PO3 Assessment of the small intestine function in the preruminant calf using lactulose, sucrose and mannitol as permeability probes.

F. André 1, N. Toulec 2

1 Laboratoire du Jeune Ruminant, INRA, 65, rue de Saint-Brieuc, F-35042 Rennes Cedex.
2 Laboratoire d’Immunopathologie Digestive, INSERM, Centre Hospitalier Lyon-Sud, F-69310 Pierre-Bénite.

The urinary excretion ratio of lactulose and mannitol by patients suffering from food allergy increases by 2-3 folds after oral allergen challenge (André et coll., 1987). Young calves are known to develop immune-mediated digestive disturbances when fed milk replacers containing insufficiently refined soybean protein sources (Sissons, 1982). A study was therefore designed to assess lactulose and mannitol as permeability markers in calves. Since they are devoid of sucrose, sucrose which is cheaper was compared to lactulose.

Eighteen Holstein male calves bought at two weeks of age were allotted to three groups 5 weeks later. They were fed milk replacers (60 g DM/kg BW) containing either skim milk protein (SMP) or a mixture of SMP (35%) and soybean protein (SBP 65%) for 15 weeks. Antigenic (ASP) and non-antigenic (NASP) soy products were compared. Sugar probes (0.1-0.2 g/kg BW) were fed with the morning meal after 2 and 8 weeks of treatment (period 1 and 2). Urine was collected totally for 6 hours and sugars assayed by gas-liquid chromatography (André et coll., 1987). The urinary clearance ratio of sugars tested was not found to be affected by protein source. SBP antigenicity or calf age. This is in general agreement with the surprisingly low effects of these factors observed on digestibility parameters and pancreatic function in that trial (unpublished).

Sucrose seemed to behave as lactulose since urinary clearances (2%) were similar. However, they were not significantly correlated. The oligoside excretion pattern appeared to differ consistently from that by humans (0.25-1 and 11-15%) the oral load excreted for lactulose and mannitol respectively; André et coll. (1987). Such discrepancies might arise from protocol, diet, species and age differences.

In conclusion, the calf small intestine permeability was not affected by protein source, soy protein antigenicity and calf age in the present trial. Sucrose seemed to be suitable for replacing lactulose in measuring intestinal permeability in calves using a dual-sugar technique.

References


PO7 Molecular isolation of genes expressed in the differentiated epithelium of the pig small intestine.

G. Pefozzi 1, D. Bariti 2, C. Murgia 1, R. Begbie 2, T.P. King 3, D. Kelly 4

1 Istituto Nazionale della Nutrizione, Via Ardeatina 546, I-00178 Rome.
2 Rowett Research Institute, UX-AB 9SB Aberdeen.

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 fetal 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 fetal 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 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 retinoil 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. This work was supported by grants from CNR (target project “Biotechnology and Bioinstrumentation”) the National Research Council of Italy (special project RAISA, sub-project 4) and the Scottish Office Agriculture and Fisheries Department.

PO9 Proportion of digestive tract: a comparison of two Spanish sheep genotypes (Churra vs Merina).

P. Frutos, P.R. Revesado, A.R. Mantecón, J.S. González, M.D. Carro.

Estación Agrícola Experimental de León, CSIC Apdo 788, E-24080 León.

The two major sheep genotypes of Northern Spain are Churra and Merina, which differ in traditional management systems which could be important in the digestive diet utilization. The experiment was carried out with the aim to know the differences between the two sheep genotypes (Churra vs Merina) in the proportions of the digestive tract, when animals have different body condition score and two levels of intake of medium quality hay are offered.
Twenty-four mature ewes (Churra vs Merina) were used according to a 2 x 2 factorial design represented by 2 levels of intake (LI, high: ad libitum and low: 0.6 ad libitum) and 2 body composition scores (BCS, good: > 3 and poor: < 2). All animals were individually penned throughout, food offered and refused was weighed daily and live-weight was recorded twice weekly. After experimental period (five weeks), all ewes were slaughtered and the live-weight and the empty components of the digestive tract after the removal of fat were weighed. The means of empty body weight (EBW) were 39.4 kg and 27.9 kg for good and poor BCS in Churra genotype, and 39.8 kg and 28.6 kg for good and poor BCS in Merina genotype. There were differences (P < 0.05) between breeds as proportion of EBW, with a lower value in Merino genotype for the total digestive tract (TDT, 0.0625 vs 0.0755), and reticulum-umen (Rr, 0.0248 vs 0.0305). There were differences (P < 0.01) between BCS groups, as proportion of EBW, with a lower value in the good BCS for TDT (0.0601 vs 0.0834), Rr (0.0248 vs 0.0256), Omum (Om, 0.0031 vs 0.0048), Abomasum (Ab, 0.0057 vs 0.0077), Small intestine (St, 0.0119 vs 0.0170), Large intestine (Li, 0.0127 vs 0.0187) and Caecum (Ca, 0.0016 vs 0.0023). There were differences (P < 0.05) between LI as proportion of EBW, with a lower value in the low LI group for TDT (0.0667 vs 0.0743), Om (0.0034 vs 0.0044), Ab (0.0060 vs 0.0072) and Ca (0.0017 vs 0.0021).

In conclusion, genotype, body condition score and level of intake have an important effect on the components of the digestive tract as a proportion of EBW. This work was supported for the CICYT (project GAN 90-0906).

References

### P10 Developmental and nutritional regulation of intestinal fucosylation processes.


Department of General and Medical Biochemistry, INSERM-CNRS U 189, Lyon-Sud Medical School, BP 12, F-69921 Oullins Cedex.

The rat intestine undergoes a concert of profound morphological and enzymatic changes between the second and the third after birth, corresponding to the weaning period. These developmental modifications result in a functionally mature intestine containing the digestive enzymes necessary to cope with the carbohydrate-rich diet of adulthood such as sucrase, maltase or isomaltase, as well as other enzymes: aminopeptidase or alkaline phosphatase, for instance [1]. Most of these enzymes are glycoproteins located in the brush-border membranes. The mechanism of regulation of the profound developmental variations of intestinal glycosylation patterns are poorly known. The purpose of the present study was to gain insight into the regulation mechanisms of the intestinal fucosylation processes, focusing on the participation of the endogenous protein inhibitor of fucosyltransferase activity [2] and on the enzymes of GDP-fucose metabolism. During the weaning period (about day 19 of rat life) intestinal maturation is accompanied by a drastic increase in the fucose content of glycoconjugates, concomitant with an increase in fucosyltransferase activities. The regulation of this fucosylation process appears to be a more complex phenomenon which involves several systems controlling fucosyltransferase substrate: the GDP-fucose pyrophosphatase activity markedly decreases at weaning while the transformation of GDP-mannose into GDP-fucose (responsible for the synthesis of 90% of the GDP-fucose cellular pool [3] rises early at day 18, preceding the increase in fucosyltransferase activity. The accumulation of GDP-4-keto-6-deoxymannose at days 14 and 18 indicates that the epimerase-reductase reaction is a limiting factor for GDP-fucose availability before weaning. An endogenous protein inhibitor of fucosyltransferase activities displays an opposite development pattern as compared to fucosyltransferase activity [4].

The comparison of the activities of these systems in 22-day-old rats either weaned or submitted to prolonged nursing suggests that the profound modifications occurring as weaning depend on the age of the animal, with an additional regulation by nutritional factors. The inverse relationship between fucose (or fucosyltransferase activity) and the endogenous protein inhibitor, both during normal weaning or after dietary manipulation, supports the hypothesis of a physiological role of this molecule.

<table>
<thead>
<tr>
<th></th>
<th>18 day-old</th>
<th>23 day-old</th>
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<tr>
<td>Glycoprotein fucose content</td>
<td>0.740 ± 0.156</td>
<td>1.660 ± 0.398</td>
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<tr>
<td>Microsomal fucosyltransferase</td>
<td>0.198 ± 0.059</td>
<td>1.341 ± 0.398</td>
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<tr>
<td>Soluble fucosyltransferase</td>
<td>0.049 ± 0.015</td>
<td>0.300 ± 0.034</td>
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<tr>
<td>Fucosyltransferase inhibitor</td>
<td>860.4 ± 282.7</td>
<td>143.9 ± 71.60</td>
</tr>
</tbody>
</table>

Activity changes of fucosylation pathway components at weaning. Results are given as means ± SD of duplicate determinations (obtained in four independent experiments). in mmol/g for fucose, pmol mg. min for fucosyltransferase activities and Units mg for the inhibitor. Animals were weaned about day 19.

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**Fig. 1.** Developmental pattern of the enzymes involved in GDP-fucose metabolism. a: GDP-fucose pyrophosphatase activity. b: Transformation of GDP-mannose into GDP-fucose.

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**Fig. 2.** Weaning vs Gut-enzymes. a: GDP-fucose production (pmol/min/mg protein). b: Weaning vs Gut-enzymes. a: GDP-fucose production (pmol/min/mg protein).