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Research Article

Diet influences the colonisation of *Campylobacter jejuni* and distribution of mucin carbohydrates in the chick intestinal tract

F. Fernandez[a,*], R. Sharma[b], M. Hinton[a] and M. R. Bedford[c]

[a]Division of Food Animal Science, Department of Clinical Veterinary Science, University of Bristol, Langford, North Somerset, Bristol, BS40 5DU (UK), Fax + 44 1934 853145, e-mail: fresie.fernandez@bristol.ac.uk
[b]Human Morphology, University of Southampton, Basset Crescent East, Southampton, SO16 7PX (UK)
[c]Finnfeeds International Ltd, Ailesbury Court, High Street, Marlborough, Wiltshire, SN8 1AA (UK)

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Abstract. The objectives of this study were to evaluate the effect of diet on the colonisation by *Campylobacter jejuni* of the chick caeca, and to determine whether the viscosity of the intestinal contents and mucin carbohydrates were altered by the diet. The diets investigated were maize based, wheat-based or wheat-based supplemented with xylanase. The xylanase-supplemented diet reduced the viscosity and lowered the numbers of *Camp. jejuni*. Feeding the enzyme-supplemented diet increased the amount of neutral and sulphated mucins in the goblet cells of the small and large intestines and caecum. An abundance of sulphated and carboxylated mucins was seen in the surface goblet cells of the large intestine with the maize- and wheat-based diets. Both the diet supplemented with xylanase and the maize diets increased crypt-surface glycosylation of the sialic acid residues. The analysed data from the combined sites showed significant differences in the amount of neutral and acidic mucins when comparing the wheat and the wheat plus xylanase diets. However, no changes were shown in the staining intensity of sulphated mucins between the three diets. Significant differences in the glycosylation of sialic acid and in the *N*-acetylglucosamine residues were shown between dietary groups. These results provide evidence that the wheat diet supplemented with xylanase leads to greater changes in the mucin composition and carbohydrate expression of goblet cell glycoconjugates, which are associated with a reduction in intestinal viscosity and decreased numbers of *Camp. jejuni*.

Key words. *Campylobacter*; chick; intestine; mucin; xylanase.

Fresh poultry meat, milk and contaminated water are recognized to be important sources of intestinal infection by *Campylobacter jejuni* in humans, resulting in acute gastroenteritis through to a severe systemic disease [1–3]. In recent years, many more cases of gastroenteritis associated with *Camp. jejuni* than with salmonellas have been reported [4–6]. Microscopical and bacteriological techniques have shown that *Camp. jejuni* is more likely to colonise the caeca of mice, pigs and chickens than other parts of their intestinal tract [7–10]. However, little is known about this preferential colonisation by *Camp. jejuni* within the mucous layer of the caecal crypts. Nor is it known whether the association of this bacterium with the intestinal mucosa alters mucin biosynthesis within the goblet cells.

* Corresponding author.

Several investigators have shown that the bacterial species within the mucous layer degrade mucin glycoproteins, both in vivo [11, 12] and in vitro [10, 13]. Some bacterial species such as *Camp. jejuni* attach to the intestinal epithelium by fimbriae which 'recognize' glycoconjugate receptors in the mucous and/or brush-border membranes [14, 15]. However, no direct evidence for the ability of *Camp. jejuni* to induce changes in the secretory pattern of the intestinal mucins, or to breach the mucosal barrier, has yet been obtained.

Mucin glycoproteins, synthesised and secreted by goblet cells, are responsible for the protective properties of the mucus gels in the gastrointestinal tract [16]. In addition to this protective function, mucin glycoproteins are both a selective barrier to nutrient absorption [17, 18] and a substrate for large numbers of endogenous and pathogenic bacteria [19].

Dietary changes modify the crypt-villus architecture and alter the mucin composition of the intestinal tract [16, 20]. Recent studies in our laboratory with chicks have shown that the constituents of feed modify goblet cell glycoconjugates in the intestinal tract [21]. It would therefore seem pertinent to investigate whether or not the constituents of poultry feed, by modifying mucin composition, could alter the colonisation of the intestinal bacteria, and thus protect chicks against infection by pathogenic organisms such as *Camp. jejuni*.

Lectins are glycoproteins which bind to specific carbohydrate groups of glycoconjugates [22]. Previous studies of the chicken intestinal mucosa used conventional histochemical techniques, plus labelled lectins, to locate such carbohydrate residues in both the colonic and caecal epithelium [21, 23–25]. However, no specific data are available on any modifications to the carbohydrate composition of the gastrointestinal mucosa which may occur during intestinal infection by *Camp. jejuni*.

This study was designed to examine the effects of diet on caecal colonisation by *Camp. jejuni* in the chick, and to determine whether the viscosity of the intestinal contents and mucin carbohydrates were altered by the composition of diets. The effects were investigated by feeding three commercial diets to broiler chicks experimentally infected with *Camp. jejuni*, and then determining changes (i) to the colonisation by *Camp. jejuni* along the intestinal tract, (ii) to the viscosity of the jejunal supernatant, (iii) in the synthesis and secretory levels of neutral, carboxylated acidic and sulphated mucins and (iv) in the binding of lectins to the carbohydrate residues of the goblet cell mucins.

The reported data may help in understanding the mechanisms by colonisation of *Camp. jejuni*, and the roles of diet and mucin carbohydrates in intestinal infections of chicks.

Materials and methods

Diets. Three diets, supplied by Finnfeeds International Ltd, were used (table 1). Two were wheat based, one of which was supplemented with 0.1% xylanase, a commercial enzyme preparation (AVIZYME-1300) obtained from *Trichoderma longibrachiatum* (EC number 3.2.1.8). The third diet was maize based.

The wheat described in this study as soft wheat is a feed wheat used in most feed studies [7, 26, 27]. The detailed nutritional values and the digestibility of the cereals are provided by the Board of Agriculture & Natural Resources [28].

Challenge organisms. A marker strain of *Camp. jejuni*, of chicken origin, resistant to 50 μg/ml nalidixic acid was obtained from the Veterinary Laboratory Agency, Addlestone, UK. Chicks were challenged orally with approximately 10^4 colony forming units (CFU) of

Table 1. Composition of diets.

Ingredients	Wheat-based (g/kg)	Wheat supplemented with 0.1% xylanase (g/kg)	Maize-based (g/kg)
Soft wheat	588.3	587.3	–
Maize	–	–	553.8
Soybean ml 48	324.9	324.9	373.0
Soya oil	44.9	44.9	29.6
Salt	3.0	3.0	3.0
Sodium bicarbonate	1.2	1.2	1.6
DL-Methionine	1.4	1.4	1.3
Limestone	13.7	13.7	12.2
Di-calcium phosphate	12.6	12.6	15.5
Vitamins/minerals*	10.0	10.0	10.0
Enzyme (xylanase)	–	1.0	–

* Vitamins/minerals premix provided the following (mg/kg of diet): retiniol, 3.7; cholecalciferol, 1.2; α-tocopherol, 30; thiamine, 2; riboflavin, 7; d-pantothonic acid; 5; cyanocobalamine, 1.5; niacin, 50; folic acid, 1; biotin, 200; iron, 80; copper, 10; manganese, 100; cobalt, 0.5; zinc, 80; iodine, 1; selenium, 0.2; molybdenum, 0.5. The vitamin/minerals supplement did not contain antimicrobial growth promoters or anticoccidial agents.

Table 2. Characteristics of FITC-conjugated lectins.

Lectin origin	Abbreviation	Carbohydrate-binding specificity	Inhibitory sugar
Triticum vulgaris	WGA	GlcNac(β1,4GlcNAc)$_{1-2}\beta$1,4GlcNeuAc	GlcNAc
Dolichos biflorus	DBA	GalNacα1.3GalNAc > GalNAcα1,3Gal	D-GalNAc
Maackia amurensis	MAA	NeuAc2,3Galβ1,4GlcNAc	δ
Ulex europaeus	UEA-I	L-Fucoseα1,2Galβ1,4GlcNAcβ1.6	α-L-fucose

Gal, galactose; Glc, glucose; GlcNAc, *N*-acetylglucosamine; GalNAc, *N*-acetylgalactosamine; NeuAc, neuraminic (sialic) acid; δ, neuraminidase.

Camp. jejuni by giving them 0.2 ml of a 10^{-3} dilution of an overnight brucella broth (BBL-11088) culture.

Experimental design. Fifty-six, 1-day-old broiler chicks (Ross-1) purchased from a commercial hatchery (M. Millard, Bath, UK) were randomly divided into three groups designated A, B and C. Initially, each group was placed in separate cardboard boxes in sets of 5 or 6. The boxes were placed at least 90 cm apart. On day 5, the chicks were challenged orally with a 10^4 CFU suspension of *Camp. jejuni*, using peptone water (Oxoid CM 9) as diluent. On day 10, chicks from the three groups were transferred to three separate pens isolated from each other, their floors covered with 2 cm of wood shavings. All three groups were given their respective diets ad libitum from day 1 (group A, wheat-based diet; group B, wheat-based supplemented with 0.1% xylanase; group C, maize-based diet), with the ambient temperature maintained at an appropriate level for their age. Animals were killed humanely after 33 days.

Sampling procedures and culture for *Camp. jejuni*. The contents of the small intestine, caeca and large intestine were sampled aseptically, using a swab, then inoculated onto campylobacter blood-free selective agar (Oxoid CM 739) with cefoperazone selective supplement (CCDA; Oxoid SR 155) further supplemented with 50 μg/ml nalidixic acid (CCDA + Na) to allow only the growth of the marker strain of *Camp. jejuni*. Plates were then incubated under microaerophilic conditions (85% N_2, 10% CO_2 and 5% O_2) at 42 °C for 2 days. The campylobacters were identified on the basis of colonial morphology, Gram stain, motility, catalase and oxidase reactions, and by API-CAMPY strips (Bio-Merieux, SA). The growth of campylobacters on the agar was scored on a scale of 0–4, with the scores corresponding, approximately, to $< 10^1$, 10^2–10^3, 10^4–10^5, 10^6–10^7 and 10^8 cfu/g, respectively.

Shredded paper from the boxes used to transport the chicks from the hatchery was also cultured. Approximately 10 g from each was placed in 90 ml of brucella broth, then incubated at 42 °C for 2 days under microaerophilic conditions. These broths were subcultured onto CCDA and CCDA + Na agar plates, then incubated under microaerophilic conditions at 42 °C for 2 days. Suspect campylobacter colonies were identified as above.

Viscosity measurements. The contents of the jejunum were collected and centrifuged at 12,000 *g* at 25 °C for 10 min. The viscosity of the supernatant (0.5 ml) was measured using a digital viscometer equipped with a CP40 cone and plate head, model DVII (Brookfield Engineering Laboratories, Massachusetts). The results are expressed in centipoise units (cPs), as described by Bedford and Classen [26].

Preparation of tissues and histochemical procedures. Four birds were randomly selected from each dietary group. Samples from the mid-region of the small intestine (jejunum), caecum and large intestine were taken from each bird, immediately fixed in 10% phosphate-buffered formalin followed by embedding in paraffin wax. From each site, serial 5-μm sections were treated with the following procedures to identify the mucosubstances.

1) The periodic-acid Schiff (PAS) reaction for staining unsubstituted α-glycol-rich neutral mucins [29].

2) 1% Alcian blue, pH 2.5 (AB 2.5) for localization of the carboxylated and/or sulphated type of acidic mucins [30].

3) 1% Alcian blue, pH 1.0 (AB 1.0) for the selective characterization of sulphomucins.

Each section was then 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 digested with 0.1% trypsin (Difco) in 0.05 M TBS for 30 min at 25 °C. After a wash in TBS, 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 with the biotinylated lectins (Vector Laboratories, Sigma) at a concentration of 10 μg/ml 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 in TBS, then incubated in diaminoben-

zidine-tetrahydrochloride (DAB) in Tris-HCl buffer, pH 7.3, with 0.3% hydrogen peroxide, for 10 min. The control experiments were carried out by omitting the lectin and by incubating the sections with the lectins and their appropriate sugars (0.3 mol end concentration).

Sections were lightly counterstained with Harris haematoxylin, mounted in DPX and examined by light microscopy.

From each slide (of mucosubstances and carbohydrate residues), four fields were examined. Results were recorded according to the staining intensities and intensity of lectin reactivity in individual goblet cells, being graded semiquantitatively from non-reactive cells (0), occasional (1), few (2), moderate (3) and numerous (4) reactive cells. Photographs were taken using Kodak T-MAX 400 black and white film.

Statistical analysis. Results were evaluated using the S-plus 2000 (Release 2) statistical analysis package. The *Camp. jejuni* scores and jejunal viscosity measurements were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's multiple-comparison test. Probabilities less than or equal to 0.05 were considered significant [31].

The combined data for each stain were analysed separately. The ANOVA used a model of scores as a function of the diet plus gut, plus site within the gut, plus bird within diet. The assumptions necessary for ANOVA were met despite the discrete scoring system.

Results

No campylobacters were found on the cultures from the shredded paper in the boxes used to transport the chicks.

Microbiological studies. Results comparing the effect of diets on *Camp. jejuni* colonisation along the chick intestinal tract are summarised in table 3. The growth scores of *Camp. jejuni* from caecal samples of birds fed the xylanase-supplemented diet were significantly lower ($p < 0.01$) (mean 2.3 ± 0.11) than those fed wheat (mean 2.8 ± 0.12) or maize (mean 2.6 ± 0.12) diets.

Lower growth scores, while not statistically significant, were also recorded in the contents of the small and large intestine of birds fed the enzyme-supplemented diet compared with those of chicks fed the other two diets.

Viscosity studies. table Table 3 shows the viscosity measurements of the jejunal supernatant from chicks infected with *Camp. jejuni* fed the three different diets. A significantly ($p < 0.01$) lower viscosity was seen in animals fed the enzyme-supplemented (mean 2.9 ± 0.19 cPs) and the maize diets (mean 3.2 ± 0.22 cPs) diets compared with those from chicks fed the unsupplemented wheat diet (mean 3.9 ± 0.35 cPs).

Histochemical studies. The effect of diet was examined by comparing the staining patterns of the surface, upper and lower crypt goblet cells of the small intestine, caecum and large intestine of chicks infected with *Camp. jejuni* fed either wheat, wheat plus xylanase or maize diets (table 4).

In the small intestine of chicks fed the diet supplemented with xylanase, the mucin granules in the surface and upper crypt goblet cells contained more neutral mucins. The AB staining at pH 1.0 demonstrated more sulphated mucins in the surface and upper crypt goblet cells of chicks fed either the supplemented wheat or the maize diet, compared with those fed the wheat diet.

In the caecum, sulphated mucins were predominant in the surface and upper crypt goblet cells (fig. 1), with carboxylated acidic mucins abundant in the surface goblet cells of birds fed the wheat diet supplemented with xylanase.

In chicks given wheat supplemented with xylanase (figure fig. 2) and maize diets, the staining intensity of the neutral mucins in the surface goblet cells of the large intestine was stronger than that seen in chicks fed the wheat diet. The Alcian blue staining at pH 1.0 showed a higher sulphated mucin content in the surface, upper and lower crypt goblet cells of the large intestines of chicks fed the supplemented wheat diet, compared with those given only wheat or maize diets. In the large intestine of birds fed diets based on wheat or maize, the intensity of carboxylated mucins was higher in the surface goblet cells of the large intestine compared with the supplemented diet (fig. 3).

Table 3. Intestinal colonisation of *Camp. jejuni* and viscosity of the jejunal supernatant in broiler chicks.

Diets	*Camp. Jejuni*			Viscosity (jejunum)
	small intestine	caecum	large intestine	
Wheat based	1.7 ± 0.16 (20)	2.8 ± 0.12 (20)	2.4 ± 0.11 (20)	3.9 ± 0.35 (9)
Wheat plus xylanase	1.4 ± 0.11 (19)	2.3 ± 0.11 (19)[a]	2.3 ± 0.10 (19)	2.9 ± 0.19 (10)[a]
Maize based	1.5 ± 0.18 (17)	2.6 ± 0.12 (17)	2.4 ± 0.12 (17)	3.2 ± 0.22 (8)

[a] The units were scored on a scale of 0–4 (see Materials and methods). Viscosity values are expressed in centipose units (cPs). Values are mean $\pm$ SE per dietary group. Means within a columm with no common superscript differ significantly ($p < 0.01$). Values in parentheses represent the numbers of birds examined.

Table 4. Effect of diet on histochemical characteristics of intestinal mucins in chicks inoculated with *Camp. jejuni*.

Intestine and cell type	Wheat-based			Wheat-based supplemented with 0.1% xylanase			Maize-based		
	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	2	3	2	4	2	3	2	2	2
Upper crypt goblet cells	2	2	2	3	2	3	2	3	3
Lower crypt goblet cells	2	3	3	3	3	3	3	3	3
Caecum									
Surface goblet cells	1	1	1	2	2	3	2	1	1
Upper crypt goblet cells	2	2	2	2	2	3	2	2	2
Lower crypt goblet cells	1	2	2	1	2	2	1	2	2
Large intestine									
Surface goblet cells	2	4	3	4	3	4	4	4	3
Upper crypt goblet cells	3	3	2	3	3	3	3	3	2
Lower crypt goblet cells	3	3	3	3	3	4	4	4	2

Numbers indicate staining frequency on a semiquantitative scale ranging from 0 to 4 (see Materials and methods).
PAS, Periodic acid-Schiff reaction for staining neutral mucins; AB 2.5, Alcian blue (pH 2.5) for localisation of carboxylated and sulphated-type acidic mucins. AB 1.0, Alcian blue (pH 1.0) for the selective characterisation of sulphomucins.

The influence of diet on the histochemical staining patterns of each of the three stains of the combined intestinal sites was assessed by comparing changes between diets, as represented in table 5. Analysis combining the intestinal sites and a comparison between diets (wheat/wheat plus xylanase, wheat/maize, and wheat plus xylanase/maize diets) showed significant differences in the composition of neutral mucin goblet cells, indicated by the periodic acid-Schiff reactivity. In contrast, the Alcian blue staining at pH 1.0 showed no significant changes in sulphated mucin contents. However, differences in the acidic mucins were significant, shown by the Alcian blue staining at pH 2.5, when comparing the wheat and wheat plus xylanase diets.

Lectin cytochemistry. The influence of diet was examined by comparing the pattern of lectin binding to four specific carbohydrates in the goblet cells of the small intestine, caecum and large intestine of infected chicks fed the three different diets. Semiquantitative results are represented in table 6.

Binding of lectins to *Dolichos biflorus* (DBA) and *Ulex europaeus* (UEA-1), which are specific for *N*-acetylgalactosamine (GalNAc) and α-fucose residues, respectively, was not observed. In all sections incubated with biotinylated lectins in the presence of specific inhibitory monosaccharides, lectin binding was inhibited or greatly reduced after this absorption.

***Triticus vulgaris* (WGA) staining.** With birds fed either the wheat- or maize-based diets, but not in those fed the supplemented diet, WGA binding was observed in the surface goblet cells of the small intestine. In the wheat diet, WGA was also present in the upper crypt goblet cells when compared with the other two diets (fig. 4).

In the caecum, WGA binding was stronger in the surface goblet cells of the birds on the supplemented diet, while stronger binding in the upper crypt goblet cells was seen in the chicks fed the maize diet.

In the large intestine of chicks fed either the supplemented or the maize diets, the upper crypt goblet cells were more intensely stained with WGA compared with the upper crypt goblet cells of the birds fed the wheat diet.

***Maackia amurensis* (MAA) staining.** In the birds fed a wheat diet, the small intestine goblet cells showed no MAA binding sites, nor was there binding in the surface goblet cells of the chicks fed the supplemented diet.

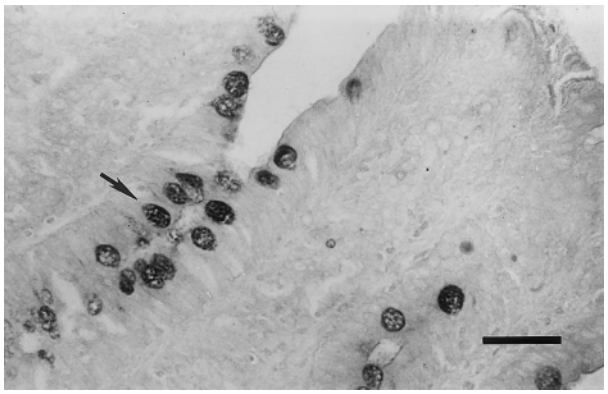

Figure 1. Caecum from a chick fed wheat plus xylanase stained with Alcian blue at pH 1.0. Arrows indicate goblet cells in the surface and upper crypt containing carboxylated acidic mucins (bar, 83 μm).

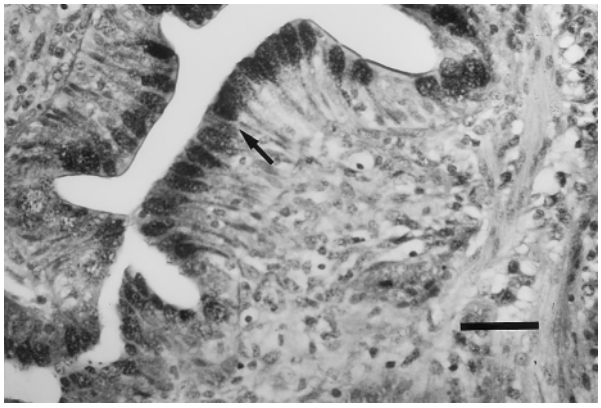

Figure 2. Large intestine from a chick fed a maize-based diet stained by periodic acid-Schiff. Increased staining of the surface goblet cells are shown by the arrow (bar, 83 μm).

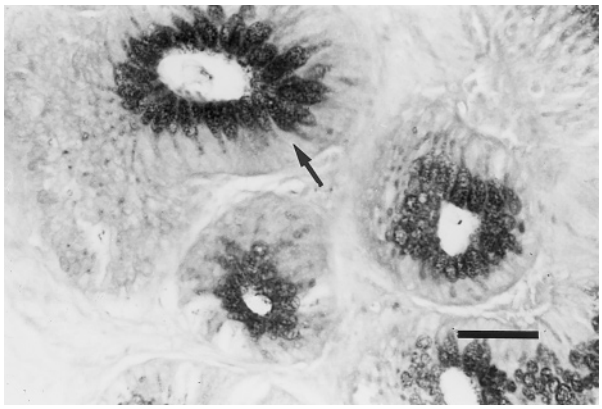

Figure 3. Large intestine from a chick fed a wheat diet, stained with Alcian blue at pH 2.5 showing (arrow) increased intensity of the surface goblet cells (bar, 83 μm).

However, MAA was present in both the upper crypt and lower goblet cells of chicks fed either the maize or the supplemented diets.

In the caecum of chicks fed either the supplemented or the maize diets, MAA-binding sites were more strongly expressed in the surface, upper and lower crypt goblet cells (fig. 5) than in the chicks fed the wheat diet. However, in the large intestine, only the surface and upper crypt goblet cells were more intensely stained with MAA.

The effect of diet on the WGA- and MAA-binding patterns of the combined intestinal sites was assessed by comparing differences between diets (table 7). When comparisons were made between the wheat/wheat plus xylanase, maizewheat plus xylanase and wheat/maize diets, significant differences in WGA- and MAA-binding sites were seen throughout the intestinal tract. The exception was the binding of WGA which did not differ significantly between the chicks fed wheat and xylanase-supplemented diets.

Discussion

The viscosity of the intestinal contents of chicks is partially dependent on the principal constituents of the diet, but can be modulated by including in the feed small amounts of additives, such as enzymes [27, 32, 33]. Recent studies in our laboratory have shown that a diet supplemented with xylanase changes intestinal viscosity and modifies the mucin composition of the chick intestinal tract [21]. There are, however, no data available on the effects of diets and microflora on the secretory patterns of the intestinal mucins of chicks infected with *Camp. jejuni*.

This study was undertaken to evaluate whether the mucin composition of goblet cells is influenced by intestinal colonisation by *Camp. jejuni* and/or by the nature of diets. Our results show that both dietary xylanase and *Camp. jejuni* change the intraluminal viscosity of the jejunum and the carbohydrate composition of the chick intestinal goblet cells.

The previously reported effect of adding xylanase to a wheat-based diet in reducing jejunal viscosity in the chick intestinal tract [21] is in agreement with the results of the present study, which showed a reduction in intestinal viscosity in chicks given a wheat-based diet supplemented with 0.1% xylanase compared with those given either a wheat- or maize-based diet. Additionally, we also observed an association between the reduction in intestinal viscosity and a decrease in the numbers of *Camp. jejuni*.

Though a reduction in jejunal digesta viscosity with dietary xylanase has previously been described [26, 27, 33], the relationship between viscosity and intestinal colonisation with *Camp. jejuni* is still not well understood. However, xylanase possibly produces a less stable environment within the intestinal lumen which leads to the elimination of the campylobacters at a faster rate because of quicker transit times. Our results associating lower intestinal digesta viscosity with reductions in *Camp. jejuni* colonisation are consistent with the findings that multiplication of intestinal microflora increases when the transit time of the digesta are increased by the presence of viscous polysaccharides [34].

Although the effects of diet on the synthesis and secretory levels of mucin glycoproteins of chicken have been previously reported [21], this study examined the effect

of diets and *Camp. jejuni* on the mucin composition by comparing the distribution pattern of neutral, carboxylated and sulphated mucins in goblet cells along the intestinal tract of chicks given either a wheat-based, wheat-based supplemented with 0.1% xylanse or a maize-based diet. Dietary xylanse increased sulphated mucins in the surface and upper crypt goblet cells of the small intestine, caecum and large intestine, while producing less carboxylated mucins in the surface of the small and large intestine, compared with the wheat-based and maize diets.

Although the mechanisms by which xylanase affects mucin synthesis are not clear, one possibility is that xylanase enhances mucin output from the goblet cells by lowering the viscosity of the intestinal contents. Results from this study confirm previous observations that consumption of dietary xylanase by decreasing intestinal viscosity stimulates mucin production in the small intestine, caecum and large intestine [21]. Our observations are also consistent with the finding that consumption of dietary bran stimulates mucin production in the small intestine and the colon, so enhancing protection of the mucosa [19, 35].

Although lectin histochemical studies have been used to investigate the distribution of glycoconjugates on the intestinal epithelium [16, 23, 24], no data on the effects of diet-microbial interaction on the carbohydrate residues of the chick intestinal goblet cells have been reported. The absence of DBA and UEA-1 observed in this study confirm the data of Suprasert and Fujioka [24], which indicated that goblet cells in the chick intestinal tract lack GalNAc and L-fucose residues.

In this study, an increase in intensity of WGA-binding sites on the surface and crypt goblet cells of the caecum and large intestine, and a decrease in the surface crypt goblet cells of the small intestine of chicks fed the supplemented wheat diet suggest that xylanase modifies the *N*-acetylglucosamine (GlcNAc) residues of goblet

Table 5. Estimation of the differences and the confidence intervals between the mean scores for each diet, broken down by stain.

Diets	PAS				AB 2.5				AB 1.0			
	Estimate	SE	Lower bound	Upper bound	Estimate	SE	Lower bound	Upper bound	Estimate	SE	Lower bound	Upper bound
Wheat-based/ wheat plus xylanase	−0.616	0.0607	−0.7590	−0.473	−0.4380	0.06	−0.579	−0.2960	-5.56×10^{-2}	0.0656	−0.210	0.0988
Wheat-based/ maize-based	−0.387	0.0607	−0.5300	−0.244	−0.0486	0.06	−0.190	0.0925	-5.56×10^{-2}	0.0656	−0.210	0.0988
Wheat plus xylanase/maize-based	0.229	0.0607	0.0863	0.372	−0.3890	0.06	0.248	0.5300	4.20×10^{-16}	0.0656	−0.154	0.1540

For stain details see legend to table 4.

Table 6. Lectin reactivity of goblet cell mucins in chicks inoculated with *Camp. jejuni*.

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

Carbohydrate binding capacity: WGA, to GlcNac (β1,4GlcNAc)$_{1-2>}\beta$1,4GlcNeuAc; MAA, to NeuAc2,3Galβ1,4GlcNAc. Values indicate staining frequency on a semiquantitative scale ranging from 0 to 4 (see Materials and methods).

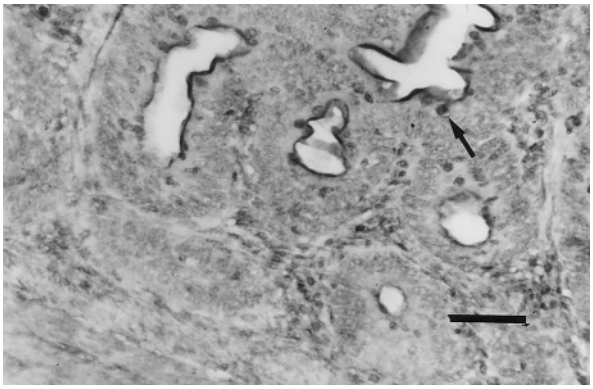

Figure 4. WGA binding in the small intestine from a chick fed a wheat based diet. The goblet cell mucins in the upper crypt (arrow) are labeled (bar, 83 μm).

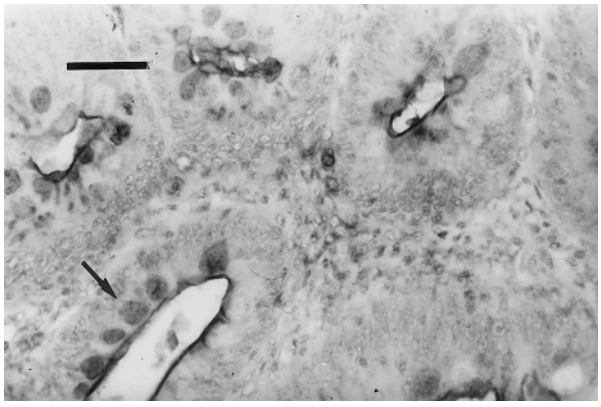

Figure 5. Large intestine from a chick fed a wheat plus xylanase diet, stained with MAA. The surface and upper crypt goblet cells (arrow) are labelled (bar, 83 μm).

cell mucins along the proximal and distal parts of the chick intestinal tract.

The carbohydrate moieties of intestinal mucins have been reported to prevent attachment by *Salmonella typhimurium* and *Yersinia enterocolitica* [36, 37]. In this study, the more marked WGA binding in the caecum and large intestine of chicks on a diet supplemented with xylanase could indicate that *N*-acetyglucosamine residues may be involved in protecting the epithelium against enteropathogens in the distal part of the chick intestinal tract. This observation is consistent with the finding that a diet supplemented with xylanase modifies the crypt surface glycosylation of *N*-acetylglucosamine residues [21].

In contrast to our previous study [21] in which the goblet cells from the caecum and large intestine of chicks fed a diet supplemented with xylanase were devoid of sialic acid residues, the present study demonstrated MAA-binding sites in the caecum and large intestine of chicks fed the supplemented wheat diet. These observations, therefore, indicate that diet and *Camp. jejuni* induce changes resulting in the addition of sialic acid residues to the glycoconjugate core of goblet cell mucins. While the significance of this effect is not yet clear, *N*-acetylneuramic acid present in *Camp. jejuni* [38] may possibly act as an additional substrate for dietary xylanase in the chick intestinal tract.

In conclusion, the data presented in this study provide evidence that a wheat diet supplemented with xylanase modifies the mucin composition and carbohydrate expression of goblet cell glycoconjugates. These changes are associated with a reduction in intestinal viscosity, and decreased the numbers of *Camp. jejuni*. In addition, xylanase enhances sialic acid residues in goblet cell mucins. This observation provides possibilities for using lectins as markers to determine *Camp. jejuni* colonisation in the chick intestinal tract. Our data on the effects

Table 7. Estimation of the differences and the confidence intervals between the mean scores for each diet, broken down by lectin.

Diets	WGA				MAA			
	Estimate	SE	Lower bound	Upper bound	Estimate	SE	Lower bound	Upper bound
Wheat-based/wheat plus xylanase	−0.118	0.0634	−0.267	0.0311	−1.080	0.0713	−1.240	−0.909
Wheat-based/maize-based	−0.347	0.0634	−0.496	−0.1980	−1.470	0.0713	−1.630	−1.300
Wheat plus xylanase/maize-based	−0.229	0.0634	−0.378	−0.0800	−0.389	0.0713	−0.557	−0.221

Carbohydrate binding capacity: WGA, to GlcNac (β1,4GlcNAc)$_{1-2>}\beta$1,4GlcNeuAc; MAA, to NeuAc2,3Galβ1,4GlcNAc.

of *Camp. jejuni* on goblet cell mucins may serve as a basis for further studies aimed at identifying mechanisms of intestinal colonisation by *Camp. jejuni*, and understanding the role of diet in the improvement of food safety.

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