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Original Research Article

Interaction between xylanase and a proton pump inhibitor on broiler chicken performance and gut function

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ABSTRACT

Three hundred thirty-six Ross 308 male broiler chicks were used in a 21-d study to explore performance and gut function when treated with a proton pump inhibitor (PPI; 0 or 89 mg/kg) in a 2 × 2 factorial arrangement with a xylanase (Xyl; 0 or 0.1 g/kg) to determine if the beneficial activity of arabinoxylan (AX) depolymerisation, through arabinoxylo-oligosaccharides (AXOS) production, starts in the upper gastrointestinal tract. Treatment with the PPI started from d 14, and by d 21 animal performance had deteriorated ($P < 0.001$). An interaction was observed between PPI and Xyl for feed conversion ratio (FCR) ($P < 0.05$), whereby the combination reduced the negative effect of PPI on FCR. Application of PPI raised digesta pH in the gizzard and caecum ($P < 0.05$), increased protein concentrations in the lower gut ($P < 0.05$) and reduced intake of digestible nutrients ($P < 0.05$). Caecal concentrations of indole, p-cresol, ammonia and the ratio of total volatile fatty acid (VFA) to butyric acid were increased with PPI ($P < 0.05$), indicating enhanced protein fermentation. Xylanase activity in the digesta were greatest in the caeca, especially when Xyl was supplemented ($P < 0.001$). The concentration of total soluble AX was greater in the gizzard and ileal digesta with Xyl supplementation ($P < 0.05$), supporting the depolymerisation action of xylanase even under acidic conditions. These data suggest xylanase may function in the gizzard even though pH is not optimal for activity and emphasises the importance of chlorohydric acid secretions in ensuring overall optimum gut function. AX depolymerisation benefits animal performance although it is still unknown how the AXOS produced with xylanase supplementation in the upper gastrointestinal tract influence the microbial populations and overall gut functionality.

1. Introduction

The mode of action of xylanase has been widely discussed, with viscosity reduction being one of the primary means by which it increases nutrient availability and growth performance (Aftab and Bedford, 2018; Bedford, 2002; Choct et al., 2004; González-Ortiz et al., 2016). In addition, the release of xylo-oligosaccharides (XOS) and arabinoxylo-oligosaccharides (AXOS) in the gastrointestinal tract (GIT), as a result of dietary arabinoxylan (AX) degradation by xylanase, may also play an important role in improving animal performance (Bedford, 2018). The beneficial effects of XOS and AXOS, either produced in situ or supplied in the feed, can be explained by fermentation to beneficial volatile fatty acids (VFA), butyrate in particular, resulting in improved GIT function and consequently performance (De Maesschalck et al., 2015). However, recent data suggests this production of XOS may be optimised at low pH (Morgan et al., 2017), contrary to what has been understood to date as a result of the relatively neutral optimum pH activity of most feed xylanases. The greatest conversion of AX into AXOS by xylanase was observed at pH 2.5 under in vitro conditions (Morgan et al., 2017). If this is also true in the animal, this would open a new conceptualisation for xylanase mode of action.

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In the broiler chicken the gizzard is the first major organ involved in digestion of consumed feed. Pepsinogen and chlorohydric acid (HCl) are secreted by the proventriculus and mixed with feed material in the gizzard (Svihus, 2014). Gastric acid is essential for lowering pH and enabling the conversion of pepsinogen to the active enzyme pepsin for protein digestion. Real-time pH measurements have shown gizzard pH fluctuates rapidly and extensively, dropping below pH 1.0 and rising above pH 4.0 (Lee et al., 2017, 2018a, 2018b), depending on factors such as feed consumption, stress, dietary additives, and individual bird variation. These fluctuations in acid secretion expose the feed to a rapidly changing environment which encourages the dissolution and digestion of different components of the diet including proteins and minerals, and the hydrolysis of other constituents such as fibre and starch.

The elevation of the pH in the stomach can be achieved with antiacids like sodium bicarbonate and proton pump inhibitors. While sodium bicarbonate is quite an unstable compound to regulate pH, proton pump inhibitors (PPI) like omeprazole or its derivatives could be a feasible option to manipulate the acid production in chickens as previously described by Guinotte et al. (1995). The intramuscular administration of omeprazole in young chicks for 3 consecutive days clearly demonstrated to increase gizzard pH. Therefore, the hypothesis of this study was that if a xylanase is producing AXOS and/or XOS in the upper GIT at acid pH, then elevation of gastric pH with a PPI should reduce this effect and thus the functionality of the enzyme hydrolysing AX. The interaction between the diminished acid production in the gizzard and xylanase supplementation was evaluated on digesta pH, dry matter (DM) and protein digestibility, fermentation profile and soluble sugars in various segments of the GIT, in addition to animal performance.

2. Materials and methods

2.1. Birds and housing

All experimental procedures were in compliance with the European Union guidelines for the care and use of animals in research (EU, 2010). A total of 336 Ross 308 one-day-old male broilers were purchased from a local hatchery (DanHatch, Finland). Upon arrival, birds were placed immediately in 28 floor pens in an environmentally controlled room, with 12 birds per pen. Each pen was 1.125 m^2 in size and was equipped with a pan feeder and a nipple waterer, and chopped straw and clean wood shavings were used as litter. Test diets and water were provided ad libitum throughout the trial. The room was preheated to 32 °C 2 d prior to the commencement of the study and kept at 32 °C for the first 2 d. Then the room temperature was gradually reduced to 22 °C over the rearing period. From d 0 the dark hours were increased daily by 1 h from 24 h light until the light–dark cycle was 18 h light and 6 h dark daily.

2.2. Experimental treatments

Wheat and soybean meal (SBM) were used as primary ingredients to formulate the experimental diets that met or exceeded nutrient recommendations for broilers fed in one phase from 0 to 21 d of age (FEDNA, 2008). The compositions of the experimental diets are shown in Table 1. One basal diet was made, then split equally into 4 subsamples each of which were supplemented on top with the experimental products: 1) control diet, without any added supplement; 2) Xyl diet, with 100 g/t of xylanase (Econase XT 25P, AB Vista, Marlborough, UK; 160,000 U/kg); 3) PPI diet, with 89 mg/kg Esomeprazole; and 4) Xyl + PPI diet, resulting in 4 experimental treatments. Experimental diets did not contain any coccidiostat, antibiotic or any other growth promoter. The PPI treatment did not receive any additive from d 0 until d 14 and the Esomeprazole was administered only in the last week of the study, from 14 d of age onwards. The Xyl + PPI treatment received in the diet the xylanase from d 0 to 21 and the PPI was administered only from 14 d of age onwards. Titanium dioxide (TiO_2) was included at 0.3% as an indigestible marker in all the experimental diets when the PPI was administered in the feeds.

Table 1
Ingredients, calculated composition (as-fed basis) and analysed composition (as-fed basis) of the experimental diet (%).

Item	Content
Ingredients	
Wheat	58.96
Soybean meal	35.70
Soya oil	2.41
Salt	0.28
Limestone	1.17
Monocalcium phosphate	0.61
Lysine HCl	0.14
DL-Methionine	0.25
Threonine	0.07
Vitamin and mineral premix[1]	0.40
Phytase[2]	0.01
Calculated composition	
AME, MJ/kg	12.35
Crude protein	24.0
Dig. Lys	1.25
Dig. Met + Cys	0.92
Dig. Thr	0.82
Dig. Try	0.26
Dig. Arg	1.46
Dig. Val	0.95
Calcium	0.90
Available phosphorous	0.45
Sodium	0.16
Analysed composition[3]	
Crude protein	24.9
Fat	4.9
NDF	9.3
Starch	33.4
Ash	7.1
Phytic acid	0.3
Calcium[4]	0.88
Phosphorous[4]	0.60

AME = apparent metabolizable energy; NDF = neutral detergent fibre.
[1] Mineral and vitamin premix provided per kg of feed: calcium 1,256 mg, zinc 65 mg, manganese 50 mg, iron 25 mg, copper 8.0 mg, iodine 0.5 mg, selenium 0.2 mg, all-rac-α-tocopheryl acetate 60.0 mg, niacin 40.2 mg, panthotenic acid 15.0 mg, riboflavin 6.0 mg, pyridoxine 4.0 mg, retinol 3.6 mg, menadione 3.0 mg, thiamine 2.5 mg, folic acid 1.0 mg, biotin 0.15 mg, cholecalciferol 0.11 mg, cobalamin 0.03 mg.
[2] Quantum Blue 5G (5,000 U/kg; AB Vista, Marlborough, United Kingdom).
[3] Predicted by near-infrared spectroscopy (AB Vista, Marlborough, United Kingdom).
[4] Phosphorous and calcium were analysed by wet chemistry.

2.3. Experimental procedures

Birds were weighed on a pen basis on d 0, d 14 and d 21 of age, to measure mean body weight (BW) and calculate body weight gain (BWG) for each period and cumulatively. Feed intake (FI) was determined by pen, and mortality was checked twice daily, and the weights of dead birds were used to adjust the feed conversion ratio (FCR). On d 21, 5 birds from each of the 28 floor pens were randomly selected and euthanized by cervical dislocation. The total GIT was removed immediately from the abdominal cavity. The pH of digesta was determined by direct insertion of the semimicro electrode (VWR, Helsinki, Finland) with the tip diameter of 6 mm. For this,

each section of the GIT for this analysis was cut, and the pH probe pushed to the lumen. Digesta contents from the gizzard, ileum and caeca were then immediately collected by opening each section with scissors and the digesta was gently squeezed to the sampling tube, pooled on a pen basis and snap frozen in dry ice. Homogenised samples were then stored at −20 °C for subsequent analysis of protein, DM, putrefaction end products, short-chain fatty acids (SCFA), xylanase activity and soluble sugars. For the analysis of the protein bands, aliquots of ileal digesta were stored at −20 °C.

2.4. Sample analyses

The proximate analysis of wheat and SBM included moisture, crude protein (CP), fat, ash, starch, neutral detergent fibre (NDF), total non-starch polysaccharides (NSP), total insoluble NSP, total AX and phytic acid and were estimated by near-infrared spectroscopy (NIR) (Foss DS2500, Hilleroed, Denmark) according to the calibrations described by Gomes et al. (2020) (Table 2). The apparent metabolizable energy (AME) of wheat and urease activity, total lysine, reactive lysine and standardized ileal digestibility of amino acids (AA) from SBM were also predicted by NIR. Diets were analysed for xylanase and phytase activity by an ELISA method using Quantiplate Kits (Enzyme Services & Consultancy, Innovation & Technology Centre, Ystrad Mynach, UK) supplied by Envirologix (Enzyme Services & Consultancy, Innovation & Technology Centre, Ystrad Mynach, UK). The chemical composition of the diets included CP, fat, NDF, starch, ash, phytic acid, calcium and phosphorous, and were also predicted by NIR (Table 1). Phosphorus and calcium contents of all relevant samples were analysed by inductively coupled plasma optical emission spectrometry (ICP-OES; AOAC 985.01) (AOAC, 2005). Dry matter (934.01) and crude protein (984.13) were analysed in feed and ileal digesta (AOAC, 2005) and used to calculate the intake of digestible DM and digestible protein. TiO_2 was analysed according to Short et al. (1996).

Soluble protein concentration in the gizzard, ileal and caecal digesta was determined using a Bicinchoninic Acid (BCA) Kit for Protein Determination (Ref. 71,285-3) following the instructions of the manufacturer (Sigma Aldrich, Espoo, Finland).

For sugar analysis in the gizzard, ileal and caecal digesta, 0.5 g of digesta was diluted in 4 mL of ice-cold water which had erythritol as an internal standard and vortexed for 5 min to extract soluble carbohydrates. Insoluble material was then spun down by centrifugation at 3,000 × *g* for 5 min. Two 200-μL aliquots were withdrawn from the supernatant, one for the analysis of soluble monosaccharides (simple sugars) and the other for the analysis of total soluble carbohydrates. For the total carbohydrates the sample was subjected to acid hydrolysis by adding 200 μL of 2 mol/L H_2SO_4, sealing the vessel and incubated at 100 °C for 2 h. Then the hydrolysate was neutralized by adding 800 μL of 1 mol/L NaOH solution. For the analysis of monosaccharides, the original 200-μL sample was not hydrolysed, but diluted with 1,000 μL of the corresponding premixed H_2SO_4–NaOH solution to reach the same ionic strength as the hydrolysed sample. Four hundred microliters of the sample preparations were mixed with 800 μL of methanol to precipitate inorganic impurities, then 200 μL of the supernatant was evaporated to dryness. The monosaccharides in samples were converted to the corresponding oximes and derivatized with N,O-bis(trimethylsilyl)trifluoroacetamide as described by Zhang et al. (2018). Finally, the samples were analysed by gas chromatography mass spectroscopy (GC–MS) (Agilent 7890-5975C GC-MSD equipped with a ZB-5 60 m × 250 μm × 0.25 μm column). Data were collected with a single ion mode and procedural calibration standards were used in quantification.

Table 2
Predicted composition[1] (%, as is) of wheat and soybean meal (SBM) used in the experimental diets.

Item	Wheat	SBM
Moisture	12.07	12.32
Protein	12.67	47.74
Fat	2.51	2.05
Ash	1.48	6.28
Starch	58.86	6.14
NDF	9.95	11.50
Total NSP	8.81	15.91
Insoluble NSP	6.91	12.08
Total A + X	5.49	3.73
Phytic P	0.24	0.45
AME, MJ/kg	12.89	–
Total lysine		2.90
Reactive lysine		2.76
SID total lysine		2.49
SID methionine		0.64
SID cysteine		0.61
SID threonine		1.88
SID isoleucine		2.00
SID leucine		3.46
SID phenylalanine		2.35
SID tyrosine		1.64
SID histidine		1.20
Urease, U		0.01

NDF = neutral detergent fibre; NSP = non-starch polysaccharides; A + X = arabinose + xylose; AME = apparent metabolizable energy; SID = standardized ileal digestible.
[1] Predicted by near-infrared spectroscopy (Gomes et al., 2020).

Xylanase enzyme activity from gizzard, ileal and caecal digesta samples were analysed with the endo-xylanase assay kit XylX6 Method (Ref. K-XylX6-2V) (Megazyme, Wicklow, Ireland) according to the manufacturer's instructions. Briefly, 0.1 g of each digesta sample was stirred with 1 mL of 0.90% sterile NaCl solution in a vortex mixer for 1 min. Aliquots of 0.05 mL XylX6 reagent solution were directly dispensed to the bottom of glass tubes and the tubes and analysed digesta samples were pre-incubated at 40 °C for approximately 3 min. To each tube containing XylX6 solution, 0.05 mL of digesta samples were added to the bottom of the tube, stirred on a vortex mixer and tubes were incubated at 40 °C for 10 min. At the end of the incubation period, 1.5 mL of stopping reagent were added and tube contents were stirred on a vortex mixer. The contents were transferred into a 2 mL Eppendorf tube and centrifuged for 10 min at 20,000 × *g*. The absorbance of the supernatants of the reaction solutions and the reagent blank was read at 400 nm against distilled water. Endo-xylanase activity (U/mL) was calculated by the formula:

$$\text{Endo-xylanase activity (U/mL)} = \frac{\text{Absorbance (reaction)} - \text{Absorbance (blank)}}{\text{Incubation time (10 min)}} \times \frac{\text{Total volume in cell (1.6 mL)}}{\text{Aliquot assayed (0.05 mL)}} \times \frac{1}{\varepsilon \text{mM}} \times \frac{\text{Extraction volume (1 mL)}}{\text{Sample volume (0.1 mL)}}$$

where εmM of 4-nitrophenol was 18.1 mmol/L in the current method.

Caecal digesta samples were analysed for SCFA. The SCFA were analysed as free acids by gas chromatography, using pivalic acid as an internal standard (Apajalahti et al., 2019). Briefly, 1 mL of H_2O was mixed with 1 g of caeca content, and then 1 mL of 20 mmol/L pivalic acid solution was added as an internal standard. After mixing, 1 mL of perchloric acid was added and SCFA were extracted by shaking the mixture for 5 min. After centrifugation, perchloric acid in the supernatant was precipitated by adding 50 μL of 4 mol/L KOH in 500 μL of supernatant. After 5 min, saturated oxalic acid was added and the mixture incubated at 4 °C for 60 min and then centrifuged at 18,000 × *g* for 10 min. Samples were analysed by GC using a glass column packed with 80/120 Carbopack B-DA/4% Carbowax 20 M stationary phase (Supelco, Bellefonte, PA, USA), using helium as the carrier gas and a flame ionization detector. The SCFA measured were lactic acid and VFA which in turn comprised of acetic, propionic, butyric, iso-butyric, 2-methyl-butyric and iso-valeric acids. The sum of isobutyrate, 2-methyl butyrate and iso-valerate results in branched-chain fatty acids (BCFA).

Caecal digesta samples were analysed for aromatic putrefaction end products and ammonia. Indole, skatole (3-methylindole), p-cresol and phenol were analysed by using deuterated phenol, 2-methylphenol and 5-methylindole as internal standards. Samples were mixed 1:3 with 3 mol/L phosphate buffer pH 7 and then extracted with methanol-ethyl acetate (1:4) by vigorous shaking. The phases were separated by centrifugation and the upper organic phase analysed by GC–MS. For the ammonia analysis digesta samples were diluted 1:20 in distilled water, shaken at room temperature for 10 min and centrifuged at 18,000 × *g* for 15 min. Ammonia was analysed from the supernatant by a colorimetric method based on the reaction of phenol and hypochlorite with NH_3, leading to colour formation, the intensity of which was measured with a spectrophotometer (Weatherburn, 1967).

Soluble proteins present in the ileal digesta were separated by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). Digesta samples were thawed at room temperature and 0.1 g of each sample was mixed with 1 mL of 0.90% sterile NaCl solution and stirred on a vortex mixer for 1 min. Ten microliters of this suspension were mixed with and equal volume of 2 × Tris-Glycine SDS Sample Buffer with added dithiothreitol 50 mmol/L and were heated at 85 °C for 2 min. The samples were immediately chilled on ice for 5 min and loaded on to Novex WedgeWell 8% to 16% Tris-Glycine gel (Invitrogen by Thermo Fisher Scientific, USA). Electrophoretic separation of proteins was performed with Mini Gel Tank in 1X Novex Tris-Glycine SDS running buffer (Invitrogen by Thermo Fisher Scientific, USA). Electrophoresis was run at 225 V for 36 min. To visualize the proteins, the SDS-PAGE gel was washed in water and stained in a RAPIDstain (Biosciences, USA). Molecular weights of protein fractions were estimated by their relative positions against a 10 to 250 kDa protein marker (PageRuler Plus Prestained Protein Ladder, Thermo Scientific USA).

2.5. Statistical analyses

The calculation of sample size was performed using the freely available EPGP programme from Pesti (University of Georgia, United States). Based on a previous study (González-Ortiz et al., 2019), the means for BWG of the control and xylanase treatments were 1,103 and 1,135, respectively, with a pooled standard deviation (SD) of 37. Thus, 4 replicates per treatment are needed to detect a 6% difference between means with 80% power using a *t*-test at a significance level of 0.05. The proposed sample size in this experiment was 7 replicates per treatment which should enable detection of a 6% difference or greater at $P < 0.05$ with a power of 87%. Performance, digesta pH, protein concentration, xylanase activity, intake of digestible nutrients, putrefaction end-products, fermentation activity through SCFA and soluble sugars were subjected to two-way analysis of variance using JMP 15 Pro (SAS). Pen was the experimental unit for all the parameters measured. The non-parametric Wilcoxon test was used for mortality rates comparison between experimental treatments. Means were separated only when the treatment *P*-value was significant and then by using the least significant difference test. Statements of significance were based on *P*-value of equal to or less than 0.05.

3. Results

Analysed enzyme activities in feed samples were all close to expected (Table 3).

3.1. Growth performance

Overall mortality was 0.59%, and no differences were observed between the experimental treatments ($P > 0.05$) (data not shown). The effects of experimental treatments on growth performance on d 0 to 14, d 14 to 21, and d 0 to 21 are shown in Table 4. No interactions were observed in BW, BWG or FI in any of the periods measured. Up to d 14, Xyl did not improve bird performance ($P > 0.05$). Treatment with PPI from d 14 to 21 reduced ($P < 0.001$) the final BW from 824 to 606 g/bird (−26%) which resulted in a reduction in BWG from d 14 to 21 ($P < 0.001$) and in the overall BWG ($P < 0.001$). PPI supplementation decreased FI from d 14 to 21 ($P < 0.001$) and consequently FI for the overall period ($P < 0.001$). Treatment with PPI from d 14 to 21 increased FCR by 57 points ($P < 0.001$). A significant interaction was observed for d 0 to 21 FCR ($P = 0.045$) whereby the negative effect of PPI was reduced somewhat by Xyl addition.

Table 3
Analysed enzyme activities in diets.

Treatments[1]			Starter (d 0 to 21)	
Identification	Xylanase	PPI	Phytase[2], U/kg	Xylanase[3], U/kg
CTR	No	No	692	<2,000
PPI	No	Yes	671	<2,000
Xyl	Yes	No	587	17,000
Xyl + PPI	Yes	Yes	791	17,500

PPI = proton pump inhibitor.
[1] Treatments: CTR (control diet), PPI (CTR diet supplemented with Esomeprazole, a proton pump inhibitorb from d 14 to 21), Xyl (CTR diet supplemented with 16,000 U/kg of xylanase from d 0 to 21), and Xyl + PPI (CTR diet supplemented with both xylanase and PPI, the latter supplemented only from d 14 to 21).
[2] One unit of phytase is defined as the amount of enzyme required to release 1 μmol of inorganic P per minute from sodium phytate at 37 °C and pH 5.5.
[3] One unit of xylanase is defined as the amount of enzyme that produces 1 nmol reducing sugars from birchwood xylan in 1 s at 50 °C and pH 5.3.

Table 4
Effects of dietary treatments[1] on the performance of broiler chickens at different ages.[2]

Xylanase	PPI	Body weight, g			Body weight gain, g/bird			Feed intake, g/bird			Feed conversion ratio, g/g		
		0 d	14 d	21 d	0 to 14 d	14 to 21 d	0 to 21 d	0 to 14 d	14 to 21 d	0 to 21 d	0 to 14 d	14 to 21 d	0 to 21 d
Xylanase effect													
-Xyl		42.28	365	714	323	349	672	422	537	958	1.309	1.652	1.450
+Xyl		42.74	368	716	325	348	673	422	530	952	1.299	1.594	1.432
PPI effect													
	-PPI	42.46	371	824[a]	328	454[a]	782[a]	429	607[a]	1,036[a]	1.308	1.339[b]	1.326
	+PPI	42.56	362	606[b]	320	244[b]	563[b]	415	460[b]	875[b]	1.300	1.906[a]	1.556
Xylanase × PPI													
-Xyl	-PPI	42.16	369	830	327	461	788	427	608	1,035	1.306	1.320	1.314[c]
	+PPI	42.40	361	599	318	238	556	417	465	882	1.313	1.984	1.586[a]
+Xyl	-PPI	42.76	372	818	329	446	776	431	606	1,037	1.310	1.359	1.339[c]
	+PPI	42.73	364	613	321	249	570	413	455	868	1.287	1.829	1.526[b]
Pooled SD		1.00	21	46	21	33	46	23	38	51	0.042	0.139	0.053
P-value													
Xylanase		0.230	0.706	0.944	0.759	0.901	0.955	0.981	0.658	0.765	0.505	0.275	0.379
PPI		0.779	0.302	<0.001	0.303	<0.001	<0.001	0.121	<0.001	<0.001	0.624	<0.001	<0.001
Xylanase × PPI		0.722	0.979	0.470	0.993	0.313	0.471	0.631	0.754	0.682	0.353	0.076	0.045

PPI = proton pump inhibitor; SD = standard deviation.
[a, b, c]Values within a column with different superscripts are significantly different ($P < 0.05$).
[1] Treatments: CTR (control diet), PPI (CTR diet supplemented with Esomeprazole, a proton pump inhibitor, from d 14 to 21), Xyl (CTR diet supplemented with 16,000 U/kg of xylanase from d 0 to 21), and Xyl + PPI (CTR diet supplemented with both xylanase and PPI, the latter only supplemented from d 14 to 21).
[2] Mean values for 7 replicate pens with 12 birds per replicate pen.

3.2. pH and xylanase activity of digesta

Analysed pH and xylanase activities of digesta of broiler chickens are presented in Table 5. No interactions were observed for either parameter in any of the gastrointestinal sections measured on d 21. While no differences in the digesta pH were found for Xyl supplementation, birds treated with PPI had higher gizzard ($P < 0.001$) and caecal ($P < 0.05$) pH. Xylanase activity was higher in ileal ($P < 0.001$) and caecal ($P < 0.001$) digesta of birds supplemented with Xyl, but the PPI did not influence the amount of active xylanase protein in any of the gastrointestinal sections measured at 21 d of age.

3.3. Intake of digestible nutrients, soluble protein concentration and non-digested proteins profile of digesta

The intake of digestible nutrients from d 14 to 21 and the soluble protein concentration of digesta of broiler chickens measured at 21 d of age are presented in Table 6 and Table 7, respectively. No interactions or Xyl effects were observed on the intake of digestible DM or protein. Treatment with PPI, however, decreased the intake of digestible DM by approximately 39% ($P < 0.001$) and that of digestible protein ($P < 0.001$) by more than 50%. Regarding the soluble protein concentration of digesta, no interactions were observed in any of the sections measured on d 21. Supplementation of Xyl did not influence the concentration of soluble protein in the

Table 5
Effects of dietary treatments[1] on the digesta pH and xylanase activity in broiler chickens measured at 21 d of age.[2]

Xylanase	PPI	Digesta pH			Xylanase activity, U/g DM		
		Gizzard	Ileum	Caecum	Gizzard	Ileum	Caecum
Xylanase effect							
-Xyl		2.86	5.87	6.48	0.027	0.047[b]	3.492[b]
+Xyl		2.94	5.95	6.49	0.040	0.293[a]	6.466[a]
PPI effect							
	-PPI	1.80[b]	6.00	6.37[b]	0.025	0.186	4.921
	+PPI	4.00[a]	5.83	6.60[a]	0.042	0.154	5.037
Xylanase × PPI							
-Xyl	-PPI	1.79	5.97	6.35	0.029	0.045	3.650
	+PPI	3.93	5.77	6.62	0.024	0.049	3.334
+Xyl	-PPI	1.81	6.03	6.40	0.021	0.327	6.192
	+PPI	4.06	5.88	6.59	0.060	0.260	6.741
Pooled SD		0.926	0.288	0.229	0.033	0.068	0.713
P-value							
Xylanase		0.823	0.457	0.896	0.279	<0.001	<0.001
PPI		<0.001	0.126	0.014	0.205	0.227	0.675
Xylanase × PPI		0.867	0.830	0.660	0.088	0.187	0.129

DM = dry matter; PPI = proton pump inhibitor.
[a, b]Values within a column with different superscripts are significantly different ($P < 0.05$).
[1] Treatments: CTR (control diet), PPI (CTR diet supplemented with Esomeprazole, a proton pump inhibitor, from d 14 to 21), Xyl (CTR diet supplemented with 16,000 U/kg of xylanase from d 0 to 21), and Xyl + PPI (CTR diet supplemented with both xylanase and PPI, the latter only supplemented from d 14 to 21).
[2] Mean values for 7 replicate pens with the pool of 5 birds per replicate pen.

Table 6
Effects of dietary treatments[1] on the intake of digestible nutrients by broiler chickens from d 14 to 21.[2]

Xylanase	PPI	Intake of digestible nutrients, g	
		DM	Protein
Xylanase effect			
-Xyl		262	141
+Xyl		259	145
PPI effect			
	-PPI	324[a]	196[a]
	+PPI	197[b]	90[b]
Xylanase × PPI			
-Xyl	-PPI	313	183
	+PPI	211	99
+Xyl	-PPI	335	209
	+PPI	184	81
Pooled SD		45	46
P-value			
Xylanase		0.885	0.813
PPI		<0.001	<0.001
Xylanase × PPI		0.171	0.214

DM = dry matter; PPI = proton pump inhibitor; SD = standard deviation.
[a, b]Values within a column with different superscripts are significantly different ($P < 0.05$).
[1] Treatments: CTR (control diet), PPI (CTR diet supplemented with Esomeprazole, a proton pump inhibitor, from d 14 to 21), Xyl (CTR diet supplemented with 16,000 U/kg of xylanase from d 0 to 21), and Xyl + PPI (CTR diet supplemented with both xylanase and PPI, the latter only supplemented from d 14 to 21).
[2] Mean values for 7 replicate pens with the pool of 5 birds per replicate pen.

Table 7
Effects of dietary treatments[1] on soluble protein concentration of digesta of broiler chickens measured at 21 d of age.[2]

Xylanase	PPI	Soluble protein concentration, mg/g DM		
		Gizzard	Ileum	Caecum
Xylanase effect				
-Xyl		49.48	111.0	287.2
+Xyl		47.09	115.8	300.9
PPI effect				
	-PPI	47.16	103.5[b]	275.0[b]
	+PPI	49.42	123.3[a]	313.1[a]
Xylanase × PPI				
-Xyl	-PPI	49.94	99.5	270.8
	+PPI	49.03	122.5	303.6
+Xyl	-PPI	44.38	107.6	279.2
	+PPI	49.81	124.1	322.6
Pooled SD		8	15	49
P-value				
Xylanase		0.465	0.415	0.463
PPI		0.488	0.002	0.049
Xylanase × PPI		0.333	0.581	0.774

DM = dry matter; PPI = proton pump inhibitor; SD = standard deviation.
[a, b]Values within a column with different superscripts are significantly different ($P < 0.05$).
[1] Treatments: CTR (control diet), PPI (CTR diet supplemented with Esomeprazole, a proton pump inhibitor, from d 14 to 21), Xyl (CTR diet supplemented with 16,000 U/kg of xylanase from d 0 to 21), and Xyl + PPI (CTR diet supplemented with both xylanase and PPI, the latter only supplemented from d 14 to 21).
[2] Mean values for 7 replicate pens with the pool of 5 birds per replicate pen.

gizzard, ileal or caecal digesta, while treatment with PPI increased soluble protein concentration by 19% in the ileal ($P < 0.01$) and 14% in the caecal ($P < 0.05$) digesta. SDS-PAGE analysis revealed specific protein bands with a molecular weight of 35 and 20 kDa in ileal digesta (Fig. 1) of birds fed PPI. However, these bands were either less concentrated or not present at all in digesta taken from non-PPI treated birds. Supplementing Xyl did not appear to alter the soluble protein profiles of ileal digesta.

3.4. Putrefaction end-products

No interactions were observed in any of the caecal putrefaction end-products measured at 21 d of age (Table 8). Birds fed the diets with PPI had higher indole ($P < 0.001$), p-cresol ($P < 0.001$) and ammonia ($P < 0.05$) compared to non-PPI supplemented birds whilst xylanase had no effect on these parameters.

3.5. Fermentation activity

The effects on SCFA in the caecal digesta on d 21 is presented in Table 9. No interactions were observed for total VFA, lactic acid, VFA-to-butyric acid ratio or the VFA-to-BCFA ratio. PPI treatment increased the VFA-to-butyric acid ratio ($P < 0.01$) and reduced the VFA-to-BCFA ratio ($P < 0.001$) of caecal digesta.

3.6. Soluble sugars

The gastrointestinal section, if gizzard, ileum or caeca, influenced the proportions of monomeric sugars and complex sugars (at least of more than 2 degrees of polymerisation [DP]) (Fig. 2). The proportion of monomeric sugars increased from the upper gastrointestinal sections to the caecal digesta, while the relative concentrations of complex sugars were gradually reduced along the gastrointestinal sections. No interactions between Xyl and PPI were observed for the relative concentrations of complex sugars and monomeric sugars. Xyl supplementation did not influence the proportion of the sugars in any of the intestinal sections measured ($P > 0.05$) (Fig. 2A), while PPI-treated birds had reduced ($P < 0.05$) proportions of complex sugars and increased ($P < 0.05$) monomeric sugars in the gizzard compared to control birds (Fig. 2B).

The total soluble sugars (monomeric sugars + complex sugars) in gizzard, ileal and caecal digesta of broilers chickens measured at 21 d of age are presented in Table 10. No interactions were observed for any of the soluble sugars on any of the gastrointestinal sections measured. Broiler chickens treated with PPI had increased levels of all the measured soluble sugars in the gizzard ($P < 0.01$), while ileal total soluble sugars, glucose, galactose and mannose were reduced, and all soluble sugars were decreased in the caeca ($P < 0.05$) with the exception of inositol. Xyl supplementation increased the content of xylose and arabinose in gizzard and ileal digesta ($P < 0.05$), but not in the caecal content. Xyl-supplemented birds also had higher inositol levels in the ileal digesta ($P < 0.05$). Total soluble sugars in the caecal digesta were not influenced by Xyl supplementation ($P > 0.05$).

The complex sugars, including oligomers of at least 2 DP and other polymeric structures, in gizzard, ileal and caecal digesta of broiler chickens are presented in Table 11. No interactions were observed for any of the complex sugars on any of the gastrointestinal sections measured. Treatment with PPI increased all complex sugar content in the gizzard digesta ($P < 0.05$) with the exception of inositol. Ileal concentrations of arabinose, xylose + arabinose, mannose, galactose, glucose and the total complex sugars were reduced ($P < 0.05$) with PPI. All complex sugars with the exception of inositol were reduced in the caecal digesta ($P < 0.05$) with PPI treatment. Xyl supplementation increased xylose, arabinose and their sum in the gizzard ($P < 0.05$) and ileal ($P < 0.05$) digesta. Total complex sugars concentrations in the gizzard digesta were also increased with Xyl supplementation whilst it had no effect on any of the in the caecal digesta ($P > 0.05$).

4. Discussion

4.1. Effects of proton pump inhibitor on animal performance and gut function

The model used in this experiment to reduce the acid production in the chicken stomach through the supplementation of Esomeprazole resulted in clear differences between the non-PPI supplemented versus the supplemented birds. Esomeprazole, the *S*-isomer of the racemate omeprazole, is a PPI very commonly used in humans to treat acid-related disorders, including gastro-oesophageal reflux disease and erosive oesophagitis and their symptoms (Cardile and Romano, 2012). Treating birds with PPI during the last 7 d of the trial significantly increased gizzard pH from 1.8 to 4.0 indicating that PPI had effectively reduced HCl secretion in broilers. As a consequence, PPI administration had negative consequences on animal performance at d 21 by depressing FI, BWG and FCR. In Guinotte et al. (1995), it was demonstrated that injection of omeprazole at 50 μmol/kg BW significantly increased gizzard pH of broilers from pH 1.77 to 2.59 in the control to pH 4.50 to 5.60 with omeprazole administration. Moreover, when omeprazole was injected for 3 consecutive days at 100 μmol/kg BW significantly reduced weight gain and feed consumption in chicks at d 18.

The inability of the proventriculus to secrete HCl and activate pepsin consequently affected protein digestion. Higher concentrations of protein were observed in the ileal and caecal digesta of PPI-supplemented birds which coincided with a 39% lower intake of digestible DM and 54% reduced intake of digestible protein. In addition, SDS-PAGE analysis revealed protein bands at approximately 35 and 20 kDa in the ileal digesta of birds treated with PPI. These bands were also clearly visible in the diet but were absent in the digesta of non-PPI treated birds. Although these bands were not

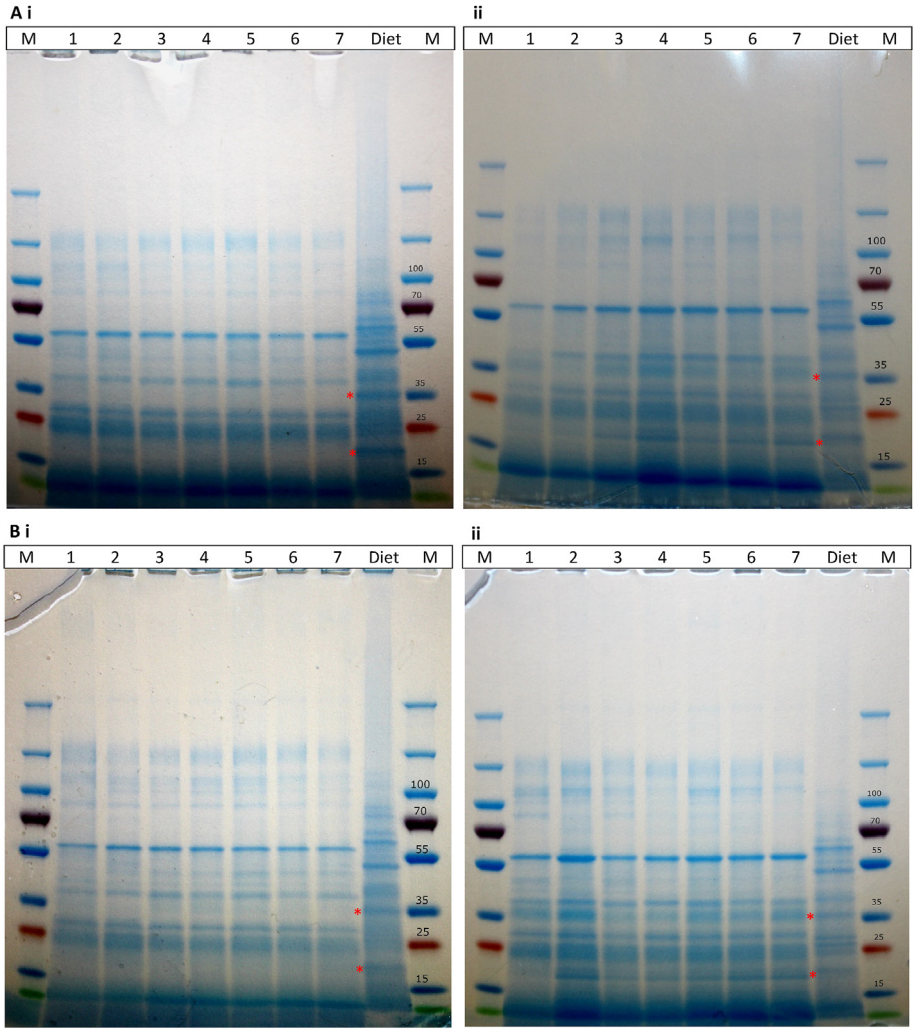

Fig. 1. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of ileal digesta taken from broilers fed diets without (A) or with (B) xylanase supplementation and no treatment (i) or treatment (ii) with a proton pump inhibitor (PPI). Gels present the profiles of ileal digesta pools of 7 replicate pens and the respective diet. Lane M shows the 10 to 250 kDa molecular weight marker. Asterisks denote where bands are missing or less concentrated in the non-PPI treated groups but present with PPI treatment.

Table 8
Effects of dietary treatments[1] on putrefaction end products in the caecal digesta of broiler chickens measured at 21 d of age.[2]

Xylanase	PPI	Indole, mg/g DM	Phenol, mg/g DM	p-Cresol, mg/g DM	Skatole, mg/g DM	NH_3, mmol/L
Xylanase effect						
-Xyl		24.35	6.88	9.80	0.28	1.56
+Xyl		28.40	7.43	9.49	0.29	1.95
PPI effect						
	-PPI	17.42[b]	7.25	2.25[b]	0.28	1.43[b]
	+PPI	35.32[a]	7.05	17.05[a]	0.30	2.08[a]
Xylanase × PPI						
-Xyl	-PPI	15.34	6.90	2.22	0.29	1.26
	+PPI	33.35	6.85	17.38	0.28	1.86
+Xyl	-PPI	19.51	7.61	2.27	0.28	1.60
	+PPI	37.28	7.25	16.71	0.31	2.30
Pooled SD		8.59	1.93	8.03	0.05	0.73
P-value						
Xylanase		0.243	0.475	0.923	0.549	0.174
PPI		<0.001	0.790	<0.001	0.443	0.028
Xylanase × PPI		0.971	0.840	0.911	0.298	0.856

DM = dry matter; NH_3 = ammonia; PPI = proton pump inhibitor; SD = standard deviation.
[a, b]Values within a column with different superscripts are significantly different ($P < 0.05$).
[1] Treatments: CTR (control diet), PPI (CTR diet supplemented with Esomeprazole, a proton pump inhibitor, from d 14 to 21), Xyl (CTR diet supplemented with 16,000 U/kg of xylanase from d 0 to 21), and Xyl + PPI (CTR diet supplemented with both xylanase and PPI, the latter only supplemented from d 14 to 21).
[2] Mean values for 7 replicate pens with the pool of 5 birds per replicate pen.

Table 9
Effects of dietary treatments[1] on the fermentation activity in the caecal digesta of broiler chickens measured at 21 d of age.[2]

Xylanase	PPI	Caecal digesta			
		Total VFA, mmol/L	Lactic acid, mmol/L	VFA-to-Butyric acid ratio	VFA-to-BCFA ratio
Xylanase effect					
-Xyl		58.93	7.59	6.32	69.15
+Xyl		61.05	7.14	6.16	78.49
PPI effect					
	-PPI	61.19	7.68	5.84[b]	114.68[a]
	+PPI	58.78	7.05	6.64[a]	32.96[b]
Xylanase × PPI					
-Xyl	-PPI	58.82	8.08	6.07	104.22
	+PPI	59.04	7.11	6.57	34.09
+Xyl	-PPI	63.57	7.27	5.61	125.15
	+PPI	58.52	7.00	6.71	31.84
Pooled SD		7.96	1.51	0.665	37.5
P-Value					
Xylanase		0.499	0.441	0.538	0.525
PPI		0.441	0.298	0.005	<0.001
Xylanase × PPI		0.400	0.560	0.254	0.432

VFA = volatile fatty acids (acetic, propionic, butyric, valeric, iso-butyric, 2-me-butyric and iso-valeric); BCFA = branched-chain fatty acids (isobutyrate, 2-methyl butyrate and isovalerate); PPI = proton pump inhibitor.
[a, b]Values within a column with different superscripts are significantly different ($P < 0.05$).
[1] Treatments: CTR (control diet), PPI (CTR diet supplemented with Esomeprazole, a proton pump inhibitor, from d 14 to 21), Xyl (CTR diet supplemented with 16,000 U/kg of xylanase from d 0 to 21), and Xyl + PPI (CTR diet supplemented with both xylanase and PPI, the latter only supplemented from d 14 to 21).
[2] Mean values for 7 replicate pens with the pool of 5 birds per replicate pen.

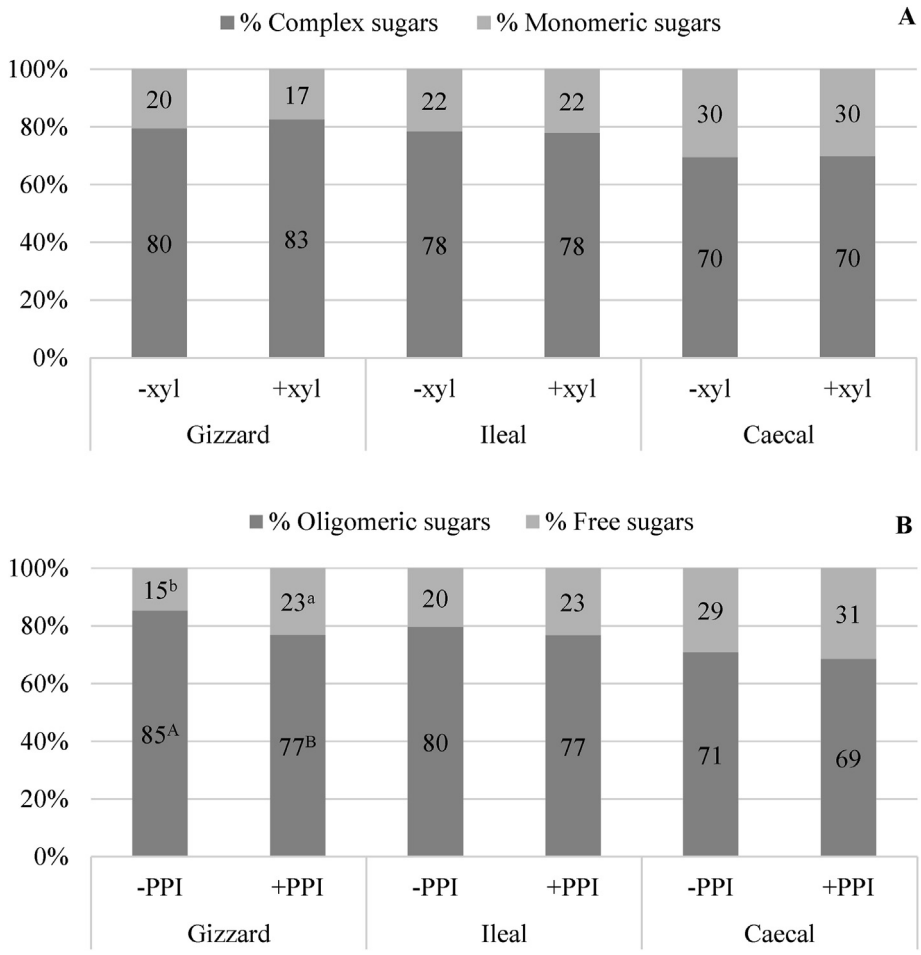

Fig. 2. Relative amount of complex sugars and monomeric sugars (%) in gizzard, ileal and caecal digesta of broilers fed diets supplemented with (+Xyl) or without (-Xyl) xylanase (A) and treated (+PPI) or not (-PPI) with a proton pump inhibitor (B). For each group of sugars, different superscripts in a gastrointestinal section significantly differ at $P < 0.05$.

Table 10
Effect of dietary treatments[1] on the total soluble sugars (g/kg DM) including monomeric sugars and complex sugars in the gizzard, ileal and caecal digesta of broiler chickens measured at 21 d of age.[2]

Item		Xylanase effect		PPI effect		Xylanase × PPI				PooledSD	*P*-value		
		-Xyl	+Xyl	-PPI	+PPI	-Xyl -PPI	-Xyl +PPI	+Xyl -PPI	+Xyl +PPI		Xylanase	PPI	Xylanase × PPI
Gizzard	Xylose	2.27[b]	4.35[a]	2.57[b]	4.05[a]	1.59	2.94	3.54	5.16	1.047	<0.001	0.001	0.735
	Arabinose	1.75[b]	2.81[a]	1.76[b]	2.80[a]	1.29	2.20	2.22	3.40	0.735	0.001	0.001	0.626
	Xylose + Arabinose	4.01[b]	7.16[a]	4.32[b]	6.85[a]	2.89	5.14	5.76	8.56	1.772	<0.001	0.001	0.688
	Mannose	0.64	0.70	0.55[b]	0.79[a]	0.52	0.76	0.58	0.81	0.148	0.318	<0.001	0.890
	Galactose	4.74	5.21	4.18[b]	5.77[a]	4.00	5.48	4.36	6.06	1.247	0.331	0.003	0.816
	Glucose	9.89	9.99	7.10[b]	12.78[a]	6.79	12.98	7.41	12.57	2.461	0.912	<0.001	0.587
	Inositol	0.58	0.61	0.46[b]	0.74[a]	0.39	0.77	0.53	0.70	0.181	0.672	0.001	0.138
	Total	19.87	23.67	16.62[b]	26.92[a]	14.60	25.13	18.64	28.70	5.381	0.074	<0.001	0.907
Ileum	Xylose	10.05[b]	17.22[a]	15.21	12.07	11.68	8.43	18.74	15.70	5.381	0.002	0.144	0.961
	Arabinose	7.81[b]	11.73[a]	11.13	8.41	9.28	6.34	12.98	10.47	3.455	0.007	0.053	0.875
	Xylose + Arabinose	16.81[b]	28.95[a]	26.34	19.42	20.95	12.67	31.72	26.18	9.072	0.002	0.055	0.693
	Mannose	4.22	4.76	5.23[a]	3.75[b]	5.07	3.37	5.40	4.12	1.341	0.307	0.009	0.695
	Galactose	24.60	26.29	31.58[a]	19.31[b]	31.75	17.44	31.40	21.17	8.719	0.620	0.001	0.551
	Glucose	29.87	28.14	35.41[a]	22.60[b]	37.24	22.51	33.58	22.70	11.012	0.687	0.006	0.655
	Inositol	3.85[b]	4.74[a]	4.52	4.06	4.01	3.69	5.04	4.43	1.022	0.035	0.256	0.715
	Total	80.40	92.87	103.08[a]	70.20[b]	99.01	61.80	107.15	78.60	27.402	0.250	0.005	0.686
Caeca	Xylose	2.65	2.89	3.50[a]	2.05[b]	3.60	1.71	3.39	2.38	1.486	0.680	0.016	0.437
	Arabinose	2.00	2.26	2.59[a]	1.68[b]	2.56	1.45	2.62	1.90	0.856	0.435	0.010	0.546
	Xylose + Arabinose	4.66	5.15	6.09[a]	3.72[b]	6.16	3.15	6.01	4.29	2.326	0.581	0.013	0.473
	Mannose	2.28	2.41	2.63[a]	2.06[b]	2.57	1.99	2.68	2.13	0.598	0.575	0.020	0.959
	Galactose	7.43	7.75	8.43[a]	6.74[b]	8.35	6.52	8.52	6.97	1.959	0.676	0.032	0.853
	Glucose	11.91	11.18	13.41[a]	9.68[b]	14.12	9.70	12.70	9.66	3.347	0.570	0.007	0.590
	Inositol	2.57	2.18	2.15	2.60	2.39	2.75	1.91	2.45	0.744	0.179	0.123	0.764
	Total	28.84	28.66	32.70[a]	24.80[b]	33.58	24.11	31.83	25.50	7.653	0.950	0.012	0.593

DM = dry matter; PPI = proton pump inhibitor; SD = standard deviation.
[a, b]Values within a row with different superscripts are significantly different ($P < 0.05$).
[1] Treatments: CTR (control diet), PPI (CTR diet supplemented with Esomeprazole, a proton pump inhibitor, from d 14 to 21), Xyl (CTR diet supplemented with 16,000 U/kg of xylanase from d 0 to 21), and Xyl + PPI (CTR diet supplemented with both xylanase and PPI, the latter only supplemented from d 14 to 21).
[2] Mean values from pooled samples from 5 birds from each of the 7 replicate pens per treatment.

Table 11
Effect of dietary treatments[1] on the complex sugars[2] (g/kg DM) in the gizzard, ileal and caecal digesta of broiler chickens measured at 21 d of age.[3]

Item		Xylanase effect		PPI effect		Xylanase × PPI				Pooled SD	*P*-value		
		-Xyl	+Xyl	-PPI	+PPI	-Xyl -PPI	-Xyl +PPI	+Xyl -PPI	+Xyl +PPI		Xylanase	PPI	Xylanase × PPI
Gizzard	Xylose	2.14[b]	4.15[a]	2.47[b]	3.83[a]	1.51	2.78	3.42	4.88	0.999	<0.001	0.002	0.799
	Arabinose	1.57[b]	2.53[a]	1.64[b]	2.47[a]	1.20	1.94	2.08	2.99	0.616	0.001	0.002	0.717
	Xylose + Arabinose	3.38[b]	6.69[a]	4.11[b]	5.96[a]	2.71	4.04	5.50	7.87	1.808	<0.001	0.012	0.451
	Mannose	0.56	0.60	0.46[b]	0.71[a]	0.45	0.68	0.48	0.73	0.152	0.497	<0.001	0.849
	Galactose	4.20	4.68	4.00[b]	4.88[a]	3.84	4.56	4.16	5.20	1.015	0.228	0.034	0.696
	Glucose	6.95	7.14	5.59[b]	8.50[a]	5.18	8.72	6.00	8.28	1.410	0.731	<0.001	0.256
	Inositol	0.07	0.08	0.09	0.07	0.06	0.08	0.11	0.05	0.077	0.678	0.578	0.165
	Total	15.50[b]	19.20[a]	14.24[b]	20.46[a]	12.23	18.77	16.25	22.14	3.632	0.015	<0.001	0.820
Ileum	Xylose	10.12[b]	16.79[a]	15.05	11.86	11.55	8.69	18.55	15.02	5.477	0.006	0.156	0.879
	Arabinose	7.62[b]	10.89[a]	10.68[a]	7.83[b]	9.03	6.21	12.34	9.45	3.457	0.026	0.049	0.977
	Xylose + Arabinose	15.61[b]	27.68[a]	25.73[a]	17.56[b]	20.58	10.65	30.89	24.47	9.274	0.002	0.028	0.622
	Mannose	4.12	4.62	5.08[a]	3.66[b]	4.93	3.31	5.24	4.00	1.358	0.362	0.015	0.721
	Galactose	23.49	24.55	30.91[a]	17.12[b]	31.30	15.67	30.51	18.58	9.219	0.775	0.001	0.617
	Glucose	16.99	17.81	22.02[a]	12.78[b]	22.76	11.22	21.27	14.34	7.926	0.797	0.008	0.471
	Inositol	0.31	0.07	0.18	0.20	0.42	0.20	−0.06	0.19	0.851	0.475	0.959	0.500
	Total	62.64	74.72	83.92[a]	53.44[b]	79.98	45.30	87.85	61.59	26.801	0.268	0.009	0.696
Caecal	Xylose	1.84	1.89	2.37[a]	1.36[b]	2.47	1.21	2.28	1.50	0.961	0.894	0.010	0.514
	Arabinose	1.29	1.55	1.70[a]	1.14[b]	1.58	1.00	1.82	1.28	0.485	0.165	0.005	0.926
	Xylose + Arabinose	3.13	3.44	4.07[a]	2.49[b]	4.04	2.21	4.10	2.78	1.429	0.570	0.008	0.638
	Mannose	1.99	2.13	2.33[a]	1.80[b]	2.24	1.74	2.41	1.85	0.545	0.502	0.017	0.898
	Galactose	6.18	6.57	6.97[a]	5.78[b]	6.73	5.63	7.22	5.92	1.528	0.507	0.050	0.869
	Glucose	8.66	8.02	9.77[a]	6.91[b]	10.26	7.07	9.29	6.74	2.636	0.522	0.008	0.751
	Inositol	0.12	−0.07	0.07	−0.02	0.14	0.10	0.00	−0.14	0.346	0.169	0.512	0.697
	Total	20.08	20.09	23.21[a]	16.96[b]	23.41	16.75	23.01	17.16	5.457	0.997	0.006	0.847

DM = dry matter; PPI = proton pump inhibitor; SD = standard deviation.
[a, b]Values within a row with different superscripts are significantly different ($P < 0.05$).
[1] Treatments: CTR (control diet), PPI (CTR diet supplemented with Esomeprazole, a proton pump inhibitor, from d 14 to 21), Xyl (CTR diet supplemented with 16,000 U/kg of xylanase from d 0 to 21), and Xyl + PPI (CTR diet supplemented with both xylanase and PPI, the latter only supplemented from d 14 to 21).
[2] Complex sugars refers to the total soluble sugars minus the monomeric sugars and thus represents oligomers with a degree of polymerisation of 2 or greater.
[3] Mean values from pooled samples from 5 birds from each of the 7 replicate pens per treatment.

identified in this study, previous work has shown similar bands in soybean meal fed broilers (Recoules et al., 2017). A protein band at 18 kDa was found to be specific to soybean meal, which belong to several glycinin- and conglycinin-derived proteins, with the Kunitz trypsin inhibitor being the predominant protein fraction, followed by Glycinin G1. In the same study, endogenous proteins involved in protein digestion were identified in a band at 36 kDa (Recoules et al., 2017). The concentration of both of these fractions appeared to increase from the duodenum to the jejunum, the major site of protein digestion and absorption, and then decrease again by the ileum, particularly that of the 36 kDa band which almost disappears. In the current study, these protein bands remained in the ileal digesta when birds were treated with PPI which might reflect enhanced pancreatic secretion of digestive enzymes and inefficient protein digestion along the small intestine. This is also supported by the higher soluble protein concentrations measured in ileal digesta of broilers treated with PPI. These findings demonstrate that protein digestion in the small intestine cannot fully compensate for inefficient digestion in the gastric phase with PPI and that the increased ileal soluble protein content may be due to increased levels of undigested dietary protein or endogenous secretions produced in an attempt to digest the excess protein.

A large part of the undigested bypass protein will serve as a substrate for protein fermentation in the caeca. Protein fermentation can yield several specific end-products such as ammonia (NH_3), BCFA, amines, phenols and indoles (Apajalahti and Vienola, 2016; Qaisrani et al., 2014) and these may have a deleterious effect on the gut environment and performance of animals (Thomke and Elwinger, 1998). In this study, increased concentrations of indole, p-cresol and NH_3 in the caeca of PPI-supplemented birds were observed. The presence of these putrefactive compounds in the caeca of broilers indicates the fermentation of tyrosine to p-cresol and tryptophan to indole (Windey et al., 2012) by specific groups of bacteria such as Clostridiaceae, Bacteroidaceae and *Staphylococci* (Terada et al., 1994). Moreover the deamination of excess AA results in the production of ammonia, a toxic waste product of microbial fermentation with a very high energy cost for the animal resulting in poor performance (Namroud et al., 2008). Further evidence of caecal protein fermentation is given by the lower VFA-to-BCFA ratio in PPI fed birds which suggests a displacement of fibre fermentation by protein fermentation (Cho et al., 2020). Fermentation of valine, isoleucine and leucine results in the formation of BCFA (iso-butyrate, 2-methyl butyrate and isovalerate, respectively). The presence of BCFA in the hindgut which are produced by many bacteria including *Bacteroides* spp., *Propionibacterium* spp., *Streptococcus* spp. and *Clostridium* spp. (Gilbert et al., 2018), has been associated with post-weaning diarrhoea in pigs (Kim et al., 2008) and a similar response is thought to occur in poultry (Marks and Pesti, 1984). On the other hand, the main metabolic end-product of fibre fermentation in the caeca are VFA (acetic acid, propionic acid and butyric acid), representing more than 80% of the total SCFA in broilers (González-Ortiz et al., 2019). Butyric acid is the most important VFA because it is the preferred energy source for colonocytes and it maintains intestinal epithelial cell integrity (Lopetuso et al., 2013). In this study, the elevated VFA-to-butyric acid ratio in the caecal contents of the PPI-treated birds indicates butyric acid production relative to other VFA was proportionally depressed.

The total soluble sugars and the complex sugars were increased in the gizzard with PPI application suggesting that fibre solubilisation was enhanced in the upper gut when gastric pH was increased. Under PPI administration it may be expected that the microbial load in the upper GIT increased, as acid-sensitive bacteria were able to grow and possibly degrade fibre components more anterior in the intestinal tract than is normally the case. Alternatively, the increased gizzard pH may have forced a feedback loop for a longer retention time of feed in the upper GIT in an effort to increase digestibility, which would hold back more feed in the crop where fermentation may result in greater fibre hydrolysis. In contrast, the content of sugars in the ileal and caecal digesta were reduced with PPI supplementation suggesting reduced release of sugars or enhanced ileal and caecal fermentation of the soluble sugars or both. Under such circumstances, it can be hypothesized that the caecal microbial communities would move to a greater dependence on protein compared with fibre as an energy source (Apajalahti and Vienola, 2016). Whilst some colonic bacteria depend obligatorily on AA, others depend on both AA and sugars for their energy and metabolism requirements, with a distinct preference for sugars if both are available (Roberfroid, 2007).

4.2. Effects of xylanase and the interaction with the proton pump inhibitor on animal performance and gut function

Increased nutrient digestibility and growth performance has been demonstrated in broiler chickens fed wheat-based diets supplemented with a xylanase (González-Ortiz et al., 2016), however in this study, Xyl had no effect on weight gain or feed efficiency in d 21 broiler chickens, except in the presence of PPI where Xyl improved FCR. There are several factors that may have influenced this result, likely related with the availability of substrate for xylanase and the length of the study (related to the age of birds) (Lee et al., 2017).

In general, today's wheat varieties have lower viscosities than they did 20 years ago. Previous studies have shown a positive relationship between enzyme efficacy and NSP concentration in different wheat cultivars (Smeets et al., 2018). The content of total AX in wheat used in the diet was low to normal according to previous data from the literature (Gomes et al., 2020; Knudsen, 1997). Therefore, this suggests that viscosity may not have been a significant issue in this study. In comparison, under the right conditions xylanase has been shown to significantly reduce digesta viscosity, and as a result increase digestibility and retention of nutrients such as protein, leading to improvements in weight gain and feed efficiency of young broilers (Esmaeilipour et al., 2011). This might also explain why Xyl supplementation under the conditions of the current study had no effect on intake of digestible DM or protein. Although viscosity reduction may have not been a major response to Xyl in this study, another important role of xylanase is the production of AXOS, a substrate for microbial fermentation in the caeca. However, age has a fundamental role in the maturation of the GIT and the ability of microbiota in the hindgut to ferment AX (Bautil et al., 2019) which may explain the limited benefits of the xylanase in this study since it only went to 21 d of age. At such a young age the caeca may have not reached full maturity to fully benefit from the substrates made available by Xyl supplementation to elicit a boost in performance (Lee et al., 2017).

Xylanase activity in the gizzard was relatively low and comparable between treatments and no differences were observed on the relative proportion of total soluble sugars vs. the complex sugars in the upper GIT. However, supplementing Xyl significantly increased the amount of soluble xylose and arabinose, which would indicate enhanced solubilisation of insoluble dietary AX. Since solubilisation of other soluble sugars were unaffected by Xyl supplementation it could be assumed that this activity was specific to AX. These results may support the work of Morgan et al. (2017) who observed that the greatest conversion of AX into XOS or AXOS was observed at pH 2.5. Zhang et al. (2014) also found higher soluble xylose and arabinose concentrations in the gizzard digesta of Xyl-supplemented birds. Even though the consequences for the animal of the production of these sugars in the upper GIT are not known yet, these

results suggest that the AX solubilisation activity by Xyl starts earlier than traditionally thought.

Xylanase activity in the ileal and caecal digesta were around 6 and 2 times higher, respectively, in birds fed Xyl compared to the non-supplemented birds. Similarly, Silva and Smithard (2002) showed that xylanase activity in the small intestine was representative of the quantities added in the feed. In this study, the xylanase activity results coincided with an increased concentration of total soluble xylose, arabinose and inositol in the ileal digesta in particular with an increased concentration of complex sugars including xylose and arabinose. Monomeric xylose and arabinose in the ileal or the caecal digesta were not increased with Xyl supplementation (data not shown) highlighting the preferential output of complex sugars from DP 2 or greater with this xylanase product as suggested in other studies (Dale et al., 2019; Melo-Durán et al., 2021; Morgan et al., 2019). As expected, the optimal pH in the small intestine favours the activity of xylanase, either supplemented in the feed or derived from the activity of the microbiota. It has been shown that many ileal species can produce such xylanases and in particular several *Bifidobacterium* species have an inherent appetite for AX, producing endogenous xylanase and other fibrolytic enzymes (De Vuyst and Leroy, 2019; Van Craeyveld et al., 2008). The supplementation of xylanase-end products such as XOS to weaned piglets has also been shown to increase the activity of xylanase in the small intestine and in the colon (Marinho et al., 2007), suggesting these products stimulate the microbiome to produce this enzyme in what appears to be a positive feedback. Furthermore, the noted increase in inositol concentrations observed in the ileal digesta of birds supplemented with Xyl suggests a more complete destruction of phytic acid, perhaps due to greater accessibility of dietary phytase to phytate as a result of phytate released from the fibre matrix (Kuhn et al., 2017; Lee et al., 2018b).

The concentration of total soluble sugars or the complex sugars in the caecal content were not influenced by Xyl supplementation which is in agreement with Zhang et al. (2014), even though there was considerably higher xylanase activity found in this site. The 20 fold + increments in the xylanase activity noted in the caeca of both control and xylanase treated birds compared with the ileum is likely attributed to microbial production of xylanase. Nonetheless, birds supplemented with Xyl still had significantly higher activities in the caeca compared to non-supplemented birds, which could be a combination of the supplemented xylanase and enhanced microbial xylanase production in response to the presence of greater quantities of substrate provided by exogenous xylanase action in the proximal tract. The fact that soluble sugar concentrations were not altered may indicate the utilisation of the soluble sugars was as rapid or even faster than their production when xylanases was fed.

5. Conclusion

In conclusion, the current study indicates that arabinoxylan solubilisation starts in the upper gastrointestinal section through xylanase supplementation. Elevation of gizzard pH with a proton pump inhibitor also allowed for increased arabinoxylan solubilisation in this section of the gastrointestinal tract, although it is unclear whether this is due to the physico–chemical interaction with gastric juices or microbial activity. Xylanase supplementation partially compensated for the negative effects of the proton pump inhibitor on feed efficiency. This is of particular importance in the utilisation of xylanase as the buffering capacity of some ingredients may have little impact on its activity. This study also highlights the significant impact of acid secretion on gastric protein digestion, especially with respect to the consequences of the delivery of undigested protein to the lower gut. Incomplete gastric protein digestion cannot be compensated for by pancreatic enzyme release with the consequence not only being impaired protein digestion and utilisation, but also the generation of toxic metabolites in the lower intestine from the excess protein provided as a result of maldigestion. This work highlights the need to consider the buffering capacity of the diet very carefully, particularly in antimicrobial growth promoter free diets, where the consequence of excess limestone, for example, may result in significantly greater risk of putrefaction of proteins in the caeca. In diets devoid of antimicrobial growth promoters, such effects could result in significant disease outbreaks.

Author contributions

Gemma González-Ortiz, Michael R. Bedford, Kirsi Vienola and **Juha Apajalahti** designed the study. **Kirsi Vienola, Kari Raatikainen, German Jurgens, Juha Apajalahti** were involved in the development of the study and the analysis of the samples. **Gemma González-Ortiz** carried out the statistical analysis of the data and all authors contributed in the data interpretation. **Gemma González-Ortiz** wrote the first draft of the manuscript. All authors critically revised the manuscript and gave final approval of the document before submission.

Declaration of competing interest

Gemma González-Ortiz, **Sophie A. Lee** and **Michael R. Bedford** work for AB Vista, who supplied the xylanase (Econase XT) used in this trial. **Kirsi Vienola**, **Kari Raatikainen**, **German Jurgens** and **Juha Apajalahti** declare there is no conflict of interest.

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