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Lectin histochemical characterisation of the porcine small intestine around weaning

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Abstract

The present study was undertaken to characterise the carbohydrate profile of the porcine small intestine using lectin histochemistry during the period from 3 days prior to weaning to 9 days post-weaning. A total of 56 piglets weaned at 4 weeks of age were included in the experiment. The most prominent changes in the glycosylation pattern were observed in the goblet cells. The highest lectin reactivity of the goblet cells in the crypts was observed 7 days post-weaning which suggests that the protective effect of the mucus layer against pathogenic bacteria is increasing during the postweaning period. The staining pattern of the apical membrane remained unchanged during the experimental period. This indicates that the glycosylation process in the goblet cells is rapidly inducible whereas changes in the glycosylation pattern of the apical membrane requires more time. The glycosylation pattern of both goblet cells and apical membrane differed between the positions of the small intestine. As glycoconjugates can act as attachment sites for microorganisms, these differences in the distribution of sugar residues may be one explanation for the site-specificity of certain pathogens.

Keywords: Lectin histochemistry; Pig; Small intestine; Weaning

1. Introduction

The surface epithelium of the gut is extensively glycosylated (Taatjes and Roth, 1991). Membrane proteins and membrane lipids are glycosylated before being embedded in the brush border membrane and all secreted mucins are carbohydrate-rich glycoproteins. The glycosylation pattern is dependent on a number of factors such as animal species, age, site in the intestines, position along crypt-villus axis, state of differentiation and maturation, diet and bacterial status.

Weaning of piglets causes profound changes in the morphology of the small intestine in pigs (Pluske, 2001) and during the weaning period changes in a number of the above mentioned factors take place in the gastrointestinal tract of pigs and it induces changes in the glycosylation pattern. Shedding of the cells lining the villi and increased proliferative activity in the crypts are well-described events (Hedemann et al., 2003) and this means that the proportion of fully differentiated, mature epithelial cells decreases and the number of immature cells increases. At weaning, the diet of the pigs is changed from easy digestible mother's-milk to a carbohydrate-rich diet mainly of vegetable origin. Studies in rats (Sharma and Schumacher, 1995) and pigs (Brunsgaard, 1998) have shown that dietary fibre influences the glycosylation pattern. Weaning has a significant effect on the microbial population of the piglet (Franklin et al., 2002; Jensen, 1998). It has been shown in rats that the gut flora influences the mucin composition of the colon (Szentkuti and Enss, 1998) and in pigs the use of probiotics influenced the mucins of the intestine (Baum et al., 2002).

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Terminal structures of gut surface glycosyl side-chains can be determined by the use of lectins, carbohydrate-binding proteins of non-immune origin. The technique has been used to obtain knowledge on the effect of age, weaning (Gelberg et al., 1992; Jaeger et al., 1989), diet (More et al., 1987) and infection (Choi et al., 2003) on the glycosylation pattern of the intestine in pigs.

The objective of the present experiment was to provide a temporal characterisation of the carbohydrate profile of the porcine intestine during the period immediately after weaning which may provide valuable information on the functional status of the gut.

2. Materials and methods

2.1. Animal care and housing

The protocol used in this experiment complied with the Danish Ministry of Justice concerning animal experimentation and care of experimental animals.

Fifty-six piglets from the herd at the Danish Institute of Agricultural Sciences, Research Centre Foulum were used in the experiment. Pigs were selected for the experiment 1 week prior to weaning. The pigs, originating from 21 litters, were divided into seven groups consisting of two or three pigs per litter from three litters. Before weaning, sows and their litters were housed in farrowing pens in an environmentally regulated building. The pigs had no access to creep feed while they were nursing. The pigs were between 25 and 33 days old at weaning. On the day of weaning the pigs were transferred to a nursery unit at 0800. Pigs from three litters were mixed and placed in one pen. The age difference within the groups never exceeded 4 days. A standard weaning diet (composition as-fed: wheat 33.0%, barley 32.9%, soy meal 10%, fish meal 10%, skimmed milk powder 10%, animal fat 2%) was fed and the pigs were given ad libitum access to feed and water.

2.2. Sample collection

One piglet per group was killed 3 days prior to weaning (day −3), on the day of weaning (day 0) and on day 1, 2, 3, 5, 7 and 9 after weaning.

The pigs were removed from their pen at 1100 and killed immediately by i.p. injection of an overdose of sodium pentobarbital (80 mg/kg bodyweight). The abdominal cavity was opened and the entire gastrointestinal tract was removed. Tissue samples for microscopy were sampled at distances of 10%, 50% and 90% of the length of the small intestine from the pylorus to the ileo-caecal valve. The samples were immediately transferred to 4% neutral buffered formaldehyde (Bie & Berntsen, Rødovre, Denmark).

2.3. Lectin histochemistry

After 48 h in 4% buffered neutral formaldehyde, the tissue samples were carefully cleaned of remaining digesta using saline (154 mM NaCl) and then transferred to a fresh solution of buffered neutral formaldehyde. Subsequently the samples were dehydrated and infiltrated with paraffin wax. Four slides were prepared from each sample, and each slide contained a minimum of four sections cut at 4 μm, at least 50 μm apart.

The lectins used in the present study, the abbreviation used to identity them and their sugar specificity, are given in Table 1. Lectin histochemistry was performed according to Pusztai et al. (1995).

The slides processed for lectin histochemistry were evaluated for staining frequency and intensity of the goblet cells/mucous cells and the apical membrane of the epithelial cells. The evaluation was done separately on the epithelial cells in the villi and the crypts. The values used for scoring the frequency of cells with positive lectin reactivity were (1) no cells, (2) between 0% and 25% of cells, (3) between 25% and 75% of cells, and (4) more than 75% of cells. The values used for scoring intensity of lectin reactivity were (1) none, (2) weak, (3) moderate, and (4) heavy staining. The scorings were done using a 25× objective on the light microscope and were all done by the same observer.

2.4. Statistical analyses

A lectin score was calculated as the product of staining intensity and proportion of stained cells of each segment in each pig. Scores were analyzed as repeated measurements with age as the between animal effect and segment as the within animal effect using a mixed model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + \beta\gamma_{jk} + \varepsilon_{ijk} \quad (1)$$

in which Y_{ijk} is the dependent variable, μ is the overall mean, α_i is the systematic effect of group ($i = 1,2,3,4,5,6,7$), β_j is the systematic effect of segment ($j = 10, 50, 90$), γ_k is the systematic effect of age ($k = -3,0,1,2,3,5,7,9$), $\beta\gamma_{jk}$ is the systematic interaction between segment and age and $\varepsilon_{ijk} \sim N(0,\sigma^2)$ represents the unexplained error.

The assumption of normality of the scores can be justified as follows: The number of stained cells within a fixed area of tissue can be regarded as a sum of variables x, where $x = 1$ if cell is stained and 0 otherwise. Hence by the central limit theorem, the sum is approximately normal distributed, and hence so are the scores. Inspection of the residuals from the model (1) indicates that the variance can be assumed homogeneous and this justifies the use of model (1).

Table 1
Lectins used in this study and their carbohydrate specificities[a]

Lectin	Abbreviation	Carbohydrate specificity
Galanthus nivalis	GNA	α1-3 D-mannose
Aleuria aurentia	AAA	α1-6 fucose
Maackia amurensis	MAA	NeuAc α2-3Gal
Sambucus nigra	SNA	NeuAc α2-6Gal/GalNAc

[a] The lectins were supplied by Boehringer Mannheim GmbH, Mannheim, Germany.