0	0·7	4·3	0·9	6·4	1·8
Small intestinal length (cm)	120·6	4·1	119·6	3·9	130·3	7·1	125·7	5·4

Values with unlike superscripts are significantly different (Student's *t* test; $P<0{\cdot}05$; *n*10).

Johnson, I. T., Gee, J. M. & Mahoney, R. R. (1984). *British Journal of Nutrition* **52,** 477–487.
Lund, E. K. & Johnson, I. T. (1990). *Proceedings of the Nutrition Society* **49,** 39A.

Growth of bifidobacteria in continuous culture. By X. WANG and G. R. GIBSON (Introduced by J. H. CUMMINGS), *MRC Dunn Clinical Nutrition Centre, 100 Tennis Court Road, Cambridge CB1 1QL*

For various health-promoting reasons, an increase in numbers and activities of bifidobacteria in the colon is desirable. In many instances these bacteria are now being added to foodstuffs such as yoghurt and milk. However, it is unclear whether such additions exert any effect in the colon. Moreover, culture viability may be affected, particularly with increasing shelf life of the product. An alternative approach is to stimulate populations of bifidobacteria already resident in the gut by dietary addition of carbohydrates which may specifically favour their growth. Various substrates, including oligofructoses and inulin, are being considered in this respect. In the present study we assessed the suitability of such compounds using single-stage continuous culture systems. The chemostats (500 ml working volume) were operated anaerobically using varying dilution rates (0·08–0·3/h), pH values (5·5–7·0) and both carbon- and nitrogen-limited conditions. Substrates used in the experiments were glucose, oligofructose and inulin (160 g/l), with an inoculum of mixed faecal bacteria. Bifidobacterial counts in the chemostats ranged from 7·6–9·7 $\log_{10}$cfu/ml growth medium, with highest numbers occurring whilst oligofructose was used as the growth substrate and during conditions of high dilution rate, low pH and high C availability.

In an attempt to reproduce some of the physical and nutritional characteristics of the proximal and distal colon, a three-stage continuous culture system was inoculated with mixed faecal bacteria. The fermenters had increasing working volumes (320–500 ml) and were arranged in series. Culture medium was pumped into vessel 1 (pH 6·0) which sequentially fed vessels 2 (pH 6·5) and 3 (pH 7·0). The system was allowed to stabilize for 2 weeks, after which time additional carbohydrate (15 g/d) was pumped into vessel 1.

Initially, this was sucrose (days 0–15), followed by oligofructose (days 16–30) and sucrose (days 31–45). Samples were periodically removed to count bacteria. The addition of oligofructose caused a marked increase in populations of bifidobacteria in comparison with total anaerobes, total aerobes, lactobacilli, Gram-positive cocci, bacteroides, clostridia and coliforms, particularly in vessel 1.

Results in the present study indicate, using different *in vitro* systems, that bifidobacteria numbers may potentially be increased by simple dietary additions, and that this effect is enhanced during conditions which resemble events that occur in the proximal colon.

Growth of selected bacterial genera in a three-stage continuous culture system with sucrose or oligofructose as additional carbon source

(Values are $\log_{10}$cfu/ml and are means of triplicate determinations)

Stage of culture system . . .	Sucrose (days 0–15)			Oligofructose (days 16–30)			Sucrose (days 31–45)		
	1	2	3	1	2	3	1	2	3
Total aerobes	7·3	7·4	7·2	7·6	7·5	7·6	7·2	7·4	7·4
Coliforms	6·9	6·9	6·8	7·4	7·2	7·2	7·2	7·2	7·2
Gram+ve cocci	7·2	7·3	6·5	7·1	7·1	7·2	6·6	6·4	6·1
Total anaerobes	9·3	9·2	8·7	9·3	9·5	9·3	9·8	9·0	8·4
Bifidobacteria	6·8	6·7	6·6	8·8	7·9	7·6	6·9	6·9	6·9
Lactobacilli	5·9	5·3	5·2	5·8	5·2	6·2	6·1	4·6	5·4
Bacteroides	8·9	8·9	8·3	8·9	9·0	8·8	9·0	8·9	8·0
Clostridia	7·0	7·5	6·9	8·5	8·3	7·4	8·6	7·8	6·7

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High bacterial counts in the proximal small intestine: an unexpected finding in normal cats. By K. JOHNSTON[1], R. M. BATT[1], A. LAMPORT[1], O. BALLEVRE[2], and *E. FERN*[2], [1]*Department of Small Animal Medicine and Surgery, Royal Veterinary College, London and* [2]*Nestec Ltd. (Friskies Research), Lausanne, Switzerland*

Small intestinal bacterial overgrowth (SIBO) is emerging as an important cause of chronic diarrhoea in dogs and could potentially be a problem in cats. Since little is known about the flora of the small intestine in normal cats, baseline information is needed in order to diagnose SIBO in this species. It was the aim of the present study to quantify the bacterial flora in the proximal small intestine of normal cats and to correlate these findings with the results of a hydrogen breath test.

Studies were performed on seven clinically healthy experimental cats fed on a commercial canned cat food (25% carbohydrate, 40% protein, and 27% fat). Undiluted duodenal juice was obtained by use of a sterile plastic tube passed through an endoscope and subjected to bacteriological culture on selective media under aerobic and anaerobic conditions. Total bacterial counts ranged from $2{\cdot}2\times10^5$ to $1{\cdot}6\times10^8$ colony forming units per ml (cfu/ml) with anaerobic counts between $7{\cdot}5\times10^4$ and $1{\cdot}1\times10^8$ cfu/ml. These counts fulfil the established criteria for SIBO in other species (King & Toskes, 1979; Batt & McLean, 1987).

These same seven cats were then used to measure breath hydrogen after a meal. The cats were fasted overnight, 100 g of the same commercial canned cat food was given and exhaled breath samples were collected every 30 min for 6 h. Basal breath hydrogen concentrations were 2 (SEM 0·5) ppm increasing to 25 (SEM 6) ppm at 6 h and did not begin to rise until approximately 300 min after the meal. The timing of the rise implies that unabsorbed carbohydrate had reached the colon and was acting as substrate for colonic bacteria to produce hydrogen gas, indicative of defective absorption of carbohydrate. This may be due to the inclusion of relatively indigestible carbohydrate sources in the diet. Alternatively, compromised intestinal absorption could be due to subclinical intestinal damage by the bacteria.

The presence of relatively high bacterial counts in the duodenum of normal cats may provide an opportunity to explore relationships between small intestinal bacterial overgrowth and clinical nutrition and may have important implications for the pathogenesis of chronic small bowel disease in this species.

Batt, R. M. & McLean, L. (1987). *Gastroenterology* **93,** 986–993.
King, C. & Toskes, P. (1979). *Gastroenterology* **76,** 1035–1055.

Adaptation to high-fat diets: Effect on plasma cholecystokinin (CCK) and food intake. By S. J. FRENCH, R. FADZLIN, B. MURRAY, R. D. E. RUMSEY and N. W. READ. *Centre for Human Nutrition, University of Sheffield, Northern General Hospital, Sheffield S5 7AU*

Previous studies have shown that gastric emptying of a fatty meal is accelerated after a 2 week period on a high-fat diet (Cunningham *et al.* 1991) suggesting down-regulation of the nutrient responsive mechanisms that inhibit gastric emptying. Infusion of CCK delays gastric emptying and is thought to inhibit eating behaviour. The aim of the present study was to investigate whether feeding a high-fat diet for 2 weeks resulted in adaptive changes in plasma CCK levels and food intake.

Twelve male subjects (aged 21–40 years) took part in the study. Each completed a 2 week dietary inventory both before and after the consumption of a high-energy–high-fat diet (19·15 MJ (4579 kcal)/d; 58% of energy from fat) for 2 weeks. Formal measurements of food intake from a pre-selected appetizing evening meal were carried out immediately before and after the high-fat diet, and blood samples for bioassay of CCK were collected after a standard breakfast (beefburger, bacon, beans, tomatoes, bread and butter) the following morning.

Following the high-fat diet, there was a small non-significant increase in food intake from the pre-selected meal (6·91 (SE 0·61) *v.* 6·40 (SE 0·54) MJ (1653 (SE 147) *v.* 1530 (SE 129) kcal) $P=0·1$ (Student's paired *t* test)), and a significant increase in the average daily food consumption during the 2 weeks following the diet (10·24 (SE 0·49) *v.* 9·58 (SE 0·61) MJ (2448 (SE 117) *v.* 2290 (SE 147) kcal)/d: $P=0·05$ (paired *t* test)). Plasma CCK responses to the standard breakfast were also raised following the diet (1285·0) (SE 153) *v.* 896·7 (SE 78·2) pM/min; 3 h integrated CCK production post- *v.* pre-diet; $P<0·01$ (paired *t* test)) with the major differences observed at 90 and 120 min following the meal.

These data show that overeating for 2 weeks can lead to an adaptive increase in food consumption and in postprandial CCK responses. This suggests that the increase in food intake is not related to down-regulation of intestinal nutrient receptors but may instead be related to a down-regulation in putative CCK receptors responsible for food intake. The elevated CCK levels might suggest a corresponding down-regulation in CCK receptors responsible for feedback inhibition of CCK release. These data support the hypothesis that prolonged overeating can lead to lower satiation which in turn sustains overeating.

Cunningham, K. M., Daly, J., Horowitz, M. & Read, N. W. (1991). *Gut* **32,** 483–486.

Moderate exercise prior to ingestion of a high-fat meal decreases postprandial lipidaemia.

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Exercise may ameliorate the potentially atherogenic effects of postprandial lipidaemia by enhancing the activity of lipoprotein lipase in skeletal muscle. The purpose of the present study was to examine the influence of a prior bout of moderate exercise on postprandial lipidaemia. Eight normolipidaemic adults (four male, four female) age 26·1 (SEM 1·6) years ingested a high-fat meal on two occasions, in a balanced cross-over design. In one trial 2 h of exercise was undertaken the previous day (12–15 h before ingestion); in the other no exercise was undertaken (control). Food intake was weighed and recorded for 2 d before the first trial and replicated for the 2 d before the second trial. The test meal consisted of cereal, fruit, nuts and cream (4968 kJ, 17 g protein, 85 g fat, 95 g carbohydrate; Schlierf *et al.* 1987). Exercise consisted of cycling (1 h) and jogging/walking outside (1 h) at 60% of predicted maximal heart rate (monitored using short-range telemetry).

Subjects reported to the laboratory after an overnight fast and a capillary blood sample was obtained from a pre-warmed hand. The meal was then ingested and subjects rested quietly for the following 6 h. Further capillary blood samples were obtained 1·5, 3·0, 4·5 and 6·0 h after ingestion. Blood samples were centrifuged and plasma stored at –20° until analysed for triacylglycerol using an enzymic colourimetric method (Boehringer Mannheim). Indices of lipidaemia were compared between trials using the Wilcoxon matched-pairs test. Fasting plasma triacylglycerol concentration was lower for the exercise trial than for the control trial (0·96 (SEM 0·19) mmol/l *v.* 1·25 (SEM 0·09) mmol/l; $P<0.05$), as was peak concentration (1·63 (SEM 0·21) mmol/ *v.* 2·21 (SEM 0·26) mmol/l; $P<0·01$) and the total lipidaemic response determined as the area under the triacylglycerol concentration *v.* time curve, normalized to zero h level (2·67 (SEM 0·61) mmol/l × h *v.* 3·80 (SEM 0·95) mmol/l × h; $P<0·01$). We conclude that prolonged moderate exercise reduces fasting plasma triacylglycerol concentrations and postprandial lipidaemia in healthy adults.

Schlierf, G., Dinsenbacher, A., Kather, H., Kohlmeier, M. & Haverbosch, W. (1987). *Metabolism* **36**, 726–730.

Tracer investigation of taurine metabolism in cats. By O. BALLÈVRE, C. PIGUET, A. STAEMPFLI, G. L. CZARNECKI and K. ACHESON, *Nestec Ltd. (Friskies Research), P.O. Box 44, CH-1000 Lausanne 26, Switzerland*

Taurine is an essential amino acid for domestic cats because of its high requirement for bile acid conjugation and limited *de novo* synthesis. Although no significant degradative pathways have been described in mammals, dietary requirement for taurine appears to be two–three times higher than its excretion in urine. Therefore, to investigate the physiological basis of dietary taurine requirements in cats, we studied its metabolism using a bolus dose of [^{15}N]taurine.

Domestic short hair cats were randomly assigned to two groups fed on either dry (*n* 8) or canned (*n* 6) food containing adequate taurine concentration (1000 ppm and 2000 ppm respectively). After 6 weeks adaptation the animals were given one meal of their respective diets to which [^{15}N]taurine had been added before processing. Samples of urine collected daily over the next 10 d were analysed for ^{15}N enrichment of taurine (by Fast-Atom-Bombardment Mass Spectrometry) and of total nitrogen, ammonia and urea (by Elemental Analyser–Isotope Ratio Mass Spectrometry). The enrichment of each compound in the daily pooled urine sample was assumed to reflect the mean enrichment of the whole-body pool on this day.

Taurine kinetics were calculated using stochastic analysis of a one-pool model at steady-state in which taurine production rate (TAU PR) was taken as being equal to: (a) taurine intake (I) + taurine synthesis (S); (b) taurine urinary excretion (E) + taurine degradation (D); (c) taurine disposal rate (TAU DR). Taurine conversion to urea was calculated from taurine and urea enrichment, assuming that urea excretion reflected urea flux.

Taurine half-life was 3·8 d in both groups. TAU PR was significantly lower in dry- than in canned food-fed animals (99 (SEM 6) and 267 (SEM 62) μmol/kg per day respectively). These values represented approximately 90% of taurine intake for both groups indicating that endogenous synthesis was almost zero and that absorption rate was between 80 and 90% of the intake. The percentage of taurine intake excreted in urine was significantly higher in the dry than in the canned diet group (55 (SEM 4) and 35 (SEM 3) % respectively), but the absolute excretion rate was almost equivalent. Taurine degradation (TAU PR –E) was also significantly higher with the canned diet. More than 70% of this degradation occurred by formation of urea in both cases. Very little ^{15}N was excreted in ammonia in the urine in either groups. These preliminary results suggest that as there are no known endogenous pathways for taurine in the cat, gut bacterial flora may be responsible for the degradation observed. There were also indications that the composition of the diet played an indirect role in this degradation.

The chronic and acute effects of tryptophan (TRP) on hormones of the enteroinsular axis.

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Adaptation to different diets causes modifications in the responses of gastric inhibitory polypeptide (GIP), insulin and glucose (Ponter *et al.* 1991). TRP stimulates GIP and insulin secretion in the rat (Tsiolakis & Marks, 1984). The present study was designed to investigate whether TRP has acute and chronic effects on hormones of the entero-insular axis in the piglet.

Eighteen piglets weaned at 12 d were used. Within each litter, two dietary regimens were represented. A semi-purified diet was formulated to be deficient in TRP, with respect to requirements, (T1; 1·63 g/kg) and a further diet was produced by the addition of L-TRP to the basal diet (T2; 2·62 g/kg). The diets were given at the same energy level by intragastric catheter in liquid form (feed:water, 1:3). After adaptation to the dietary regimens, two acute intragastric loads were given: a glucose load (3·28 g glucose/kg$^{0.75}$) and a glucose plus tryptophan load (3·28 g glucose/kg $^{0.75}$ and 0·0441 g TRP/kg$^{0.75}$). The intragastric loads were standardized on a volume and osmolarity basis. Blood samples were taken from an indwelling jugular catheter at intervals for 180 min and analysed for insulin, GIP and glucose.

The log transformed mean integrated GIP, insulin and glucose concentrations are shown in the Table.

Infusion . . .	Glucose plus TRP		Glucose			
Diet . . .	T1	T2	T1	T2	RSD	*P* value
GIP (ng/l per min):						
0-15 min	7·86^{b}	8·21^{a}	7·78^{bc}	7·60^{c}	0·18	<0·001
0-180 min	7·99^{a}	8·15^{a}	7·73^{b}	7·66^{b}	0·18	<0·001
Insulin (mU/l per min):						
0-180 min	2·57	2·70	2·59	2·86	0·47	NS
Glucose (nmol/l per min):						
0-180 min	1·74^{b}	1·78^{b}	1·90^{a}	1·87^{a}	0·14	<0·05

^{a,b,c} Mean values with unlike superscripts were significantly different (ANOVA).

Plasma GIP concentrations were affected both acutely and chronically while glucose concentrations were affected acutely by TRP. Adaptation to a TRP-supplemented diet appeared to sensitize the GIP response to TRP. The addition of TRP to the glucose load reduced plasma glucose while it increased GIP. GIP may have potentiated the disposal of the glucose after the test or TRP may have had a direct effect on glucose disposal. In this experiment under hyperglycaemic conditions TRP did not increase insulin concentrations.

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Tsiolakis, D. & Marks, V. (1984). *Hormone and Metabolic Research* **16** 226–229.

Differential effects of dietary proteins on protein synthesis activity in several tissues of newborn lambs. By P. PATUREAU MIRAND, M. C. VALLUY, L. MOSONI and G. BAYLE, *Laboratoire d'Etude du Métabolisme Azoté, Institut National de la Recherche Agronomique, 63122 Theix*

Colostrum feeding results in higher protein synthesis activity than milk feeding in the ileum and skeletal muscles of piglets (Burrin *et al.* 1992) and in the small intestine of newborn lambs (Patureau Mirand *et al.* 1990), but not in the liver in either species. Our aim was to determine whether these differential effects could be related to protein intake or quality. We compared protein synthesis activity in small intestine, liver, longissimus dorsi, lung, spleen and pancreas of newborn lambs fed either bovine colostrum (BC), bovine milk (BM) or bovine milk supplemented (37 g/l) with whey protein hydrolysate (MW) or casein hydrolysate (MC). For each diet, a total amount of 660 g divided into six meals was fed to four lambs from the first hour to 17 h after birth. Protein synthesis activity was determined when lambs were 18 h old by measurement of [^{3}H]-valine incorporation according to the flooding-dose procedure. Absolute synthesis rates (ASR) in the small intestine and fractional rates (FSR) of protein synthesis in the other tissues are given in the Table. In the small intestine of newborn lambs, FSR was not suitable as a measure of protein synthesis because of colostral protein (IgG) retention in the wall. Therefore we calculated ASR.

Diet . . .	BC	BM	MW	MC	SE
Protein intake (g/kg BW) . . .	13·2	2·75	5·22	5·22	
Small intestine*	57·9^a	33·4^b	57·5^a	45·3^c	7·0
Liver†	90·3^a	70·4^b	83·0^{ab}	96·3^a	10·7
Longissimus dorsi†	15·1^a	15·6^a	21·4^b	22·0^b	2·7
Lung†	39·1^a	36·7^a	46·6^b	37·6^a	3·8
Spleen†	53·4^a	37·1^b	48·7^a	43·0^{ab}	6·6
Pancreas†	168	203	202	185	33

* ASR (μmol valine incorporated/h per kg body-weight); † FSR (%/d).

^{a,b,c} Values in the same row with unlike superscripts were significantly different (Duncan's multiple range test.) ($P<0·05$).

Milk supplementation with whey hydrolysate increased protein synthesis in small intestine and lungs to levels which were as high (small intestine) as or higher (lungs) than with colostrum. Casein supplementation also stimulated protein synthesis in liver to levels at least as high as those seen with colostrum. Feeding milk supplemented with either hydrolysate increased protein synthesis in muscle relative to colostrum or milk feeding. No dietary effects could be detected on FSR in pancreas. These data show that protein synthesis activity in tissues is differentially affected by protein intake and quality in newborns.

Burrin, D. G., Shulman, R. J., Reeds, P. J., Davies, T. A. & Gravitt, K. R. (1992). *Journal of Nutrition* **122,** 1205–1213.

Patureau Mirand, P., Mosoni, L., Levieux, D., Attaix, D., Bayle, G. & Bonnet, Y. (1990). *Biology of the Neonate* **57,** 30–36.

Nutritional value of lentil and effect of cooking on free amino acid tissue concentrations of growing rats. By E. COMBE, *Laboratoire d'Etudes du Métabolisme Azoté INRA Theix – 63122 Ceyrat* and M. CVIRN; *Instituta za prerhano, Oddelek za zivinorejo, Universa v Ljubljana, Slovenie*

Lentil seeds (var. Anicia) provide 280 g protein (N × 6·25) kg together with slow digestible sugars, fibres, minerals, and low level of antinutritional factors. In order to check the nutritional value of this legume, either raw or cooked lentils were used as the only food protein in the experimental diet and compared to the casein control diet. The diets were isoenergetic (18·8 MJ/kg dry matter). The apparently digestible protein content was slightly higher in the lentil diets than in the casein diet (145 *v.* 122 g/kg DM) to meet most of the amino acid requirements. Additional essential amino acids were used to balance the amino acid supply. Thirty male Sprague Dawley rats (*n* 10, body-weight 74 (SE 4) g for each group) received *ad lib.* one of the diets for 17 d. The growth rate and DM intake were measured for the last 10 d and N balance determined for the last 5 d. At the end of the experimental period, blood and muscle free amino acids were determined.

Growth rates and DM intakes were not significantly different between the lentil or casein groups. However, the protein efficiency ratio was lower in the case of the raw (1·91 (SE 0·17)) and cooked (2·09 (SE 0·22)) lentil diets than that of the casein controls (3·17 (SE 0·31)). Apparent digestibility of N from lentil also was lower than that from casein. This has already been shown with other legumes (Combe *et al.* 1991).

In blood, the high levels of free arginine and glycine found in the two lentil groups were in agreement with their levels of digestible intake. In contrast, the low levels of free lysine, histidine and methionine in the blood were not in good agreement with their digestible intakes. The present results raise the suspicion that some of these essential amino acids are not fully available for the circulating pool.

	Raw lentil		Cooked lentil		Casein	
Muscle free AA (mg/g)	Mean	SE	Mean	SE	Mean	SE
TAA	115[2]	20	184[b]	22	126[a]	5
EAA	64[a]	15	113[b]	19	74[a]	6
Glycine	25[a]	2	39[b]	5	24[a]	3

[a,b] Values with unlike superscripts were significantly different (ANOVA); $P<0·05$. TAA, sum of free amino acids; EAA, sum of threonine, lysine, valine, leucine, isoleucine and arginine.

The Table shows that cooking the lentils modified the free amino acid concentrations in muscles. The concentrations of threonine, lysine, arginine and branched-chain amino acids together with glycine were significantly increased in the group fed the cooked lentils compared with the raw lentil group. This suggests that free amino acid concentrations in the muscle, which are mostly intracellular, are more sensitive to cooking-induced modifications of seed components than the systemic free amino acid concentrations.

Combe, E., Achi, T. & Pion, R. (1991). *Reproduction, Nutrition, Développement* **31**, 631-646.

Metabolic characteristics of isolated colonocytes: modulation by dietary fibre or by the colonic microflora. By C. CHERBUY, B. DARCY-VRILLON, M. T. MOREL, A. BORLET, F. BLACHIER and P. H. DUEE, *Unité d'Ecologie et de Physiologie du Système Digestif, INRA, F78352 Jouy-en-Josas*

The metabolism of energy-yielding substrates in colonic epithelial cells and its possible variations have not been extensively documented. The objective of the present work was to assess the effect of the following factors on colonocyte metabolism: dietary fibre level (high *v.* low) in the pig, and bacterial status (germ-free *v.* conventional) in the rat.

Colonocytes from pigs or rats previously accustomed to their diets were isolated by a method adapted from Roediger & Truelove (1979) using EDTA and hyaluronidase (*EC* 3.2.1.35) buffers, and incubated (37°, 30 min) with ^{14}C- or ^{3}H-labelled substrates. Cell viability was high at the onset of incubation (90 (SEM 1)% in the pig, *n* 22; 82 (SEM 2)% in the rat, *n* 12), and metabolic parameters remained linear for at least 60 min at 37°.

In all cases, the capacity to utilize n-butyrate (10 mM) was 0·4–0·6 nmol/min and 10^6 viable cells. The capacity to convert butyrate into $^{14}CO_2$ was 2–3 times higher than that measured for glutamine (5 mM) or glucose (5 mM). Moreover, butyrate oxidation accounted for 45–65% of butyrate metabolism, the remainder being represented by ketone body generation.

In pig colonocytes, glucose and glutamine were extensively utilized (respectively 1 and 2 nmol/min and 10^6 viable cells), but predominantly not for oxidative purposes. With both diets glycolysis was the major pathway responsible for glucose disappearance, but the glycolytic capacity was 25% lower in pigs fed on the high fibre diet. Whatever the diet, butyrate exerted a sparing effect on both glycolysis and glucose oxidation. Glutamine metabolism was accompanied by ammonia, glutamate, aspartate, and alanine production, and was only slightly affected by dietary fibre.

In rat colonocytes, the capacity for glucose utilization, measured with [5-^{3}H] glucose, was decreased by 40% in germ-free animals, accompanied by a corresponding decrease in the glycolytic capacity. Conversely, the capacity for glutamine utilization, and ammonia production was increased by 40% in germ-free rats, though glutamine oxidation was not affected.

Taken together, these data suggest that the metabolic substrates studied play specific roles in colonocytes. The oxidative capacity for butyrate remained high whatever the nutritional or bacterial status, whereas the capacity for glucose or glutamine utilization could be modulated. We hypothesize that these modulations respond to specific needs in the cells that are presently under investigation.

Roediger, W. E. W. & Truelove, S. C. (1979). *Gut* **20**, 484–488.

Alteration by lymphokines of the epithelial function and barrier capacity of the human colon carcinoma cell line HT29-C119A. By M. HEYMAN, A. HIRRIBAREN, A. L'HELGOUAC'H and J. F. DESJEUX, *INSERM U290, Hôpital St Lazare, 75010 Paris*

In various intestinal diseases, particularly food protein intolerance in infants, lymphocyte infiltration of the lamina propria might be an important factor causing intestinal dysfunction via lymphokine release. The effects of such lymphokines were therefore measured on chloride secretion and macromolecular transport in intestinal HT29-C119A cells. Cells were grown on transwell filters and serosally exposed for periods of 2, 24 or 48 h to phytohaemagglutinin-activated (or non-activated) human lymphocyte culture supernatants used at 1 or 5% (v/v) concentration. The concentrations of interferon γ, tumour necrosis factor α and interleukin-6 in supernatants were (4·72 (SD 1·63), 3·17 (SD 0·14) and 4·47 (SD 1·12) ng/ml respectively). The intestinal function was studied in Ussing chambers; chloride secretion was assessed as the variations of short-circuit current (I_{sc}) and the barrier capacity was monitored by the electrical resistance of the monolayers (R) and intact (Ji) or degraded (Jd) HRP fluxes. Lactate dehydrogenase (LDH) release in the presence of various concentration of lymphokines was assessed as a test of cellular injury.

The results indicated that lymphokines do not directly stimulate the electrogenic chloride secretion by HT29-C119A cells as shown by the lack of increase in I_{sc} after serosal exposure to lymphokine-enriched supernatants. However, long-term (48 h) stimulation led to significant decrease in I_{sc} (6·79 (SD 0·65) *v.* 2·90 (SD 1·16) $\mu A/cm^2$ in control and treated cells respectively) and to a degradation of the barrier capacity as attested by the decrease in R (158 (SD 10) in controls to 36 (SD 9) ohms/cm^2) and the increase in Ji (167 (SD 33) in controls to 874 (SD 206) ng/h per cm^2 in lymphokines-treated cells) and Jd (1113 (SD 114) to 2327 (SD 234) ng/h per cm^2).

This decreased barrier capacity was associated with an increase in LDH release (141 (SD 8) and 283 (SD 13) UI/h per cm^2 in 1 and 5% lymphokine-treated monolayers *v.* 70 (SD 14) UI/h per cm^2 in control monolayers).

The results indicate that lymphokines do not directly stimulate the intestinal chloride secretion but decrease the electrolyte transport and the barrier capacity to macromolecules via cellular and paracellular pathways after a prolonged exposure.

Interleukin-2 and T-lymphocytes in mice fed on a legume diet. By J. LARRALDE, M. L. ESPARZA and J. A. MARTINEZ, *Department of Physiology and Nutrition, University of Navarra, 31008 Pamplona, Spain*

Immune response depends upon nutrient intake, therefore immunocompetence has been repeatedly used to assess nutritional status under different nutritional situations (Chandra, 1992). On the other hand, feeding animals on diets containing legumes as the main source of protein may induce some physiological, biochemical and immunological disturbances, which have been attributed to the occurrence of some anti-nutritional factors and to a low utilization of their high protein content (Larralde & Martinez, 1989). In this context, the present trial was focused on the study of the influence of a legume diet (peas) on cell-mediated aspects of immunity.

Male Swiss mice weighing about 35 g were assigned in two dietary groups, which were fed on casein (control) or peas (legume) as the only source of protein; the diets were balanced for principal nutrients. The percentage of spleen T-lymphocytes after labelling with monoclonal antibody conjugated with fluorescein isothiocyanate (FITC) was assayed by flow-cytometry, while the measurement of interleukin-2 (IL-2) involved a bioassay employing an IL-2-dependent CTTL-2 cell line (Hudson & Hay, 1989).

	Control		Pea	
	Mean	SEM	Mean	SEM
Average daily gain (g/d)	0·33	0·03	0·23*	0·03
Spleen (% body-wt)	0·61	0·05	0·36***	0·05
Thymus (% body-wt)	0·06	0·01	0·09**	0·01
T-lymphocyte (%)	41·2	2·4	54·7*	3·9
IL-2 (U/10^6 cells)	5·94	0·95	1·10***	0·51

Significantly different from control values: *$P<0·05$, **$P<0·01$, ***$P<0·001$.

No differences in food intake were found. Our measurements indicate that both growth rate and spleen weight were markedly depressed in the legume-fed mice, while thymus weights were significantly increased, suggesting a direct effect on the immune organs.

IL-2 is essentially an autocrine immunoregulator produced by T-cells which is involved in the maintenance and proliferation of different cell types of the immune system (Hudson & Hay, 1989). Our data show that IL-2 values are lower in those animals fed on diets containing the legume compared with controls, as has been previously found in experimental zinc deficiency (Moulder & Steward, 1989) and dietary protein deprivation (Chandra, 1992). Also an increase in the proportion of T-cells has been reported to be increased in dietary-restricted animals (Hishinuma *et al.* 1990). Our results could be ascribed to the occurrence of some anti-nutritional factors contained in the legume diet with a specific effect on the immune response, or to a lower protein utilization. Where results different from ours have been obtained the reason may be differences in diets and/or duration of treatment (Chandra, 1992).

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Antigenic soya-bean protein digestion in the dairy calf at weaning. By H. M. TUKUR, J. P. LALLES and R. TOULLEC, *Laboratoire du Jenne Ruminant, INRA, 65 rue de Saint-Brieuc, 35042 Cédex*

Glycinin (Glyc) and β-conglycinin (βcong), major globulins of soya-bean meal (SBM), appear to induce digestive disturbances in preruminant calves fed SBM milk replacers and are resistant to digestion (Sissons, 1982). In ruminating calves, systemic antibodies have been reported, and a preliminary study has indicated the survival of these antigens along the digestive tract (Lallès *et al.* 1991). In the present work an attempt was made to quantify those antigens that escape digestion along the gut of the ruminating calf.

Five fistulated Holstein male calves were fed on milk up to 6 weeks of age, then they were weaned onto a concentrate-rich diet containing SBM between 7–10 weeks (Lallès & Poncet, 1990). Thereafter the SBM diet was exclusively fed up to 20 weeks. When dry feed was first offered at 7 weeks calves were given 600 ml rumen contents from mature sheep via a rumen cannula. Digesta were collected throughout the trial and immunoreactive Glyc and βcong were assayed by ELISA (Lallès *et al.* 1991).

The duodenal flows of both Glyc and βcong were very low right from the time of weaning, and decreased to even lower levels after weaning. The amounts of these proteins reaching the ileum and faeces, though detectable, was so low that it may have no nutritional significance. In fact the ileal flow of Glyc represented about 0·02% of ingested Glyc compared to the value of 10% reported for preruminant calves (Mathis *et al.* 1992). This could be attributed to the efficiency of the rumen in degrading dietary proteins from an early age.

			Flow (% of intake)					
	Intake (g/d)		Duodenal		Ileal		Faecal	
Age (weeks)	Glyc	βcong	Glyc	βcong	Glyc	βcong	Glyc	βcong
9–10	3·9	1·4	0·03	9·1	0·01	0·03	0·003	0·010
15–16	7·1	2·6	ND	0·1	0·03	0·11	0·004	0·010
19–20	11·0	4·1	ND	1·4	0·02	0·05	0·005	0·010
SEM	0·8	0·3	0·10	2·0	0·004	0·01	0·001	0·003

ND, not detected (detection limit of ELISA: 0·5 μg/g DM).

In conclusion, much less antigenic soya-bean protein escapes digestion and reaches the intestine in the ruminant than in the preruminant calf. This small amount of immunoreactive protein could lead to the stimulation of the immune system (Lallès *et al.* 1991), but it may not be enough to provoke the usual hypersensitive reactions observed in preruminant calves.

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Pancreas enlargement induced in rats by long-term consumption of dietary legume proteins. By G. GRANT, P. M. DORWARD, S. BARDOCZ and A. PUSZTAI, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Short-term exposure of young rats to dietary soya bean (*Glycine max*) or kidney bean (*Phaseolus vulgaris*) results in rapid enlargement of the pancreas (Pusztai, 1989; Grant, 1990). This is due to the action of seed lectins and trypsin inhibitors in the diet. In the present study the long-term effects of consumption of diets containing different levels of these anti-nutritional factors upon rat pancreatic weights were evaluated.

Hooded Lister rats were pair-fed for up to 800 d on diets (100 g protein/kg) containing lactalbumin (control), raw soya-bean (2·4 g Kunitz trypsin inhibitors, 0·8 g Bowman-Birk trypsin inhibitors and 0·6 g lectin/kg diet) or lupin seed (*Lupinus angustifolius*; negligible levels of trypsin inhibitor or lectin) as the sole source of dietary protein. Kidney bean (0·4 g Bowman-Birk trypsin inhibitors and 2·4 g lectin/kg diet) comprised 17% of the protein in a fourth diet with the remainder being supplied by lactalbumin.

Enlargement of the pancreas occurred in young rats within 24 h of exposure to dietary soya bean and this growth, due to hypertrophy and hyperplasia, continued for up to 150 d. During the next 200 d, although the pancreas remained enlarged, no additional macroscopic changes were evident. Subsequently, after 350 d, there was a second period of very rapid pancreatic growth. During this phase of enlargement preneoplastic changes and pancreatic tumours developed in 8–15% of the rats.

Extensive pancreatic growth was also found in young rats given the raw kidney bean diet. However, in contrast to the findings with soya bean, the trophic effect diminished with time and by 300 d little enlargement of the pancreas was evident.

Pancreatic weights in rats consuming the lupin seed diet were similar to those in control rats at all stages of the study.

The reasons for the second period of pancreatic growth observed in soya bean-fed rats remain unknown. However, since soya bean diets (high trypsin inhibitor content, low lectin content) induced this change, whereas kidney bean (high lectin content, low trypsin inhibitor content) and lupin seed (low lectin content, low trypsin inhibitor content) did not, the second phase of pancreatic growth was probably mediated primarily by the trypsin inhibitors.

It is possible that ageing and onset of senescence, prolonged exposure to soya bean, or both leads to changes in pancreatic metabolism which result in the organ becoming highly susceptible to the action of trypsin inhibitors.

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Use of chemical treatments to reduce antinutritional effects of tannins of salseed (*Shorea robusta*) meal in diets of broiler chickens. By S. MAHMOOD and R. SMITHARD, *University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU*

The improved growth performance reported for rats fed on diets based on sorghum treated with water, acid or alkali compared with diets based on untreated sorghum has been ascribed to removal or deactivation of tannins. Experiments, using salseed meal (SSM) as the tannin-containing feedstuff, have been conducted to investigate the effects of several treatments on the tannin content of the meal, on the growth performance of birds, on the activities of digestive enzymes and on protein digestion when treated or untreated SSM was included in broiler diets. Salseed meal was mixed (820 ml/kg) with either distilled water (pH 5·3), 0·67M acetic acid (pH 2·4) or 0·67M sodium hydrogen carbonate (pH 8·2), incubated in a closed container at 37° for 12 h, then dried by forced draught at 37°. In the untreated, water-, acid- and alkali-treated SSM the contents of hydrolysable tannins (68·3, 37·4, 42·7, 26·6 g/kg), condensed tannins (12·4, 8·1, 7·3, 2·1 g/kg) and trypsin-inhibiting activity (0·96, 0·16, 0·15, 0·16 U/g) showed that tannin activity was lessened by the treatments, particularly with alkali.

Diets (33·6 g N/kg; 12·5 MJ metabolizable energy/kg) containing treated or untreated SSM (300 g/kg) or a control diet containing no SSM were fed to broiler chickens (initial age 3 weeks). For the determination of N digestibility the diets, restricted to ensure that the same amount of each diet was consumed, were given to five groups of eight broiler chickens for a period of 2 weeks. In the second week seven total collections of excreta were made, each one over a different 12 h period. For the production study, which also yielded the measurements of enzyme activities and organ weights, the diets were offered *ad lib.* to five groups of eight broiler chickens for a period of 4 weeks. The results of the two experiments are shown in the Table.

Diet . . .	Control	SSM treatment				
		None	Water	Acid	Alkali	SE
Gain: feed (g/g)	0·56[a]	0·42[c]	0·47[b]	0·43[b]	0·48[b]	0·01
Apparent N dig[y]	0·592[a]	0·421[b]	0·475[b]	0·410[b]	0·482[b]	0·012
Pancreas wt (g/kg)	1·73[a]	2·59[d]	2·26[bc]	2·43[cd]	2·15[b]	0·08
Jejunal digesta enzymes (U/g dry digesta)						
Trypsin (*EC* 3.4.21.4)	3·52[a]	1·51[c]	1·85[bc]	1·83[bc]	2·15[b]	0·2
Amylase (*EC* 3.2.1.1)	2362[a]	456[c]	723[bc]	827[b]	520[bc]	87
Jejunal mucosal enzymes (U/g mucosa)						
Dipetidase (*EC* 3.4.13.11)	91·9[a]	49·6[d]	56·3[cd]	57·3[c]	66·7[b]	2·4

[a,b,c,d,] Within rows, values with unlike superscripts were significantly different: $P<0·05$.

None of the treatments entirely eliminated the adverse effects of salseed. Reducing the tannin content lessened the adverse effect on feed conversion, pancreatic hypertrophy and proteolytic activity. Overall, alkali treatment was the most effective treatment.

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Effect of pea intake on bone protein synthesis and immunoreactive IGF. By J. A. MARTINEZ[1], R. MARCOS[1], M. T. MACARULLA[2] and J. LARRALDE, [1]*Department of Physiology and Nutrition, University of Navarra, 31008 Pamplona, Spain* and [2]*Department of Nutrition and Food Science, University of País Vasco, 01007 Vitoria, Spain*

Legumes supply a considerable number of nutrients. However, it has been reported that some diets containing these raw seeds as the sole source of protein may induce impairment of growth and other physiological, biochemical or immunological alterations (Larralde & Martínez, 1989).

In this context, male Wistar rats weighing about 98 g were fed on a legume diet (*Pisum sativum* L.) in order to evaluate the possible involvement of bone protein turnover and plasma somatomedins in the nutritional outcome.

Animals were fed on casein (control) or legume (pea) as the source of protein (130 g/kg) for 12 d. Protein synthesis was measured by using the phenylalanine flooding dose method, while the immunoreactive somatomedin (Som C) was analysed by an immunoassay, as previously reported (Martínez *et al.* 1991).

	Control		Pea	
	Mean	SEM	Mean	SEM
Average daily gain (g/d)	7·0	0·3	3·4***	0·5
Tibia weight (g)	0·50	0·03	0·46***	0·02
Tibia K_s (%/d)	46·5	4·0	29·2**	3·4
Som C (U/ml)	12·3	0·7	3·5***	0·5

Significantly different from control: **$P<0·01$; ***$P<0·0001$.

Compared with control animals, average daily gain, tibia weight and bone protein synthesis (K_s) were markedly reduced in the legume-fed animals. Even more dramatic decreases in K_s in muscle has been reported when rats were fed diets containing faba beans (Larralde & Martínez, 1989). The differences could be ascribed to the fact that faba beans contain higher levels of anti-nutritional factors than peas.

The measurement of somatomedin has been recently used as a method for protein quality evaluation and protein nutritional status (Cossack, 1991). The values of somatomedin correlated well with protein synthesis rates ($r=0·73$; $P<0·01$), which may suggest that somatomedin concentrations are not only involved in the observed growth inhibition but could specifically mediate stunting in response to the legume intake through an interaction with bone turnover (Takahashi *et al.* 1990).

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Soya-bean glycinin and ß-conglycinin resistance to digestion in the preruminant calf. By C. MATHIS, J. P. LALLES, I. CAUGANT and R. TOULLEC, *Laboratoire du Jeune Ruminant, INRA, 65 rue de Saint-Brieuc, 35042 Rennes Cédex*

Calves fed liquid diets containing insufficiently processed soya-bean protein (SBP) show a poor nitrogen apparent digestibility often associated with gut disturbances (Sissons, 1982). The aim of the present study was to clarify the respective contributions of glycinin and ß-conglycinin to such a digestibility decrease by measuring their flow at the ileum of the preruminant calf.

Six Holstein male calves were fitted with an abomasal catheter and an ileo-caecal cannula at 6 weeks of age. After recovery, they were fed (58 g DM/kg $BW^{0.75}$ per d) randomly for 2 weeks on each of three milk replacers whose protein was provided either by skim-milk powder (SMP) or by a mixture (50:50) of SMP and SBP. SBP was a heated flour (ASP) or an ethanol-treated concentrate (NASP). Since immunoreactive glycinin and ß-conglycinin were detected in ASP but not in NASP the former was considered to be antigenic *in vitro*. Ileal digesta were collected over the last 4 d of each period. The apparent digestibility of N was determined. Immunoreactive soya-bean globulins were assayed in feeds and digesta by ELISA.

As expected, the apparent digestibility of N was the highest for SMP (mean 0·90 (SD 0·02)) and the lowest for ASP (0·85 (SD 0·04)), that for NASP diet being intermediate (0·87 (SD 0·04)) but only the difference between SMP and ASP was significant ($P<0·05$). Immunoreactive glycinin and ß-conglycinin were detected neither in SMP nor in NASP ileal digesta. The latter result suggests that the globulins were renatured during the digestion process *in vivo*. By contrast, when the calves were fed ASP, glycinin and ß-conglycinin reached the ileum in substantial amounts representing 10 and 1% of their respective intakes. Therefore, glycinin appeared to be less digested than ß-conglycinin and also than legumin, the 11S protein from pea (Bush *et al.* 1992). At the ileum, apparent digestibility of N was negatively correlated with glycinin (but not ß-conglycinin) flow for ASP-fed calves, showing that this decreased digestibility was partly explained by a drop in true digestibility of protein. A kinetic study during the first 10 h following the morning meal showed that both globulins were present in appreciable amounts in digesta from the fourth hour on, as observed in the case of pea legumin (Bush *et al.* 1992).

In conclusion, our results indicate that significant amounts of globulins from antigenic soya-bean sources, especially glycinin, escape intestinal digestion thus contributing to a reduction in the apparent digestibility of N in the preruminant calf. Further studies are in progress to analyse α-conglycinin digestion and to determine which peptides from these globulins are resistant to enzymic breakdown in the preruminant calf.

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Effect of soya-bean trypsin inhibitors in the diet on trypsin activities in pancreatic tissue and ileal digesta and trypsin inhibitor activity in small intestinal digesta of piglets.

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Numerous studies with various animal species have shown that raw soya-bean meal increases the secretion of proteases from the pancreas into the small intestine. These effects are probably associated with the content of trypsin inhibitors in the diet. The relationship between levels of the trypsin inhibitor activity (TIA) in the feed and the magnitude of these effects is insufficiently understood. Also, there is a lack of information regarding the activity of the trypsin inhibitor (TI) during passage through the small intestine.

An experiment was carried out to study the influence of increasing amounts of TI in the feed on trypsin activity and on TIA in different parts of the small intestine of young growing pigs. Thirty-six pigs, each fitted with a PVTC-cannula at the end of the ileum, were fed on six different diets with equal amounts of protein and starch, but differing in TIA. Two different soya-bean concentrates with a different level of TI, together with casein as the protein source, were used (Table 1).

Table 1. *Amounts of protein sources in the experimental diets (g/kg protein)*

Diet . . .	1	2	3	4	5	6
Soya-bean concentrate A	500	1000	495	500	–	–
Soya-bean concentrate B	–	–	103	250	500	1000
Casein	500	–	402	250	500	–
Calculated TIA*	0·234	0·469	0·469	0·787	1·107	2·214

* mg trypsin inhibited/g air dry material.

After a period of digesta collection, the piglets were killed and pancreatic tissue, duodenal (1·5 m distal to the stomach) and ileal (1·5 m proximal to the cannula) chyme were collected for enzyme and TIA determinations. Trypsin activity was measured according to Bergmeyer (1974), after activation by zymogen (pancreatic and duodenal samples). TIA was determined according to van Oort *et al.* (1989). Ileal digestibility values of dry matter and crude protein were not significantly different between the groups. Results of the enzyme and TI measurements are given in Table 2.

Table 2. *Trypsin and trypsin inhibitor activity*

Diet . . .	1	2	3	4	5	6
Trypsin activity*						
Pancreas‡	2867	3022	2802	3172	3195	3666
Ileum‡	314	338	399	301	214	362
Trypsin inhibitor activity†						
Duodenum§	0·230	0·110	0·110	0·230	0·396‡	0·704‡
Ileum§	0·620	0·400	0·380	0·570	0·406‡	0·402‡

* Units /g freeze-dried material; † mg trypsin inhibited /g air dry material; ‡ determined per animal; § determined in pooled samples except where indicated.

Pancreas weight was not affected by increasing levels of soya-bean trypsin inhibitor activities in the diet. The trypsin activity in the pancreatic tissue tended to be higher for the groups with more TIA. The TIA in duodenal chyme showed a slight increase with higher dietary TIA levels, but not in ileal chyme.

The enzyme and trypsin inhibitor activities (Table 2) were not significantly ($P>0·05$) different across treatment, with levels of soya-bean trypsin inhibitors varying from 0·234 to 2·214 mg TIA/g product in the feed.

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D-Xylose absorption test for malabsorption in tapeworm-infected sheep. By J. N. SWART, H. J. FOURIE and P. C. VAN SCALKWYK, *Department of Animal Science, University of the Orange Free State, Bloemfontein, South Africa*

One of the best established and most reliable screening tests for human malabsorption syndromes involves the use of D(+)– xylose (Hill *et al.* 1970; McNeely, 1984).

The possible detrimental effect on nutrient utilization in the small intestine of a pure tapeworm (*Moniezia expansa*) infestation in lambs was investigated by using the D-xylose absorption test. Five control (uninfested) and four infested lambs were used. The xylose solution (0·5 mg/kg body-weight) was administered directly into the abomasum. Plasma xylose concentration (Tietz *et al.* 1985) was determined at intervals of up to 8 h after administration.

Time-period of peak xylose absorption (h)	Plasma xylose levels (mg/100 ml)			
	Control		Infested	
	Mean	SD	Mean	SD
–4	5·8	0·53	5·0	1·48
–3	9·5	2·08	8·5	3·20
–2	13·6	4·38	11·5	3·54
–1	18·2	3·35	14·9	4·91
Peak	33·1[c]	2·30	19·9[d]	1·45
1	23·4[a]	5·10	16·1[b]	2·29
2	17·3[a]	4·47	11·8[b]	3·94
3	11·3	4·95	9·3	5·57
4	7·5	3·00	7·1	4·02
5	2·4	1·37	3·2	0·15

Significant differences between groups: [a,b]$P<0·05$; [c,d]$P<0·001$.

Results demonstrate a significantly lower absorption of xylose in the infested group up to 2 h after peak levels. This is a reflection of a detrimental effect on the integrity of the surface area of the small intestine by the tapeworm infestation. It can, therefore, be assumed that high *Moniezia expanza* infestations adversely affect nutrient absorption and, therefore, normal growth of lambs.

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Antigen specificity and cross-reactivity of fifteen monoclonal antibodies against porcine pancreatic α-amylase II, and its AB and C domains. By JOSETTE FUERI, CHRISTIAN FUERI, GENEVIEVE FERREY, JEAN-CLAUDE CHAIX and GUY MARCHIS-MOUREN, *Laboratoire de Biochimie et de Biologie Moléculaire de la Nutrition, Case 342, Faculté des Sciences, Université d'Aix-Marseille III, Avenue Escadrille Normandie-Niemen, 13397 Marseille Cédex 13*

α-Amylase (α-1,4-glucan-4-glucanohydrolase; *EC*.3.2.1.1), a major digestive enzyme secreted by the pig pancreas, catalyses the hydrolysis of internal glucosidic bonds in starch (Desseaux *et al.* 1988). Porcine pancreatic α-amylase (PPA) exists as two isoforms, PPA I and PPA II distinguished by their pI. The 496-residue sequence of PPA I has been established (Pasero *et al.* 1986), partial amino-acid sequence of PPA II was found identical to PPA I (unpublished results). The precise nature of the difference between PPA I and PPA II is still not known. From crystallographic studies it appears that both isoforms contain an A/B catalytic (β/α) 8 barrel domain (1–403) and a ß-stranded C-terminal domain (410–496) which participates as the second substrate binding site (Buisson *et al.* 1987). Recent attempts to determine the respective roles of these domains have been performed by limited proteolysis of PPA with subtilisin. The cut occurs at bond 369–370 in the loop connecting ß8 to α8. Two fragments have been obtained: a large one (41 kD) containing the major part of the A/B domain, and a small one (14 kD) containing the C-domain (Desseaux *et al.* 1991*a,b*). All α-amylases and triose phosphate isomerase are members of the (ß/α) 8-barrel enzyme family.

In the present work we have been looking for monoclonal antibodies (mabs) which might help us to understand how starch binds to amylase and how the glucosidic chain is guided to the active site. Highly-productive hybridoma secreting mabs specific for porcine α-pancreatic amylase II were established. Fifteen clones were selected. The mabs produced (K_D = 1·68–11·2 nM) were checked for cross-reactivity with six heterologous antigens, namely porcine pancreatic α-amylase I, barley amylase, human pancreatic α-amylase, Taka amylase and triose phosphate isomerase, using direct ELISA assay. Mabs were classified into seven groups; in a few groups mabs cross-reacted with a single heterologous antigen either porcine pancreatic amylase I (six mabs) or barley amylase (two mabs) or human pancreatic amylase (three mabs). Two other groups cross-reacted with two heterologous antigens either porcine I and human or porcine I and barley amylases. Only one mab out of fifteen cross-reacted in direct ELISA binding to all amylases and triose phosphate isomerase. Using a sandwich ELISA test only three mabs were found to bind porcine amylase II present at high concentration. Results consistent with direct porcine amylase binding were obtained from binding inhibition assays. Analysis by the additivity test showed that three mabs, B10.10, B1.11, C6.4 recognize distinct epitopes while the epitopes for the other pairs tested are either overlapping or at least close to each other. Finally, mabs binding specifically either to the AB or to the C domain fragment, or to both fragments, have been obtained. Up to now, none of the mabs tested has been found to inhibit amylolytic activity.

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Response of pancreatic lipase to graded levels of dietary tallow in the weaned piglet. By T. C. REIS DE SOUZA[1], J. PEINIAU[2] and A. AUMAITRE[2], [1]*Escola de Medicina Veterinaria UFBA, 40210 Salvador, Bahia, Brazil* and [2]*Station de Recherches Porcines, INRA, 35590 Saint-Gilles*

It is generally accepted that in simple-stomached animals the biosynthesis of pancreatic enzymes is stimulated by an increase in the level of particular dietary components. Starch and protein levels are known to increase the production and the secretion of amylase and chymotrypsin, respectively. A similar mechanism has been observed for pancreatic lipase in pigs receiving high levels of dietary lipids. But the effect on pancreatic lipase of graded levels of lipids in the diets of piglets has not been reported.

Thirty-three piglets weaned at 3 weeks of age were used to examine the response of pancreatic lipase: (1) to graded levels of 0, 40 and 80 g tallow/kg diet (Expt. 1) and (2) to emulsifying agents, lecithin and biliary salts, incorporated at the level of 15 and 6 g/kg respectively, to a diet containing 80 g tallow/kg (Expt. 2). Experimental diets were fed for 7 weeks (Expt. 1) and 5 weeks (Expt. 2). Specific activities of lipase (Rathelot *et al.* 1975) and amylase (Corring & Saucier, 1972) were determined in the pancreatic tissues of animals killed after a 12 h fast.

The specific activity of lipase (Y1; IU/mg of protein) increased curvilinearly with the level of dietary tallow (X; %) according to the following equation: $Y1 = 12{\cdot}54 - 3{\cdot}61\ X - 0{\cdot}33\ X^2$ (n 15; $R^2 = 0{\cdot}81$). The average values were 12·3, 21·7 and 20·0 (SEM 1·13) lipase IU/g protein for 0, 40 and 80 g added tallow respectively. Conversely the specific activity of amylase (Y2; IU/mg of protein) decreased linearly with increasing level of tallow (X; %) as follows: $Y2 = 280 - 20{\cdot}8\ X$ (n 15; $R^2 = 0{\cdot}94$). The average values were 282, 202 and 117 (SEM 19) amylase IU/g protein for 0, 40 and 80 g added tallow respectively. The average value of the true faecal digestibility of lipids estimated using the two diets containing 40 and 80 g tallow/kg (Expt. 1) was constant and amounted to 0·783 and 0·765 (SEM 0·075) respectively.

Compared to a control diet containing no emulsifying agent, addition of lecithin or biliary salts had no significant effect on the specific activity of either pancreatic lipase or amylase (Expt. 2). The apparent faecal digestibility of fat did not differ significantly between diets, being 0·799; 0·777 and 0·749 (SEM 0·045) for the diets containing mixed tallow, tallow plus lecithin or tallow plus biliary salts, respectively.

The results confirm the adaptative response of pancreatic lipase to graded levels of tallow in the piglet and can be used as indicators of the optimum level of fat in the starter diet.

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Pancreatic nutritional adaptation in calves: effect of pea flour incorporation in the milk substitute. By G. LE DREAN, I. LE HUEROU-LURON, V. PHILOUZE, R. TOULLEC and P. GUILLOTEAU, *Laboratoire du Jeune Ruminant, INRA, Rennes*

Pea flour incorporation in milk substitutes is limited by the calf's capacity to digest pea carbohydrates and by reactions of hypersensitivity induced mainly by globulins. Hydrothermal treatments modify starch and proteins which become more digestible and denature lectins and protease inhibitors, but they do not affect oligosides (Nunes Do Prado *et al.* 1989). Concerning the pea flour utilization by preruminant calves, nutritional adaptation of the pancreas has never been studied. The present experiment was conducted to measure the pancreatic response to pea flour incorporation in milk substitutes.

Twenty-two Holstein 50-d-old calves were given, for 88 d, milk substitutes whose proteins were provided either exclusively by dairy products (T) or partially (16·5 g/kg) by a raw dehulled (R) or a flaked whole (F) pea flour.

With the R diet, live weight was lower than with the T or F diets (185 (SD 3), 202 (SD 3) and 199 (SD 2) kg, respectively; $P \leqslant 0{\cdot}01$), feed efficiency also was lower (Toullec *et al.* 1992). In contrast, pancreas weight was decreased in both R and F groups compared with the T group (131 (SD 12), 125 (SD 9) and 146 (SD 8) g, respectively; $P \leqslant 0{\cdot}05$). This effect was not related to the presence of antitryptic factors as suggested by Gorrill & Thomas (1967) since they were inactivated in the F product. However, pancreatic weights (724 (SD 40), 709 (SD 63) and 629 (SD 43) mg/kg live weight in T, R and F groups respectively) were not significantly changed. Pancreatic protein, RNA and DNA concentrations as well as trypsin and chymotrypsin specific activities were not altered, but amylase activity was higher with the R and F diets than with the T diet (8·4 (SD 0·9), 7·8 (SD 0·7) and 4·6 (SD 0·4), respectively; $P \leqslant 0{\cdot}01$). This unexpected change could be due to a stimulatory effect of the pea carbohydrates. The three diets contained a similar level of starch, but their nature was different (cereal starch for T diet).

Flaked pea flour seemed to be better tolerated with regard to calf growth while the pancreatic function appeared to adapt to the incorporation of pea flour. However, this adaptation was equivocal and insufficient to cope with the utilization of the raw product.

Groups . . .	T		R		F	
	Mean	SE	Mean	SE	Mean	SE
Pancreatic contents (mg/g pancreas)						
Proteins	170	5	164	10	157	4
RNA	13·8	0·7	12·7	0·8	14·1	0·7
DNA	4·4	0·2	4·9	0·3	4·8	0·3
Specific activities (U/mg protein)						
Amylase	4·6	0·4[a]	8·4	0·9[b]	7·8	0·7[b]
Trypsin (*EC* 3.4.21.4)	48·9	5·9	60·4	13·3	47·6	6·4
Chymotrypsin (*EC* 3.4.21.1)	26·6	3·5	39·3	9·3	27·3	5·1

[a,b] Mean values with unlike superscripts were significantly different: $P<0{\cdot}05$.

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Effects of inulin on goblet cell distribution and mucin type in the hindgut of heteroxenic rats. By J. C. MESLIN[1], N. FONTAINE[1] and C. ANDRIEUX[2], [1]*LNSA*, [2]*LEPSD INRA CRJ 78352 Jouy en Josas Cédex*

It has been previously observed (Andrieux *et al.* 1991) that a 'human-like' diet containing 100 g/kg inulin (a fructo-oligosaccharide poorly digested but fermented by the microbial flora) lowered the caecal contents pH, increased caecal weight and short-chain fatty acid concentration when given to heteroxenic rats compared with the same diet containing 100g sucrose/kg.

The aim of our study was to determine the effects of the same diets upon goblet cell distribution and mucin type in the hindgut of heteroxenic rats having a human methanogenic bacterial flora.

Male 3-month-old F344 rats were fed on diets over a period of 3 weeks. Rats were then killed and samples of mucosa and contents were taken from the caecum and proximal and distal colon, and put into isopentane cooled with liquid nitrogen and stored at –80°. Cryostat sections (10 μm) were cut at –20°, fixed with formaldehyde vapours and stained with periodic acid-Schi (PAS; for neutral mucin), alcian blue (AB; pH 2·5 for acid mucin), and a high-iron-diamine (HID) reaction without prior oxidation (for sulphomucin). Goblet cell number of each mucin type was counted for each anatomical site and expressed per crypt section.

	PAS+		AB 2·5+		HID+	
	Mean	SE	Mean	SE	Mean	SE
Sucrose diet						
Caecum	14·5	0·3	11·5	0·2	12·7	0·2
Proximal colon	16·2	0·4	16·7	0·4	14·4	0·5
Distal colon	14·5	0·4	15·1	0·5	18·1	0·4
Inulin diet						
Caecum	17·1	0·2**	11·6	0·2	16·2	0·3**
Proximal colon	19·7	0·6**	16·7	0·2	15·0	0·4
Distal colon	14·1	0·4	17·4	0·3**	16·1	0·5**

Significantly different from sucrose diet: $^{**}P<0·001$.

Compared with heteroxenic rats fed sucrose, the inulin diet significantly increased the number of neutral and sulphated mucin-containing cells in the caecal crypts; the number of acid mucin-containing cells was unchanged. In the proximal colon, the increase in goblet cell number was observed only for neutral mucin-containing cells. In the distal colon, there was an increase in acid and a decrease in sulphated mucin-containing cells, resulting in an unchanged total goblet cell number.

Further studies are in progress for detection of mucin types in the contents by biochemical assays.

Inulin fermentation alters goblet cell distribution and mucin type, mainly in the caecum. Other experimental conditions are needed to dissociate the effects of pH and short-chain fatty acid concentration upon goblet cell number and mucin type.

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Amino acid losses in ileostomy fluid on a protein-free diet. By M. F. FULLER[1], ANNE MILNE[1], C. I. HARRIS[1], T. M. S. REID[2] and R. KEENAN[3], [1]*Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB,* [2]*Department of Medical Microbiology, Aberdeen Royal Infirmary, Aberdeen AB9 2ZD* and [3]*Department of Surgery, Aberdeen Royal Infirmary, Aberdeen AB9 2ZD*

Essential amino acids are required for maintenance to replace obligatory losses and, in the absence of dietary protein, are supplied from the net breakdown of body protein. Amino acids are lost obligatorily through both metabolism and secretion of intact amino acids from the epithelia. Secretions from the skin constitute only a small component of nitrogen loss (Calloway *et al.* 1971) but losses via the gastrointestinal tract may be much more substantial. Although a large proportion of the protein secreted into the gut may be recovered, that which passes into the caecum appears to contribute little or nothing to amino acid need (Zebrowska *et al.* 1973; Wünsche *et al.* 1984). Results obtained with pigs suggest that these losses may constitute the major component of maintenance amino acid needs (Fuller, 1991). We have therefore attempted to assess the importance of gastrointestinal losses in humans by measuring amino acid excretion in ileostomy fluid. Preliminary results are presented for seven subjects.

The volunteers were aged 29-68 years and had a mean weight of 66 kg. They had uncomplicated ileostomies for ulcerative colitis and were in good general health. They were given for 4 d an essentially protein-free diet (<0·5 gN/d) supplying 1·3 times their predicted BMR; it was given as three meals daily with 0·33 g polyethylene glycol (PEG) at each meal.

Ileostomy fluid was collected at frequent intervals and stored at 4° before freezing. Each 24 h collection was analysed separately. On the third and fourth days the subjects were given an antibiotic (Ciprofloxacin 500 mg BD) to suppress microbial activity. Samples of ileostomy fluid were taken each day for microbiological examination.

Daily dry matter and N losses in the ileostomy fluid were only slightly reduced by antibiotic despite a more than hundredfold reduction in bacterial numbers; the mean DM output was 22 g/d and the mean N loss 715 mg/d. Mean daily losses (mg) of some essential amino acids were; thr 293, val 214, ileu 129, leu 236, phe 140 (tyr 146), lys 302, his 144. Expressed as a proportion of adult maintenance needs (FAO/WHO/UNU, 1985), these range from 0·2 for histidine to 0·63 for threonine. The total N excretion in urine and ileostomy fluid was 3·6 g/d (55 mg/kg per d) for which the N lost in ileostomy fluid accounted for one-fifth.

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Analytical characterization by reverse-phase HPLC of products obtained by *in vitro* enzymic hydrolysis of whey proteins with gastric and pancreatic proteases. By T. LENGAGNE[1], T. EFSTATHIOU[1], L. ROGER[1] and F. MENDY[2], [1]*NUTRINOV, Rue de Saint-Brieuc, Rennes* and [2]*Parc de Béarn, Saint-Cloud*

The bovine whey proteins, α-lactalbumin and ß-lactoglobulin are of high nutritional quality. Together with bovine casein, they are the most commonly used substitutes for human milk proteins. However, those proteins, and especially ß-lactoglobulin, are held responsible for intolerances and allergic manifestations in infancy. Those reactions might be attributed to an insufficiency of proteolytic equipment (Jakobson *et al.* 1983). If trypsin and chymotrypsin activities are rapidly reaching adult levels, it seems that a down-regulation of acid–peptic digestion and low quantities of elastase 2 may play a role in protein intolerance (particularly for ß-lactoglobulin) before 18 months. This protease, elastase 2, has been reported to be a cationic endoprotease distinct from the anionic enzyme elastase 1. Both show elastolytic activity but use different ways; while elastase 1 hydrolyses Ala-Ala and Ala-Gly peptide bonds leaving alanine as the main or single C-terminal amino acid, elastase 2 hydrolyses the bonds formed between leucine, phenylalanine or tyrosine with glycine or alanine, leaving three new C-terminal amino acids: leucine, phenylalanine and tyrosine.

The specificity of elastase 2 was investigated using synthetic substrates, revealing that it preferentially hydrolyses peptides containing leucine, methionine, and phenylalanine in the P_1 position when there is proline in the P_2 position. This specificity would be a good explanation for the effect of elastase 2 on globular proteins like ß-lactoglobulin. This contains similar sequences (Ala_{37}-Pro_{38}-Leu_{39}, Ala_{142}-Leu_{143}-Pro_{144}-Met_{145}) responsible for curves and hydrophobic pockets in the polypeptidic chain. To ascertain the role of these proteases on whey proteins, a technique of reverse-phase HPLC has been used. It allows us to perform mapping of products obtained by different methods of *in vitro* hydrolysis. Chromatograms reveal that introduction of a gastric incubation (HCl-pepsin) before trypsin–chymotrypsin hydrolysis leads to a reduction of nearly 100% in the quantity of residual α-lactalbumin, and that the presence of elastase during the pancreatic hydrolysis (trypsin–chymotrypsin) involves modifications of some peptide ratios (without real increase of free amino-acids), including disappearance for one of them.

With acknowledgments to Dr J. M. Kahn of Sandoz Nutrition for the financial contribution to this work.

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The use of ruminal indigestible neutral detergent fibre to study intradiurnal variation of digestion and outflow of rumen dry matter (RDM) in sheep fed *ad lib.* on lucerne hay. By R. BAUMONT and J. JAMOT, *Station de Recherches sur la Nutrition des Herbivores, INRA Centre de Clermont-Fd/Theix, 63122 St Genès-Champanelle*

Although difficult to measure, intradiurnal variation of rumen dry matter outflow rate (RDMOR) is important to understand the physical limitation of forage intake. A method of RDMOR measurement is proposed using ruminal indigestible neutral detergent fibre (RINDF).

Four ruminally-fistulated wethers were fed *ad lib.* twice daily (09.00, 21.00 hours) on lucerne hay (digestibility 0·64). Daily intake, time spent eating and ruminating were recorded during 5 d (Baumont *et al.* 1990) and rumen contractions during 48 h. During the subsequent 2 weeks, rumen dry matter pool size (RDMPS) was measured by manually emptying the rumen at 09.00, 13.00, 17.00 and 21.00 hours and samples of rumen dry matter (RDM) were dried and ground, and placed the following week in nylon bags in the rumen for 48 h. NDF residue was determined to estimate RINDF pool size. RDM removal rate (RDMRR: through digestion and outflow) during a time interval (0,T) was calculated as

$$\text{either} \quad \frac{\text{RDMPS}_0 + \text{Intake}_{(0,T)} - \text{RDMPS}_T}{T}$$

$$\text{or} \quad \left(\frac{I}{T}(\text{RDMPS}_0 + \text{Intake}_{(0,T)} - \text{RDMPS}_T)\right).$$

Assuming that RINDF can leave the rumen by outflow only, RDMOR during (0,T) was calculated as RINDF removal rate divided by the mean concentration of RINDF in rumen content during (0,T).

The RDMOR estimated by RINDF (0·58 (SD 0·06) g/min) is in agreement with results obtained by direct duodenal flow measurements (Malbert & Baumont, 1989). RDMRR was higher between 09.00 and 13.00 hours than between 13.00 and 21.00 hours (1·35 (SD 0·10) *v.* 0·92 (SD 0·07) g/min, $P<0·10$). RDMOR was also higher ($P<0·10$) between 09.00 and 13.00 hours (0·91 (SD 0·13) g/min) than between 13.00 and 21.00 hours (0·41 (SD 0·07) g/min). The increase in RDMOR was variable between animals (CV 28%). There was no relationship between RDM digestion rate (0·44 (SD 0·14) *v.* 0·51 (SD 0·10) g/min) for the same time periods and the corresponding values for RDMRR and RDMOR. Animals spent 63·5% of the time eating between 09.00 and 13.00 hours and the increase in RDMOR during these hours could be explained by increased ruminal motility (1·38 (SD 0·04) *v.* 0·98 (SD 0·06) contractions/min, $P<0·05$) and outflow per contraction (0·66 (SD 0·10) *v.* 0·42 (SD 0·09) g/contraction, $P<0·30$).

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Distribution and post-prandial variations of the activity of polysaccharide-degrading enzymes in rumen micro-organisms. By C. MARTIN, B. MICHALET-DOREAU, *Station de Recherches sur la Nutrition des Herbivores, Unité de la Valeur Alimentaire, INRA Theix, 63122 St Genès-Champanelle*

The digestion of polysaccharide components in the ruminant is effected by the combined actions of enzymes produced by a complex microbial population, which is able to degrade and utilize plant structural and storage polysaccharides. The aim of the present study was to determine the distribution of polysaccharidases (amylase (*EC* 3.2.1.1), carboxymethyl-cellulase and xylanase (*EC* 3.2.1.32)) between the liquid-associated bacteria (LAB), protozoa (LAP) and solid-associated bacteria (SAB), and to follow the post-prandial variations in the specific activities of these enzymes.

Four Jersey cows fitted with rumen cannulas were given once daily a diet consisting of 700 g hay + 300 g barley/kg at 8 kg DM/d. LAB, LAP and SAB were isolated under anaerobic conditions from rumen contents collected 1 h before feeding and at 2, 5, 8, and 11 h after feeding on 2 successive days (Williams & Strachan, 1984). Enzymes of these three microbial populations were extracted by sonication. Specific activities of polysaccharidases were expressed in the Table as nmol reducing sugar released/mg protein per min. The effect of time after feeding on specific enzyme activity of each microbial population was tested by ANOVA with two main effects, time and animal.

Enzyme	Microbial population	Time-period after feeding (h)					
		−1	2	5	8	11	SE
Amylase (*EC* 3.2.1.1)	LAB	0·0	0·0	5·6	4·0	1·9	0·8
	LAP	14·1	28·7	22·1	21·2	29·6	3·7
	SAB	14·7	34·9	35·5	31·6	22·3	2·7
Carboxymethyl-cellulase	LAB	0·1	0·0	0·3	0·2	0·4	0·1
	LAP	2·5	5·2	8·4	5·2	8·9	1·5
	SAB	6·8	2·9	4·2	5·0	6·2	0·6
Xylanase (*EC* 3.2.1.32)	LAB	0·7	0·2	1·9	1·2	1·6	0·3
	LAP	10·6	19·6	25·1	7·6	14·9	3·9
	SAB	69·2	24·8	29·0	35·0	37·4	6·4

The present study confirmed that polysaccharide-degrading enzymes were more active in the SAB (Williams & Strachan, 1984) than in either of the other fractions, but the LAP also contributed significantly to amylolytic and carboxymethylcellulase activities. Activity of the LAB was negligible. The post-prandial variations in the specific activity of amylase were broadly similar within the sub-populations. In contrast, there were marked differences in the activity profiles of cell wall polysaccharide-degrading enzymes within the LAP and SAB. Protozoa were more active at 2 and 5 h after feeding, whereas the SAB were most active at the end of the post-prandial period (−1 h).

Manipulation to modify level and period of enzymic activity may, therefore, lead to understanding of ruminal polysaccharide utilization.

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Effect of lipid supply in the diet of cows on calcium and magnesium pools in the rumen. By A. FERLAY and M. DOREAU, *INRA, Laboratoire de la Sous-Nutrition, Theix, 63122 Saint Genès-Champanelle*

Lipid addition to ruminant diets often decreases fibre digestion in the rumen, especially when diets are deficient in divalent cations. The positive effect of the association between lipids and divalent cations, principally calcium, could be due to: (1) the formation of insoluble soaps in the rumen, (2) the enhancing effect of Ca on adhesion of bacteria upon feed particles.

In order to determine if the level of divalent cations could be a limiting factor in ruminal digestive efficiency, diurnal variations in ionized and total Ca and magnesium concentrations in the rumen were studied using a control diet (C; 18 g fatty acid/kg dry matter (DM)) and two diets supplemented with rapeseed oil (74 g fatty acid/kg DM) given either in continuous infusion (LC) or as a single infusion (LS) daily at 08.15 hours. The three diets had the same content of Ca and Mg and were offered at 09.00 and 16.00 hours. The effects of these diets on digestibility and ruminal digestion are described elsewhere (Doreau & Ferlay, 1992). Ionized Ca was defined as Ca present in the ultrafiltrate (1000 ***g*** for 30 min) obtained from the supernatant (20 000 ***g***, 5 min at 4°) of ruminal fluid, and total Ca as Ca present in the rumen contents. The same fractions were used for Mg. Ca and Mg were measured by atomic absorption spectrophotometry.

Total Ca and Mg in DM did not differ between diets or vary with time of sampling. Ionized Ca (ICa) was decreased ($P<0{\cdot}05$) by lipid supplementation except at 00.00 and 04.00 hours and depended on the time of sampling; ionized Mg (IMg) was not affected by diet or the time of sampling. Assuming classical values for DM and non-particle-associated water pools in the rumen, the fractions of the total Ca and Mg in the ionized forms were: Ca 5·9% and 1·8%, and Mg 26·9% and 23·8% for the control and oil-containing diets, respectively.

	Time of sampling						
	08.00	10.00	12.00	16.00	20.00	00.00	04.00
ICa (μg/ml)							
C	29·8[a]	137·3[c]	91·0[c]	46·2[c]	147·0[a]	59·1	19·6
LC	0·6[b]	17·4[d]	31·5[d]	12·7[d]	44·8[b]	27·1	0·1
LS	5·5[b]	68·8[d]	40·0[d]	1·7[d]	49·9[b]	35·0	5·6
SE	2·5	8·2	5·7	4·5	9·6	5·0	2·0
IMg (μg/ml)							
C	44·1	135·9	98·2	77·4	212·6	129·6	19·3
LC	20·5	63·1	132·0	84·8	139·9	116·2	19·0
LS	33·4	123·5	115·6	111·2	198·2	136·2	19·0
SE	6·4	7·7	13·2	12·8	20·1	9·3	5·0

Within columns values with unlike superscripts were significantly different: [a,b] $P<0{\cdot}05$; [c,d] $P<0{\cdot}01$.

These results show: (1) the concentration in ICa can limit fibre digestion when lipids are added to the diet, (2) the behaviour of Mg is different although it could theoretically form soaps with fatty acids.

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Short-chain fatty acids (SCFA) and colonic motility: influence on segmental motor patterns. By P. E. SQUIRES, R. D. E. RUMSEY, and N. W. READ, *Department of Biomedical Science, The University, Sheffield S10 2TN*

Absorption of SCFA from the colon is metabolically significant. SCFA also suppress general colonic motility (Squires *et al.* 1992*a*) and it has been suggested that the level of SCFA absorption is regulated by the effects on motility. The present study examines whether SCFA alter a colonic motility pattern dominated by segmentation (1 mM Loperamide; Squires *et al.* 1992*b*).

Male Wistar rats were anaesthetized (60 mg Sagatal/kg given intraperitoneally) and the large intestine was isolated and perfused vascularly with Krebs bicarbonate (O_2:CO_2, 95:5, v/v; 1·2 ml/min, 37°) via the superior mesenteric artery with hepatic portal outflow. The tissue was suspended horizontally between two volume transducers. Serosal strain gauges (RB Products, USA) recorded contractile activity in the caecum (tail (CT) and body (CB)), proximal (PC), proximal-to-middle (PM), middle-to-distal (MD) and distal colon (DC) for an initial 30 min luminal infusion with Krebs buffer and subsequent 30 min infusion with Krebs containing acetic (66 mM), propionic (26 mM) and butyric (8 mM) acids plus 1 mM-Loperamide (Sigma). Each period was subdivided into 10 min intervals. Data are expressed as mean % change in activity, recorded as the area under the response from each gauge site or transducer. The number of migrating contractions in each interval was also recorded.

Activity preceding the addition of the test solution was similar to that with buffer only, except for MD in the 2nd 10 min interval (89 (SE 8)%; $P<0·05$). Immediately following administration there was a significant decrease in colonic contractile activity; PC (57 (SE 8)%), PM (41 (SE 5)%), MD (42 (SE 9)%) and DC (46 (SE 7)%; $P<0·05$ respectively), similar to the effect of SCFA alone. There was no change in the number of migrating contractions, although the amount of fluid expelled from the distal colon decreased in line with the transient fall in colonic contractility. The present study has failed to demonstrate that SCFA promote segmental activity and, therefore, probably absorption in the rat colon. On the contrary, the data suggest that a mixture of SCFA blocks the prolonged segmental activity in the proximal colon demonstrated in response to Loperamide.

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Contribution of intestinal microflora to lysine requirements in non-ruminants. By D. TORRALLARDONA, C. I. HARRIS, E. MILNE and M. F. FULLER, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Although there is microbial activity in those sections of the gastrointestinal tract in which absorption of amino acids takes place and evidence from amino acid labelling that the gastrointestinal microflora makes a contribution to the amino acid needs of non-ruminants, its quantitative significance is still in question. We present preliminary results of work to quantify the absorption of microbial lysine.

One rat (114 g) was fed for 10 d on a protein-free diet containing fermentable sugars together with [^{15}N]ammonium chloride; another rat was given the same with unlabelled ammonium chloride. The carcass of each, excluding the digestive tract, was homogenized and the microbial fraction of the faeces was separated by successive centrifugation. Lysine was isolated by ion-exchange chromatography from the carcass and the microbial fraction and ^{15}N-enrichment in each was measured by isotope ratio mass spectrometry. Freedom from ^{15}N-contamination was checked by isolating lysine from the control rat hydrolysed in the presence of [^{15}N]ammonium chloride. The total lysine content of the carcass was also measured.

One 18 kg pig was kept in a metabolism cage and fed a low-protein diet containing [^{15}N]ammonium chloride and [^{14}C]polyglucose for 10 d. Lysine was isolated from the carcass and from the microbial fraction of digesta taken from the ileum at slaughter and the ^{15}N- and ^{14}C-enrichments of both were measured. The results are shown in the Table.

	Lysine enrichment (ape or dpm/μmol)		Body lysine content (g)
	Body	Microbial	
Rat: (^{15}N)	0·0467	1·0836	1·65
Pig: (^{15}N)	0·0030	0·0757	196·6
(^{14}C)	0·289	9·441	

If it is assumed that the animals could not incorporate either label into lysine and that all labelled lysine was therefore of microbial origin, then, ignoring any loss or degradation of lysine in the 10 d and assuming that the bacterial sample is representative, the absorbed microbial lysine can be estimated as body lysine content multiplied by body lysine enrichment divided by microbial lysine enrichment. These estimates are, for the rat, 7·1 mg/d and for the pig, 779 mg/d from ^{15}N and 602 g/d from ^{14}C.

These results suggest a significant supply of microbial lysine in pigs and rats. In the rat labelling of lysine could have resulted from coprophagy, but with the pig this was prevented.

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Effect of inulin on the fermentation profile in rats associated with human flora from low-, high- and non-methane producers. By C. ANDRIEUX, S. LORY, N. ROLAND and O. SZYLIT, *Laboratoire d'Ecologie et de Physiologie du Système Digestif, unité MBS, INRA, 78352 Jouy en Josas Cédex*

Methane concentration of expired air varies between subjects from none to a high level. Differences in bacterial fermentation could affect the breakdown of dietary carbohydrates such as inulin and alter the production of methane. Inulin is a fructo-oligosaccharide known to be undigested by endogenous enzymes but fermented by the bacterial flora of the digestive tract.

Influence of inulin on fermentation profile was studied in three groups of rats born germ-free and associated with different human flora either from a non-methane or from low- and high-methane producers. The ability of the human flora to keep its fermentation capacity when inoculated into germ-free rats has been tested previously by Andrieux *et al.* (1991). Rats were reared in isolators and fed on either a control diet or a diet containing 100 g inulin/kg. Hydrogen and methane production were measured in a respiratory chamber and other bacterial metabolites, SCFA and lactic acid, were analysed in the caecal contents.

Methane production . . .	None				Low				High			
Diet . . .	Control		Inulin		Control		Inulin		Control		Inulin	
Gas production (ml/d per 100 g body wt)	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Hydrogen	0·06	0·02	2·7	0·5	0·07	0·02	0·9	0·04	0·17	0·07	0·9	0·5
Methane					0·25	0·15	0·02	0·01	3·8	0·8	9·0	0·7

Hydrogen excretion was low and quite similar in all control groups whereas methane production differed strongly between groups, from none to a high methane production. Inulin increased hydrogen excretion more in non-methane- than in methane-producing rats. Methane excretion was increased in high-methane producers and strongly decreased in low-methane producers.

In the caecum of control groups, SCFA was twofold higher and lactic acid lower in non-methane- as compared to methane-producing rats. In low- and non-methane producers, inulin decreased the caecal pH below 5·5 and increased lactic acid production 4–6 times. SCFA increased more in methane than in non-methane producers, but the butyrate proportion was significantly enhanced in all groups.

Capacity of bacterial flora to produce methane seems to affect inulin fermentation profile, in favour of lactic acid production in low- and non-methane producers and methanogenesis in high-methane producers. This should be taken into account to explain the individual differences in reaction to ingestion of undigestible carbohydrates.

Andrieux, C., Lory, S., Dufour-Lescoat, C., de Baynast, R. & Szylit. O. (1991). *Food Hydrocolloids* **5**, 49–56.

Effects of colonic fermentation on respiratory gas exchanges following a glucose load in man. By P. RITZ, D. CLOAREC, M. BEYLOT, M. CHAMP, B. CHARBONNEL, S. NORMAND and M. KREMPF, *Laboratory of Human Nutrition, Hôtel Dieu, Nantes, and INSERM U 197, Lyon*

In humans, the amount of energy produced from the oxidation of short-chain fatty acids (SCFA) is unknown and could modify the metabolic utilization of energetic fuels (e.g. carbohydrates and lipids). However, this process is usually considered as minor and neglected. We have addressed this assumption in six normal subjects receiving orally either 50 g of naturally ^{13}C-enriched glucose or 50 g of the same glucose plus 20 g of purified lactulose. Their respiratory gas exchanges, breath hydrogen, methane and $^{13}CO_2$ concentrations, plasma glucose, insulin and free fatty acid concentrations were monitored for 8 h.

	O_2 consumption (l/8 h)		CO_2 production (l/8 h)		△ RQ (area)	
	Mean	SE	Mean	SE	Mean	SE
Glucose	114·9	7·8	96·7	5·4	12·46	2·7
Glucose + lactulose	114·0	7·0	98·4	5·5	26·25[a]	3·3

[a] Significantly different from glucose: $P<0·01$.

No differences in oxygen consumption, breath $^{13}CO_2$ production, or plasma concentrations of blood glucose, FFA and insulin could be found, between the two experiments. The rise in CO_2 production above baseline ($\triangle CO_2$, 8·97 (SD 1·6) *v.* 3·56 (SD 0·8) litres/8 h; $P<0·05$) and breath H_2 concentration were significantly greater with the lactulose as well as the change in respiratory quotient (△ RQ; area under the curve, 26·25 (SD 3·3) *v.* 12·46 (SD 2·7); $P<0·01$). This suggests that the fermentation process induced by lactulose generates extra fuels going through an oxidation pathway. Therefore, the classical equations used to calculate carbohydrate and lipid oxidation, and energy expenditure from indirect calorimetry data which do not include the contribution of SCFA are probably not valid when colonic fermentation is taking place. Indeed, in this experiment, we could have overestimated glucose oxidation (12·5%) if the fermentation process were not considered.

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Adaptation to two doses of lactulose by human colonic flora in continuous culture. By V. DUCROS[1], M. DURAND[1], P. BEAUMATIN[1], G. HANNEQUART[1], C. CORDELET[1], and J. P. GRIVET[2], [1]*Laboratoire de Nutrition et Sécurité Alimentaire, INRA, 78352 Jouy-en-Josas Cédex* and [2]*Departement de Physique, Université d'Orléans, 45071 Orléans Cédex*

The aim of the present study was to examine the effect of two doses of lactulose on the fermentation pathways at different intervals during a 12 d adaptation period in a continuous *in vitro* incubation system. The four 1-litre vessels of the system were inoculated with homogenized human faeces (130 g/l) collected from a non-methanogenic subject (provided by L. Abensour, Hôpital St. Lazare, Paris). After 8 d adaptation to the complex medium two vessels were fed daily 10 g and the other two 20 g of lactulose. The experiment was then continued for 12 d. At the end of the experiment the flora from each group of vessels was assayed for reductive acetogenesis measured by ^{13}C NRM in batch culture (Durand *et al.* 1992). The daily short-chain fatty acids (SCFA) and H_2 production and the pH measured after 3–4 d and 9–11 d adaptation are shown in the table.

Lactulose dose . . .	10 g/d				20 g/d			
Adaptation (d) . . .	3–4		9–11		3–4		9–11	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
SCFA								
Total (mmol/d)	132	7	132	6	154	8	62	32
Molar ratio (%)								
Acetate	55	4	50	2	25	3	93	2
Propionate	13	1	13	1	5	1	2	1
Butyrate	27	4	32	2	69	4	3	1
Lactate (mmol/d)	0		0		Trace		139	20
H_2 (mmol/d)	36	6	37	3	84	15	Trace	
pH	6·2	0·04	6·4	0·03	5·9	0·1	4·6	0·01

The reductive acetogenic activity was high when the flora had been adapted to the low lactulose level (16·3 and 6·0 mM-$^{13}CO_2$ incorporated into acetate and butyrate carbons, respectively) whereas it was much lower with the flora adapted to the high lactulose (2·4 mM-$^{13}CO_2$ incorporated into acetate).

The changes of metabolic pathways observed during the period of adaptation with 20 g/d per litre were consistent with those reported in man consuming 20 g lactulose twice daily, namely a fall in H_2 excretion and increase in caecum acetate and lactate concentrations (Florent *et al.* 1985). The present results suggest that in this case acetate is formed by heterofermentative lactic acid bacteria rather than by reductive acetogens. In contrast, it appears that lactulose at a low level results in a stable fermentation pattern supporting acetate synthesis from CO_2 by non-methanogenic flora.

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Phenotype modulation in butyrate-treated rat colon carcinoma cell lines. By PASCALE PERRIN[1], FABIENNE VAVASSEUR[1,3], NATHALIE LABARRIERE[1], PATRICK GASSER[2], JEAN P. GALMICHE[2], FRANCIS BORNET[3] and KHALED MEFLAH[1], [1]*INSERM CJF 90.11, Institut de Biologie, 9 quai Moncousu, 44035 Nantes Cédex 01,* [2]*Laboratoire Fonctions Digestives et Nutrition, CHU Nord, 44035 Nantes Cédex 01* and [3]*Department of Nutrition and Health, Eridania, Beghin-Say Group, 54 Avenue Hoche, 75008 Paris*

Dietary fibre, resistant starch and indigestible sugars are fermented by the anaerobic bacteria in the large bowel, resulting in the production of short-chain fatty acids (SCFA), mainly butyric, propionic and acetic acids, the former being the principal source of energy for colonocytes. It has been proposed that n-butyric acid could play an important role in the normal growth and differentiation of the colonic mucosa *in vivo*, and differential ratios of SCFA favouring n-butyric acid might modify the growth of colonic neoplasms and perhaps prevent their genesis, as suggested by epidemiologic studies. Because sodium butyrate (NaB), acting as a 'differentiating agent', has been reported to reverse *in vitro* some of the transformed characteristics of numerous cancer cell lines, NaB or derivatives could be of chemotherapeutic interest for colon cancers, and the management or prevention might also be facilitated through diet-controlled SCFA production in the colon. The present study set out to test the effect of NaB on two rat cell lines selected from a unique chemically-induced colon adenocarcinoma, and which differ by their state of differentiation and their subcutaneous tumorigenicity in the syngeneic BDIX rat. *In vitro* effects of NaB on the two cell lines were examined both on morphological criteria and relative to differentiation and tumorigenicity markers, i.e. alkaline phosphatase (ALP; *EC* 3.1.3.1), dipeptidylpeptidase IV (DPP-IV; *EC* 3.4.14.5), α (1→2) fucosyltransferase (*EC* 2.4.1.69) activities and expression of glycosidio antigens. ALP and DPP-IV, specific for the small intestine and the foetal colon, have been reported as markers for enterocytic differentiation of numerous colonic tumour cells, as were blood-group ABH glycosidic antigens. Upon sodium butyrate treatment the two cell lines presented a dose-dependent decrease of proliferation and a morphological evolution towards a more differentiated epithelial phenotype, but modulation of differentiation markers as ALP and DPP-IV, α (1→2) fucosyltransferase and the related blood-group antigens was non-linearly dependent on sodium butyrate concentration, and varied according to the cell line. However, when considered together, the differentiation markers suggested for the two cell lines a progress towards a more differentiated status that reached a maximum for 2·5 mmol/l NaB treatment, less differentiated features appearing when higher NaB concentrations were used. The present study points out the *in vitro* differentiating effect of NaB on the two cell lines, and NaB concentration, as crucial criteria for modulative effects on these colonic tumoural cells.

Ruminal fermentation of neutral detergent fibre and nitrogen in legume, grass and mixtures by growing steers. By BARBARA P. GLENN (Introduced by G. B. HUNTINGTON), *US Department of Agriculture, Agricultural Research Service, Ruminant Nutrition Laboratory, Beltsville, Maryland, USA*

The objective was to evaluate associative effects of lucerne (*Medicago sativa* L.) with orchardgrass (*Dactylis glomerata* L.) on ruminal fermentation. Second growth of lucerne (270 g dry matter (DM)/kg) and orchardgrass (240 g DM/kg) were direct-cut harvested and ensiled with 1·3 g HCHO/kg and 1·7 g HCOOH/kg on a wet weight basis. Diets of lucerne:orchardgrass were (DM basis) 100:0, 75:25, 50:50, 25:75 and 0:100. Data are listed in this order. Diets had 460, 479, 508, 541 and 587 g neutral detergent fibre (NDF)/kg DM and 26, 27, 28, 31 and 31 g N/kg DM. Holstein steers were raised as one group and allocated to two digestion experiments. In Expt 1, five steers (body-weight (BW) 273 (SE 9) kg) fitted with ruminal and proximal duodenal cannulas were fed diets 12 times/d at restricted intake (R) of 70 g DM/kg $BW^{0·75}$ in a 5×5 Latin square. In Expt 2, each diet was offered *ad lib.* (ALIB) to three steers (BW 355 (SE 11) kg) in a completely randomized design.

In Expt 1 (R), NDF intake increased linearly ($P<0·001$). Ruminal NDF fermentation (0·495, 0·619, 0·640, 0·672, 0·721) increased linearly ($P<0·05$) with increasing intake of grass. For each diet, all digestible fibre was fermented in the rumen.

The fractional contribution of digested NDF to digested DM increased linearly ($P<0·05$) in both trials from 0·37 to 0·67 in Expt 1 (R) and from 0·41 to 0·78 in Expt 2 (ALIB). In Expt 1 (R), there was a cubic effect ($P<0·05$) on efficiency of microbial N synthesis with increasing intake of grass (24, 22, 25, 34, 31 g microbial N/kg truly fermented organic matter) but there was no effect on intestinal N flow or total digestibility. In Expt 2 (ALIB), DM intake decreased linearly ($P<0·001$) from 8·5 to 6·1 kg/d with increasing intake of grass. In Expt 2 (ALIB), there were quadratic effects on digested N ($P<0·05$) and tissue N retention ($P<0·10$). Extent of fermentation of NDF was lower for legume than grass but, for both, all digestible NDF was fermented in the rumen. At restricted intake, the greater microbial efficiency as more grass was offered may be related to its high extent of fermentation and N content. The associative effects for ruminal microbial N synthesis and tissue N retention indicate synergism in ruminal fermentation of NDF and N from mixtures of two herbage species by steers fed on forage diets.

***In vitro*-fermentation of purified fibre from brown algae by human faecal bacteria.** By C. MICHEL[1], J.-L. BARRY[2], M. LAHAYE[3], C. BONNET[2] and S. MABEAU[1], [1]*Centre d'Etude et de Valorisation des Algues, BP 3–22610 Pleubian,* [2]*INRA – Laboratoire de Technologie Appliquée à la Nutrition, BP 527–44026 Nantes Cédex 03* and [3]*INRA – Laboratoire de Biochimie et Technologie des Glucides, BP 527–44026 Nantes Cédex 03*

Previous fermentability studies of total fibre from brown marine algae, with human faecal bacteria, led to discrepancies between substrate disappearance and bacterial metabolism. This result was surprising in so far as these two variables were usually closely correlated (Auffret *et al.* 1991). The aim of the present work was to identify, among total algal fibre, the polysaccharide(s) involved in this original fermentative pattern.

Total fibre from Pheophyceae consists of Na-alginates, laminarans, fucans sulphates and cellulose. The latter is well known so only the soluble purified fibres were studied: Na-alginates (A; Sigma), laminarans (L; Sigma), fucans sulphate (F; Ifremer) and beet fibre (B), used as reference, were incubated with human inoculum for 24 h (Barry *et al.* 1989). Fermentation products (gases, short-chain fatty acids (SCFA)) and residues were quantified. Polysaccharide fermentability was estimated as percentage of individual sugar disappearance (bacterial utilization coefficient; BUC), and as theorical organic matter fermented (TOMF), based on SCFA concentrations, according to Van Nevel & Demeyer (1970).

	Beet fibres	Laminarans	Na alginates	Sulphated fucans
Fermentability (proportion initial DM)				
BUC	78·2	90·1	86·8	0
TOMF	73·4	80·2	57·4	11·8
SCFA (mol proportions)				
Acetate	67	61	82	—
Propionate	20	23	13	—
Butyrate	13	16	5	—

Butyrate production from L was significantly ($P<0·05$) higher than from A. This SCFA is known to be involved in metabolic epithelial cell growth and motor properties of dietary fibre, so physiological properties of L may be different.

BUC and TOMF values for B, L and F only were closely correlated ($y = 1·1\ x\ 3·31$; r 0·90) suggesting that they involve classical pathways of bacterial fermentation. Because the BUC and TOMF results from A were not consistent with that of the other fibre types, it would appear to be the constituent of algal fibre responsible for the discrepancies in fermentative behaviour noted above. Alginates are polymers of mannuronic and guluronic acids; the latter is not known in the other fibre constituents and it may lie at the origin of the unusual fermentative behaviour.

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The effect of sucrose fatty esters on metabolism of human faecal bacteria. By RACHEL L. MILLER, NIAMH A. SCULLY and C. HENDERSON, *The Robert Gordon University, Queen's Road, Aberdeen AB9 2PG*

Wakita & Hoshino (1987) showed that sucrose fatty esters (SFE) containing 70% of fatty acid as monoester were hydrolysed by, and altered fermentation patterns of, rumen microbial populations.

In the present study four SFE were used (Ryoto Sugar Ester: Mitsubishi Kasei Foods Corp). These were S–170, containing 100% di-, tri- and polyesters of which stearate was 70%; B–370, 80% di-, tri- and polyesters of which behenate was 70%; O–1570, 70% monoester of which oleate was 70%; P–1570, 70% monoesters of which palmitate was 70%.

Faecal samples were diluted in anaerobic buffer solution and incubated with SFE emulsions (final concentrations 0·5 or 0·05 g/l) for 4 h at 37°. Pre-gelatinized wheat starch (5 g/l; Expt A) or sugar-beet fibre (20 g/l; Expt B) provided fermentable substrate. Faecal samples from two female donors were used for Expt A and Expt B respectively.

The four SFE were tested concurrently at the two levels of inclusion. Each experiment was replicated four times using separate faecal specimens. Fermentation products were analysed by gas–liquid chromatography after high-speed centrifugation of incubation mixtures. Data were corrected for pre-incubation levels of fermentation products in the faecal samples.

Fermentation products (mmol/l) in Expt A (0·5 g SFE/l)

Fermentation product . . .	Ethanol		Acetate		Propionate		Butyrate	
Sucrose fatty ester	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	0·52	0·23	29·68	1·90	6·87	1·75	9·27	1·32
Oleate ester	1·58*	0·73	35·40*	2·81	9·23*	1·19	13·30*	3·65
Palmitate ester	1·76***	0·67	35·26*	2·73	9·23*	1·13	13·97*	4·02
Behenate ester	1·69	1·06	31·08	1·62	8·30	0·74	11·69	3·27
Stearate ester	1·09	0·98	33·75	3·83	8·71	1·50	12·05	4·49

Significantly different from control by two-way analysis of variance: *$P<0·05$; ***$P<0·001$.

In Expt A significant increases in fermentation products were observed in the presence of 0·5 or 0·05 g/l sucrose oleate and sucrose palmitate. In the presence of sucrose behenate and sucrose stearate the increases in fermentation products were not significant. In Expt B there were no significant effects of SFE.

SFE were donated by Mitsubishi Kasei Food Corporation and sugar beet fibre by British Sugar Corporation.

Wakita, M. & Hoshino, S. (1987). *British Journal of Nutrition* **58,** 493–502.

Esterification of oleic and arachidonic acids in mouse jejunum. By Y. MATHIEU, A. BERNARD and H. CARLIER. *Laboratoire de Physiologie de la Nutrition, Ecole Nationale Supérieure de Biologie Appliquée à la Nutrition et à d'Alimentation, Université de Bourgogne, 1, Esplanade Erasme 21000 Dijon*

Studies carried out in mouse jejunal organ culture revealed some differences between esterification processes of oleic and arachidonic acids. To bring to light these differences, monoacylglycerol–acyltransferase activities were measured on microsomal preparations of mouse jejunal mucosa.

In a first set of experiments, explants of 2·5-month-old mouse jejunum were incubated for 15 min at 37° in 10 ml of a stirred culture medium enriched with lipids (1·2 mM-[^{14}C]oleic acid or arachidonic acid-oleic acid-monopalmitin 1:1:1 mol:mol:mol) emulsified in sodium taurocholate (2·4 mM). Synthesized labelled phospholipids and triacylglycerols of the lipoproteins secreted in the incubation medium were extracted and analysed by thin-layer chromatography.

In a second set of experiments, explants of 2·5-month-old mouse jejunum were cultured for 15 min in Falcon dishes in the presence of the same culture medium enriched in lipids and cultured for 6 h in a free lipid medium. Synthesized labelled phospholipids and triacylglycerols both remaining in explants and released in the culture medium were analysed at the end of the culture.

	Synthetic activity of jejunal explants from 2·5-month-old mouse (nmoles synthesized/mg intestinal protein)			
	Phospholipid		Triacylglycerol	
	Mean	SEM	Mean	SEM
C18:1 (*n* 5)	35	1	84	10
C20:4 (*n* 6)	37	3	57*	6

* Significantly different from C18:1; $P<0·05$.

In the two cases, at the same phospholipid synthesis level, triacylglycerol formation was significantly lower with arachidonic acid compared with oleic acid. Such results are in agreement with previous works in rats (Chen *et al.* 1985; Pavero *et al.* 1992).

Age (months)	Monoacylglycerol–acyltransferase specific activities (nmol CoA released/min per mg microsomal protein) in 2·5-, 12- and 24-month-old mice (*n* 4)					
	2·5		12		21	
	Mean	SEM	Mean	SEM	Mean	SEM
Microsomal protein (mg)	2·2	0·3	3·5	0·8	5·5†	0·4
Oleoyl CoA (*n* 4)	276	21	337	36	155	62
Arachidonoyl CoA (*n* 4)	59**	2	48**	18	29**	5

** Significantly different from oleoyl CoA, $P<0·01$.
† Significantly different in same row, $P<0·05$.

In 2·5-, 12- and 21-month-old mice monoacylglycerol-acyltransferase activity was measured spectrophotometrically by the method of Rodgers (1969) using oleoyl and arachidonoyl-CoA. Assays showed a lower activity of arachidonoyl-CoA than oleoyl-CoA. These results support previous observations regarding specific modalities in esterification processes according to the nature of fatty acid. Furthermore, these differences in the activity of the monoacylglycerol–acyltransferase between the two acyl-CoAs persist with age without significant modification for 21-month-old *v.* 2·5-month-old mice.

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Propionate presents effects similar to those of butyrate on HT29 cell growth and differentiation. By L. GAMET[1], D. DAVIAUD[2], C. DENIS-POUXVIEL[2], C. REMESY[1], J.-C. MURAT[2] and H. PARIS[2], [1]*Laboratoire des Maladies Métaboliques, INRA de Clermont-Ferrand/Theix, F-63122 St. Genès Champanelle* and [2]*INSERM U317, Bât. L3, CHU Rangueil, F-31054 Toulouse*

Carbohydrates of the fibre fraction are extensively broken down by the microflora in the large intestine. This fermentation process results in the production of short-chain fatty acids (SCFA), chiefly acetic, propionic and butyric acids. Protective effects of dietary fibre against colon cancer are partly explained by the fact that it dilutes the gut contents and speeds up the transit time, thus diminishing the concentrations of carcinogens or promotors and the time of exposure (Weisburger, 1991). However, the end-products of fibre fermentation (such as SCFA) may have specific effects, and butyrate has given rise to a great deal of interest. Besides its role as an energy fuel for the colonic mucosa (Roediger, 1982), butyrate has been found to be an antiproliferative and differentiating agent in various cancer cell-lines *in vitro* (Kruh *et al.* 1991). However, growth regulatory properties of the other SCFA have been relatively overlooked.

The effects of SCFA on growth and differentiation of the human colonic adenocarcinoma cell line HT29 have been compared and the present results show that, under present culture conditions, both propionate and butyrate inhibited the growth of HT29 cells, whereas acetate had no significant effect. The antiproliferative effect of propionate or butyrate was associated with an inhibition of fetal calf serum-induced activation of ornithine decarboxylase (ODC; *EC* 4.1.1.17), a key enzyme of polyamine metabolism. Inhibition of cell growth by either propionate or butyrate could not be reversed by putrescine addition, which reveals that these SCFA are not solely acting on the ODC-polyamine system. Propionate and butyrate also elicited an increase of alkaline phosphatase (*EC* 3.1.3.1) activity, which reflects a more differentiated phenotype than that of untreated control cells. SCFA might induce some changes in the plasma membrane components; it appeared indeed that propionate and butyrate could, in the HT29 cell line, depress α2-adrenoreceptor number and increase the amount of G_1 proteins. The level of expression of α2-adrenoceptors could be correlated with the amounts of its mRNA, suggesting that these SCFA exert their effect at the transcriptional level. Recent data support the hypothesis of a relationship *in vivo* between intestinal cell differentiation and the expression of α2-adrenoceptors and G_1 proteins. Our results confirm the effect of propionate on the regulation of differentiation. Moreover, since α2-adrenoceptors mediate the anti-secretory and pro-absorptive effects of catecholamines, and since G_1 proteins are in some cases involved in ion channels regulation, it is suggested that propionate and butyrate, by this means, could regulate electrolyte transport through the colonic epithelium.

Taken together, our results suggest that propionate, like butyrate, may play an important role in the physiology of the colon and could partially account for the protective effect of dietary fibre with respect to colon carcinogenesis.

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Weisburger, J. H. (1991). *Seminars in Oncology* **18,** 316–336.

Rapid modulation of lactate utilization by inhibition of glycolysis in isolated hepatocytes from fed rats. By C. MORAND, C. REMESY and C. DEMIGNE, *Laboratoire des Maladies Métaboliques, INRA de Clermont-Ferrand/Theix, F-63122 St. Genès Champanelle*

Lactate is a major gluconeogenic substrate in the liver, particularly during starvation, and it plays a key role in glucose homeostasis. Most of the *in vitro* studies dealing with the control of hepatic lactate utilization have been carried out in starved animals. In fed animals the liver is a minor site for lactate utilization although its concentration in the portal vein is higher than in the other vascular areas, due to intestinal glycolysis. The hepatic utilization of lactate may be controlled by its rate of transport across the plasma membrane, by cellular metabolism or by changes in acid-base equilibrium (Fafournoux *et al.* 1985; Sestoft & Marshall, 1988; Felipe *et al.* 1991).

In the absence of lactate in the medium, hepatocytes from fed rats released lactate and pyruvate, and the addition of lactate shifted the lactate balance from net release to net utilization, with a threshold of about 3 mM (in the presence of 10 mM glucose). In order to avoid interferences from pyruvate efflux, the initial lactate:pyruvate (L:P) ratio in the medium has been appropriately adjusted to get a steady ratio throughout the experiments. Increasing concentrations of lactate in the medium stimulated its own utilization by inhibiting hepatic glycolysis (estimated by the release of ^3H_2O from [6-^{3}H]glucose). Addition of oleate to liver cells elicited: (1) a net reduction of the release of lactate and pyruvate in basal conditions; (2) a decrease in the threshold of lactate utilization (down to 1·5 mM); (3) a marked stimulation of lactate utilization at physiological concentrations (2·3 mM). Oleate acted by decreasing the cellular concentrations of pyruvate as a result of an inhibition of glycolysis and of a rise in gluconeogenesis from C3-units. It turns out that fatty acids mimick the effect of glucagon on lactate metabolism mediated by inhibition of glycolysis, but not those corresponding to a stimulation of glycogenolysis.

The impact of changes in pH has also been investigated on the utilization of lactate by hepatocytes from fed rats. In acidotic conditions (pH 7·15 and [HCO_3-]10 mM), the threshold of lactate utilization was considerably lowered, with a shift from 3 mM (pH 7·45) to about 1 mM (pH 7·15). Furthermore, for the low concentrations of lactate (<1 mM), lactate production by liver cells was markedly reduced, reflecting a 50% inhibition of the glycolytic flux. Acidosis also enhanced the utilization of lactate up to 5 mM (+50%).

In conclusion, it appears that there are possibilities of rapid modulation of lactate utilization in hepatocytes from fed rats, depending chiefly on the presence of factors which inhibit glycolysis, such as cAMP-dependent hormones, but also fatty acids or acidosis.

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Effect of baked beans on steroid metabolism of hypercholesterolaemic pigs. By N. M. B. COSTA[1], A. G. LOW[1], A. F. WALKER[1] and R. W. OWEN[2], [1]*Department of Food Science and Technology, University of Reading, Whiteknights, Reading, Berks, RG6 2AP* and [2]*PHLS Centre for Applied Microbiology and Research, Division of Biotechnology, Porton Down, Salisbury, Wilts SP4 0JG*

The hypocholesterolaemic effect of baked beans has been demonstrated in pigs (Costa *et al.* 1991; Shutler *et al.* 1988) and in normocholesterolaemic subjects (Shutler *et al.* 1989), but the mechanism of action has not been established. The present study investigated the effect of baked beans on steroid metabolism of pigs fed a Western-type diet. Three groups (BL, NP and IR) of four pigs were made hypercholesterolaemic by feeding a semi-purified control diet, high in saturated fatty acids, supplemented with 10 g cholesterol/kg for 14 d. The IR pigs were previously prepared with an ileo-rectal anastomosis to nullify the function of the large intestine. After the period on the control diet, IR and intact (NP) pigs were placed on a similar diet, in which baked beans replaced part of the basal diet (300 g baked beans/kg diet on dry-matter basis), for a further 28 d. Group BL was kept on control diet throughout the experiment. NP pigs fed on baked beans showed considerable differences compared with the other groups, as follows: (a) reduced plasma cholesterol; (b) higher concentration of cholesterol in bile; (c) higher concentration of bile acids, especially secondary bile acids in bile; (d) reduced elimination of bile acids in faeces, especially secondary bile acids; (e) higher excretion of coprostanol and lower elimination of cholesterol in faeces.

Group . . .	BL	NP	IR	SED
Plasma cholesterol (mmol/l)	6·24	3·97	5·58	1·075
Bile				
Cholesterol (mg/ml)	0·78	1·58	0·99	0·388
Bile acids (mg/ml): total	$8·97^{a}$	$14·63^{b}$	$6·69^{a}$	1·884
secondary	$3·80^{b}$	$7·58^{c}$	$0·34^{a}$	0·598
Daily faecal output: cholesterol (g)	$6·70^{b}$	$3·72^{a}$	$9·16^{c}$	0·892
coprostanol (g)	$2·18^{b}$	$4·06^{c}$	$0·32^{a}$	0·360
primary bile acids (g)	$0·00^{a}$	$0·00^{a}$	$1·75^{b}$	0·183
secondary bile acids (g)	$1·08^{b}$	$0·44^{a}$	$0·33^{a}$	0·166

Values in same row with unlike superscripts are significantly different (Student's *t* test): $P<0·05$, df 9.

These findings suggest that baked beans potentiate bacterial fermentation and steroid degradation in the large intestine. Simultaneously, it seems to speed up the entero-hepatic circulation of bile acids and cholesterol. The high concentration of bile acids and cholesterol in bile may promote a feedback inhibition on hepatic cholesterol synthesis and, hence, reduce plasma cholesterol. Further investigations, however, are necessary to make a firm conclusion.

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Metabolic and endocrine changes induced by heat exposure in chickens. By P. A. GERAERT[1], J. C. F. PADILHA[2] and S. GUILLAUMIN[1], [1]*INRA Station de Recherches Avicoles, 37380 Nouzilly* and [2]*CNPq–Universidade Federal Santa Catarina, 88049 Florianopolis, Brasil*

When exposed to heat, birds, like mammals, decrease their food intake in order to reduce metabolic heat production. However, the reduced growth rate observed in hot conditions is only partly attributable to decreased food intake (Mitchell & Goddard, 1990). To understand metabolic and endocrine changes involved in heat exposure, male chickens were exposed to 22 or 32° (constant) for 14 d starting at 2 or 4 weeks of age. A group of pair-fed birds received amounts of food equal to that consumed at 32°. Blood samples were taken at the end of each period.

Results presented in the Table showed that reduction of growth rate due to high temperature reached 6% between 2 and 4 weeks of age, and 22% between 4 and 6 weeks of age for the same intake. In fasted birds, no difference appeared in glycaemia or triacylglycerolaemia. However, in fed chickens, heat exposure significantly increased plasma glucose concentration while triacylglycerolaemia was higher in pair-fed birds (22°). Plasma thyroxine (T4) and triiodothyronine (T3) levels were decreased in heat-exposed birds compared with pair-fed birds ($P<0{\cdot}001$). Indeed, thyroid hormone levels would be more related to energy balance or feed efficiency than to energy intake (Danforth & Burger, 1989).

		22° *ad lib.*		22° pair-fed		32° *ad lib.*	
		Mean	SD	Mean	SD	Mean	SD
				2–4 week period			
Food intake (g/d)		94·3	6·0	80·9	1·3	80·9	7·8
Weight gain (g)		705	57	614	27	580	73
Glucose (g/l):	Fasted	1·94	0·11	1·95	0·07	1·79	0·16
	Fed	2·21	0·14	2·34	0·16	2·51	0·41
Triacylglycerols (g/l):	Fasted	0·48	0·12	0·45	0·10	0·51	0·10
	Fed	1·42	0·22	1·68	0·31	1·50	0·32
				4–6 week period			
Food intake (g/d)		154·9	12·3	117·4	2·9	118·3	18·8
Weight gain (g)		1155	134	845	61	659	169
Glucose (g/l):	Fasted	1·92	0·09	2·05	0·13	1·92	0·17
	Fed	2·26	0·06	2·36	0·14	2·42	0·16
Triacylglycerols (g/l):	Fasted	0·27	0·06	0·25	0·06	0·26	0·07
	Fed	1·28	0·32	1·35	0·20	1·05	0·37
T3 (nmol/l):	Fasted	1·60	0·37	1·43	0·29	1·44	0·38
	Fed	3·51	0·95	3·59	0·84	1·47	0·46
T4 (nmol/l):	Fasted	21·6	3·1	18·4	3·2	18·6	3·2
	Fed	13·2	1·9	13·1	2·0	9·6	1·9

Sensitivity to exogenous insulin was also determined at 48 d of age. While in fasted chickens heat exposure induced decreased response, fed, heat-stressed chickens appeared more sensitive to insulin than pair-fed birds at 22°.

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Adaptation of gastric lipase in mini-pigs fed on a high-fat diet. By M. ARMAND[1], P. BOREL[1], P. H. ROLLAND[2], M. SENFT[1], M. ANDRÉ[1], H. LAFONT[1] and D. LAIRON[1], [1]*Unité 130-INSERM (National Institute of Health and Medical Research), 18 avenue Mozart, 13009 Marseille* and [2]*Unité 278-INSERM, 27 boulevard Jean Moulin, 13005 Marseille*

Whereas lots of investigations have been conducted on the adaptation of pancreatic lipase (*EC* 3.1.1.3) to dietary fat, only limited data were available until recently concerning the nutritional adaptation of pre-duodenal lipases. Only three studies performed in young rats (Hamosh, 1978), adult rats (Armand *et al.* 1990) and adult rabbits (Borel *et al.* 1991) have established the existence of an adaptation of lingual or gastric lipase activities in response to increased dietary fat. Pre-duodenal lipase is predominantly gastric lipase in both pigs and humans. Thus, the present study was performed in mini-pigs to obtain the first comparative data on the adaptation of gastric lipase and colipase-dependent pancreatic lipase to dietary fat in an omnivorous animal model.

Nine adult male mini-pigs of the Pitmann-Moore strain, weighing 14–21 (mean 18·3 (SD 2·6)) kg at the beginning of the experiment, were used. These were divided into two groups which were fed for 3·5 months on either a low-fat diet (four pigs) or a high-fat diet (five pigs). The low-fat diet (500 g/d) was a standard food for pigs providing 13·5 MJ/kg supplied by fat (5·6%), proteins (18·6%) and carbohydrates (75·7%). The high-fat diet (400 g/d) contained additional sources of fat. It provided 21·2 MJ/kg, with 60·5% supplied by fat. Thus, the fat content was 20 g/kg in the low-fat diet (vegetable fat) and 340 g/kg in the high-fat diet (vegetable fat and butter fat). After a 24 h fast the mini-pigs were anaesthetized and the pancreas and stomach were removed. The methods used for gastric lipase, pancreatic lipase and colipase activity assays have been previously described (Borel *et al.* 1991).

Feeding pigs for 3–5 months on the high-fat diet significantly increased the final body-weights ($P<0·05$) but did not change the fundus-area mucosa and pancreas weights, or the protein content in both tissues. The gastric lipase activity was essentially found in the fundus-area mucosa (71–134 units/g tissue), compared with the cardiac area (4–12 units/g tissue), the body area and the antro-pyloric area (no detectable values). When the amount of fat present in the diet increased from 2% to 34%, the activity (units/g mucosa) of gastric lipase located in the fundus area and the specific activity of the enzyme significantly increased by 88·7% and 85·2%, respectively. The calculated lipase activity present in the whole fundus-area mucosa significantly increased by 84·9%. Feeding mini-pigs 340 g fat/kg significantly increased the pancreatic lipase activity by 63·3% and 111·3% for activity/g tissue and for specific activity, respectively. The lipase activity of the whole pancreatic gland significantly increased by 76·4%. The specific activity of colipase (units/mg protein) significantly increased by 70·8% in the high-fat-fed group but the colipase:lipase ratio decreased from 0·822 to 0·680.

In conclusion, we have found that the extent of adaptation of gastric and pancreatic lipase to dietary fat were very similar while the relative importance of either enzyme did not change after adaptation. Given this body of evidence obtained in rats, rabbits and pigs, it can be pointed out that gastric lipase adaptation may well occur in humans. This might be especially important in pathologic situations in which pancreatic lipase is severely lacking due to pancreatic insufficiency such as cystic fibrosis or chronic pancreatitis, and in full-term and pre-term newborns.

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Kinetics of the adaptation of rat pancreatic hydrolases to a high-protein diet containing soya bean and fish. By E. F. LHOSTE, M. FISZLEWICZ, T. TRANCHANT, A. M. GUEUGNEAU and T. CORRING, *Unité d'Ecologie et de Physiologie du Système Digestif, INRA, 78350 Jouy-en-Josas*

The effect of high dietary protein on the rat pancreas is well known. To date the quality of protein has only been addressed in terms of biological value, but most studies have been performed using casein which is not the major constituent of the human diet.

In the present experiment we studied the kinetics of adaptation of enzyme biosynthesis and mRNA expression in the pancreas of Fischer rats fed on diets containing 200 or 500 g protein/kg. The protein component of the diets consisted of a mixture (50:50) of fishmeal and soya-bean protein isolate. Rats were adapted to 200 g protein/kg diet for 1 week before receiving the 500 g protein/kg diet. They were killed after being fed for 0–7 d on the latter. For each enzyme, protein biosynthesis and mRNA expression were studied. Specific activities were assayed in rats fed on the experimental diets for 1 week.

Day . . .	0		1		2		3		5		7	
Biosynthesis (% of total):												
AMY (*EC* 3.2.1.1)	25·9		31·2		22·0		27·9		20·7		19·2	
LIP (*EC* 3.1.1.3)	5·9		5·4		4·1		6·2		6·9		5·7	
SERPROT	17·4		22·5		23·0		25·6*		25·5*		26·2*	
mRNA (% of day 0):	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
AMY	119	15	94	18	76*	7	66*	9	64*	7		
LIP	110	9	128	22	143	10	179*	26	174*	23		
CTG (*EC* 3.4.21.1)	118	10	104	15	130	15	154	5	169*	20		
TRP (*EC* 3.4.21.4)	125	9	118	10	137*	9	152**	17	144*	13		
ELA I (*EC* 3.4.21.11)	113	9	123	30	135	11	135	7	159	24		
ELA II (*EC* 3.4.4.7)	139	13	107	10	154	12	178*	47	195**	21		
PCPA 2 (*EC* 3.4.17.1)	133	24	165	47	92	5	134	39	110	10		
PCPB (*EC* 3.4.17.2)	126	11	121	15	114	11	133	15	120	11		

Significantly different from day 0: *$P<0·05$; **$P<0·01$.

In response to the high-protein diet, amylase (AMY) biosynthesis and mRNA were progressively reduced. Lipase (LIP) biosynthesis was not modified but mRNA levels were increased. Serine protease (SERPROT) biosynthesis was enhanced due to an increase in chymotrypsinogen (CTG), trypsinogen (TRP) and elastase II (ELA II) mRNA. Carboxypeptidases (PCP, A2 and B) biosynthesis and mRNA were not modified.

Amylase and protease specific activities followed the same pattern as their respective mRNA. Lipase specific activity was not modified.

Adaptation of the exocrine pancreas to a high-protein diet in germ-free and conventional rats. By I. CATALA, M. FISZLEWICZ, E. F. LHOSTE and T. CORRING, *Unité d'Ecologie et Physiologie du Système Digestif, INRA, 78350 Jouy-en-Josas*

The present study was conducted to evaluate the possible role of intestinal microflora in the adaptation of the exocrine pancreas to a high-protein diet containing a protein mixture from two different sources (fishmeal and soya-bean protein isolate). Two groups of twelve germ-free rats and two groups of twelve conventional rats were fed over 21 d on either the control diet (200 g protein/kg diet) or a high-protein diet (500 g/kg). The eventual alteration in the specific activity of the endopeptidases trypsin, chymotrypsin and elastase, the exopeptidases carboxypeptidase A and B and the expression of their corresponding pancreatic mRNA was quantified.

When animals were fed on the control diet the presence of intestinal microflora led to a twofold increase in trypsin specific activity and an enhanced expression of chymotrypsin and elastase mRNA. There was an adaptation of proteolytic pancreatic enzymes when animals received the high-protein diet, whatever the bacterial status. As far as chymotrypsin is concerned, the adaptation was associated with a modified mRNA expression.

Specific activities (SA; units/g of protein) and mRNA expression (cpm/μg mRNA) of pancreatic endopeptidase in germ-free (GF) and conventional (CV) rats

	Diet (g protein/kg)	Trypsin		Chymotrypsin		Elastase	
		GF	CV	GF	CV	GF	CV
SA	200	0·38***	0·60	22·7	23·4	0·89	0·80
	500	0·61***	1·00†††	44·6**	34·0†††	1·15	1·15†††
mRNA	200	121	148	922***	2898	225***	617
	500	124	127	1625***	3211	253***	700

Significantly different from CV rats: $^{**}P<0·01$; $^{***}P<0·001$.
Significantly different from 200 g protein/kg diet: †††$P<0·001$.

When animals were fed on the control diet, the presence of the intestinal microflora was associated with an increase in both specific activity of carboxypeptidase A (9·3 (SD 20)) and mRNA expression of carboxypeptidase B (322 (SD 967)). The high-protein diet did not modify the specific activities of either carboxypeptidase. The molecular regulation of pancreatic adaptation to the high-protein diet remains to be demonstrated.

Depending on the enzyme, the intestinal microflora acts at different levels in the biosynthesis mechanism. This does not seem to be altered by the protein concentration of the diet.

Pharmacological and biochemical evidence for differential expression of A- and B-subtype CCK-gastrin receptors in the calf pancreas during development. By V. LE MEUTH[1], V. PHILOUZE[1], M. FORMAL[1], I. LE HUEROU-LURON[1], N. VAYSSE[3], C. GESPACH[2], P. GUILLOTEAU[1] and D. FOURMY[3], [1]*INRA, Rennes,* [2]*INSERM U55, Paris* and [3]*INSERM U151, Toulouse*

The status of CCK-gastrin receptors was studied in pancreatic plasma membranes isolated from newborn calves, from milk-fed calves at 28 and 119 d (PR) and from weaned calves at 119 d (R).

At all stages, Scatchard plot analysis of binding data were compatible with two affinity classes of binding sites: high affinity, low capacity sites and low affinity, high capacity sites. Affinity and capacity of the high affinity sites were significantly higher ($P<0{\cdot}05$) in 119 d-weaned calves (R; kd = 0·13 (SEM 0·03) nM, B_{max} = 53 (SEM 12) fmol/mg protein) than in newborn (kd = 0·23 (SEM 0·02) nM, B_{max} = 2·43 (SEM 0·07) fmol/mg protein) and in 119 d milk-fed animals (P; kd = 0·35 (SEM 0·08) nM, B_{max} = 18 (SEM 5) fmol/mg protein).

At birth, IC_{50} for ^{125}I-BH-(Thr^{28}, Nle^{31})-CCK-9 binding were: (±) L 364718: 0·6 nM > CCK-9: 1·5 nM > JMV-179: 6·7 nM > G17 ns: 130 nM > (±) L 365260: 220 nM; PD 135158 > 100 nM. At 28 d there were: CCK-9: 1 nM > G17 ns: 1·4 nM > PD 135158: 6·7 nM > (±) L 365260: 10 nM > (±) L 364718: 140 nM > JMV-179: 620 nM. At 119 d (P) there were: CCK-9: 0·7 nM > G17 ns: 2·6 nM > PD 135158: 4·9 nM > (±) L 365260: 6·7 nM > (±) L 364718: 85 nM > JMV-179: 924 nM. At 119 d (R) there were: CCK-9: 0·3 nM > G17 ns: 0·5 nM > PD 135158: 3 nM > (±) 365260: 16 nM > (±) L 364718: 190 nM > JMV-179: 670 nM. Binding was inhibited by GTP[S] at all stages (IC_{50}: 0·07–2·6 μM).

We further identified the CCK receptor by photoaffinity labelling with ^{125}I-ASD-(Thr^{28}, Nle^{31})-CCK-9, followed by SDS-PAGE. At birth, the labelling occurs mainly on a component of 85–95 kDa which is the usual mass of the CCK-A receptor in other species. At 28 d, two components seem to be labelled: a first one at 85–95 kDa and a second one at 42–47 kDa. At 119 d (P) and (R) labelling occurs at 42–47 kDa.

We conclude that in neonatal calf pancreas CCK receptor is mainly of A-subtype whereas at 28 and 119 d (P or R) a predominant B-subtype is found. These receptors are functionally coupled to G protein(s) and have distinct mass. Prolonged milk feeding in animals between 28 d and 119 d seems to prevent CCK receptor expression at the cell membrane level. This report, which is the first about differential expression of A and B pancreatic CCK-G receptors during development, raises the question of the status of pancreatic receptors in higher mammals.

Adult and old rats retain sensitivity for stimulation of muscle protein synthesis by amino acid. By L. MOSONI, M. L. HOULIER, P. PATUREAU MIRAND, G. BAYLE and J. GRIZARD, *Laboratoire d'Etude du Métabolisme Azoté, Institut National de la Recherche Agronomique, Theix, 63122 Ceyrat*

A loss of skeletal muscle protein is well described in old age, resulting from an imbalance between muscle protein synthesis and degradation. However, in studies measuring basal muscle protein turnover *in vivo* only slight differences were obtained between adult and old animals (Lewis *et al.* 1984; Mays *et al.* 1991). Our approach was to stimulate muscle protein synthesis by amino acids and insulin in order to detect age differences in muscle responsiveness. Conscious male rats, aged 12 (adult) and 24 (old) months, were infused for 90 min with either saline (9 g NaCl/l; C), amino acids (AA), or amino acids with insulin and glucose (AAI). Gastrocnemius protein fractional (FSR) and absolute (ASR) synthesis rates were measured during the last 15 min of infusion (flooding dose of valine with L-(2,3,4)-^{3}H-valine). Gastrocnemius weight, protein, ASR and FSR are given in the Table.

	Adult			Old		
	n	Mean	SE	*n*	Mean	SE
Gastrocnemius:						
Weight (g)	16	3·2	0·1	15	2·2*	0·1
Protein (mg)	16	557	20	15	397*	23
ASR (mg/d)	16	36·9	1·7	15	35·9	1·5
FSR (%/d):						
C	6	5·9	0·2	4	7·8*	0·5
AA	5	7·1†	0·2	4	9·2*†	0·4
AAI	5	7·0†	0·2	6	9·2*†	0·4

* Significantly different from adult: *$P \leq 0{\cdot}05$. Significantly different from C, within each age group: †$P \leq 0{\cdot}05$.

Old rats showed a decline in muscle weight and protein mass. ASR were not different in adult and old rats but FSR were higher in old rats because of muscle atrophy. When variability related to atrophy was taken into account (variance–covariance analysis), we showed that amino acid infusion with and without insulin stimulated gastrocnemius FSR to a similar extent in adult and old rats, and that insulin had no additional effect, whatever the age, which is consistent with results obtained in adult rats by Baillie & Garlick (1991).

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Threonine dehydrogenase (*EC* 1.1.1.103) in growing pigs fed on threonine-deficient diets: effect of glutamic acid or protein addition. By N. LE FLOC'H, B. SÈVE and Y. HENRY, *INRA, St Gilles, 35590 L'Hermitage*

The aim of the present work was to investigate the interaction between threonine deficiency in the diet and protein or glutamic acid supplementation on growth, food intake and threonine metabolism. In pigs, the major pathway for the degradation of threonine seems to be via threonine dehydrogenase (TDG) which leads to glycine formation *in vivo*, and aminoacetone production *in vitro* (Ballèvre *et al.* 1991).

Fifty-four crossbred gilts (Pietrain × Large White) weighing 40 kg were allotted to six experimental treatments. Basal diets (1 and 4) were low-protein diets (126 g/kg). In diets 2 and 5 the content of protein was 156 g/kg. In diets 3 and 6, glutamic acid was added to match the additional non-essential amino acids in diets 2 and 5. In diets 1, 2 and 3, adequate supplements were used to meet the requirements for essential amino acids except threonine which was maintained at 4·2 g/kg diet. The requirement for threonine was covered in diets 4, 5 and 6 through the addition of 1 g L-threonine/kg diet. Pigs were slaughtered at 102 (SD 3·1) kg. TDG activity was measured in the liver as the rate of aminoacetone formation (Bird, 1980).

Threonine concentration (g/kg diet) . . .	4·2			5·2				
Diets . . .	1	2	3	4	5	6	Effects†	
Weight gain (kg/d)	0·77	0·74	0·90	1·02	1·02	0·98	T**	T×G*
Food intake (kg/d)	2·41	2·39	2·61	2·71	2·65	2·57	T**	T×G*
TDG‡	32·8	46·7	73·5	57·1	87·7	72·6	T**	G**
(U/kg BW)							P*	TPG**

T, threonine addition; P, protein addition; G, glutamic acid addition; TPG, contrast comparing the differences of response to protein and glutamic acid at each level of threonine. Level of significance: $*P<0·05$; $**P<0·01$.

† Analysis of variance using the GLM procedure of SAS.

‡ TDG activity (μmol aminoacetone formed/min).

Supplementation of a threonine-deficient diet with glutamic acid led to an increase in weight gain and food intake. Protein addition to either diet did not elicit similar increases in weight gain or food intake. This result suggests that glutamic acid may partly replace threonine in a threonine-deficient diet. Glutamic acid addition was more effective than protein addition in increasing TDG activity when threonine was limiting but the reverse was true when threonine was adequate. This result is consistent with previous data showing an increase in TDG activity according to protein supplementation (D. Bercovici and B. Sève, unpublished results). These data imply that threonine metabolism and glutamic acid are not independent but the mechanism of this interaction remains unclear.

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Bioavailability and pharmacokinetics of synthetic lysine (LYS) and methionine (MET) in pigs. By J. VAN DER MEULEN[1], J. G. M. BAKKER[1] and L. P. JAGER[2], [1]*Research Institute for Livestock Feeding and Nutrition (IVVO-DLO)* and [2]*Central Veterinary Institute (CDI-DLO), Lelystad, The Netherlands*

Increasingly pig diets are supplemented with synthetic amino acids (AA). Digestibility trials indicate that synthetic AA disappear completely from the small intestine. However, it has been suggested that plasma profiles of synthetic AA differ from those of protein-bound AA, resulting in an imbalance of AA in the body. The object of the present study was to investigate the absorption and elimination kinetics of synthetic MET and LYS and to determine their bioavailability.

Four barrows (37 kg), fitted with indwelling jugular vein catheters, were fed once daily on a barley–tapioca diet at a level just exceeding maintenance requirement. Experiments of 3 consecutive days each were carried out. In the first Expt the barrows received a dose of 0·9 mmol MET/$kg^{0·75}$ orally (day 2) and intravenously (day 3), while on day 1 (control) no MET was administered. The second Expt was carried out in the same way, but MET was replaced by 2·1 mmol LYS/$kg^{0·75}$. The amount of MET or LYS the pigs received in the last 2 d of the Expts exceeded their requirement, but did not exceed the concentration in commercial pig feeds. Each day regular blood samples were taken and plasma AA levels were determined. Plasma curves after intravenous and oral administration were corrected for the control levels of day 1. Individual plasma concentration *v.* time curves were analysed and basic pharmacokinetic parameters were calculated by using the PCNONLIN software package. The bioavailability was calculated by dividing the area under the plasma curve (AUC) after oral administration by the AUC after intravenous administration.

Plasma concentration *v.* time curves after intravenous injection of both MET and LYS could be well described by a two-compartment model, while the oral curves were adequately described by a one-compartment model with first-order absorption. The intravenous administration resulted in a mean (SEM) residence time (MRT) of 5·02 (0·41) and 10·67 (1·18) h and plasma clearance (CL) of 0·32 (0·04) and 0·18 (0·04) l/h per $kg^{0·75}$, for MET and LYS respectively. After oral administration there was a lag time of 0·61 (0·08) h before absorption of LYS started, while there was no lag time for MET. The absorption half-life for MET and LYS was 0·74 (0·43) and 4·32 (1·45) h, respectively. This indicates that absorption of both MET and LYS takes a few hours and is faster for MET. The faster absorption of MET is in agreement with the sequence of disappearing essential AA from different sources of dietary protein in isolated loops of the small intestine (Buraczewski, 1980). After oral administration MRT were 7·45 (0·46) and 12·62 (0·95) h and CL was 0·34 (0·03) and 0·24 (0·02) l/h per $kg^{0·75}$, for MET and LYS respectively. So LYS, once absorbed, remains for a longer time in the systemic circulation than MET. The bioavailabilities of MET and LYS were 69 (6) and 86 (8)%, respectively. As it has been reported that synthetic AA are completely digested, this indicates that added synthetic MET is metabolized by the intestinal microflora, the intestinal wall and/or the liver to a far greater extent than added synthetic LYS.

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Effect of dietary methionine concentration on plasma free amino acid levels in genetically lean and fat chickens. By A. M. CHAGNEAU, T. COCHARD, S. HAMZAOUI, M. LARBIER and B. LECLERCQ, *INRA, Station de Recherches Avicoles, 37380 Nouzilly*

Genetically lean chickens use dietary amino acids (AA) more efficiently than fat chickens in the synthesis of body fat and feather proteins (Leclercq, 1983; Leclercq & Guy, 1991). However, this difference may be limited to only a few AA families. In an experiment, using diets supplemented with methionine, to determine the sulphur AA (SAA) requirement of lean (LL) and fat (FL) chickens, plasma samples were collected from birds in a fed state and free AA profiles determined by liquid chromatography.

Lysine, glutamic acid, histidine and serine were found at significantly higher concentrations in LL birds than in FL birds (Table). Branched-chain AA, aromatic AA, SAA and arginine were found at higher concentrations in FL birds than in LL birds (Table). No differences were observed for aspartic acid, glycine, alanine and total AA. Methionine supplementation decreased free AA levels, with the exception of arginine and leucine.

This suggests that the metabolism of AA families differs between genotypes. Therefore, the 'ideal' AA profiles of dietary protein will differ between genotypes.

AA concentration (mg/100 ml plasma)

SAA (g/kg diet) . . .		5·4	5·8	6·2	6·6	7·0	Genotype effect	Diet effect	Inter-action
Branched-chain	LL	7·85	7·74	7·68	6·96	7·12	***	*	NS
	FL	9·18	10·17	8·78	8·60	8·18	***	*	NS
SAA	LL	2·20	2·31	2·25	2·87	2·47	***	***	**
	FL	2·15	3·98	3·35	3·76	4·14	***	***	**
Lysine	LL	23·8	24·0	19·9	14·5	14·0	***	***	NS
	FL	20·2	15·5	12·0	11·3	10·0	***	***	NS
Arginine	LL	5·51	5·21	6·15	6·15	7·12	***	NS	NS
	FL	7·12	8·10	7·84	7·78	8·19	***	NS	NS
Aromatic AA	LL	4·66	5·45	5·15	4·90	4·65	**	*	NS
	FL	5·39	6·33	6·09	5·76	5·58	**	*	NS
Glutamic acid	LL	3·08	2·93	3·14	2·65	2·75	***	NS	NS
	FL	2·69	2·73	2·48	2·56	2·44	***	NS	NS
Glycine	LL	6·51	6·37	5·92	5·11	4·87	NS	***	NS
	FL	6·75	6·79	5·82	5·56	4·83	NS	***	NS

Significance levels: *$P<0·05$; **$P<0·01$; ***$P<0·001$.

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The ontogenic development of the active form of hepatic pyruvate dehydrogenase (*EC* 1.2.4.1) during the sucking–weaning transition. By LARA S. R. BLANN and KEITH SNELL, *School of Biological Sciences, University of Surrey, Guildford GU2 5XH*

During the sucking–weaning transition hepatic metabolism adapts to the changing dietary conditions from high-fat–low-carbohydrate milk to high-carbohydrate–low-fat solid food. Glycogen stores are low throughout the sucking period as a result of their rapid depletion at parturition and thus glucose levels are maintained in the body by high rates of gluconeogenesis. Pyruvate dehydrogenase (PDH) activity is inhibited by high levels of acetyl CoA produced by fat oxidation, thereby preventing futile cycling of gluconeogenic precursors through PDH and pyruvate carboxylase. At weaning it appears that replenishment of glycogen stores may precede lipid synthesis. PDH plays a critical role in switching from the glycogenic pathway to the lipogenic pathway in adult rats and, thus, its activity profile around the sucking–weaning transition is of interest in view of the changing dietary situation.

The active form of PDH in crude Wistar rat liver extracts was measured spectrophotometrically (Caterson *et al.* 1982) using arylamine acetyltransferase following enzyme at 30°. Weaning was imposed at 20 d by removal of the mother from pups.

Activity is shown to be low before weaning at 20 d and to rise by 23 d to adult values (Table). There appears to be a 24–72 h delay in the increase in enzyme activity at weaning indicating that gluconeogenesis is still dominant at that time. These results suggest a lag in the switch from gluconeogenic and glycogenic to lipogenic and glycolytic pathways and, therefore, provide evidence for the replenishment of glycogen stores in advance of lipid synthesis during the metabolic adaptation to the sucking–weaning transition.

Age (d) . . .	14	16	18	20	21	23	25	27	Adult
Activity (mU/g wet wt)	39·8	69·8	59·0	48·6	56·9	305·2**	356·8**	275·0**	424·1**
SEM	10·0	4·6	4·4	6·5	8·4	33·0	47·4	31·4	23·4

ANOVA F; 10, 48 = 20·61, $P<0·01$.
Significantly different from values for 21 d and below: $^{**}P<0·01$ (Duncan's Multiple Range Test).

We are grateful to Drs. Mark Holness and Mary Sugden for advice and to the Wellcome Trust for financial support.

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Simulation of the effects of diet on the contribution of rumen protozoa to degradation of fibre in the rumen. By JAN DIJKSTRA and SEERP TAMMINGA, *Wageningen Agricultural University, Department of Animal Nutrition, Haagsteeg 4, 6708 PM Wageningen. The Netherlands*

A mixed population of rumen micro-organisms is involved in fibre degradation. In contrast with the considerable knowledge on predominant rumen cellulolytic bacteria, the role of rumen protozoa in fibre degradation is still controversial (Coleman, 1986). In various *in vitro* studies, protozoa have been reported to be responsible for 4–40% of total rumen microbial fibre digestion (Williams, 1989). Results of biochemical, cultural and microscopic studies indicate that the contribution of protozoa to fibre degradation depends on the complex interactions between protozoa, bacteria and dietary characteristics. Integration of these relationships is possible through mathematical representation of the processes involved (Thornley & Johnson, 1990).

The aim of the present study was to establish the role of the rumen protozoa in NDF degradation, using a mathematical model of rumen fermentation. The model is an extension of the dynamic, deterministic model described by Dijkstra *et al.* (1992). The model is driven by continuous inputs of nutrients and comprises nineteen state variables, each representing a pool size in the rumen. Parameters were assigned values based on results of well-defined experiments, where available, or alternatively were assigned values on a priority basis, given relevant experimental observations.

Simulations using inputs of nutrients representing mixed diets, based on grass silage (g/kg DM: NDF, 550; starch and sugars, 80; N, 25) and in which the proportion of concentrate (g/kg DM: NDF, 200; starch and sugars, 430; N, 25) was varied between 0 and 90%, fed at 15 kg DM/d (for a cow live weight 550 kg) showed increased protozoal OM in the rumen and increased protozoal contribution to NDF degradation at increased concentrate levels of the diet (Table). The results of the model indicate that diet composition affects the contribution of protozoa to NDF degradation in the rumen and help to quantify this contribution.

Effect of supplementation of grass silage diets on model estimates of digestion characteristics

Proportion of concentrate in diet (%) . . .	0	30	60	90
NDF duodenal flow (kg/d)	2·4	2·2	2·0	1·8
Total non-ammonia N duodenal flow (g/d)	311	307	315	340
Ruminal digestion of NDF (%)	68·5	64·7	57·2	45·3
Total microbial OM in rumen (kg)	1·86	1·73	1·48	1·29
Protozoal OM in rumen (kg)	0·16	0·36	0·45	0·47
Protozoal contribution to NDF degradation (%)	6·4	9·1	16·3	32·1

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Ribosomal DNA-targeted hybridization probes for the detection, identification and quantitation of anaerobic rumen fungi. By J. DORÉ[1], A. G. BROWNLEE[2], L. MILLET[1], I. VIRLOGEUX[1], M. SAIGNE[1], G. FONTY[1] and P. GOUET[1], [1]*Laboratoire de Microbiologie, INRA CR Clermont-Theix, 63122 Saint-Genès-Champanelle* and [2]*Division of Animal Production, CSIRO, Blacktown NSW 2148, Australia*

Anaerobic fungi are normal inhabitants of the rumen and hindgut of herbivorous mammals. Their potential contribution to plant matter degradation has been well documented in recent reviews (Fonty *et al.* 1991), as well as their interest in animal nutrition based on their amino acid composition. Nevertheless, the evaluation of their overall biomass in the digestive tract is still impaired by the absence of appropriate quantitation tools to account for both their free (zoospore) and immobilized (rhizoid) development stages. Besides, the recognition of the diversity within this group of micro-organisms seems far from complete as indicated by the frequent and debated proposal of new taxa. Based on sequences of 18S rRNA and rDNA obtained for five strains of anaerobic rumen fungi (ARF) representing four genera (Doré & Stahl, 1991; Bowman *et al.* 1992), we have developed a set of hybridization probes. The aims of the work reported here were to: (1) confirm target-sequence conservation by sequencing polymerase chain reaction (PCR)-amplified rDNA from several new strains; (2) test the specificity of probes; (3) assess the validity of specific probes for the diagnostic and quantification of ARF.

Of the five genera recognized so far, we have used three strains of *Anaeromyces,* six of *Orpinomyces,* seven of *Piromyces*, four of *Caecomyces* and eight strains of *Neocallimastix.* Only a subset of the corresponding DNA extracts from pure culture lyophilizates were used for PCR amplification and comparative sequencing. All DNA extracts were dotted on nylon membranes and hybridized with the various ^{32}P-labelled probes. Molecular target quantitation was compared to other techniques for growth measurement of a pure strain of ARF on a solid substrate. Finally, rumen contents of gnotobiotic lambs before and after specific association with ARF were used to test a total ARF hybridization probe.

The total ARF probe targeting a variable region of the eukarya 18S rDNA showing complete homology between the five strains characterized by complete sequencing was shown similarly to recognize DNA extracts of all strains tested by dot-blot hybridization. It could detect as little as 5 pg of a PCR fragment for which it served as amplification primer. Hybridization conditions have been developed for use with DNA extracts from rumen content of lambs. A very low background signal was obtained using ARF-free rumen contents and a clear qualitative detection signal was obtained using rumen contents of ARF-associated animals. The total ARF probe allowed us to follow quantitatively the growth of a pure strain of ARF on a solid substrate during the first few days of incubation, and preliminary results indicated that correlation with chitin assays and gas production as conventional measures of fungal growth were satisfactory (r=0·98 and 0·99 respectively).

Two genus-specific probes have been developed for the genera *Anaeromyces* and *Caecomyces*. Their specificity was based on the sequence data available and confirmed using all DNA extracts available from our culture collection in hybridization tests. The other genera *Orpinomyces* and *Neocallimastix* were both recognized by a less specific probe. Finally, based on hybridization and sequencing information for the 18S rDNA-target region characterized, a molecular variability within the genus *Piromyces* has been clearly observed.

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Permanent rumen fistulas in the thoracic region in calves. By G. BERRA[1], M. C. ANTOGNOLI[1], A. MAGGIO[2], J. PEREIRA[2] and P. GUILLOTEAU[3], [1]*Instituto de Patobiologia, Centro de Investigacion en Ciencias Vetarinarias, INTA Castelar, Argentina,* [2]*Universidad de Lomas de Zamora, Argentina* and [3]*INRA Rennes*

Oesophageal groove closure has been studied by different methodologies based on indirect techniques (Guilhermet *et al.* 1975; Brugere *et al.* 1987). In the present work a new method for direct visualization of oesophageal groove movements through a permanent rumen fistula situated in the thoracic region in calves is presented.

Five Holstein male, 20-d-old calves, weighing 45–50 kg, were kept in confinement and fed twice/d with 2 litres of milk. Animals were deprived of food intake 24 h before surgery. Anaesthesia was induced with Ketalar (10 mg/kg) given intravenously and maintained with halothane and oxygen through an endotracheal tube. With the animal in right recumbency, trichotomy and disinfection of the left costal area was carried out. A dorsoventral skin incision of 15 cm length was made on the 9th rib in the left costal area. Muscular planes were incised to reach the outer surface of the 9th rib; the length of the first wound also allowed access to the outer surfaces of the 8th and 10th ribs. Periostea were removed. Resection of a fragment of each rib was made from 3 cm below costovertebral joints to costocontral unions. The diaphragm was incised on its costal insertion and fixed by stitches to a point coinciding with the 7th rib. It was necessary to move the spleen back from the rumen and diaphragm for rumen fixation to the thoracic wall. The rumen was sutured to the costal wall in a circle of 5 cm diameter making the stitches coincide with the points of the hours of a clock. The rumen was open 10 d after surgery when cicatrization and rumen adhesion to the costal wall had taken place. A flexible rumen cannula (3 in diameter, up to 2 in wall thickness) was placed in it.

Total length of surgery was about 1–10 h. Feed ingestion was normal 2 d after surgery in all the animals. All calves increased their respiratory frequency during the following 7–10 d. Peripheral incision tissues showed an inflammatory reaction between 3 and 7 d post-surgery, but a good adherence of ruminal wall to rib took place between the 8th and 10th day. Little loss of rumen contents through the wound occurred after the 3rd week, as in abdominal rumen fistulas. Permanent ruminal fistulation in the thoracic region implies surgery with a certain level of complexity, such as intubation for artificial respiration, displacement of diaphragm and spleen and costal extirpation. Maintenance after surgery is similar to that in animals with abdominal rumen fistulas. Practical benefit of this technique is that it allows the direct observation of the oesophageal groove movements in several situations, such as reactions provoked by artificial stimulus, different conditions in their natural habitat, administration of different kinds of drugs, etc. It can also be used in salivary secretion studies, in determination of consumption and bite size, in the parasitology field for the estimation of larvae consumption on pasturage, and to evaluate the efficacy of antiparasitic drugs, especially benzamide-derived ones which have questionable activity in the rumen because of partial closure of the oesophageal groove.

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The behaviour of Co-EDTA- and Yt-labelled hay in the gastrointestinal tract of sheep. By J. GASA, A de VEGA, C. CASTRILLO, J. BALCELLS and J. A. GUADA, *Departamento de Produccion Animal y Ciencia de los Alimentos, Facultad de Veterinaria, Universidad de Zaragoza, Spain*

Estimation of digesta mean retention time (MRT) in the gut of ruminants from compartmental analysis of markers requires both identification of mixing pools and understanding of the behaviour of different markers. In a factorial design, four pairs of twin ewe lambs 12 months old (38·3 (SD 1·37) kg LW) were fed a chopped (C; 44·8% digestible organic matter (DOM) or ground and pelleted (P; 40·9% DOM) lucerne hay at 90% of previously-recorded voluntary intake (65 and 88 g DM/kg body-weight$^{0.75}$ for C and P, respectively). Diets were offered in twelve equal meals at intervals of 2 h. After 5 d in which a daily dose of 200 mg Co-EDTA liquid-phase marker and 6 g Yt-labelled hay (50 mg of Yt) was given by mouth, in twelve separate doses, pairs of animals were slaughtered every 2 h. The contents of reticulo rumen (RR), omasum (OM), abomasum (Ab), lower gut, caecum (HG1), ascending colon (HG2) and transverse colon, descending colon and rectum (HG3) were removed, weighted and sampled. Since marker concentration did not differ among the last three sections, a single hindgut mixing compartment was considered (HG). The MRT values for both markers in each compartment of the tract were calculated (Faichney, 1975).

From the results, at least four mixing compartments can be identified in the digestive tract (see Table). As reported by Grovum & Williams (1973), RR and HG were the main mixing pools, residence time in Om and Ab accounting for less than 5% of the total MRT.

Mean retention time (h)

	RR		Ab		Om		HG	
Diet	Co	Yt	Co	Yt	Co	Yt	Co	Yt
C	13·95	24·82	0·56	0·81	0·82	1·24	7·74	6·84
P	6·47	15·79	0·30	0·56	0·32	0·71	6·88	7·55
SE	0·71	1·64	0·16	0·25	0·20	0·26	0·80	0·85
Significance	**	*	NS	NS	NS	NS	NS	NS

NS, not significant; * $P<0·05$; ** $P<0·001$.

Both diet and type of marker affected MRT in the RR, the differences between diets being 7·5 ($P<0·01$) and 9·0 ($P<0·05$) h for Co and Yt respectively. Mean values recorded at Ab and Om followed the same tendency ($P<0·1$). By contrast, differences in HG MRT between diets or markers were negligible, probably owing to the fact that markers do not behave independently in the hindgut (Faichney & Boston, 1983). Furthermore, and as previously demonstrated by these authors, it appears from the results on diet P that the RR is not always the pool with the longest MRT for Co-EDTA.

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Effects of phenolic acids on ruminal proteolytic bacteria. By D. DEBROAS and G. BLANCHART, *ENSAIA – Laboratoire de Zootechnie, INRA. 2, avenue de la Forêt de Haye, 54500 Vandoeuvre*

Proteins bound to the plant cell wall are hardly hydrolysed by the ruminal micro-organisms. One of the most cited hypotheses is that cell wall polysaccharides decrease protein accessibility by proteolytic enzymes. Degradation of plant cell wall nitrogen (NDIN) is higher when the cell wall fraction (NDF) is more degradable (Lindberg, 1981), but the influence of end-products of cell wall compounds, such as the lignin monomers, on proteolytic bacteria has never been tested. The quantities of these products liberated during 7 d ruminal fermentation of Bermuda grass blade are: p-coumaric acid, 68·5%; ferulic acid, 90% (Akin & Benner, 1988). These lignin monomers present *in vitro* an inhibitory effect on the growth of cellulolytic bacteria (Chesson *et al.* 1982).

In the present study we have tested the effect of p-coumaric acid and ferulic acid, at two concentrations (0·1 and 0·2% (w/v)), on the growth of two ruminal bacteria isolated in our laboratory: *Bacteroides ruminicola* S17/3 and *Butyrivibrio fibrisolvens* S23. The bacteria were isolated in our laboratory using the anaerobic roll-tube technique (Hungate, 1950; Bryant, 1972).

In the control, the maximum growth rates of *Bacteroides ruminicola* S17/3 was 0·36/h and became 0·28 and 0·27/h in the presence of 0·2% of ferulic acid or p-coumaric acid. An inhibitory effect of growth was also observed for *Butyrivibrio fibrisolvens* S23. The inhibition of *Bacteroides ruminicola* S17/3 was maximal with 0·1% of phenolic acids. The maximum growth rates of *Butyrivibrio fibrisolvens* S23 diminished when the lignin monomer concentration increased. Both lignin monomers used in the present study presented an inhibitory effect toward maximum growth rate of both proteolytic strains. During the cell wall degradation, concentration of phenolic acids became higher and could have a negative influence on the growth of proteolytic bacteria. In conclusion, solubilization of phenolic acids can represent a limiting factor of NDIN degradation.

Maximum growth rate (/h) of Bacteroides ruminicola *S17/3 and* Butyrivibrio fibrisolvens *S23*

		Ferulic acid concentration (%)		p-Coumaric acid concentration (%)	
	Control	0·1	0·2	0·1	0·2
B. ruminicola S17/3	0·36	0·29	0·28	0·27	0·27
B. fibrisolvens S23	0·27	0·25	0·21	0·24	0·19

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Proteins from different cultivars and varieties of lupins resistant to *in vitro* rumen digestion. By H. TAI and R. S. BUSH, *Agriculture Canada, Research Station, Fredericton, N.B., Canada, E3B 4Z7*

Sweet white lupins (*Lupinus albus*) are beginning to find acceptance by some producers in the Atlantic provinces of Canada. To date, most of the local impetus for lupin production has been derived from the agronomic characteristics of the plant under our growing conditions. Only a limited amount of literature exists examining the biological value of this crop in livestock feeding regimens. If specific proteins are not digested in the rumen they must be digested in the lower gut to be of any use to the ruminant. To choose the correct varieties of lupin, it is essential to know the proteins that are found in each variety so that those proteins that might be more readily digested can be assessed.

Extracts were prepared from eight cultivars of *L. albus*, and two cultivars each of *L. angustifolius* and *L. luteus*. Rumen fluid was collected from a fistulated cow, transported to the lab under CO_2 atmosphere, filtered and 5 ml added to flasks containing 20 ml artificial saliva and 35 mg extract protein. Samples (1 ml) were taken at 0, 1, 2, 3, 4, 5, 6, 7, 9 and 23 h and mixed with 0·01 ml saturated $HgCl_2$.

Electrophoresis of the extracts on agarose gels revealed three distinct globular storage proteins (conglutins α, ß and γ). Under denaturing conditions with ß-mercaptoethanol and SDS, twenty or more distinct protein bands were apparent. The cv. Ultra had major bands at 45, 40, 37, 25 and 17 kD at 0 h. There was significant digestion of the 45 kD proteins by 3 h and the 30 kD proteins by 5 h. The extract contained traces of a 20 kD doublet band (19 and 17 kD) which progressively increased to 9 h and was one of the few perceptible bands in the 24 h sample. There was another protein at 30 kD which increased from 2–9 h. Western transfers tested with anti-α and γ rabbit antisera did not react with proteins as small as the 20 kD doublet. The anti-γ antiserum reacted with numerous bands in the initial sample, however, by 1 h only two bands at high molecular weight were found. The anti-ß antiserum reacted with the many of the smaller molecular weight proteins and peptides including the 20 kD doublet. The Western transfer is much more sensitive than Coomassie protein stain and reactive bands were observed for the larger proteins even though they could not be visualized.

Another albus variety, Kiev Mutant, was similar to Ultra. Two proteins <40 kD were much less readily digested than the 45 kD proteins. A number of small peptides <20 kD–10 kD were accumulated through 8·5 h.

The *L. angustifolius* cv Danja was different. There were more large proteins >45 kD in the extract than in the albus extracts. Two proteins (60 and 65 kD) were present throughout the digestion. The smaller of these appeared to increase to 9 h. One protein at about 40 kD was maintained without increase or decrease. The 20 kD doublet proteins and smaller peptides increased throughout the digestion.

Having shown that some proteins and peptides that are not readily digested from lupin extracts by rumen bacteria, the proteins and peptides must be identified. In the ruminant, these indigestible proteins may add to the rumen bypass protein.

Proteins <40 kD have been sufficiently digested to decrease greatly their immune reactivity. Proteins from *L. albus* were more readily digested than those from the other varieties studied. The value of the rumen bypass proteins will depend on their relative digestion in the lower digestive tract.

Rumen osmotic pressure, water flux and volatile fatty acid (VFA) absorption in sheep. By F. D. DeB. HOVELL and S. LÓPEZ, *Rowett Research Institute, Bucksburn, Aberdeen, AB2 9SB*

Water movement through the rumen wall is defined mainly by osmotic pressure (OP) (Engelhardt, 1970). This water flux affects the absorption of nutrients in the rumen and the liquid outflow to the omasum. Four lambs nourished by intragastric infusion of nutrients (270 mmol VFA/h and buffer into the rumen, casein into the abomasum) were used in a Latin square experiment to study the effect of rumen OP on water flux. On experimental days, casein infusion and drinking water were withdrawn. The control OP (261 mOsmol/kg) was that obtained with infusions of VFA and buffer only. Rumen OP was increased by also infusing NaCl, and was held constant for 9·5 h at 356, 425 or 499 mOsmol/kg. Rumen fluid samples were taken at 0 h, 2 h, and every 1·5 h thereafter. Rumen volumes and liquid outflows were estimated using PEG and Cr-EDTA. Liquid outflow rate (F; ml/h) increased linearly ($P<0·01$) with OP as $F=1·36 \times OP$ (SE 0·29–82) (n 16, $r^2=0·61$).

Treatment (OP; mOsmol/kg)	261-OP	356-OP	425-OP	499-OP	SE
NaCl infusion (g/h)	Nil	5·3	8·2	12·8	
Outflow rate (ml/h)	281	398	492	599	26·9
Water absorption rate (ml/h)	93	–7	–103	–206	31·4
Rumen volume (l)	4·0	3·9	4·6	5·1	0·20
Rumen pH	6·1	5·7	5·5	5·2	0·13
Total VFA concentration (mmol/l)	111	109	118	128	9·7
VFA absorption (mmol/h)	220	232	217	191	7·4

There was a net absorption of water into the plasma with 261-OP, no appreciable net transepithelial flux of water for 356-OP, and increasing net fluxes into the rumen at 425-OP and 499-OP, resulting in a linear relationship between the net flux of water (W; ml/h) across the rumen wall (calculated as the difference between liquid infused and outflow) and OP, given by $W=440–1·28$ OP (SE 0·30) (n 16, $r^2=0·57$). Rumen volume was increased at higher OP. Rumen pH decreased as OP was increased even though VFA and buffer infusion rates were held constant. VFA absorption was greatest with 356-OP, and then reduced as OP was further increased. The results on water flux are in agreement with published results obtained with rumen pouches (Engelhardt, 1970), or with normally-fed sheep fasting on the day of measurement (Warner & Stacy, 1972).

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Histological consequences of different surgical procedures for ileorectostomy in pigs. By J. REDLICH[1], U. HENNIG[1], J. P. LAPLACE[2], W. B. SOUFFRANT[1], J. M. V. M. MOUWEN[3], R. BERG[4], [1]*Oskar Kellner Institute, D-2500 Rostock, Germany,* [2]*INRA Recherches Porcines, F-35590 Saint-Gilles,* [3]*Faculty of Veterinary Medicine, NL-3508TD Utrecht, the Netherlands* and [4]*Humboldt University, D-1040 Berlin, Germany*

Ileal (i.e. precaecal) digestibility allows one to estimate the digestion in the small intestine in pigs. Among different methods available for digesta collection, ileorectal anastomosis (IRA) was developed with four possible surgical procedures: either end-to-end (EE) or end-to-side (ES) with or without preservation of the ileo-caecal valve (EEV, EE, ESV, ES respectively). The aim of the present work was to investigate the possible changes of microscopical anatomy in pigs submitted to these four different procedures, in comparison with intact animals (INT).

Samples of the small intestine were taken *in vivo* under deep anaesthesia, 147 d after surgery: D at 10 cm distal to the pylorus; J1 at 20 cm distal to the duodeno-colic ligament; J2 at 100 cm and I at 10 cm proximal to the ileo-caecal valve. They were treated according to a classical procedure and computer-assisted histometric examinations were performed. The deviation from normal (mean in INT pigs) was expressed for all IRA groups using a scale based on the SD value calculated for INT pigs (atrophy and hypertrophy mean respectively a decrease or an increase higher than 1, 2 or 3 SD values).

The length of the villi and the depth of the crypts in the EE and EEV groups did not differ from INT pigs, while some differences occurred in ES and ESV pigs. The number of goblet cells, normal in ES and ESV, was significantly increased in the jejunum and ileum of EE and EEV pigs (50–60% increase). The area of lymphatic follicles was the largest in INT pigs, smaller in ES and ESV, and the smallest in EE and EEV pigs; the preservation of the valve lowers the lymphatic area in both EE and ES procedures. The total thickness of the intestinal muscle layers was reduced in ES pigs over the whole small intestine. In contrast to that it was increased in the ileum of EEV and ESV pigs due to hypertrophy of the circular layer; this could be the consequence of the proximity of ileo-caecal and anal sphincters.

The results suggest that the digestive ability of the small intestinal mucosa for main nutrients should be preserved in EE and EEV pigs whose villi and crypts are normal. However, as pointed out by the goblet cell numbers, there is probably a large increase of water absorption ability in these pigs deprived of normal colic function. In addition, the smaller size of lymphatic area indicates a reduced contact of their ileal mucosa with microbial antigens. As a conclusion, the EE procedure seems to be an appropriate model to measure ileal digestibility of main nutrients in pigs.

Colon simulation technique as an *in vitro* model to study the effects of diet on microbial production of enzymes potentially involved in colon cancer. By K. STÜCK and G. BREVES, *Department of Veterinary Physiology, University of Giessen, D 6300 Giessen, Germany*

Epidemiological studies have shown that the incidence of colon cancer may be related to dietary habits. A critical factor may be a combination of high fat and low fibre intake. Dietary components may affect intestinal microflora, which may produce co-carcinogenic or carcinogenic compounds, or both. Substrates for bacterial transformation can reach the colon either with the diet or in bile (Rowland *et al.* 1985).

It was the aim of the experiments to use a semi-continuous *in vitro* incubation as a model to study the effects of dietary fibre and fat content on basic variables of microbial metabolism in the colon and on different microbially-produced enzymes. The incubations were carried out by applying the colon simulation technique (Ositec) described by Breves & Dreyer (1991). The inocula were obtained from caecally-fistulated pigs. The donor animals were either fed on a standard grain diet with 25% of energy being present as fat (Diet 1) or they were fed on a diet with 8·7% of dietary dry matter as crude fibre (Diet 2). In each *in vitro* experiment pH values, production of short chain fatty acids (SCFA), ammonia concentrations and digestibilities of organic matter (OM) were measured as indicators of microbial metabolism. Activities of ß-glucosidase (ß-glco), ß-glucuronidase (ß-glcu) and nitroreductase (nitre) were determined.

Increasing the carbohydrate content and decreasing the fat content of the diet resulted in lower digestibilities of OM, lower NH_3 levels and SCFA production and higher pH values, and induced lower activities of ß-glucosidase, ß-glucuronidase and nitroreductase.

Effect of diet on microbial metabolism and enzymatic activities (n 5)

		pH	SCFA (mmol/d)	OM digestibility (%)	NH_3 (mmol/l)	ß-glco (U/d)	ß-glcu (U/d)	Nitre (U/d)
Diet 1	Mean	6·40	6·88	53·3	6·9	0·68	1·15	0·49
	SD	0·43	0·43	1·5	0·09	0·06	0·09	0·03
Diet 2	Mean	6·53	4·83	51·8	6·2	0·46	0·66	0·28
	SD	0·02	0·21	1·8	0·04	0·005	0·01	0·006

Diet 1 energy content: fat 25·0%; protein 20·5%; carbohydrate 54·5%.
Diet 2 energy content: fat 9·9%; protein 22·2%; carbohydrate 67·9%.

The results indicate that both the basic microbial metabolism of the gut flora and the microbial production rate of different enzymes can be modified by the diet. Thus, these results are the basis for further experimental studies on the effects of dietary components on microbial metabolism which may be involved in synthesis of tumour-promoting substances in the hindgut.

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Measuring intestinal crypt cell proliferation by confocal microscopy. By T. C. SAVIDGE[1], M. W. SMITH[1], J. A. WALKER-SMITH[2] and A. D. PHILLIPS[2] (Introduced by A. M. PRENTICE), [1]*AFRC IAPGR, Babraham, Cambridge CB2 4AT* and [2]*Queen Elizabeth Hospital for Children, London E2 8PS*

Changes taking place in crypt cell proliferation in response to hyperphagia, food restriction and damage caused by the presence of food allergens indirectly affect the ability of the intestine to digest and absorb nutrients. Previous methods available to investigate these important responses required the use of radioactive nucleotide analogues or metaphase arrest agents to quantify proliferating crypt cells. In humans, especially children, the use of such techniques present unacceptable ethical constraints and measurements of crypt cell production rates (CCPR) are largely obtained by counting the percentage of dividing cells in crypt sections or squashes. These techniques are, however, severely hampered by the limitations of having to count cells in two and three dimensions (3-D) respectively. The present work describes a novel technique specifically adapted to confocal laser scanning imaging microscopy, that is able to count accurately cells in intact 3-D crypt structures obtained from routine investigative proximal intestinal biopsies.

Histologically-normal-appearing jejunal biopsies were obtained from four children (two male, two female; aged 21–80 months), were washed in NaCl solution (9 g NaCl/L; 4°) and fixed in 10% formal saline (pH 7·4;RT) for 48 h. Biopsies were then incubated with 20 g Triton-X/l (30 min;RT), followed by 10 μg propidium iodide/ml phosphate-buffered saline for 10 min with slight agitation. Micro-dissected crypts were then analysed using a Biorad MRC 500/600 confocal microscope (CM). CCPR were also calculated from haematoxylin and eosin stained longitudinal sections (LS) from eleven control biopsies (six male, five female; aged 4–43 months).

A direct comparison of the two different techniques demonstrated that, although crypt lengths were identical (33 cells/crypt column), counting LS overestimated the crypt population by 17% (740 (SEM 14) *v.* 634 (SEM 30 cells/crypt; $P<0{\cdot}05$) and the incidence of mitosis, and hence CCPR, by 161% (14·6 (SEM 0·8) *v.* 5·6 (SEM 0·4) cells/crypt per h corrected for Tannock's factor; $P<0{\cdot}001$). The number of mitoses measured using CM agrees with previously published results utilizing crypt squashes, but CM also confers the ability to count accurately total cell populations and position mitotic figures in 3-D. Thus, adaptive alterations to crypt proliferation demonstrates the inadequacy of Tannock's correction factor to compensate accurately for counting CCPR in longitudinal sections. The CM technique and maturation compartments can be calculated from the construction of 3-D mitotic index distribution curves. This new method for analysing crypt cell proliferation can now be applied to any study involving nutritional manipulations in animals or humans.

Differential effects of epidermal growth factor and transforming growth factor-α on gastrointestinal epithelial cell proliferation. By R. A. GOODLAD[1], C. Y. LEE[1], M. A. GHATEI[2], S. R. BLOOM[2] and N. A. WRIGHT[1], [1]*Imperial Cancer Research Fund, Histopathology Unit, 35–43 Lincoln's Inn Fields, London WC2A 3PN* and [2]*Department of Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 0NN*

The fifty-three amino acid peptide, epidermal growth factor (EGF), is a powerful stimulator of intestinal epithelial cell proliferation and shares the same receptor on intestinal cells as transforming growth factor-α (TGF-α) leading to the suggestion that the true ligand for the EGF receptor may be TGF-α. Isomolar amounts of EGF (60 μg/rat per d) or of TGF-α were infused into parenterally-fed rats (Goodlad, 1987) for 3 d, after which the rats were injected with vincristine and killed at timed intervals; the accumulation of arrested metaphases over time in microdissected crypts was determined to calculate the crypt cell production rate (CCPR). Plasma hormone levels were determined by radioimmunoassay.

	TPN control (*n* 10)		TGF-α (*n* 10)		EGF (*n* 10)	
	Mean	SEM	Mean	SEM	Mean	SEM
Plasma hormone (pmol/l)						
Gastrin	12·4	0·7	19·7**	1·7	16·6**	1·1
PYY	49·2	2·5	71·8*	9·9	128·4***	17·7
Enteroglucagon	32·4	5·8	41·0	10·4	62·8**	7·8
CCPR (cells/crypt per h)						
Fundus	2·09	0·55	1·34	0·71	2·33	0·49
Antrum	2·08	0·44	1·85	0·25	3·47*	0·44
Mid small intestine	15·02	2·26	21·01	3·01	31·33**	4·14
Proximal colon	5·64	1·23	6·90	2·09	13·23**	1·51
Mid colon	3·08	1·41	5·89	3·48	13·80**	2·67
Distal colon	4·12	3·60	15·65	7·47	26·37**	4·32

Significantly different from control: *$P<0·05$; **$P<0·01$; ***$P<0·001$.

Both agents increased proliferation in the gastrointestinal tract when compared with the parenterally-fed control group; however, the trophic effect of EGF was more pronounced than that of TGF-α. The hormonal response to the two peptides also differed, for while TGF-α and EGF both significantly increased plasma gastrin, TGF-α had the greater effect. Nevertheless, EGF was more potent at increasing plasma peptide YY and plasma enteroglucagon.

It is concluded that while TGF-α, like EGF, has some mitogenic effects on the gastrointestinal tract of the rat, it is a less potent mitogen but a more potent gastrin-releasing agent, presumably via its inhibitory action on gastric acid secretion.

Goodlad, R. A., Wilson, T. G., Lenton, W., Wright, N. A., Gregory, H. & McCullagh, K. G. (1987). *Gut* **28**, 573–582.

Insulin and intestinal epithelial cell proliferation. By R. A. GOODLAD[1], C. Y. LEE[1], S. G. GILBEY[2], M. A. GHATEI[2], S. R. BLOOM[2] and N. A. WRIGHT[1], [1]*Imperial Cancer Research Fund, Histopathology Unit, 35–43 Lincoln's Inn Fields, London WC2A 3PN* and [2]*Department of Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, DuCane Road, London W12 0NN*

There is evidence to implicate insulin as a moderator of intestinal epithelial cell proliferation (Goodlad & Wright, 1990). Endogenous plasma insulin was modified by dietary manipulation. Normal and sham-operated orally-fed rats were compared with rats maintained by total parenteral nutrition (TPN; Goodlad *et al.* 1987) using either a TPN diet high in glucose, or a low-glucose, hypoenergetic TPN diet.

After 7 d the rats (groups of ten) were injected with vincristine to arrest cells in metaphase and crypt cell production rate (CCPR) was determined (Goodlad *et al.* 1987). Plasma was taken for hormone radioimmunoassay.

	Orally-fed		Sham-operated		TPN		TPN-hypoenergetic	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Plasma hormone (pmol/l								
Insulin	219·0	48·9	132·9	27·9	2043·0**	480·0	454·0	115·0
Gastrin	53·8	6·0	62·0	17·4	22·8***	3·2	14·3***	0·6
PYY	103·6	10·3	132·0	30·7	68·3*	8·4	31·3***	3·2
Enteroglucagon	176·2	38·7	130·0	30·0	30·0***	6·4	15·4**	4·7
CCPR (cells/crypt per h)								
Mid small intestine	25·3	3·9	25·4	3·8	11·0**	1·0	9·1**	0·8
Mid colon	6·4	3·1	6·2	4·0	4·3	2·2	4·0	1·9

Significantly different from orally fed: *$P<0·05$; **$P<0·01$; ***$P<0·001$.

Sham operation had no effect on either plasma hormone levels or on CCPR. The standard TPN diet was associated with significantly reduced levels of gastrin, peptide YY (PYY) and enteroglucagon. Gastrin and PYY were further reduced by the hypoenergetic diet. Plasma insulin was much increased in the TPN but not in the TPN-hypoenergetic group. Small intestinal CCPR was reduced in the TPN groups, but no difference was observed between the standard and the low energy TPN diets.

The lack of difference in CCPR between the two TPN diets, despite the massive elevation of plasma insulin, strongly suggests that insulin does not have a substantial role in the control of gastrointestinal epithelial cell renewal.

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Goodlad, R. A., Wilson, T. G., Lenton, W., Wright, N. A., Gregory, H. & McCullagh, K. G. (1987). *Gut* **28,** 573-582.

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Molecular isolation of genes expressed in the differentiated epithelium of the pig small intestine. By G. PEROZZI[1], D. BARILA[1], C. MURGIA[1], R. BEGBIE[2], T. P. KING[2] and D. KELLY[2] [1]*Istituto Nazionale della Nutrizione, Via Ardeatina 546, 0178 Rome, Italy,* and [2]*Rowett Research Institute, Aberdeen AB2 9SB*

The functionally-differentiated epithelium of the intestinal mucosa plays a key role in the absorption of nutrients and in their vectorial transport to the circulation. This epithelium is an excellent model system to study the factors involved in cellular differentiation. As a first step towards the isolation of stage-specific genes from pig intestine, cDNA clones from other species have been used as hybridization probes to define the timing of expression of enterocyte-specific functions during foetal development in the pig. The use of pig intestine as an experimental model for molecular studies has several advantages over its extensively-used murine counterparts, such as the possibility of purifying larger quantities of mRNA, even from early developmental stages, and an intestinal maturation programme during foetal life that more closely resembles that in humans.

Research is in progress for the isolation of pig cDNA clones to be used as species-specific probes in *in situ* hybridization experiments requiring higher stringencies. A cDNA library was constructed on the bacteriophage vector Lambda ZAPII, using poly(A)+ RNA extracted from mature pig intestine, and therefore representative of the pattern of genes expressed in differentiated intestinal epithelial cells. The genes encoding cellular retinol binding protein II (CRBP II) and fatty acid binding protein (L-FABP) have been isolated from this library. Determination of the DNA sequence of these genes and comparison with the human and rat sequences reveals a higher degree of homology of these two proteins with their human counterparts. The intestinal pig cDNA library will now be used to isolate differentiation-specific genes with a subtractive hybridization approach.

The effect of pattern of nutrient supply during lamb growth on the digestive tract proportions. By T. MANSO, A. R. MANTECÓN, M. A. CHASO, P. LAVIN and T. CASTRO, *Estacion Agrícola Experimental (CSIC). Apdo 788. 24080 León, Spain*

The effect of level of intake during the milk-fed period and energy:protein (E:P) relationships during the post-weaning period on the digestive tract components of Churra lambs was investigated.

Four lambs were slaughtered at 2 d old (I group, 2·87 (SD 0·19 kg) empty body weight; EBW) and twenty-four lambs were assigned to a 2×2 factorial design defined by two levels of intake (1·5 and 0·9 MJ GE/$kg^{0·75}$ per d) during the milk-fed period, and two diets (HP, lucerne hay and barley plus 200 g fish-meal/kg; LP, lucerne hay plus barley). In both diets a vitamin–mineral supplement was used. The hay and concentrate were independently offered *ad lib*.

Four lambs of each level of intake (high-WH and low-WL) were slaughtered at weaning (4 weeks of milk-feeding) at 8·79 (SD 0·18; WH) and 5·62 (SD 0·46; WL) kg EBW and sixteen post-weaning lambs were slaughtered (F group) at 16·57 (SD 0·63) kg EBW.

At slaughter, EBW was estimated and empty digestive components after removal of fat were weighed. The results are calculated as proportion of EBW.

There were differences ($P<0·05$) between the I group and the weaning groups in the relative size of the components of the digestive tract with a higher value in the I group for the omasum (OM; 0·0032(I) *v.* 0·0012(WH) *v.* 0·0025(WL)), small intestine (SI; 0·0319(I) *v.* 0·029(WH) *v.* 0·0272(WL)) and caecum (0·0032(I) *v.* 0·0016(WH) *v.* 0·0018(WL)) and a lower value in the reticulo-rumen (RR; 0·0057(I) *v.* 0·0072(WH) *v.* 0·0103(WL)). There were differences ($P<0·05$) between WH and WL groups with a lower value for the RR and OM and a higher value for SI in the WH group.

In the F group there were differences ($P<0·01$) between HP and LP groups with a higher value for HP in the RR (0·0427 *v.* 0·0368) and a lower value in abomasum (AB; 0·005 *v.* 0·0059) and large intestine (LI; 0·0085 *v.* 0·0101). The effect of preweaning level of intake was significant in AB ($P<0·05$) with a lower value in WL than WH group (0·0050 *v.* 0·0058). The effect of E:P ratio on the proportions in the LI was significant ($P<0·05$) when the lambs had a low level of intake during the milk-fed period (0·0109(LP) *v.* 0·0080(HP)).

The E:P ratio during the post-weaning period affected the digestive tract proportions and this effect depended on the previous level of intake during the milk-feed period; both factors must be taken into account when digestive utilization is being considered.

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Developmental changes in intestinal glycoprotein glycosylation; relationship with diet changes at weaning and hormonal variations. By M. C. BIOL, A. MARTIN, D. RUGGIERO-LOPEZ, D. LENOIR, S. PINTORI and P. LOUISOT, *INSERM–CNRS U189, Faculté de Médecine Lyon–Sud, BP 12. 69600, Oullins*

The apical membrane of the intestinal cell is exposed to important developmental changes which culminate at weaning and require profound adaptive alterations in membrane composition and enzymic equipment. Among them, inverse modifications of the fucosylation and sialylation of glycoproteins in intestinal brush-border membranes or mucins are accompanied by a decrease of the sialyl-transferase activity between birth and weaning and a rise in fucosyl-transferase activity from weaning until adulthood (Biol *et al.* 1987, 1992). The synthesis of substrate (GDP-fucose) also rises just after weaning whereas its degradation by GDP-fucose-pyrophosphatase varies inversely with the activity of a regulatory protein inhibiting the fucosyl-transferase activity (Ruggiero-Lopez *et al.* 1991).

These large variations in the fucosylation process could be due to factors such as changes in the hormonal levels or in the diet composition at weaning time.

The effects of the drastic modification of diet composition on the fucosylation changes at weaning were studied. Animals were weaned early at 16 d of age or suckled until they were 22 d old. Normally-weaned rats were weaned at 19 d of age. Fucosylation is prematurely induced by early weaning, whereas prolonged nursing prevents the normal increase of fucosylation by acting on the fucosyl-transferase activity, its substrate degradation and the activity of its inhibitor.

	Prolonged-nursing rats (n 8)		Normally-weaned rats (n 8)	
	Mean	SE	Mean	SE
Glycoprotein fucose content (μmol/g)	1·6	0·1	2·2**	0·2
Fucosyl-transferase (pmol/mg per min)	1·9	0·2	2·7**	0·8
GDP-fucose degradation (pmol/mg per min)	340	36	85**	42
Inhibitor activity (μ/mg)	111*	17	58*	12

Significantly different (Student's t test): *P<0·050; **P<0·005.

Among postnatal changes, corticosterone and insulin levels rise at weaning and the role of these hormones was also investigated. Treatment with glucocorticoids (cortisone or hydrocortisone), injected to 10-d-old sucking rats at 10 μg/day per g for 4 d induces a high increase in fucosylation, reversible after treatment with an antiglucocorticoid (RU 486 administered at 20 μg/d per g). This increase disappears when rats are treated with glucocorticoids during or after weaning. On the other hand, treatment with antiglucocorticoid between 16 and 22 d of age does not prevent fucosylation enhancement at weaning. Moreover, adrenalectomy before the normal enhancement of the corticosterone level (at the end of the third week) does not prevent the increase of fucosylation in spite of the low level of corticosterone, indicating that glucocorticoids take only a minor part in the fucosylation variations at weaning. Insulin treatment of sucking rats also induces a marked increase in fucosylation which disappears when rats are treated during or after weaning. Injections of insulin (16 mU/d per g, twice daily for 4 d) to rats submitted to prolonged nursing allows the enhancement of the fucosylation process, prevented in prolonged-nursing control rats (untreated with insulin). Thus, insulin appears as an hormonal factor able to play a major role in intestinal fucosylation changes at weaning time.

	Fucosyl-transferase activity (pmol/mg per min)			
	Prolonged-nursing rats (n 12)		Weaned rats (n 12)	
	Mean	SE	Mean	SE
Control	2·0^{abc}	0·1	2·8^{a}	0·1
Insulin-treated	3·2^{b}	0·2	2·8^{c}	0·1

Significantly different by Anova and Newman-Keul's test.

In conclusion, the developmental regulation of glycoprotein fucosylation is a very complex process involving several enzymes and an inhibitory protein which seem to have concerted actions regulated under influence of ontogenic, hormonal or nutritional factors, or both.

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Net absorption of low molecular weight peptides by the mesenteric- and portal-drained viscera of steers. by C. J. SEAL and D. S. PARKER, *Department of Biological and Nutritional Sciences, The University, Newcastle upon Tyne, NE1 7RU*

The absorption of low molecular weight peptides across the gastrointestinal barrier and their subsequent appearance in blood is poorly documented. The results presented here form part of a study in which the net absorption of free amino acids (FAA) was measured in chronically-catheterized, forage-fed steers with or without 1 mol propionic acid/d by intraruminal infusion (Seal & Parker, 1991*a*). Carotid (C), mesenteric (M) and portal (P) plasma from three steers was centrifuged through 10 K molecular weight filters, and low molecular weight peptides (<1500 molecular weight) in filtrates were separated by reverse-phase HPLC and their amino acid composition determined after acid hydrolysis (Seal & Parker, 1991*b*).

Although increased amino acid absorption was seen in animals receiving propionate, peptide uptake was not affected by infusion of the VFA and results are shown averaged across both treatments.

	Free amino acids (mM)				Peptide-bound amino acids (mM)			
	C	M	P	SEM	C	M	P	SEM
Total	2·20	4·06	2·71	0·225	0·85	1·93	1·61	0·124
EAA	1·30	2·02	1·48	0·119	0·26	0·96	0·61	0·080
NEAA	0·90	2·03	1·23	0·111	0·59	0·97	0·99	0·079
BCAA	0·78	1·02	0·82	0·064	0·12	0·60	0·32	0·062

EAA, essential amino acids; NEAA, non-essential amino acids; BCAA, branched-chain amino acids.

Peptide-bound amino acid (PBAA) levels were significantly higher in M and P plasma ($P<0·001$). Increased net absorption of total PBAA between the mesenteric and portal veins (1·76 and 3·02 mmol/min respectively) is in contrast to free amino acid uptake (3·02 *v.* 2·03 mmol/min) indicating net absorption of PBAA but not FAA from large intestinal and stomach tissues. Total PBAA concentrations average 0·4, 0·47 and 0·59 of FAA for C, M and P plasma respectively. The pattern of amino acids was different in the two fractions; glycine, leucine and proline accounting for 0·54 of total PBAA, but only 0·25 of FAA. In contrast, valine and alanine were present in higher proportions in the FAA pool (0·27 *v.* 0·18 for PBAA pool). These results confirm the importance of the low molecular weight peptide fraction in the supply of α-amino nitrogen for tissues.

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Stimulation of calcium absorption in growing pigs treated for 2 months with porcine growth hormone (GH). By C. COLIN[1], I. DENIS[1], C. LACROIX[1], P. CAMUS[1], A. BREHIER[2], M. THOMASSET[2] and A. POINTILLART[1], [1]*LNSA – INRA 78352 Jouy-en-Josas Cédex* and [2]*U.120 Inserm, Hop. R. Debré, 75019 Paris*

In GH-treated pigs we have shown a link between bone growth and mineralization and elevated plasma levels of $1,25(OH)_2$ vitamin D (Pointillart *et al.* 1992). The present study investigates whether intestinal absorption of calcium was stimulated to adapt bone mineralization to produce accelerated bone growth during GH treatment.

Sixteen 10-week-old growing pigs (26 kg body-weight) received twice daily subcutaneous injections of 50 μg porcine GH/kg BW (eight GH-treated pigs) or its vehicle (eight control pigs) for 2 months. All animals were fed on a diet containing (g/kg) Ca 1·1, P 6, Mg 0·15 and 1000 iu vitamin D_3/kg. Apparent Ca absorption and retention was evaluated in a 10 d balance trial on six animals from each group (at the end of the feeding period). Animals were housed in individual cages and fed on similar amounts of diet. Faeces and urine were collected and pooled daily for Ca determination. At slaughter, plasma levels of Ca, P, Mg, $1,25(OH)_2D_3$ (Calcitriol, the major biologically-active metabolite of vitamin D), insulin-like growth factor 1 (IGF1) and GH were also determined. CaBP (calbindin-D 9K) is generally considered as the molecular expression at the intestinal level of the hormonal action of calcitriol. Thus, we also measured mucosal CaBP in the mid-jejunum since CaBP-activated Ca transport across the jejunal membrane might be of great importance, especially in pigs (Pointillart *et al.* 1991).

GH treatment did not alter the plasma levels of minerals. However, plasma $1,25(OH)_2D_3$ was slightly elevated (+40 5, $P<0·05$) and plasma IGF1 was doubled ($P<0·001$) in GH-treated pigs. Apparent absorbed and retained Ca were significantly ($P<0·001$) greater in GH-treated than in control pigs, when expressed as daily amounts or as a percentage of intake. Both Ca absorption and retention (% intake) were stimulated by 70 % in the GH-treated group (53 (SE 2) *v.* 31 (SE 2); Ca absorption, % intake, GH *v.* control values; $P<0·001$). Jejunal CaBP in the treated group was greater than in control pigs (12·5 (SE 1·2) *v.* 8·8 (SE 0·7) μg/mg soluble protein, GH *v.* control; $P<0·05$). Thus, GH treatment may enhance Ca utilization and thus explain the adequate mineralization of enlarged bones previously described. This effect is probably mediated by stimulating vitamin D metabolism, since there were high correlations between jejunal CaBP concentration and *in vivo* absorbed Ca (r 0·72; $P<0·02$), between CaBP and calcitriol (r 0·65; $P<0·05$), and between calcitriol and plasma IGF1 (r 0·62; $P<0·02$). These results for intact pigs with high basal levels of circulating endogenous GH (3–5 ng/ml), emphasize the clinical application of exogenous GH treatment in humans exhibiting decreased bone mineralization.

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[1-^{13}C]Leucine uptake into the portal vein of sheep receiving exogenous glucose intravascularly or intraduodenally. By F. PICCIOLI CAPPELLI, C. J. SEAL and D. S. PARKER, *Department of Biological and Nutritional Sciences, The University, Newcastle upon Tyne NE1 7RU*

Previous work from this department has suggested that amino acid uptake into the portal vein may be influenced by the supply of energy-yielding substrates in the gastrointestinal tract (Seal & Parker, 1991; Seal *et al.* 1992). The present experiment investigated the effect of changing the availability of glucose to the luminal or serosal tissues of the intestine on leucine metabolism by the portal-drained viscera of sheep.

Four Suffolk-cross wether sheep, average weight 43·6 kg, with indwelling catheters in the portal vein, mesenteric vein and a carotid artery, and a duodenal infusion catheter, were fed 924 g DM/d of a dried grass pellet diet (Seal & Parker, 1991) in twenty-four equal portions. Measurements of plateau plasma [1-^{13}C]leucine enrichment were made during the last 3 h of a 6·5 h intraduodenal [1-^{13}C]leucine infusion (50 mg [1-^{13}C]leucine 99 atom per cent excess/h) with intrajugular (IJG) or intraduodenal (IDG) infusions of glucose (2·0 mg glucose/kg body-weight per ml per min). Plasma flow in the portal vein was measured by downstream dilution of *p*-amino hippuric acid.

	Control	IJG	IDG	SEM	Contrast 1	Contrast 2
Plasma flow (l/min)	1·58	1·44	1·65	0·028	NS	**
Carotid glucose (mM)	4·18	4·86	4·68	0·129	**	NS
Carotid leucine (mM)	0·23	0·18	0·14	0·020	*	NS
Leucine absorption rate (μmol/min)	41·8	39·3	26·5	12·51	NS	NS
[1-^{13}C]leucine recovery (% infused)	60·5	82·5	52·1	15·77	NS	NS

Contrast 1, control *v.* (IJG + IDG); Contrast 2, IJG *v.* IDG: *$P<0·05$; **$P<0·01$; NS, not significant.

Plasma flow was significantly increased during intraduodenal glucose infusions. Circulating glucose concentrations were higher and plasma free leucine levels were significantly reduced during IJG and IDG infusions. Despite the fall in leucine concentrations, net leucine absorption rates and the recovery of [1-^{13}C]leucine infused into the duodenum were not affected by increasing glucose supply to the gut tissues.

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[^{15}N] and [^{14}C]glutamine fluxes across rabbit ileum in experimental bacterial diarrhoea.

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L-Glutamine is considered a potential candidate for use in oral rehydration solutions, the mainstay of treating dehydration due to diarrhoea. This possibility arises from the fact that glutamine stimulates sodium absorption across rabbit ileum and piglet jejunum. An additional advantage of glutamine utilization is its central role in intestinal energy metabolism. Glutamine is also a major nitrogen carrier and donor *in vitro* and *in vivo*. Moreover, glutamine has been reported to stimulate intestinal mucosal growth and repair. Use of glutamine could therefore be more beneficial in conditions when the intestinal mucosa and its energy balance are compromised such as diarrhoea and malnutrition.

Thus, L-glutamine (Gln) fluxes and the effects of Gln on Na^+ and Cl^- transport were studied across the ileum of weanling rabbits, either healthy (H) or infected (I) with rabbit diarrhoeagenic *Escherichia coli* (RDEC-1). Stable ([α-^{15}N]Gln) and radioisotopic ([U-^{14}C]Gln) tracers provided identical estimates of Gln transport both in H and I rabbits. RDEC-1 infection, however, decreased net Gln flux (682 (SD 147)–278 (SD 63) by ^{14}C; 739 (SD 160)–225 (SD 110) by ^{15}N nmol/h per cm^2) due to a reduction in mucosal-to-serosal flux. After addition of Gln, increases in net Na absorption ($\Delta J^{[15N_{net}]Gln}$ = 1·87 (SD 0·45; H) *v.* 0·70 (SD 0·27; I) μeq/h per cm^2) and short-circuit current (ΔI_{sc}) (1·80 (SD 0·40; H) *v.* 0·74 (SD 0·14; I) μeq/h per cm^2) were also reduced in infected rabbits. Addition of glucose after Gln, however, stimulated Na absorption further.

These results indicate that: (1) Gln is actively absorbed as the intact Gln molecule across rabbit ileum; (2) Gln stimulates an electrogenic Na absorption in a 1:2 ratio that may be further stimulated by glucose; (3) in RDEC-1 infection electroneutral NaCl absorption, intact Gln absorption, and electrogenic stimulation of Na absorption by glutamine are reduced.

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Postnatal development of glucose and galactose metabolism in isolated pig enterocytes. By B. POSHO, B. DARCY-VRILLON, M. T. MOREL, F. BERNARD and P. H. DUÉE. *Unité d'Ecologie et de Physiologie du Système Digestif, INRA CRJ, 78352 Jouy-en-Josas*

The perinatal and weaning periods are characterized by great changes in prevailing physiological and nutritional conditions, implying profound metabolic adaptations in various tissues to ensure fuel homeostasis (Girard *et al.* 1992). In the pig the maturation of the intestinal mucosa is considerable during the first postnatal days. During suckling, lactose from the milk is the only source of dietary carbohydrate, providing glucose and galactose as hydrolysis products. Thus, the purpose of the present work was to assess the capacity of isolated villus enterocytes to metabolize these hexoses during development.

Pigs were taken immediately after birth, at different stages during suckling, or after weaning. Metabolically-active enterocytes were isolated by a method adapted from Vidal *et al.* (1988), and incubated with glucose or galactose at various concentrations. At birth, glucose utilization (measured with 2 mM-glucose) amounted to 1·0 (SD 0·1) nmol/min per 10^6 viable cells. Glucose oxidation to CO_2 accounted for 25% of glucose disappearance, whereas 65% of the glucose consumed was converted into lactate, pyruvate and alanine. During the 1st week (day 1–day 2), isolated enterocytes exhibited a threefold increase in their capacity to utilize glucose. Thereafter, this capacity decreased and matched that measured after weaning (0·9 (SD 0·3) nmol/min per 10^6 cells), while glucose oxidation to CO_2 accounted for 10% of glucose consumption. At birth, intestinal cells had a high capacity to utilize galactose (1·4 (SD 0·1) nmol/min per 10^6 cells; measured with 2 mM-galactose), and oxidation to CO_2 accounted for 20% of this utilization. This high galactose utilization was maintained during the 1st week of life only, and disappeared afterwards. Similarly, galactose oxidation gradually declined after birth. In addition, enterocytes from sucking newborn pigs had a high capacity for gluconeogenesis from galactose, since the conversion of galactose into glucose accounted for 20–25% of the hexose disappearance. In cells from 2-d-old sucking pigs substrate utilization and production of metabolites were concentration-dependent. Moreover, glycolysis and gluconeogenesis capacities were not detected in cells from contemporary unsuckled pigs. Metabolic and enzymic determinations suggest a prominent role of hexokinase to explain the enhanced glycolytic capacity at 2 d.

According to the present data, pig enterocytes exhibit specific and transient metabolic characteristics, sustained during a few days after birth only.

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Diversity of membrane sialo-glycoconjugates in the developing porcine small intestine. By T. P. KING, R. BEGBIE, R. SPENCER and D. KELLY, *Rowett Research Institute, Aberdeen AB2 9SB*

Affinity cytochemistry and biochemistry were used to investigate the diversity of glycoconjugate structures in the developing porcine small intestine. Lectins reactive with sialic acid and other carbohydrate moieties were used as cytochemical probes on 1 μm resin sections of jejunal tissue from ten newborn, ten sucking (2 weeks post-partum) and ten weaned (5 weeks post-partum) Large White × Landrace piglets. The same probes were used on Western blots of jejunal membrane polypeptides previously resolved by SDS-PAGE.

Labelling with the lectin from *Galanthus nivalis* revealed the presence of high-mannose intestinal membrane glycoconjugates in all pigs examined. This form of glycosylation was strong at birth, sustained during suckling and diminished after weaning. *Sambucus nigra* agglutinin was used to identify the presence of sialic acid α2,6-linked to penultimate Gal or GalNAc moieties. Membrane sialylation was extensive at birth, declined during suckling and was much diminished after weaning. Conversely, labelling with *Ulex europeus* agglutinin I revealed a conspicuous increase in α1,2 fucosylation of microvillar glycoconjugates between the suckling and weaning periods.

In addition to these temporal glycosylation changes the affinity cytochemistry and biochemistry revealed considerable diversity of some intestinal glycoconjugates in piglets of the same age and nutritional status. This was particularly evident with the labelling patterns obtained with *Maakia amurensis* agglutinin II which identifies sialic acid α2,3 linked to Galβ 1,4GlcNAc.

Age-related and individual diversity of intestinal glycoconjugates may contribute to variations in enteric disease susceptibility in humans and their domesticated animals. Enterotoxigenic *Escherichia coli* strains are frequently associated with outbreaks of diarrhoea in pigs. In addition to small numbers of type 1 fimbriae which recognize surface mannose groups, some strains of these bacteria possess extensive surface arrays of K88 fimbriae which recognize other unidentified oligosaccharide sequences. *In vitro* incubations were undertaken using suspensions of K88ac positive *E. coli* on resin sections of jejunal tissue. Mannose-resistant attachment of K88ac fimbriae to villus surfaces was revealed by immunofluorescence microscopy. K88ac binding was evident on jejunal sections from many but not all of the thirty pigs examined but there was no correlation with the observed glycosylation patterns. This evidence suggests that sialylated or fucosylated sequences, or both, may be present on the membrane receptors for K88ac fimbriae, but such moieties are unlikely to be the key determinants for fimbrial–receptor interaction.

Effect of oral administration of an oil enriched in γ-linolenic acid in platelet aggregation and lipid composition in alcoholic patients during withdrawal. By J. F. MENEZ[1], A. MESKAR[1], C. GERARD[1], G. LE MENN[1] and B. E. LEONARD[2], [1]*CHRU A. Morvan, 29285 Brest* and [2]*Pharmacology Department, University College, Galway, Republic of Ireland*

Ethanol has been shown to inhibit the enzyme Δ 6 desaturase which is responsible for the conversion of linoleic acid to γ-linolenic acid (GLA), the immediate precursor for arachidonic acid (AA; Nervi *et al.* 1980). As a consequence of these changes, the membrane lipid composition and platelet function are impaired. The administration of an oil enriched in GLA (Glen, 1982) may bypass this inhibition of Δ 6 desaturase and help the platelet to maintain normal levels of AA in the presence of ethanol. In rats' platelets, this oil treatment attenuated some of the detrimental effects due to ethanol especially the decrease in the platelet serotonin (5-HT) uptake (Corbett *et al.* 1990). In the present report twenty-eight alcoholic patients, admitted for detoxification, were divided into three groups and treated for 9 weeks in a double-blind study with six placebo oil capsules; containing no GLA, daily (G1), or with Boracelle oil, containing 200 g GLA/kg, i.e. 600 mg/d (G2), or with Boracelle oil plus carnitine (1 g carnitine/d, G3). Blood was collected before treatment, and then 3 weeks and 9 weeks after the beginning of the treatment and analysed for platelet aggregation and membrane lipid composition. Results were analysed using paired *t* tests. Thrombin, collagen, adrenaline, ADP and AA were tested as platelet aggregation inducers. In controls (G1) the velocity and intensity of the aggregation was increased compared to day 1 when AA was used as inducer, especially 21 d after the beginning of the treatment (velocity, +65%; intensity, +40%); in contrast they were decreased (−24 and −18%, respectively, $P<0{\cdot}05$) in G2 and to a lesser extent in G3. Using thrombin as inducer the same phenomenon was noticed (+40 and +60% in G1, between day 0 and day 21; −10 and −43% in G2). In G2 the 20:3 *n*-6 (3·86 (SD 1·14) *v.* 1·77 (SD 0·15)% at day 0) and the 20:3 *n*-6:18:3 *n*-6 ratio was doubled 21 d after the beginning of the treatment. Aggregation results and fatty acid composition of platelets suggest the efficiency of this treatment in bypassing the inhibition of Δ 6 desaturase and in increasing the PGE1 content. Horrobin (1987) and Segarnick & Rotrosen (1987) have suggested the importance of PGE1 in improving the cognitive function in alcoholics. Treatment with Boracelle oil should ameliorate these functions in alcoholics but they are difficult to evaluate.

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Effect of progressive enrichment on monounsaturated fatty acids (MUFA) in the diet on serum lipids, lipoproteins and fatty acids in normolipidemic males. By B. DELPLANQUE, J. L. RICHARD and B. JACOTOT, *INSERM U 32, Hôpital Henri-Mondor, F 94010 Créteil*

Three isoenergetic diets high in fat (38% of energy intake), low in saturated fatty acids (SFA; 12%) and progressively enriched in MUFA (14 and 21%) at the expense of polyunsaturated fatty acids (PUFA), were tested in twenty-five normolipidemic monks (mean age 54 (SD 13) years and BMI 23 (SD 3)). High monounsaturated oil (Oleisol, 740 g oleic acid/kg), medium monounsaturated oil, balanced for MUFA and PUFA (440 g linolenic, 410 g oleic and 12 g α-linolenic acid/kg) and polyunsaturated oil (sunflower, 670 g linoleic acid/kg) were studied after diet periods of 8 weeks using successively these oils in turn as visible fat. The daily intake of PUFA:MUFA:SFA (42:26:34 g for poly diet, 30:38:34 g for moderate-MUFA diet, and 15:56:32 g for high-MUFA diet) was such that % SFA was kept constant but the PUFA:MUFA ratios were 1·6, 0·77 and 0·25, and the P:S ratios were 1·23, 0·88 and 0·44, respectively for PUFA, moderate- and high-MUFA diets.

The composition of plasma fatty acids (FA) and erythrocyte phospholipid FA reflected the composition of dietary FA. The FA analysis showed a progressive and significant MUFA enrichment of plasma phospholipids (PL), cholesteryl esters (CE) and triacylglycerols (TG) with moderate- and high-MUFA diets, mainly at the expense of linoleic acid. The differences were always more striking with the high-MUFA diet than with the moderate-MUFA diet and more prominent in plasma TG than in plasma CE or PL, and erythrocyte PL.

Analysis of blood lipids showed that only the high-MUFA diet induced higher levels of total cholesterol, TG, PL and LDL-C, when compared with moderate-MUFA and PUFA diets (23 (SD 8)%, 41 (SD 23)%, 16 (SD 6) %, respectively). In contrast, serum lipid concentrations after the moderate-MUFA diet were similar to those after the PUFA diet. The levels of serum lipids after the high-MUFA diet were very similar to those characteristic of the prestudy state, suggesting that the PUFA diet and moderate-MUFA diet had a greater effect on atherogenic lipid variables than the MUFA-rich diet in this normolipidemic male population. The HDL-C levels were similarly increased by both MUFA diets (14%) compared with the PUFA diet. The mean levels of apoA-I, apoA-II and apo B were progessively and significantly increased with MUFA-enriched diets, when compared with the PUFA diet.

The measure of the total mass of ultracentrifugally-isolated HDL subfractions was consistent with the measurement of HDL-C levels in that they both showed an increase in the concentrations of HDL mass and HDL-C after consumption of MUFA-enriched diets. The diet also affected the distribution of HDL particles along the density spectrum; the high-MUFA diet induced higher levels of HDL_3 particles, whereas the moderate-MUFA diet increased the levels of HDL_1 particles.

Changes in the concentration of plasma lipids, apolipoproteins and lipoproteins in normolipidemic men brought about by the progressive MUFA enrichment of a high-fat (380 g/kg) isoenergetic diet at the expense of PUFA showed that, among three diets low in saturated fats, the most favourable effects were obtained with the moderate-MUFA diet which provided approximately the same amount of unsaturated fat as the other two diets, but equally divided between MUFA and PUFA. Whereas this diet had the same lowering effect on LDL-C levels as the diet rich in PUFA, its content of MUFA probably prevented the decrease in levels of HDL-C frequently seen with diets high in PUFA.

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Short-chain fatty acids (SCFA) and colonic motility: influence on propulsive motor patterns. By P. E. SQUIRES, R. D. E. RUMSEY and N. W. READ, *Department of Biomedical Science, The University, Sheffield S10 2TN*

Absorption of SCFA from the colon is metabolically significant. SCFA also suppress general colonic motility (Squires *et al.* 1992*a*) and it has been suggested that the level of SCFA absorption is regulated by the effects on motility. The present study examines whether SCFA alter a colonic motility pattern dominated by propagated propulsion (1mM sodium rhein; Squires *et al.* 1992*b*).

Male Wistar rats were anaesthetized (60mg Sagatal/kg given intraperitoneally) and the large intestine isolated and perfused vascularly with Krebs bicarbonate (O_2:CO_2, 95:5, v/v; 1·2 ml/min at 37°) via the superior mesenteric artery with hepatic portal outflow. The tissue was suspended horizontally between two volume transducers. Serosal strain gauges (RB Products, USA) recorded contractile activity in the caecum (tail (CT) and body (CB)), proximal (PC), proximal-to-middle (PM), middle-to-distal (MD) and distal colon (DC) for an initial 30 min luminal infusion with Krebs buer and subsequent 30 min infusion with Krebs containing acetic (66 mM), propionic (26 mM) and butyric (8 mM) acids plus 1 mM sodium rhein (Madaus, Cologne). Each period was subdivided into 10 min intervals. Data are expressed as mean % change in activity (n 6), recorded as the area under the response from each gauge site or transducer. The number of migrating contractions in each interval was also recorded.

Activity preceding the addition of the test solution was similar to that with buffer only, except for MD in the 2nd 10 min interval (83 (SD 8)%; $P<0·05$). No reduction in colonic contractility was observed following administration of the test solution. The number of migrating contractions increased by more than 100% in the second and third intervals of the administration (both $P<0·05$); a similar effect to sodium rhein alone (Squires *et al.* 1992*b*), although the amount of fluid expelled from the distal colon was unchanged. The present study demonstrates that a mixture of SCFA (100 mM) does not inhibit the action of a prokinetic agent which alters the pattern of motility to one of propulsion, suggesting that SCFA themselves do not alter motility to a pattern which would indicate increased absorption.

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Fats decrease gastric emptying rate and reduce post-prandial glycaemia. By P. THOUVENOT[1], C. LATGE[2], M-H. LAURENS[1], G. KARCHER[1] and J. M. ANTOINE[3], [1]*Médecine Nucléaire, CHRU, 54000 Nancy,* [2]*Centre Recherches et Etudes Alimentaires, 19100 Brive* and [3]*BSN, Paris*

We have shown that the slower the gastric emptying rate (GER) the lower the glycaemic index of starchy foods (Mourot *et al.* 1988). The purpose of this study was to compare the GER of pasta in a meal with and without added fats, to register postprandial glycaemia, and to measure the GER of fats.

Ten healthy volunteers, five men and five women, consumed after an overnight fast, a meal either with or without added fats (84·3 g CHO, and 3·8 g–57·4 g added fats). Pasta was labelled with ^{99}Tc and fats with ^{111}In. Gastric scintigraphies were performed immediately after the meal and every 10 min over a 5 h period. Venous blood samples were collected before the meal and 30, 60, 90, 120, 180, 240 and 300 min after.

GER were expressed as the time (min) needed for the stomach to empty 10%, 50% and 80% of labelled products.

		Pasta alone		Pasta with fats		Fats	
Extent emptying (%)		Men	Women	Men	Women	Men	Women
10	Mean	20·7	20·2	29·5	25·4	34·1	28·8
	SEM	2·6	2·4	2·7	4·7	8·9	5·5
50	Mean	61·7	67·5	81·4	104·2	121·9	172·4
	SEM	3·8	4·3	6·6	12·8	9·7	16·7
80	Mean	126·7	148·1	154·3	235·3	221·9	376·5
	SEM	11·7	11·2	10·6	27·8	20·2	41·1

For glycaemic index values we used the glycaemic index of men after the no-fats meal as a reference: Meal without fats, men, 100 (SEM 13), women, 110 (SEM 8); meal with added fats, men, 65 (SEM 9), women, 78 (SEM 18).

Individual glycaemic indices were correlated with GER. The slope of the linear regression between GER and glycaemic indices was similar to the one observed with various starchy foods.

We concluded that added fats reduced the glycaemic index of pasta partly by slowing down gastric emptying rate of pasta.

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Quantitative assessment of the effect of non-starch polysaccharides on nutrient absorption in the pig: use of a transit time ultrasound flow probe. By D. E. BLAKE[1], F. G. ROBERTS[1], J. W. SISSONS[2], N. CANIBE[3], C. L. JONES[2], K. E. BACH KNUDSEN[3] and P. R. ELLIS[1], [1]*Food Research Group, Biomolecular Sciences, King's College London, Campden Hill Road, London W8 7AH,* [2]*Protein Technologies International, St. Louis, USA* and [3]*National Institute of Animal Science, Foulum, Tjele, Denmark*

It is well documented that soluble non-starch polysaccharides, such as guar gum, induce changes in gut function and carbohydrate metabolism (e.g. reduction in postprandial hyperglycaemia). It is frequently stated that guar gum has the potential to modify the rheological behaviour of digesta *in vivo* ultimately delaying the rate of nutrient absorption. However, the evidence for this has been gained from indirect measurements only. The present work was done to study the impact of guar gum (high viscosity grade; M 150, Meyhall Chemical, Switzerland) on glucose uptake using a direct method.

A method has been described for measuring the absorption of nutrients from the digestive tract in the pig (Rérat *et al.* 1980) based on an accurate determination of the portal blood flow rate and the estimation of postprandial porto-arterial differences of various nutrients. This technique has been updated by the use of a permanent ultrasonic blood flow probe (Transonic Systems, Ithaca, NY USA) fitted around the hepatic portal vein of the pig and has been used successfully in an earlier study (Roberts *et al.* 1991). In the present study two pigs (45 kg) were fitted with permanent catheters in the hepatic portal vein and mesenteric artery, and a blood flow probe. Semi-purified diets containing either wheat bread (control) or guar gum bread were given twice daily at a level of 40 g/kg body-weight per day. Each pig received each diet on two separate occasions. The proportion of guar bread added was adjusted to give a guar gum concentration of 40 g/kg total diet; wheat bread was added at a level to provide a similar amount of available starch. Blood samples were taken (7 ml per vessel) at 0, 10, 20, 30, 40, 50, 60, 90, 120, 150, 180, 210 and 240 min after feeding, for glucose and insulin measurements. From the continuous blood flow measurements and glucose and insulin concentrations it was possible to quantify the response to guar gum in the diet.

The average blood flow rate was 37 ml/min per kg body-weight. The flow probe preparation remained viable for over 60 d with no decrease in recorded blood flow rate or impairment to animal health. Total glucose absorption was reduced by 30% ($P<0{\cdot}005$) over a 4 h period following the guar bread meal, compared with the control.

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The effect of dietary fibre on oro-caecal transit time in dogs. By S. RIGBY[1], J. V. JOHNSON[1], K. PAPASOULIOTIS[2] and T. J. GRUFFYDD-JONES[2], [1]*Waltham Centre for Pet Nutrition, Leicestershire* and [2]*University of Bristol Veterinary School, Avon*

Dietary fibre decreases postprandial hyperglycaemia in man and dogs and has proved beneficial in the management of diabetes mellitus (Blaxter *et al.* 1990). Decreasing oro-caecal transit time (OCTT) could be a contributory factor in achieving this effect. The present study investigated breath hydrogen profiles as a measurement of OCTT in six male Beagles (aged 18 months) following the addition of soluble fibre (20 g guar) and insoluble fibre (20 g wheat bran) to a standard complete canned food (STD).

All diets were fed for a minimum of 5 d before measurements were performed. The protocol for breath H_2 measurement was carried out twice for each dog for each test diet, with a minimum of 48 h between tests. Readings were taken every 15 min and treated by cusum analysis to assess the rate of increase of H_2 production. OCTT was taken as a rise of 5 ppm H_2 sustained for three consecutive readings. Results are summarized in the Table.

Diet	OCTT (median; min)	OCTT (range; min)
STD	135	120–150
STD + guar	150	90–240
STD + wheat bran	128	105–195

No significant differences were detected between the three diets ($P>0{\cdot}05$; ANOVA) although the levels of fibre supplementation were considerably higher than levels which have previously been shown to reduce postprandial hyperglycaemia (Blaxter *et al.* 1990). This suggests that the method of breath H_2 may not be sufficiently sensitive to measure OCTT changes in this study, or other mechanisms may be responsible for the effect of dietary fibre in lowering postprandial glucose concentrations, or both.

Blaxter, A. C., Cripps, P. J. & Gruydd-Jones, T. J. (1990). *Journal of Small Animal Practice* **31**, 229–233.

Breast-feeding and other prognostic factors among malnourished children admitted for diarrhoea in a Madagascar hospital. By O. RAZAFINDRAKOTO[1], N. RAVELOMANANA[1], F. RANDRAIMIHARISOA[1], V. RASOARIVAO[1], V. RAMIALIMANANA[1], D. R. RAKOTOARIMANANA[1], M. RAZANAMPARANY[1], J. Y. MARY[2] and A. BRIEND[3], [1]*Services de Pédiatrie, Hôpital de Befalatanana, Antananarivo, Madagascar,* [2]*Unité INSERM 263, Paris* and [3]*ORSTOM* and *INSERM U290, Paris*

The present study examined the risk factors for mortality among 6–35 months-old malnourished children with diarrhoea enrolled in a protocol comparing standard and rice-based ORS for rehydration. Children from the present study had either a weight-for-age Z-score below –2 or a weight-for-height below 70% of National Centre for Health Statistics standard (Hamill *et al.* 1979). All had diarrhoea defined by at least three liquid stools/24 h for less than 5 d. During the 6 h following admission children were randomly allocated to receiving 100 ml/kg body-weight of standard or of rice-based oral rehydration solution. Then they received 420 kJ/kg per day of high-energy milk on the first day, progressively increased to 840 kJ/kg per day. Children (*n* 148) were followed from admission up to diarrhoea cessation. Mortality was similar (15 and 16%) in the two groups. Its relationships with different risk factors for both groups combined are presented in the Table.

Risk factors	Deaths (%)	Odds ratio (95% CI)	*P*
Sex: Boys	9	1	<0·01
Girls	25	3·5 (1·4–8·9)	
Breast feeding: Yes	8	1	<0·01
No	25	3·7 (1·4–9·6)	
Dehydration: Mild	5	1	<0·05
Moderate or severe	19	4·4 (1·0–20·0)	
Duration of diarrhoea: ≤3 days	11	1	<0·05
>3 days	25	2·7 (1·1–6·7)	
Proteinaemia: ≤40 g/l	35	1	<0·01
>40 g/l	12	0·3 (0·1–0·7)	
Weight for height: ≤66·7%	39	1	<0·01
>66·7%	11	0·2 (0·1–0·5)	

Multivariate logistic regression showed that only breast-feeding, weight-for-height and sex were significantly related to the risk of dying. Absence of breast-feeding was associated with a higher risk of dying only among girls with a low weight-for-height. The relationship between breast-feeding and mortality does not seem to be fully explained by the effects of malnutrition or dehydration.

Hamill, P. V. V., Drizd, T. A., Johnson, C. L., Reed, R. B., Roche, A. F. & Moore, W. M. (1979). *American Journal of Clinical Nutrition* **32**, 607–629.

Polyamine metabolism in enterocytes isolated from newborn pigs. By H. M'RABET-TOUIL, F. BLACHIER, L. POSHO and P.-H. DUÉE, *Unité d'Ecologie et de Physiologie du Système Digestif – INRA-CRJ – 78352 Jouy-en-Josas Cédex*

Various physiological situations of enhanced gastrointestinal mucosal growth are associated with a stimulated ornithine decarboxylase (ODC) (*EC* 4.1.1.17) activity (McCormack & Johnson, 1991), the rate-limiting enzyme in polyamine biosynthesis. In the pig the growth of the intestinal mucosa is very intense during the first days after birth (Widdowson *et al.* 1976). The intestinal metabolism of polyamines has not yet been investigated in such a model of hypertrophy. Villus enterocytes isolated from pigs (*n* 6) immediately after birth or after a 2 d suckling period were found to contain sizeable and similar amounts of putrescine, spermidine and spermine, 0·23 (SD 0·08), 0·41 (SD 0·09) and 1·24 (SD 0·08) nmol/10^6 cells, respectively. At birth, despite a relatively high ODC activity (5·77 (SD 1·25) pmol/10^6 cells per 60 min, *n* 3) as determined at 20μM-L-[1-^{14}C] ornithine, putrescine synthesis from 2mM-L-arginine or 2mM-L-glutamine was very low in isolated enterocytes (6·4 (SD 3·8) pmol/10^6 cells per 30 min, *n* 4), while spermidine and spermine production were not detectable. This could be explained by a very low L-ornithine generation from both amino acids and to an inhibitory effect of endogenous polyamines on ODC activity. After 2 d, polyamine synthesis from L-arginine remained undetectable despite a higher L-ornithine generation which was concomitant with an increased arginase activity in enterocytes. A dramatic fall in ODC activity in enterocytes isolated from 2-d sucking pigs could explain such an undetectable rate of polyamine production.

The uptake of polyamines by enterocytes was also determined by incubating $0{\cdot}3\times10^6$ cells (37°, 10 min) in the presence of 2·5mM-L-glutamine and with respective radioactive polyamines. At both stages, enterocytes were able to take up polyamines from the extracellular medium in a temperature-dependent manner.

In conclusion, the present data suggest that the *de novo* synthesis of polyamines does not play a significant role in the control of polyamine content of pig enterocytes during the postnatal period. At the same time, polyamine uptake by enterocytes would contribute to maintain a steady-state polyamine content.

McCormack, S. A. & Johnson, L. R. (1991). *American Journal of Physiology,* **260,** G795-G806.
Widdowson, E. M., Colombo, V. E. & Artavanis, C. A. (1976). *Biology of the Neonate,* **28,** 272–281.

Nutritive value of goat's and cow's milk crude protein and ileal digestibility of their amino acids by the growing pig. By C. FÉVRIER, J. MOUROT, Y. JAGUELIN and A. MOUNIER, *INRA-Station de Recherches Porcines, 35590 Saint-Gilles*

Goat's milk is often used as a basis for infant feeding but the comparison of its nutritive value with that of cow's milk is frequently made on an equivalent liquid basis and without taking into account the differences in protein and lipid contents. In this new study, for which the growing pig was used as an experimental model, the nutritive value of goat's milk was estimated by comparison with cow's milk, for the same crude protein and crude lipid levels.

A group of five pairs of male castrated littermates, weighing on average 35 kg, were fed on barley fortified with minerals and vitamins, and liquid UHT milk, either cow or goat, at 210 g/kg total dry matter of the diet, for 28 d. The apparent digestibility coefficients (aDC) were measured between the 8th and 18th days. Another group of six castrated males, each prepared with an ileo-rectal anastomosis, was used to measure the pre-ceacal aDC of the main nutrients and of the amino acids of the diets, by comparison with that of barley alone. Each pig was fed a different diet each week.

In the first group the aDC of the main nutrients were equivalent for both milks with the exception of minerals, which were better utilized with goat's milk. The same applied to pre-ceacal aDC; however, at this level a slight reduction of aDC for cystine, glycine, tyrosine and histidine in goat's milk was observed. The other amino acids were equally well utilized, particularly lysine, with an aDC of about 0·95.

In the first group, growth rates, feed efficiency and the total quantities of fat and lean tissues were equivalent. Opposite effects of the different milks on ham and loin weights were observed (ie. ham weight was higher after goat's milk, but loin weight was lower compared with cow's milk). This effect could be due to different levels of bone mineralization, which was not measured in this experiment.

It is possible to conclude from this model that the overall nutritive value of UHT liquid goat's milk is not significantly different from that of cow's milk for growth. We have in the meantime demonstrated that it acts favourably on the quality of fat tissue (Mourot *et al.* 1992).

Mourot, J., Février, C., Jaguelin, Y. & Mounier, A. (1992). *Cahiers de Nutrition et de Dietétiques* **27**, 351 (Abstr).

Nutrient digestibility and energy use in growing medium breed puppies. By H. DUMON, P. NGUYEN, LUCILE MARTIN and A. MALEK, *Department of Animal Production (Nutrition), Ecole nationale vétérinaire de Nantes, CP 3013, 44087 Nantes Cx 03*

According to the National Research Council (NRC 1985) growing dogs require after weaning about twice as much energy per unit body-weight as adult animals of the same breed. A decrease to 1·6 times is suggested when 40% adult body-weight is achieved. Estimates of the maintenance energy requirements per unit body-weight of adult dogs vary widely as a result of differences in breed, sex, age, skin insulation, environment and body-weight. The aim of the present study was to determine dietary energy needs of medium breed puppies and to determine if practical recommendations, should be adjusted in accordance with energy utilization. In this first trial, the latter was only evaluated through digestibility.

Eighteen German Short-Haired Pointers were kept in metabolism cages for 3 week periods when they were 10 (2 weeks post weaning), 19 and 28 weeks old. During each trial period the dogs were randomly assigned to one of six experimental diets, formulated with commonly-used foodstuffs. These diets were offered on a body-weight (BW) basis according *a priori* to NRC recommendations. The diets differed in crude protein (CP) content (from 150–650 g/kg, achieved by starch substitution) but not in the estimated (Atwater factors) metabolizable energy (eME) content (17·6 kJ (4·2 kcal)/g).

Weight gain ($\triangle$BW) and dry matter (DMd), organic matter (OMd), crude protein (CPd) and ether extract (EEd) digestibilities and nitrogen retention were recorded. Actual ME intake (aME) was calculated from digestible nutrients and N balance.

Age of pups (weeks) . . .	10		19		28	
	Mean	SE	Mean	SE	Mean	SE
DMd	0·758	0·016	0·784	0·017	0·784	0·009
OMd	0·788	0·010	0·818	0·011	0·849	0·006
CPd	0·721	0·017	0·774	0·013	0·854	0·008
EEd	0·755	0·024	0·815	0·021	0·837	0·016
aME/eME	0·847	0·010	0·887	0·012	0·912	0·010

The partition of aME intake can be modelled according to the following formula:

$$aME = 171 \times BW^{0.75} + 3200 \times \triangle BW + 209 \ (r^2 = 0.84)$$

These preliminary results suggest that, if ME content of the diet is estimated with standard coefficients (NRC of Atwater) assuming mean digestibility values, a significant part of the high energy requirements of the youngest weaned pups results from a low efficiency of digestive processes.

National Research Council (1985). *Nutrient Requirements of Dogs*. Washington, DC: National Academy Press.

The nutritive value of different wheat varieties for poultry. By S. P. ROSE[1], P. S. KETTLEWELL[2], S. M. REYNOLDS[1] and R. M. WATTS[1], [1]*National Institute of Poultry Husbandry* and [2]*Crop and Environment Research Centre, Harper Adams College, Newport, Shropshire TF10 8NB*

The objective of the present study was to identify whether there are differences in nutritive value for poultry between commonly used wheat varieties.

Two trials were conducted. In Trial 1, twelve wheat samples were fed to 7-d-old broiler chickens for 14 d. The wheat samples included six different varieties grown at two sites in Yorkshire and Humberside and harvested in 1990. The same six wheat varieties, grown the following year in Shropshire, were fed in the second trial. The chickens were given complete diets (210 g protein/kg) that contained 70% of the wheat sample. Nitrogen-corrected metabolizable energy (AME_n) was determined in a 4 d collection period beginning at 17 d old.

Table. The growth and food utilization of chickens given different wheat varieties

Wheat varieties . . .	Slejpner	Mercia	Haven	Dean	Apollo	Beaver	SEM
			Trial 1				
Weight gain (kg/bird)	0·5207	0·4768	0·4735	0·5889	0·5052	0·4414	0·02097
Feed:gain ratio	1·810	1·858	1·946	1·765	1·811	2·017	0·0455
AME_n (MJ/kg diet)	12·02	12·28	12·26	12·22	12·09	11·84	0·094
Hagberg falling no(s)	398	417	344	420	399	287	15·2
			Trial 2				
Weight gain (kg/bird)	0·4588	0·4856	0·4713	0·4813	0·4544	0·4963	0·02038
Feed:gain ratio	2·104	1·974	2·080	1·992	2·066	2·006	0·0719
AME_n (MJ/kg diet)	12·32	12·11	12·13	12·56	12·24	12·59	0·215
Hagberg falling no(s)	339	341	128	314	282	225	25·6

There were no significant differences in proximate analysis between any of the wheat samples. Hagberg falling numbers (HFN) were greater in the wheat samples used in Trial 1 than Trial 2. The differences were probably most influenced by weather conditions between the two growing seasons. Differences in productive performance of the chickens due to wheat variety were evident in Trial 1 but not Trial 2. HFN and the feed:gain ratio of the broilers were linearly related in Trial 1 ($r=-0·81$; $P<0·01$), but not in Trial 2 ($r=-0·28$). AME_n tended to increase ($P<0·1$) by 0·027 MJ/kg with each ten unit increase in HFN in this trial. This closely agrees with the relationship described by McNab (1991) for TME_n.

In conclusion, there were differences in the nutritive value of wheat varieties. These differences were not consistent between growing seasons or growing site. There was a relationship between HFN and feed conversion efficiency in the trial in which variety differences were evident.

McNab, J. (1991). HGCA Project Report no. 43. London: HGCA.

The effect of microbial phytase on the performance of ducks given diets with high amounts of rice bran. By D. J. FARRELL and E. MARTIN, *Department of Biochemistry, Microbiology and Nutrition, University of New England, Armidale, N.S.W. 2351 Australia*

Rice bran is used widely by the poultry industry in many developing countries but it has antinutritional factors. It contains phytic acid phosphorus which is poorly available to poultry. In the present study we included rice bran in diets of ducks grown from 19 to 40 d of age with and without a microbial phytase (Gist-brocades, Delft, The Netherlands).

The diets were formulated to duck nutrient specifications, contained only plant ingredients and were based largely on grain sorghum and soybean meal. Diets contained 1 g inorganic P/kg as $CaHPO_4$ and 6 g Ca/kg. Rice bran was substituted for grain sorghum at 300 and 600 g/kg diet. Diets were calculated to be equal in nitrogen and metabolizable energy and were with (+) or without (–) phytase at 1000 FTU/kg. Thus there were six diets × three replicates × five ducks/group. The results are given in the Table.

Rice bran (g/kg) . . .	0		300		600		LSD
Enzyme . . .	+	–	+	–	+	–	($P<0·05$)
Gain (g/d)	85·5	78·3	77·8	74·4	71·8	67·3	2·71
Food intake (g/d)	211	212	194	193	178	178	6·0
FCR	2·47	2·70	2·49	2·60	2·48	2·64	0·111
Tibia ash							
(g)	2·84	2·82	2·61	2·39	2·27	2·10	0·160
(%)	53·4	52·0	52·2	51·6	50·5	50·4	0·97

Calculated total P and available P (assumed to be 30% of phytic acid P) were (g/kg diet) 5·0, 8·5 and 12·0 and 2·1, 3·2 and 4·2 for diets with 0, 300 and 600 g rice bran/kg diet respectively.

Rice bran without enzyme decreased ($P<0·05$) growth rate and food intake but not food conversion ratio (FCR); tibia ash also declined ($P<0·05$). Overall, enzyme addition improved performance ($P<0·05$) except for food intake. Values for tibia ash (g and %) on the diet without rice bran were always higher ($P<0·05$) than on other diets. Enzyme addition may have been inadequate to make the high phytic acid P content of rice bran more available. It is concluded that phytase addition can increase utilization of rice bran by finisher ducks by making some of the phytic acid P more available. Phytase may also increase the availability of the other dietary components.

Dietary fibre and cell proliferation in the stomach. By R. A. GOODLAD[1], B. RATCLIFFE[2], C. Y. LEE[1] and N. A. WRIGHT[1], [1]*Imperial Cancer Research Fund, Histopathology Unit, 35–43 Lincoln's Inn Fields, London WC2A 3PN* and [2]*Robert Gordon Institute of Technology, Aberdeen AB9 2PG*

Addition of fermentable dietary fibre to a fibre-free diet stimulates epithelial cell proliferation in the colon of conventional but not germ-free rats (Goodland *et al.* 1990). Fibre also increased the gross weight of all regions of the gastrointestinal tract in both the groups via a general trophic effect on the muscle layers. Groups of fifteen germ-free (GF) or conventional (CV) rats were fed a fibre-free diet with or without a fibre supplement (Ispaghula:trifyba, 1:9) for 2 weeks, and were then injected with vincristine to determine the crypt cell production rate (CCPR; Goodlad & Wright, 1982).

	Germ-free				Conventional				Two-way analysis of variance. Effect of		
	Fibre-free		+ Fibre		Fibre-free		+ Fibre				
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Diet	Flora	Inter-action
Tissue wt (% total body-wt)											
Stomach	0·37	0·004	0·47	0·01	0·39	0·01	0·46	0·01	***		*
Small intestine	2·06	0·04	2·14	0·03	1·94	0·03	2·12	0·04	***		
Caecum	0·54	0·02	0·63	0·01	0·28	0·01	0·35	0·01	***	***	
Colon	0·36	0·01	0·56	0·02	0·34	0·01	0·50	0·02	***	***	
Crypt cell production rates (cells/crypt per h)											
Fundus	0·88	0·62	2·63	0·82*	0·41	0·26	3·35	0·45***			
Antrum	2·70	0·24	3·78	0·78	2·16	0·24	3·54	0·36**			
Jejunum	11·9	1·9	12·5	1·1	15·5	1·6	14·3	1·5			
Ileum	11·4	1·6	12·3	1·0	11·5	0·8	16·5	1·1**			
Proximal colon	2·8	0·5	3·5	0·6	2·2	0·5	7·7	1·0***			
Mid colon	4·2	1·3	2·9	1·0	3·1	0·5	9·2	1·4***			
Distal colon	2·9	0·8	4·4	1·3	4·7	0·7	9·4	1·4***			

Significantly different from respective fibre-free group: *$P<0·05$; **$P<0·01$; ***$P<0·001$.

The CCPR of the stomach of both the CV and the GF groups was significantly elevated by fibre suggesting that the action of fibre in the stomach differs from that of the rest of the gastrointestinal tract in that it does not require the microbial fermentation of fibre. A similar result was seen in the antrum, but not the fundus, of rats refed with fibre.

Thus, there are at least three distinct and separate effects of fibre on the gastrointestinal tract. In the hind-gut fibre dramatically stimulates epithelial proliferation, but only in the presence of the intestinal microflora; however, it also has direct, trophic effects on the intestinal muscle layers and on the mucosa of the stomach. This has some worrying implications, especially when increased fibre intake is being advocated.

Goodlad, R. A., Ratcliffe, B., Fordham, J. P., Lee, C. Y. & Wright, N. A. (1990). *Proceedings of the Nutrition Society* **49**, 37A.

Goodlad, R. A. & Wright, N. A. (1982). In *Techniques in the Life Sciences, Techniques in Digestive Physiology*, vol. P2, pp. 212/1–212/23 [T. A. Titchen, editor]. Ireland: Elsevier Biomedical Press.

***In vivo* protein synthesis in the developing ruminant stomach.** By D. ATTAIX, D. ROSOLOWSKA-HUSZCZ, E. AUROUSSEAU, G. BAYLE and M. ARNAL, *Institut National de la Recherche Agronomique, Centre de Recherches de Clermont-Ferrand-Theix, 63122 Ceyrat*

There are no reliable measurements of fractional rates of protein synthesis (K_s) in the ruminant stomach although its contribution to whole-body protein synthesis is substantial (Lobley *et al.* 1980). Almost all published data have been obtained after continuous infusion of tracer doses of labelled amino acids and depend closely on the choice of the amino acid precursor pool. We report here the influence of age and weaning on K_s in the developing ovine stomach using a flooding dose of L-[^{3}H]valine which overcomes such problems (Attaix & Arnal, 1987).

Thirty male Ile de France × Romanov-Limousin lambs were randomly allocated to two groups of six and two groups of nine animals. Lambs were either 1-, 5- or 8-week-old milk-fed, or 8-week-old weaned. *In vivo* protein synthesis was measured after an intravenous injection of 12·8 mmol of L-[3,4 (*n*) ^{3}H]valine/5 kg body-weight (1- and 5-week-old groups) or /5 kg body-weight$^{0.75}$ (both 8-week-old groups). The animals, diets and techniques used were as reported by Attaix *et al.* (1989).

Between 1 and 8 weeks of age K_s values were not different in the reticulo-rumen of milk-fed lambs and ranged between 29·6 and 25·3%/d. There was a marked decline (45%) in ribosomal capacity, i.e. total RNA:protein ratio (C_s) which was cancelled out by a significant increase (56%) in the protein synthetic efficiency, i.e. protein synthesis relative to RNA (K_{RNA}). By contrast, K_s decreased between 5 and 8 weeks of age in the omasum and abomasum, mainly due to depressed K_{RNA}. K_s values were 45·4 (SE 3·7), 45·2 (SE 2·2) and 66·7 (SE 9·7) %/d in the reticulo-rumen, omasum and abomasum of 8-week-old ruminant lambs respectively; these values were 79, 134 and 75% higher than in milk-fed animals of the same age, owing to increases in both C_s and K_{RNA}. Our findings demonstrate that development of the ruminant stomach at weaning clearly depends on increases in both RNA abundance and presumably in the efficiency of translation. However, changes in nutrients at weaning, weaning itself, or both, influence K_{RNA} in the omasum and abomasum more markedly than in the reticulo-rumen.

Attaix, D. & Arnal, M. (1987). *British Journal of Nutrition* **58,** 159–169.

Attaix, D., Aurousseau, E., Bayle, G., Rosolowska-Huszcz, D., Manghebati, A. & Arnal, M. (1989). *Journal of Nutrition* **119,** 463–470.

Lobley, G. E., Milne, V., Lovie, J. M., Reeds, P. J. & Pennie, K. (1980). *British Journal of Nutrition* **43,** 491–502.

Programming of expression of disaccharidases in the neonatal small intestine: use of a xenograft model. By N. JORDAN, S. AUSTIN and L. T. WEAVER, *MRC Dunn Nutrition Unit, Cambridge CB4 1XJ*

During development each gene must express itself at the right time in the right cell. In the small intestine (SI), timing and extent of genetic signals may be modulated by environmental (luminal and systemic) factors. Our aim was to measure the control of disaccharidase expression in sections of SI deprived of luminal influences.

Sections of mid SI from twenty-four fetal guinea-pigs (GP) were transplanted subcutaneously to seventy-five athymic mice, and removed at 2 d intervals from birth to 28 d. Sections of mid SI were also removed from seventy-two naturally-reared (control) GP at the same time intervals. Control (C) and xenograft (X) tissues were studied histologically, and biochemically for disaccharidase activities (μmol glucose/mg protein per h (U); Table).

Age (d) . . .	Fetal		2–4		6–8		14–16		22–24		26–28	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Lactase C	0·6	0·2	8·9	3·7	10·1	1·6	1·7	0·4	0·3	0·2	0·0	0·0
Lactase X			0·3	0·1	0·1	0·0	0·2	0·0	2·8	1·5	4·5	1·8
Maltase C	2·6	0·8	6·0	2·3	51·1	13·9	14·0	1·6	8·6	1·1	14·9	6·4
Maltase X			1·0	0·1	0·8	0·1	0·9	0·1	7·2	2·5	7·1	2·3
Sucrase C	0·9	0·2	1·6	0·7	5·6	1·0	3·7	1·0	3·9	0·5	1·6	1·1
Sucrase X			0·1	0·04	0·1	0·05	0·2	0·01	0·2	0·17	0·1	0·01

In contrast with the typical changes seen in control SI, xenograft lactase was poorly expressed during the first 16 d, after which there was a peak of activity (4·5 U at days 26–28), half that seen in the first week (max 10 1 U) in controls. After a small rise in the first few days, maltase was also expressed in the xenograft, rising to max of 7·2 U by the 4th week. There was negligible expression of sucrase during the study period, compared with a peak of 5·6 U at days 6–8 in the controls. These changes occurred in association with necrosis of the xenograft mucosa, from days 2–14, followed by regeneration, manifest by formation of new epithelium and the appearance of primitive villi and crypts.

At 20 d after transplantation the disaccharidases were expressed in new populations of epithelial cells, derived from cells which escaped death. Expression was in accordance with the developmental pattern seen in controls neonatally.

We suggest that an inherent ontogenic programme of disaccharidase expression is disrupted by transplantation. When the xenograft SI has regenerated and has achieved the cellular orientation and organization of 'normal' mucosa, there is restoration of the disaccharidase expression, with an early rise in lactase followed by a later rise in maltase, while sucrase may not be expressed until 30 d onwards.

Effects of processing and feed enzymes on nutrient digestibility in diets for weaned pigs.

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Hydrothermal processing (Aumaitre, 1976) and enzyme supplementation (Graham *et al.* 1988) have been shown to improve digestibility of diets for young pigs. Gamma irradiation improved the utilization of chick diets containing barley or rye (Patel *et al.* 1980).

The present study was designed to compare the effects of extrusion cooking (Diet 2), gamma irradiation (Diet 3) or supplementation with enzyme cocktails (Diets 4 and 5) on a commercial diet for weaned pigs (Diet 1). Diet 1 contained (g/kg): wheat 570, hypro soya 175, concentrate 255. The concentrate contained nutrients not provided by the wheat–soya portion. Titanium dioxide (1 g/kg) was included as a digestibility marker. Extrusion cooking was carried out using a wet extruder at a die temperature of 220°. Gamma irradiation was done using a gamma beam 650 cobalt irradiator; wheat and soya received a total dose of 60 kGy. Diet 4 contained (g/kg): amylase 0·2, xylanase 0·2, cellulase 0·2 and pectinase 0·1. Diet 5 contained an enzyme mixture of B-glucanase, pectinase, cellulase and hemicellulase (1 g/kg).

Three replicates each with two 7 d balance periods were done. The first replicate used eight littermate pigs allocated to Diets 1–4. The following two replicates each used five littermates allocated to Diets 1–5. Ileal collections were made for 8 h/d during each balance period.

The second enzyme cocktail (Diet 5) had no effect on apparent digestibilities of dry matter (DM), crude protein (CP), gross energy (GE) or organic matter (OM) at ileal or faecal levels (Table). Apparent improvements in faecal and ileal apparent digestibilities of DM, CP, GE and OM occurred with Diets 2, 3 and 4. These were statistically significant for DM (Diets 3, 4), GE (Diets 2, 3, 4) and OM (Diet 4) in faeces and for DM, GE and OM (Diets 2, 4) and for CP (Diet 2) at the terminal ileum.

Apparent digestibility

Diet . . .		1	2	3	4	5	SEM	*P*
DM	Faecal	0·838	0·850	0·851*	0·857*	0·842	0·006	0·028
	Ileal	0·669	0·704*	0·686	0·703*	0·691	0·014	0·079
CP	Faecal	0·812	0·826	0·831	0·836	0·811	0·012	0·220
	Ileal	0·740	0·786	0·763	0·766	0·731	0·015	0·022
GE	Faecal	0·835	0·853*	0·853*	0·853*	0·839	0·007	0·050
	Ileal	0·708	0·746*	0·729	0·735*	0·729	0·012	0·057
OM	Faecal	0·858	0·872	0·871	0·875*	0·863	0·006	0·023
	Ileal	0·695	0·735*	0·716	0·731*	0·720	0·012	0·023

Significantly different from Diet 1; *$P<0·05$.

It is concluded that appropriate feed enzymes can improve digestibility of wheat–soya-based diets to a similar extent to that observed with more expensive treatments such as extrusion cooking or gamma irradiation, and that the effects occur in the small intestine.

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Effect of Virginiamycin on the precaecal apparent digestibility of the major nutrients comparing a diet with high and low precaecal digestibility in pigs. By J. A. DECUYPERE, S. SPRIET and H. K. HENDERICKX, *University of Ghent, Faculty of Agricultural Sciences, Research Centre for Nutrition, Animal Production and Meat Technology, Proefhoevestraat 10–12, 9090 Melle, Belgium*

In previous experiments (Decuypere *et al.* 1975, 1991) with growing pigs (20–40 kg) Virginiamycin (V) greatly improved the apparent precaecal digestibility (APD) of the major nutrients. The effect was most pronounced for the carbohydrate and was thought to be due to the low APD of lactose, given the age of the pigs, from the milk powder used in the diets. In another experiment (Cosijn *et al.* 1984), using a diet based on maize, barley and soya-bean meal, V only slightly improved the digestibility of protein. The APD of such diets is rather high and it could be that there is little room for further improvements using growth-promoting substances.

To test this hypothesis the effect of V on the APD was studied in four ileal-cannulated pigs (26–60 kg), comparing a diet with a high (A) and low (B) APD. The main components of the diets were g/kg: A, barley 490, maize 200, extracted soya-bean meal 250; and B, barley 250, maize 100, manioc 240, extracted soya-bean meal 100, meat-and-bone meal 100, wheat shorts 100, beet pulp 50. Treatments were control (C) *v.* 50 ppm V during 2 weeks using a 4×4 Latin-square experimental design with two replications. During the 2nd week of treatment ileal contents were sampled and the APD was determined using 4N HCl-insoluble ash as marker.

The results are given in the Table and confirm the hypothesis that there was no effect of V with diet A but V significantly ($P<0{\cdot}05$) improved the APD of all nutrients except fat. The APD of crude fat in diet B was low and variable and there was no effect of V.

Apparent precaecal digestibility

	Diet A				Diet B			
	C (*n* 12)		V (*n* 12)		C (*n* 11)		V (*n* 12)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Dry matter	66·2	2·2	66·2	1·7	51·5	3·1	55·6	2·0
Organic matter	69·5	2·2	69·4	1·6	58·4	2·9	62·4	1·8
Crude protein	81·2	2·1	82·0	1·6	67·2	2·4	70·9	1·6
Crude fat	71·0	3·2	70·1	3·7	53·0	6·3	51·4	6·7
NFE (carbohydrate)	71·4	2·3	71·2	1·4	63·8	2·9	67·2	2·0

NFE, nitrogen-free extract.

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Growth factors in colostrum: receptor localization and stimulation of phosphorylation in microvillar membranes of neonatal pigs. By D. KELLY, M. MCFADYEN, C. MORGAN and T. P. KING, *Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB*

Evidence is accumulating that mammalian colostrum and milk contain biologically active factors that can influence and modify the intrinsic developmental programming of the small intestine. Potential factors include epidermal growth factor (EGF), insulin and insulin-like growth factors (IGF-1), which are known to stimulate cellular mitogenesis and differentiation in a number of cell systems following interaction with cell-surface receptors. Specific binding of ^{125}I-radiolabelled EGF and IGF-1 to the jejunal intestinal mucosa of newborn, sucking and weaned pigs was demonstrated using *in vitro* autoradiography on 20 μm cryostat sections. Binding sites for both growth factors were found on microvillar and basolateral surfaces of mature enterocytes and crypt cells. Electron microscope ligand-gold cytochemistry has been used to resolve further the localization of receptors for these growth factors.

One of the earliest cellular events subsequent to the binding of EGF and IGF-1 to membrane receptors is the activation of a tyrosine-specific protein kinase and enhanced phosphorylation of specific membrane proteins. To clarify the potential role of EGF and IGF-1 in the developing intestine an *in vitro* protein phosphorylation assay has been used to monitor the incorporation of radiolabelled phosphorus from γ^{32}P-ATP into purified microvillar membrane proteins. TCA-precipitable radioactivity was found to increase significantly in intestinal membranes stimulated with IGF-1 at nanomolar concentrations; this effect was more subtle in the case of EGF. Phosphorylated membrane proteins were resolved using SDS–PAGE followed by autoradiography. This revealed at least five proteins exhibiting enhanced phosphorylation (>200 kD, 97 kD, 57 kD, 40 kD, 26 kD) in response to IGF-1 but not EGF. Developmental differences in the response to IGF-1 were also apparent. Some of the stimulated proteins share the same electrophoretic mobility as those observed when defatted sow colostrum was applied to the purified membranes, suggesting that a component of the stimulatory activity found in colostrum may be attributable to IGF-1.

These results demonstrate that IGF-1 can elicit second messenger responses when in contact with intestinal microvillar membranes and consequently may be an important mediator of intestinal growth and differentiation. Although EGF receptors were shown to be present in the intestinal mucosa and a subtle effect was observed in TCA precipitates, phosphorylation of microvillar membrane proteins was not obvious on SDS–PAGE. This suggests that the microvillar membrane is not the major site of action of EGF. Studies are in progress to elucidate the nature of other important bioactive factors in sow colostrum.