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The influence of diet on the mucin carbohydrates in the chick intestinal tract

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Abstract. The presence of nutrients in the intestinal lumen is a major factor influencing bacterial colonization in poultry. This study was conducted to investigate the effects of poultry feed on viscosity of intestinal contents and on mucin carbohydrates by comparing jejunal supernatants and the histochemical composition of goblet cells in chicks reared to 5 weeks of age on either a conventional maize-based diet or a wheat-based diet or a wheat diet supplemented with 0.1% xylanase. Regional differences in the distribution of the neutral, carboxylated and sulphated mucins were demonstrated using conventional histochemical techniques, while a panel of lectins was used to study alterations in glycoconjugate synthesis of mucins in chicks fed different diets.

1) Feeding a diet supplemented with xylanase lowered the viscosity but increased the amount of neutral, carboxylated and sulphated mucins in the jejunum.
2) In chicks fed a maize-based diet, neutral mucins increased in the surface and upper crypt goblet cells of the small and large intestines but decreased in the caecum.
3) Feeding a diet supplemented with xylanase modified crypt-surface glycosylation of *N*-acetylglucosamine residues and resulted in loss of sialic acid residues in the small and large intestines.
These results indicate that the constituents of poultry feed, in particular the consumption of a diet supplemented with xylanase, lead to changes in intestinal viscosity and mucin composition which are associated with alterations in the goblet cell glycoconjugates of the chick intestinal tract.

Key words. Chick; diet; intestine; mucins; xylanase.

The surface epithelium of the gastrointestinal tract is lubricated and protected by a mucus layer which contains mucins or mucin-type glycoproteins synthesized and secreted by goblet cells [1]. Apart from their protective function, mucins act as a substrate for the resident flora and by aggregation facilitate the removal of pathogenic bacteria [2]. The amount and composition of mucins are in a balanced state between the degradation of luminal mucins by bacteria and their renewal by goblet cell secretions from the intestinal crypts [3]. Recent studies in our laboratory have shown that the rat intestinal mucosa adapts to different diets and microbial populations by modifying mucin composition [1], crypt-villus architecture [4] and goblet cell glycoconjugates [5]. There is, however, no information on the factors that maintain the integrity of gut mucosa and regulate the secretory pattern of intestinal mucins in chicks.

The mechanisms by which enteropathogenic bacteria such as *Salmonella typhimurium*, *Escherichia coli* and *Campylobacter jejuni* breach the mucosal barrier and colonize the alimentary tract of infected animals are only partially understood [6, 7]. However, it is clear that bacterial enteropathogens must traverse the mucus layer in order to approach and adhere to epithelial cells. Some bacterial species attach to the gut epithelium by

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Table 1. Composition of diets.

Ingredients	Group A wheat-based diet (%)	Group B wheat supplemented with 0.1% xylanase diet (%)	Group C maize-based diet (%)
Wheat–soft	54.74	54.64	-
Maize	-	-	54.25
Soybean meal 48	34.89	34.89	37.67
Soy oil	4.26	4.26	1.75
Tallow starter	2.00	2.00	2.00
Salt	0.38	0.38	0.41
D,L-methionine	0.17	0.17	0.15
Limestone	1.22	1.22	1.22
Dicalcium phosphate	1.35	1.35	1.55
Vitamins/minerals	1.00	1.00	1.00
Enzyme–xylanase	-	0.1	-

fimbriae which recognize glycoconjugate receptors in mucus and/or brush border membranes [8–10]. Since dietary factors that either affect the production of mucin or enhance its degradation would make the intestinal mucosa susceptible to pathogenic organisms [4], it is possible that appropriate modifications of poultry feed may modulate colonization of bacteria and thus protect chicks against infection.

The aim of this study is to define the effects of diet on synthetic and secretory levels of neutral, acid and sulphated glycoproteins in mucin-containing goblet cells and on goblet cell glycoconjugates along the chick intestinal tract. Lectins, because of their binding affinity for specific carbohydrate residues of cell glycoconjugates [11], were used to localize differences in the distribution of secretory glycoconjugates and to detect changes in glycosylation of mucin along the crypt-to-surface epithelial axis.

Investigating in situ changes in mucin synthesis of young rearing chicks in response to dietary manipulation requires an experimental model which simulates the commercial situation. Our model, which meets such requirements, involves rearing chicks to 5 weeks of age on unsupplemented and enzyme-supplemented diets obtained from commercial sources. This model is currently being used in our laboratory to investigate whether poultry flocks are less susceptible to *C. jejuni* infection according to the type of diet.

Materials and Methods

Birds. Broiler chicks (Ross 1) were obtained from a commercial hatchery (M. Millard, Bath, UK) and kept initially in groups in open-topped cardboard boxes (100 cm × 50 cm × 57 cm). Food was presented ad libitum in metal troughs, and water was continuously available from plastic fountain drinkers. The ambient temperature was maintained at an appropriate level for the chicks' age.

Experimental diets. Three experimental diets supplied by Finnfeeds International Ltd., which differed in constituents and texture, were used (table 1). Two diets were wheat-based, one of which was supplemented with 0.1% xylanase. The third was a maize-based diet.

Experimental design. On the day of purchase, 60 chicks were divided in three groups (groups A, B and C). Each group was placed initially in two sets of 10 in separate cardboard boxes. After 10 days, chicks were transported to a pen whose floor was covered with 2 cm of wood shavings. All three groups were given their respective diets from day 1 (group A, wheat-based diet; group B, wheat supplemented with 0.1% xylanase and group C, maize-based diet), which were continuously available thereafter. Animals were killed humanely after 33 days.

Viscosity analysis of jejunal contents. The contents of the jejunal portion of the small intestine were collected and centrifuged at 12,000*g* for 10 min at 25 °C. Viscosity in centipoise (cP) units was determined in 0.5 ml of super natant within 30 min using a Brookfield DV-II viscometer equipped with a CP40 cone and plate head.

Preparation of tissues and histochemical procedures. Samples from the mid-region of the small intestine (jejunum), caecum and proximal large intestine (2 cm from the caecum) were taken from four birds from each group and immediately fixed in 10% phosphate-buffered formalin and embedded in paraffin wax.

Serial 5-μm sections were subjected to the following procedures for the identification of mucosubstances:

1) The periodic-acid Schiff (PAS) reaction for studying unsubstituted a-glycol-rich neutral mucins.

2) 1% Alcian Blue, pH 2.5 (AB 2.5) for localization of the carboxylated and/or sulphated type of acidic mucins [12].

3) 1% Alcian Blue, pH 1.0 (AB 1.0) for the selective characterization of sulphomucins [13].

The Alcian Blue dyes and the techniques used in this study were pretested in α dot-blot assay for their suitability to label carboxylated and sulphated mucins, respectively [14].

Table 2. Characteristics of lectins.

Lectin origin	Abbreviation	*Carbohydrate-binding specificity	Inhibiting sugar
Triticum vulgaris	WGA	GlcNAc(β1,4GlcNAc)1–2 > β1,4GlcNAc > NeuAc	GlcNAc
Dolichos biflorus	DBA	GalNAcα1,3GalNAc > GalNAcα1,3Gal	D-GalNAc
Maackia amurensis	MAA	NeuAcα2,3Galβ1,4GlcNAc	†
Ulex europaeus	UEA-I	L-fucoseα1,2Galβ1,4GlcNAcβ1,6	α-L-fucose

* Gal, galactose; Glc, glucose; GlcNAc, *N*-acetylglucosamine; GalNAc, *N*-acetylgalactosamine; NeuAc, neuraminic acid (sialic acid).
† Neuraminidase.
Adapted from Danguy et al. [31] and commercial literature of Sigma Chemical Co. and EY Laboratories.

Table 3. Effect of diet on viscosity* of jejunal supernatant in chicks.

Group A Wheat-based diet	Group B Wheat supplemented with 0.1% xylanase	Group C Maize-based diet
6.3	1.9	2.2
3.4	3.3	2.0
3.5	2.0	2.6
5.1	2.5	2.8
(4.6 $\pm$ 1.4)†	(2.4 $\pm$ 0.6)†	(2.4 $\pm$ 0.4)†

* The units are shown in cP units from four animals in each group.
† Values are mean $\pm$ SD per dietary group.
For details of procedures see, Materials and methods.

Table 4. Effects of diet on histochemical characteristics of intestinal mucins in chicks.

Intestine and cell type	Wheat-based diet			Wheat supplemented with 0.1% xylanase diet			Maize-based diet		
	PAS	AB 2.5	AB 1.0	PAS	AB 2.5	AB 1.0	PAS	AB 2.5	AB 1.0
Small intestine									
Surface goblet cells	3	3	2	4	2	3	4	2	3
Upper crypt goblet cells	2	1	1	2	2	2	2	1	1
Lower crypt goblet cells	2	2	1	2	3	1	1	1	1
Caecum									
Surface goblet cells	2	1	2	1	1	1	1	1	2
Upper crypt goblet cells	2	1	1	2	2	2	1	1	1
Lower crypt goblet cells	1	0	1	1	1	1	0	0	0
Large intestine									
Surface goblet cells	3	4	4	4	3	4	4	3	3
Upper crypt goblet cells	2	2	2	3	2	3	3	2	3
Lower crypt goblet cells	1	1	1	2	1	2	3	1	2

Numbers indicate staining frequency on a semiquantitative scale ranging from 0 to 4 where 0 corresponds to no reactive cells, 1 to occasional, 2 to a few, 3 to a moderate number and 4 to numerous reactive cells. PAS = periodic acid Schiff; AB = Alcian blue.

For identification of specific carbohydrate residues, sections were hydrated through a series of graded alcohols and brought to 0.5 mol/l TBS (tris buffered saline, pH 7.4) supplemented with 0.1% calcium chloride. Sections were then trypsinized with 0.1% trypsin (Sigma) in 0.05 M TBS for 30 min at 25 °C. After a wash in TBS, the endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide in methanol. Sections were washed again in TBS and incubated for 30 min at room temperature in a Shandon Sequenza immunostaining centre with the biotinylated lectins (Sigma, EY Laboratories, Vector Laboratories) at a concentration of 10 µg ml^{-1} in TBS. The lectins used, their sources, abbreviations, sugar specificities and inhibitors are listed in table 2. After a further wash in TBS, sections were treated with ABC complex (Peroxidase standard PK-4000, Vector Laboratories, Peterborough, UK) for 30 min, washed again in TBS, and then incubated in diaminobenzidine tetrahydrochloride (DAB) in Tris HCl buffer, pH 7.3, with 0.3% hydrogen peroxide for 5 to 10 min. The control experiments were carried out by omission of the lectin and by incubation of the sections with the lectins and their appropriate inhibitory sugars (0.3 mol end concentration). Sections were lightly counterstained

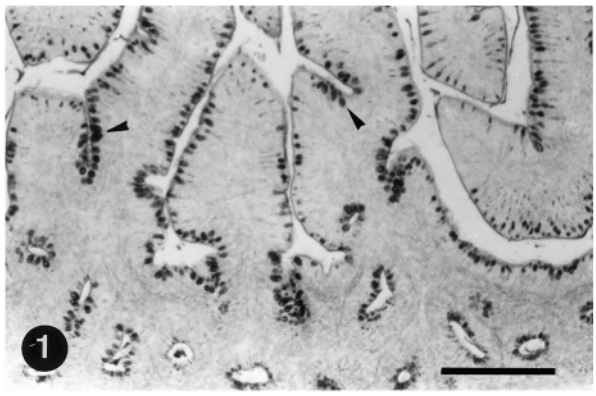

Figure 1. Small intestine of a chick fed on a wheat-based diet supplemented with 0.1% xylanase stained with Alcian Blue at pH 2.5. Arrowheads indicate surface goblet cells containing acidic mucins (bar = 25 μ).

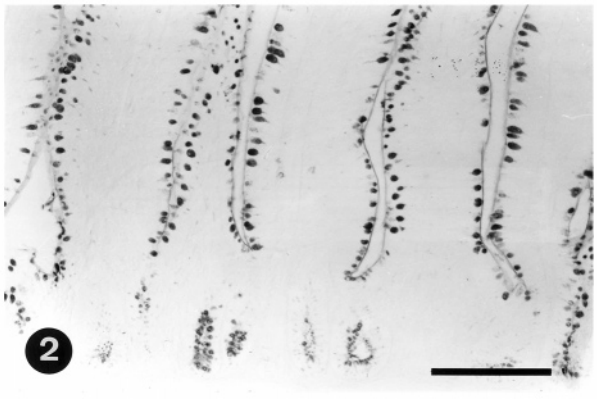

Figure 2. Goblet cells of small intestine from a chick fed a maize-based diet stained with Alcian Blue at pH 1.0 showing sulphated mucins in the surface epithelium and upper crypts (bar = 25 μ).

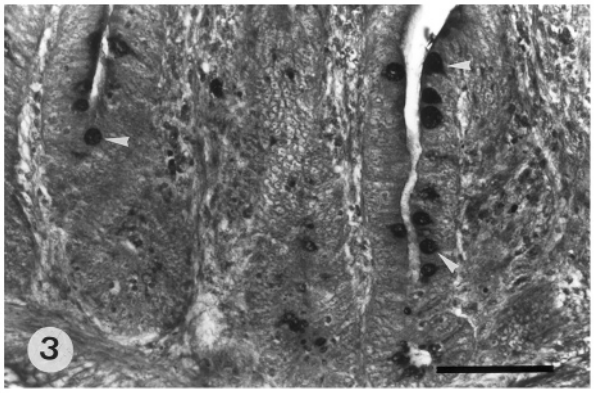

Figure 3. Caecum from a chick fed a wheat-based diet showing PAS-positive goblet cells in the surface epithelium and upper crypt (arrowheads) (bar = 25 μ).

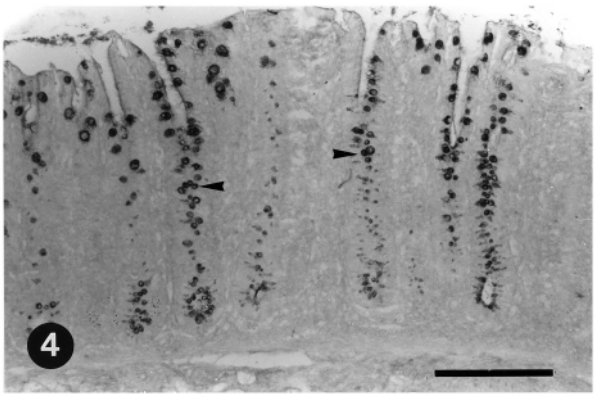

Figure 4. Mucin-containing cells of the caecum from a chick fed a wheat diet supplemented with 0.1% xylanase, stained by Alcian Blue at pH 2.5. Arrowheads indicate goblet cells in the upper crypt containing acidic mucins (bar = 25 μ).

with Harris hematoxylin, mounted in DPX and examined by light microscopy. Photographs were taken on Kodak T-MAX 400 black-and-white film.

Results were recorded according to the staining intensities and intensity of lectin reactivity in individual goblet cells and graded semiquantitatively from nonreactive cells (0) to numerous reactive (4) cells. Photographs were taken using a Kodak T-MAX 400 black-and-white film.

Results

Viscosity studies. The viscosity measurements of jejunal supernatant from each of the 12 samples examined and the mean viscosity of each of the three groups are shown in table 3. A lower viscosity of the jejunal supernatant was observed in chicks fed the enzyme-supplemented diet (mean 2.4 ± 0.6 cP) and the maize-based diet (mean 2.4 ± 0.4 cP) compared with those fed the unsupplemented wheat diet (mean 4.6 ± 1.4). One-way analysis of variance indicates that the overall differences between the means are significant ($P = 0.012$).

Histochemical studies. The influence of diet was assessed by comparing the histochemical staining pattern of intestinal mucins in chicks fed either a wheat-based diet or a wheat diet supplemented with 0.1% xylanase or a maize-based diet. The staining frequency of carbo-

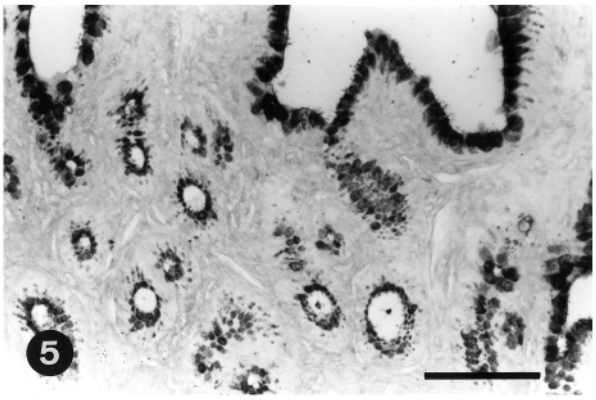

Figure 5. Large intestine from a chick fed a wheat-based diet stained by Alcian Blue at pH 2.5. Note the distribution and increased staining intensity of the surface goblet cells (bar = 25 μ).

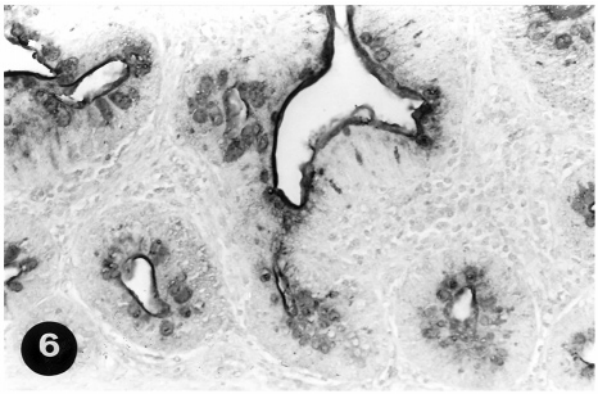

Figure 6. WGA binding in the small intestine from a chick fed a wheat-based diet. The goblet cell mucins in the upper crypts are labelled (bar = 25 μ).

hydrate-containing surface goblet cells, and upper and lower crypt goblet cells of the small intestine, caecum and the large intestine are represented semiquantitatively in table 4.

In the small intestine, the PAS reactivity of goblet cells was similar in chicks fed different diets. However, in animals fed a supplemented diet, the mucin granules in upper crypt goblet cells contained more acidic (fig. 1) and sulphated mucins. The PAS procedure and AB staining at pH 1.0 showed a lower neutral and sulphated mucin content in the lower crypt goblet cells of animals fed a maize-based diet (fig. 2) compared with those fed wheat-based diet or a wheat diet supplemented with 1% xylanase.

In the caecum, the neutral mucins were more predominant in the surface goblet cells of chicks fed the unsupplemented wheat-based diet (fig. 3), whereas carboxylated (fig. 4) and sulphated acidic mucins were abundant in the upper crypt goblet cells of animals fed the wheat diet supplemented with 0.1% xylanase. In the caecum of chicks fed a maize-based diet, overall staining intensity was lower in the surface and upper crypt goblet cells. In contrast to animals fed a wheat diet, the lower crypt goblet cells in chicks fed a maize-based diet contained no stored mucins.

The overall PAS staining intensity of goblet cells in the large intestine of chicks fed the unsupplemented wheat-based diet was less than in animals given the wheat-

Table 5. Lectin reactivity of goblet cell mucins in chicks given a wheat-based diet, wheat supplemented with 1% xylanase diet or a maize-based diet.

Intestine and cell type	Wheat-based diet		Wheat supplemented with 0.1% xylanase diet		Maize-based diet	
	WGA	MAA	WGA	MAA	WGA	MAA
Small intestine						
Surface goblet cells	3	0	1	0	1	0
Upper crypt goblet cells	2	0	1	0	1	0
Lower crypt goblet cells	2	0	2	0	1	0
Caecum						
Surface goblet cells	3	2	3	0	3	2
Upper crypt goblet cells	3	3	3	0	3	3
Lower crypt goblet cells	3	3	3	0	3	3
Large intestine						
Surface goblet cells	3	3	3	0	2	1
Upper crypt goblet cells	1	3	2	0	1	1
Lower crypt goblet cells	1	3	2	0	1	1

Numbers indicate staining frequency on a semiquantitative scale ranging from 0 to 4 where 0 corresponds to no reactive cells, 1 to occasional, 2 to a few, 3 to a moderate number and 4 to numerous reactive cells. See table 2 for lectin abbreviations.

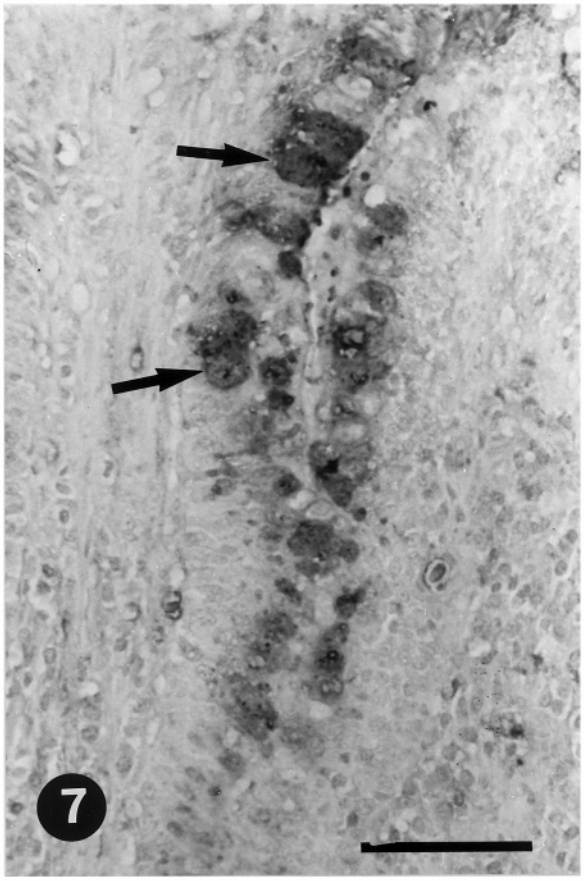

Figure 7. Labelling of a large intestinal crypt with WGA in a chick fed a wheat based diet supplemented with 0.1% xylanase. Goblet cells in the upper part of the crypt (arrows) show a more intense reaction (bar = 50 μ).

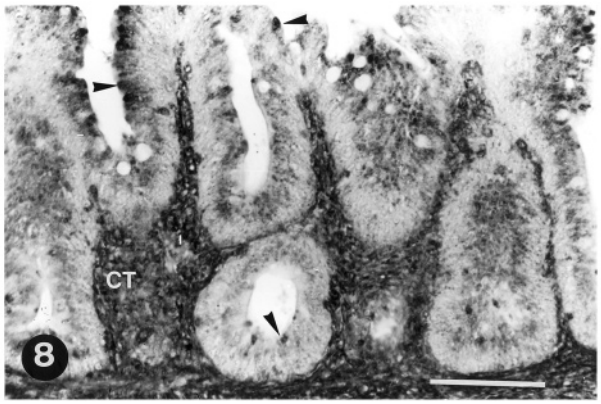

Figure 8. MAA binding of caecum in a chick fed a wheat-based diet. The surface and crypt goblet cells (arrowheads) are labelled. The mucosal connective tissue (CT) shows a strong positive reaction (bar = 25 μ).

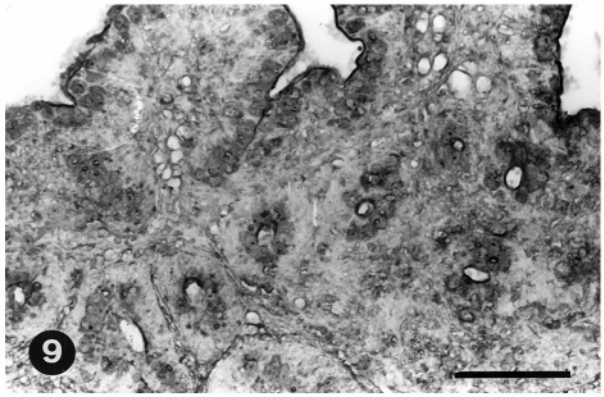

Figure 9. Large intestine stained with MAA. The surface and crypt goblet cells are heavily labelled (bar = 25 μ).

supplemented and maize-based diets. However, the surface goblet cells in the large intestine of animals fed the wheat-based diet were found to be strongly positive for AB staining at pH 2.5 (fig. 5) and 1.0, indicating the abundant presence of carboxylated and sulphated acidic mucins.

Lectin cytochemistry. The effects of diet on the expression of various carbohydrate residues in goblet cell mucins were examined by comparing the lectin-binding pattern of animals maintained on three different diets (table 5). In all sections incubated with biotinylated lectins in the presence of specific inhibitory monosaccharides, lectin binding was inhibited or greatly diminished after this absorption. Binding of lectins from *Dolichos biflorus* (DBA) and *Ulex europaeus* (UEA-I), which are specific for *N*-acetylgalactosamine (GalNAc) and α-L-fucose residues, was not observed in any animal and therefore will not be mentioned in the 'Results'.

Triticum vulgaris (WGA) staining. In the surface and upper crypt goblet cells of the small intestine, labelling with WGA was more intense in animals fed a wheat-based diet (fig. 6). No differences in WGA binding to caecal goblet cell mucins were observed between the three dietary groups. However, in the large intestine of chicks fed a wheat diet supplemented with 0.1% xylanase, WGA binding in the crypt goblet cells was considerably increased (fig. 7).

Maackia amurensis (MAA) staining. The goblet cells in the small intestine were not stained with this lectin. Although MAA reactivity was detected in both surface and crypt goblet cells of the caecum in animals fed wheat-based (fig. 8) and maize-based diets, MAA-binding sites were not seen in the caecum of animals fed the wheat-supplemented diet. As in the caecum, there were differences in the binding of MAA in the large intestine. In chicks fed a wheat-supplemented diet, the goblet cells showed no MAA-binding sites, whereas in animals fed the unsupplemented wheat diet, MAA binding was

strong (fig. 9). MAA-binding sites were weakly expressed in the goblet cells of the large intestine of chicks fed the maize-based diet.

Discussion

Although the role of dietary constituents in the regulation of mucin secretory activity of gut epithelial cells has been recognized in several species [rat: 1, 4, 5; pig: 15; hamster: 16], the relationship between nutritive factors and secretory patterns of intestinal mucosubstances in poultry is poorly understood. This study evaluates for the first time the trophic effects of diet on the composition and carbohydrate sequences of mucin-like glycoproteins in the chick intestinal tract. Using a chick experimental model system, our comparative evaluation of the independent effects of three different diets demonstrates that enzyme supplementation determines the intraluminal viscosity of jejunum and histochemical characteristics and carbohydrate expression of intestinal goblet cells.

The efficiency of utilization of wheat by poultry is limited by nonstarch polysaccharides, which elevate viscosity in the small intestine [17]. In this study, supplementation of the wheat-based diet with xylanase lowered jejunal digesta viscosity, thus enabling more rapid digestion. Although similar solubilization of nonstarch polysaccharides from supplemental enzymes has been described in previous investigations [18, 19], the significance of viscous carbohydrate complexes within the intestinal lumen of birds is still not well understood.

The influence of diet on mucin composition was examined by comparing the distribution pattern of neutral, carboxylated and sulphomucins in surface and crypt goblet cells of chicks given either a maize-based diet or wheat diets unsupplemented and supplemented with 0.1% xylanase. Compared with the other two diets, the wheat diet supplemented with xylanase produced more neutral mucins in the small and large intestines and more carboxylated and sulphated mucins in the upper crypt cells of the small intestine and caecum. The precise mechanisms by which xylanase affects mucin synthesis are not clear, but our results provide evidence of an association between lower intestinal viscosity and enhanced mucin output. Recent studies have shown xylanase activity in wheat bran [20], and our findings are consistent with previous observations that consumption of dietary bran stimulates mucin production in the small intestine [16] and the colon [2], thus enhancing protection of the mucosa.

When the effects of a wheat-based diet on goblet cell mucins were compared with the effects of the maize-based diet, there were no noticeable differences in acidic mucins either in the small intestine, caecum or large intestine. However, in animals fed the maize-based diet, neutral mucins increased in the surface and upper crypt cells of the small and large intestines but decreased in the caecum. Maize and wheat share many physicochemical properties and form conventional dietary ingredients of poultry feed. Since these two ingredients which were fed in equal amounts in two diets produced marked differences in mucin composition in this study, their mechanisms of action on goblet cell activity along the intestinal tract may be very different. The precise mechanism for mucosal response to wheat or maize in poultry feed cannot be determined from the present study, but there is evidence to suggest that inclusion of dietary maize protects chickens from pathogens and increases their survival [21].

Although binding of lectins with similar specificities for sugar residues in the colonic, caecal and ileal epithelium of chicken have been reported [22–24], this study is the first to examine the effects of dietary constituents on the terminal carbohydrate residues of goblet cell mucins along the chick intestinal tract. The absence of DBA and UEA-I binding is in agreement with results of previous studies of chicks [23], which have shown that caecal goblet cells are devoid of GalNAc and α-L-fucose residues. Our finding of changes in PAS reactive neutral mucins and AB reactive carboxylated and sulphated mucins in goblet cells during upward migration along the crypts in the chick intestinal tract are consistent with studies in the mouse [25], rat [4, 26] and rabbit [27], where similar changes associated with the position of goblet cells in intestinal crypts have been described. Coincident with the histochemical changes, the WGA binding of the large intestine was increased along the crypt-surface axis, although this pattern was not reflected in the small intestine or the caecum. However, the decreased WGA-binding sites of surface and crypt goblet cells in the small intestine, and an increase in caecum and large intestinal crypt goblet cells of chicks fed a supplemented diet, would indicate that xylanase modifies the *N*-acetylalucosamine (GlcNAc) residues of goblet cell mucins along the proximal and distal parts of the chick intestinal tract. Because the carbohydrate moieties of intestinal mucins prevent attachments of pathogenic bacteria such as *Salmonella typhimurium* [28] and *Yersinia enterocolitica* [29], strong binding of WGA to caecal goblet cells in our study suggests that in addition to other carbohydrate residues, GlcNAc-containing glycoconjugates secreted by goblet cells may be involved protecting the caecal epithelium from pathogenic enterobacteria.

The most striking difference in lectin binding between dietary groups was observed with MAA, which was strong in the caecum and large intestine of chicks fed wheat- and maize-based diets but absent in those fed a diet supplemented with xylanase. This absence of MAA reactivity most likely results from a higher sialidase activity of diet supplemented with xylanase, since it has

recently been shown that the glycosidase profile is retained by bacterial xylanase [30].

In conclusion, we have shown that dietary constituents in poultry feed modify mucin composition and induce major changes in the expression of goblet cell glycoconjugates containing terminal GlcNAc and sialic acid residues. These results, which may be attributed to interactions between diet and microflora in the intestinal lumen and to subsequent changes in the activity of several glycosyltransferases and glycosidases, require further investigation.

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