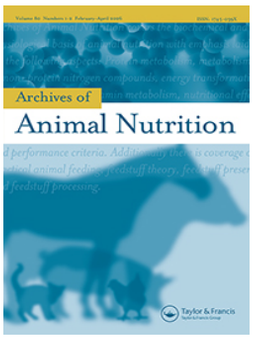

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The use of xylanase enzyme and inulin prebiotic supplementation to support broiler chicken production performance

Vasil Radoslavov Pirgozliev[a], Isobel Margaret Whiting[a], Stephen Charles Mansbridge[a], Kristina Kljak[b], Heloisa Helena de Carvalho Mello[c], José Henrique Stringhini[c], Amy E. Johnson[d], Falko Drijfhout[d], David Yovchev[e], Atanas Georgiev Atanasov[f,g,h], Zhi Yang[a,i] and Stephen Paul Rose[a]

[a]National Institute of Poultry Husbandry, Harper Adams University, Shropshire, UK; [b]Department of Animal Nutrition, Faculty of Agriculture, University of Zagreb, Zagreb, Croatia; [c]Escola de Veterinária e Zootecnia, Universidade Federal de Goiás, Goiânia, Brasil; [d]School of Chemical and Physical Sciences, Keele University, Staffordshire, UK; [e]Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria; [f]Ludwig Boltzmann Institute for Digital Health and Patient Safety, Medical University of Vienna, Vienna, Austria; [g]Laboratory of Natural Products and Medicinal Chemistry (LNPMC), Center for Global Health Research, Saveetha Medical College and Hospital, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, India; [h]Department of Biotechnology and Nutrigenomics, Institute of Genetics and Animal Biotechnology of the Polish Academy of Sciences, Magdalenka, Poland; [i]College of Animal Science and Technology, Yangzhou University, Yangzhou, Jiangsu, P. R. China

ABSTRACT

Two isoenergetic (12.05 MJ/kg ME) and isonitrogenic (approximately 200 g/kg CP) basal diets were prepared using 670 g/kg of wheat with low fibre and/or high fibre contents. Each basal diet was divided into four portions: one remained as is and fed as control (C); the second was the C supplemented with 100 FXU/kg of a commercial xylanase (XYL); the third was the C plus 20 g/kg of inulin (IN) powder; and the fourth was the C supplemented with both XYL and IN at the same inclusion rates, resulting in a total of eight experimental diets. A study was conducted from 10 to 21 days of age involving 320 female Ross 308 broiler chickens. Each diet, in meal form, was fed *ad libitum* to eight pens, five birds each, following randomisation. Supplementary XYL increased dietary nitrogen corrected apparent metabolisable energy (AMEn) ($p < 0.001$), dry matter retention (DMR), daily feed intake (FI), weight gain (WG) and reduced feed conversion ratio (FCR) ($p < 0.05$). Dietary IN tended ($p < 0.01$) to increase WG and reduce FCR and dietary wheat did not have an impact on growth performance variables ($p > 0.05$). Birds fed XYL had reduced relative weight of the pancreas ($p < 0.050$) and there was XYL by IN interaction ($p < 0.05$) on relative weight of the caeca as it was greater for those fed XYL and IN. No other changes in relative weight of the gastrointestinal tract organs were observed

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CONTACT Vasil Radoslavov Pirgozliev vpirgozliev@harper-adams.ac.uk

($p > 0.05$). Bird fed XYL or IN had greater ($p < 0.05$) butyric acid (BA) concentration in caecal content. Feeding XYL led to reduced acetic (AA) and propionic acid (PA) concentrations in caecal excreta ($p < 0.05$) and to a greater BA:AA ratio ($p < 0.001$). Dietary IN increased blood serum glutathione peroxidase (GSH-Px) ($p < 0.05$) and XYL increased concentration of hepatic coenzyme Q_{10} ($p < 0.05$). The type of dietary wheat did not have an impact on any of the studied variables, suggesting that birds were able to tolerate the fibre contents in this study. It seems that both IN and XYL, can serve as feed enhancers that potentially may promote antioxidant status of birds and help poultry to cope with various stress factors during production. The study further confirms that supplementing wheat-based diets with XYL may be a strategy to mitigate the reduction in available energy and to increase nutrient availability in broiler diets.

1. Introduction

The cost of feed is the single largest expense in poultry production, typically accounting for 60–70% of total production costs (Alqaisi et al. 2017). Nutrition is an important part of poultry production, aiming to optimise growth performance, feed efficiency, and overall health while reducing cost of production and minimising environmental impact (Pirgozliev and Bedford 2013). Cereal grains, such as wheat, are commonly used as energy sources in broiler diets due to their high starch content (Kiarie et al. 2014). However, the nutritional value of wheat can vary considerably depending on its non-starch polysaccharide (NSP) content and solubility (Pirgozliev et al. 2023). Wheat with high soluble NSP content tends to increase the viscosity in small intestinal digesta in poultry, impairing nutrient digestion and absorption resulting in reduced feed efficiency and growth performance (Whiting et al. 2017; Bedford 2018). In contrast, low-viscosity wheat cultivars typically result in greater nutrient digestibility and broiler performance due to their lower soluble NSP content and reduced impact on digesta flow.

To counter the negative viscosity effects, exogenous enzymes such as xylanase (XYL) are commonly added to poultry diets (Bedford 2018). Xylanase specifically targets arabinoxylans, the predominant NSP in cereals like wheat and barley, breaking them down into smaller oligosaccharides and reducing intestinal viscosity (Bedford 2018). This enzymatic action improves nutrient availability and may also generate prebiotic compounds that support beneficial gut microbiota (Kiarie et al. 2014; Bedford 2018). In addition to enzyme supplementation, prebiotics like inulin (IN) have gained attention for their potential to enhance gut health and nutrient absorption (Bucław 2016). Inulin, a fructan-type carbohydrate derived from plants such as chicory, resists digestion in the upper gastrointestinal tract and is fermented in the hindgut, promoting the growth of beneficial bacteria such as *Bifidobacteria* and *Lactobacilli*, increased antioxidant capacity and bird growth (Rebolé et al. 2010; Shang et al. 2018; Untea et al. 2023). Similarly, XYL can also increase caecal butyric acid (BA) concentration, antioxidant status (Pirgozliev et al. 2023) and bird growth (Bedford 2018). Feeding high viscous wheat, however, usually reduces bird's antioxidant capacity (Pirgozliev et al. 2023) and impairs growth (Whiting et al. 2017).

Simultaneous use of various feed additives and supplements in diets, including XYL and prebiotics, is common practice in commercial poultry production (Singh et al. 2021; Šimić et al. 2023). However, information on the response of broiler chickens to inclusion of prebiotics, alone or in combination with exogenous XYL is inconsistent. Rebolé et al. (2010) feeding wheat-barley based diet, found no response to XYL, or IN by XYL interaction in growth performance or caecal short chain fatty acids (SCFA) concentration in broilers, although broilers fed IN only grew better and there was greater BA caecal concentration. There was no response to xylooligosaccharides. (XOS), or XOS by XYL interaction in growth performance or caecal SCFA concentration in maize-based diets, however broilers fed XYL only had greater daily weight gain, lower feed efficiency and greater caecal SCFA production (Singh et al. 2021). Recent research (Šimić et al. 2023) with maize-based diets showed greater weight gain and feed efficiency in broilers fed a combination of XOS and XYL, compared to their individual use, but there was no response in caecal SCFA fermentation. Although many factors may have influenced the responses observed, it seems that dietary fibre, in particular soluble NSP, may have a role to play in the responses. The production of XOS in the caeca, due to XYL action, provides a source of prebiotics (Šimić et al. 2023), which may provide an alternative substrate to other supplementary prebiotics and support a wider diversity of SCFA producing microorganisms in the caeca.

Therefore, the objective of the current study was to examine the effects of IN and XYL, individually or in combination, on growth performance, dietary energy and nutrient availability, growth of GIT segments and jejunal villus morphometry, hepatic antioxidants and caecal volatile fatty acids (VFA) concentration in broiler chickens fed diets based on high soluble NSP (HSW) or low soluble NSP (LSW) wheat samples.

2. Materials and methods

2.1. Wheat samples

The chemical composition and quality parameters of the two wheat samples used in this study are shown in Table 1. The selection criteria were the similar proximate composition of the samples and the differences in soluble NSP content. One of the samples, i.e. LSW, contained 13 g/kg, whereas the other sample, HSW, contained over 33 g/kg soluble NSP.

Table 1. Analysed chemical composition of low (LSW) or high (HSW) in soluble non-starch polysaccharides wheat samples.

Item	LSW	HSW
Dry matter [g/kg]	887	885
Crude protein [g/kg]	118	126
Crude fat [g/kg]	15.4	9.8
Ash [g/kg]	15.6	17.4
Starch [g/kg]	718	674
Total non-starch polysaccharides [NSPt] [g/kg]	85.5	106.8
Soluble non-starch polysaccharides [NSPs] [g/kg]	13.0	33.5
Insoluble non-starch polysaccharides [NSPi] [g/kg]	72.5	73.3
Gross energy [MJ/kg]	15.83	15.81

2.2. Experimental diets

Two basal diets were prepared using 670 g/kg of each experimental wheat sample (Table 2). To highlight the impact of supplementary XYL, both diets were formulated to be slightly lower in crude protein (CP; 194 and 202 g/kg, respectively) and metabolisable energy (ME; 12.05 MJ/kg) than the breeder recommendations for Ross 308 broilers (Aviagen Ltd., Edinburgh, UK). Each basal diet was divided into four equal portions: one remained as is and fed as control (C); the second was the C supplemented with 100 FXU/kg of a commercial XYL (Ronozyme WX (CT), DSM, Switzerland); the third was the C plus 20 g/kg of IN powder (Bioglan®, Holland & Barrett, UK); and the fourth was the C supplemented with both XYL and IN at the same inclusion rates. This resulted in a total of eight experimental diets. All diets included 5 g/kg titanium dioxide (TiO_2) as an indigestible marker. No adjustments were made for minor differences in protein or dry matter content between the wheat samples and IN supplementation.

2.3. Husbandry and sample collection

The experiment was conducted at the National Institute of Poultry Husbandry and approved by the Harper Adams University Research Ethics Committee (Project number 0001-201901-STAFF,16 January 2019) and is reported here in accordance with the

Table 2. Ingredient composition (g/kg, as-fed) of the experimental broiler chicken diets formulated with either low (LSW) or high (HSW) in soluble non-starch polysaccharides wheat samples.

Dietary ingredients	LSW diet [kg]/100[kg]	HSW diet [kg]/100[kg]
LSW	67.00	–
HSW	–	67.00
Soybean meal (48 Crude Protein)	21.97	21.97
Soybean meal (Full Fat)	5.00	5.00
Rapeseed oil	2.00	2.00
Dicalcium phosphate	1.45	1.45
Limestone	1.25	1.25
NaCl	0.27	0.27
HCL Lysine	0.27	0.27
DL Methionine	0.39	0.39
Vitamin mineral premix[a]	0.40	0.40
Calculated analysis (as fed)		
Crude Protein g/[kg]	194	202
Metabolisable Energy MJ/[kg]	12.05	12.05
Crude Fat g/[kg]	40.1	41.8
Ca g/[kg]	8.9	8.8
Available P g/[kg]	4.6	4.6
Lysine g/[kg]	13.0	13.0
Methionine + Cysteine g/[kg]	9.3	9.3
Soluble non-starch polysaccharides [g/kg]	18.7	32.5
Insoluble non-starch polysaccharides [g/kg]	79.0	79.5
Determined values (as fed)		
Dry matter g/[kg]	900	907
Gross Energy MJ/[kg]	16.40	16.44
Crude Protein g/[kg]	189	196
Crude Fat g/[kg]	39.4	41.1

[a]The Vitamin and mineral premix contained vitamins and trace elements to meet the requirements specified by NRC (1994). All the experimental diets were designed to be low in P. The premix provided (units/kg diet): retinol 3600 µg, cholecalciferol 125 µg, α-tocopherol 34 mg, menadione 3 mg, thiamine 2 mg, riboflavin 7 mg, pyridoxine 5 mg, cobalamin 15 µg, nicotinic acid 50 mg, pantotenic acid 15 mg, folic acid 1 mg, biotin 200 µg, iron 80 mg, copper 10 mg, manganese 100 mg, cobalt 0.5 mg, zinc 80 mg, iodine 1 mg, selenium 0.2 mg and molybdenium 0.5 mg.

ARRIVE 2.0 guidelines (Percie du Sert et al. 2020). A total of 320 female Ross 308 broilers (Cyril Bason Ltd, Craven Arms, UK) were sourced from a commercial hatchery. On arrival, all chicks were housed in a single floor pen bedded with wood shavings and fed a proprietary starter diet containing 12.55 MJ/kg ME and 230 g/kg CP. At 10 days of age, birds were randomly allocated to 64 raised floor pens (60 × 60 cm; 5 birds per pen). Each pen was equipped with a front feeder tray and two nipple drinkers, with solid floors covered by cardboard. The eight experimental diets were each assigned to eight pens following randomisation. The house temperature at day old was 32°C and was gradually reduced to 20°C after birds were 20 d of age. A standard lighting programme for broilers was used, decreasing from 23:1 (hours light: dark) from day old to 18:6 at 7 days of age, which was maintained until the end of the study. The house relative humidity was maintained between 50% and 70%. Feed, in mash form, and water were available *ad libitum* during the entire study period from arrival at day old to the end of the study. Bird health and welfare were monitored daily. Body weight and feed intake were recorded at 10 and 21 days of age to calculate average daily feed intake (FI), average daily weight gain (WG), and feed conversion ratio (FCR) on a per pen basis. From 18 to 21 days, the solid floors of the pens were replaced with wire mesh. Excreta representative grab samples were collected twice per day to prevent potential N losses and the trays were cleared from excreta every day. Excreta samples were kept in freezer refrigerated, then homogenised, dried at 60°C, milled through a 0.75 mm screen, and stored for analysis.

At 21 days, one bird per pen was randomly selected, electrically stunned, and blood was collected from the jugular vein into heparin-coated tubes for glutathione peroxidase (GSH-Px) analysis. Gastrointestinal organs including proventriculus, gizzard, duodenum, pancreas, jejunum, ileum, caeca, liver without gallbladder and spleen were excised, weighed without the digesta, and expressed relative to body weight. The liver was freeze-dried, milled, and analysed for antioxidant content. Fresh caecal samples were collected for VFA analysis.

2.4. Laboratory analysis

Dry matter in feed and excreta samples was determined by drying of samples in a forced draft oven at 105°C to a constant weight (AOAC 2000; method 934.01). Crude protein (6.25 × N) in samples was determined by the combustion method (AOAC 2000; method 990.03) using a LECO FP-528 N (Leco Corp.,St. Joseph, MI, USA). Oil (as ether extract) was extracted with diethyl ether by the ether extraction method (AOAC 2000; method 945.16) using a Soxtec system (Foss Ltd., Warrington, UK). The gross energy (GE) value of feed and excreta samples were determined in a bomb calorimeter (model 6200; Parr Instrument Co., Moline, IL, USA) as previously described (Pirgozliev et al. 2006). Non-starch polysaccharides and total starch content in wheat samples, and titanium in feed and excreta were determined as described elsewhere (Whiting et al. 2017). Plasma GSH-Px was determined using a standard procedure as described elsewhere (Woods et al. 2021). The concentration of hepatic antioxidants, including coenzyme Q_{10}, vitamin E and total carotenoids, was determined as previously described (Karadas et al. 2010). The VFA concentration, including acetic acid (AA), BA and propionic (PA), in broiler caecal digesta were determined by using an Agilent 5973 N GC/MS equipped with an Agilent 6890 N GC and an Agilent 7683 autosampler (Keele University, Staffordshire,

UK). Analyses on jejunal morphometry were performed as reported elsewhere (Šimić et al. 2023).

The N-corrected apparent metabolisable energy (AMEn) of the experimental diets was determined following the method of Hill and Anderson (1958). Nutrient retention coefficients and the proportion of organ weights were calculated as described elsewhere (Pirgozliev et al. 2023).

2.5. Statistical Analysis

Performance data were analysed using Genstat (23^{dth} edition) statistical software (IACR Rothamsted, Harpenden, AL5 2JQ, UK). Comparisons among the studied variables were performed by two-way ANOVA using a 2 × 2 × 2 factorial design (Wheat × IN × XYL). Data were checked for homogeneity and normality prior to ANOVA. Results were considered significant at $p < 0.05$. Data are expressed as means and their pooled standard errors of means (SEM).

3. Results

The determined chemical composition of the experimental diets was similar, and the main difference was in the NSP content (Table 2). Table 3 presents information on growth performance variables, AMEn and nutrient retention coefficients. Feeding XYL led to increased daily FI, WG and reduced FCR ($p < 0.05$), which agreed with greater dietary AMEn and DMR. Dietary IN tended to increase daily WG ($p = 0.090$) and to reduce FCR ($p = 0.051$). There were no differences ($p > 0.05$) in growth performance, dietary AMEn and retention coefficients due to dietary NSP content. No treatment interactions were observed ($p > 0.05$) within the studied variables in Table 3. The relative weight, in %, of the GIT organs is presented in Table 4. Feeding XYL reduced the weight of the pancreas ($p = 0.012$). Regarding empty caeca weight, expressed as percent (%) from the body weight of 21 d old broiler chicken, there was XYL × IN interactions ($p = 0.010$; data not in tables) as feeding both, XYL and IN, led to heavier empty caeca (basal feed, 0.509^{a}; XYL only feed, 0.497^{a}; IN only feed 0.500^{a}; XYL+IN feed, 0.614^{b}; SEM = 0.0239). The results of the jejunal villus morphometry of the chicks are presented in Table 5. Feeding IN reduced VW compared with the rest of the treatments ($p = 0.001$). There were no pronounced differences between treatments regarding VH. Overall, the CD was deepened in birds fed HSW compared to LSW diets only but the impact of supplementary IN or XYL on CD was inconsistent. It seems that feeding XYL × HSW diet resulted in higher VH:CD ($p < 0.001$) but did not differ from LSW only diet ($p > 0.05$). Bird fed LSW had higher VH:CD ratio compared to HSW fed birds ($p < 0.001$). Feeding XYL × IN × HV led to greater jejunal epithelium height (JEH; $p = 0.016$) compared to most of the treatments. The VFA concentration in caecal digesta is presented in Table 6. Feeding XYL reduced AA and PA concentration ($p < 0.05$). However, individually XYL and IN increased ($p < 0.05$) the concentration of BA and BA: AA ratio increased ($p < 0.001$) with dietary XYL supplementation. There were no interactions observed ($p > 0.05$). Table 7 presents the results of the impact of the experimental treatments on bird antioxidant status. Feeding XYL increased the hepatic concentration of coenzyme Q_{10} ($p = 0.027$), although dietary IN increased ($p = 0.012$) blood GSH-Px in birds. No differences ($p > 0.05$) were detected within the remaining variables studied. There were no interactions ($p > 0.05$).

Table 3. Effect of wheat soluble non-starch polysaccharides content, inulin and xylanase supplementation on growth performance, energy and nutrient availability when fed to female Ross 308 broiler chickens from 7 to 21 d age.

Item[a] Treatment[b]	BW 10d [g/b]	BW 21d [g/b]	FI [g/b/d]	WG [g/b/d]	FCR [g:g]	AMEn [MJ/kg]	DMR	NR	FR
Wheat									
LSW	301	787	62.3	44.7	1.428	11.86	0.740	0.632	0.729
HSW	306	801	64.3	45.6	1.465	11.85	0.743	0.651	0.723
SEM	6.52	18.2	0.92	1.03	0.0210	0.044	0.0033	0.0083	0.0119
IN									
No	301	784	62.5	43.9	1.476	11.87	0.742	0.641	0.720
Yes	306	804	64.0	46.4	1.416	11.84	0.741	0.642	0.731
SEM	6.52	18.2	0.92	1.03	0.0210	0.044	0.0033	0.0083	0.0119
XYL									
No	297	772	61.7	43.1	1.479	11.73	0.734	0.634	0.719
Yes	310	816	64.8	47.2	1.414	11.98	0.749	0.650	0.732
SEM	6.52	18.2	0.92	1.03	0.0210	0.044	0.0033	0.0083	0.0119
Probabilities									
Wheat	0.598	0.607	0.130	0.559	0.220	0.854	0.482	0.112	0.724
IN	0.577	0.435	0.254	0.090	0.051	0.608	0.906	0.960	0.508
XYL	0.176	0.089	0.019	0.008	0.035	<0.001	0.002	0.191	0.434
Wheat × IN	0.950	0.815	0.248	0.637	0.349	0.210	0.165	0.462	0.520
Wheat × XYL	0.176	0.185	0.078	0.196	0.527	0.358	0.228	0.136	0.655
XYL × IN	0.439	0.710	0.510	0.352	0.476	0.717	0.803	0.888	0.749
Wheat × XYL × IN	0.687	0.746	0.839	0.490	0.267	0.358	0.253	0.124	0.396

[a]BW10 = body weight at 10 d old; BW21 = body weight at 21 d old; FI = daily feed intake; WG = daily weight gain; FCR = feed conversion ratio; AMEn = dietary N-corrected apparent metabolisable energy (AMEn was determined on excreta collected from 18 to 21d age); DMR = dry matter retention coefficient; NR = nitrogen retention coefficient; FR = fat retention coefficient. [b]LSW = wheat with low soluble non-starch polysaccharides content; HSW = wheat with high soluble non-starch polysaccharides content; SEM = pooled standard errors of mean; IN = inulin; XYL = exogenous xylanase; Probabilities = differences are statistically significant when $p < 0.05$.

4. Discussion

The objective of the current study was to examine the effects of IN and XYL in LSW and HSW diets on growth performance variables of chicken broilers and to compare them with changes in energy and nutrient metabolism, GIT development, antioxidant status of the birds and caecal VFA concentration. The overall BW of birds in the study was about 800 g, or approximately 16% below the Ross 308 female broiler weight for commercial flocks. This was possibly due to a combination of frequent weighing, being fed a mash diets, rather than steam pelleted feed and being kept in small groups (V. Pirgozliev, MW. Mirza, et al. 2016). This was not considered to be detrimental to the experimental objectives.

Results obtained in this study demonstrate that growth performance variables were not significantly affected by dietary inulin, which are consistent with the previous reports in broiler chickens (Ortiz et al. 2009; Wu et al. 2019). However, Yusrizal and Chen (2003) found that inulin or fructooligosaccharides (FOS) at 10 g/kg of diet improved body weight gain and FCR in female but not in male broiler chickens. Xu et al. (2003) also observed improvements in body weight gain when supplementing 4 g/kg of FOS to diet. Rebolé et al. (2010) reported that feeding 10 and 20 g/kg inulin in a wheat- and barley-based diet had a beneficial effect on body weight gain of the broilers at 21 d age, but the positive effect of 20 g/kg inulin disappeared at 35 d age. Rodrigues et al. (2011) did not find difference in growth performance variables between the control and control supplemented with IN in 28 d old broilers. Li et al. (2018) also reported no difference in

Table 4. Effect of wheat soluble non-starch polysaccharides content, inulin and xylanase supplementation on gastrointestinal organs as percent (%) from the body weight of 21 d old female Ross 308 broiler chicken.

Item[1] Treatment[2]	Bird [g]	Spleen	Liver	P&G	Pancreas	SI	Caeca[3]	GIT
Wheat								
LSW	789	0.070	2.968	2.696	0.425	5.296	0.531	8.950
HSW	803	0.071	3.076	2.752	0.426	5.167	0.529	8.870
SEM		0.0032	0.1081	0.0704	0.0119	0.1358	0.0169	0.1950
IN								
No	786	0.071	3.004	2.681	0.430	5.321	0.503	8.930
Yes	807	0.069	3.040	2.767	0.421	5.142	0.557	8.890
SEM		0.0032	0.1081	0.0704	0.0119	0.1358	0.0169	0.1950
XYL								
No	772	0.074	3.073	2.777	0.448	5.378	0.505	9.110
Yes	820	0.066	2.971	2.671	0.404	5.084	0.555	8.710
SEM		0.0032	0.1081	0.0704	0.0119	0.1358	0.0169	0.1950
Probabilities								
Wheat		0.813	0.483	0.573	0.989	0.505	0.941	0.790
IN		0.558	0.812	0.387	0.621	0.354	0.027	0.866
XYL		0.075	0.508	0.289	0.012	0.132	0.039	0.159
Wheat × IN		0.240	0.605	0.178	0.225	0.877	0.565	0.684
Wheat × XYL		0.772	0.280	0.951	0.566	0.417	0.105	0.423
XYL × IN		0.333	0.300	0.922	0.084	0.900	0.010[3]	0.698
Wheat × XYL × IN		0.503	0.992	0.331	0.729	0.253	0.879	0.239

[1]P&G = proventriculus and gizzard; SI = small intestine; GIT = total gastrointestinal tract. [2]LSW = wheat with low soluble non-starch polysaccharides content; HSW = wheat with high soluble non-starch polysaccharides content; SEM = pooled standard errors of mean; IN = inulin; XYL = exogenous xylanase; Probabilities = differences are statistically significant when $p < 0.05$. [3]There was XYL x IN interactions ($p = 0.010$; data not in tables) as feeding both, XYL and IN, led to heavier empty caeca (basal feed, 0.509[a]; XYL only feed, 0.497[a]; IN only feed 0.500[a]; XYL+IN feed, 0.614[b]; SEM = 0.0239); [a − b] Values within data presented not sharing the same superscripts differ significantly.

performance due to dietary IN. The inconsistency in responses of growth performance to dietary supplemental IN among these studies maybe due to differences in poultry hybrids, sex, rearing conditions, dietary formulations, dietary IN concentrations, and duration of its administration. Performance data from the current study also showed that the addition of XYL to the diets with different NSP contents improved growth performance variables and there were no XYL by NSP or XYL by IN interactions. The lack of XYL by wheat soluble NSP interaction agrees with recent study using the same dietary formulation (Pirgozliev et al. 2023) and confirms that the difference in the soluble NSP content of the diets, due to the different wheats, was within the tolerable range of NSP, such that increased levels of xylanase were not warranted. Rebolé et al. (2010) also did not find an interaction on growth performance of chickens fed IN and a mixture of NSP degrading enzymes.

The lack of differences in AMEn and nutrient retention coefficients in IN supplemented diets agrees with lack of growth performance differences in the present study. Biggs and Parsons (2007) found no impact of oligosaccharides, including IN, on ME and most amino acids. Alzueta et al. (2010) also reported that dietary starch digestibility and AMEn were not affected by IN supplementation, although an improvement in ileal digestibility of protein, fat and most amino acids was observed. Rodrigues et al. (2011) and Li et al. (2018) reported no difference in dietary AMEn and nutrient retention coefficients in chickens fed IN. Julendra et al. (2021) also reported no difference in AMEn in diet containing 15 g/kg IN and the control. Feeding XOS to broiler chickens,

Table 5. Effect of wheat soluble non-starch polysaccharides content, inulin and xylanase supplementation on intestinal villus morphometry of 21 d old female Ross 308 broiler chicken.

Item[1] Treatment[2]	VH [µm]	VW [µm]	CD [µm]	VH:CD	JEH [µm]
Wheat					
LSW	855	156	200	4.57	44.2
HSW	919	170	216	4.64	44.6
SEM	19.9	5.7	2.6	0.099	1.28
IN					
No	900	177	197	4.89	45.1
Yes	874	149	219	4.32	43.8
SEM	19.9	5.7	2.6	0.099	1.28
XYL					
No	918	157	210	4.68	42.4
Yes	856	169	206	4.52	46.5
SEM	19.9	5.7	2.6	0.099	1.28
Wheat IN					
LSW No	825^{a}	169	189	4.72	45.3
HSW No	974^{b}	184	206	5.06	44.9
LSW Yes	884^{a}	143	210	4.42	43.2
HSW Yes	864^{a}	156	227	4.22	44.4
SEM	28.1	8.1	3.7	0.140	1.81
Wheat XYL					
LSW No	911	151	196^{a}	4.89^{b}	45.9^{bc}
HSW No	925	164	224^{c}	4.47^{ab}	38.9^{a}
LSW Yes	799	161	203^{ab}	4.24^{a}	42.6^{ab}
HSW Yes	913	176	208^{b}	4.80^{b}	50.4^{c}
SEM	28.1	8.1	3.7	0.140	1.81
XYL IN					
No No	960^{b}	169	194	4.80	47.9
Yes No	839^{a}	185	200	4.98	42.2
No Yes	876^{a}	146	218	4.25	45.0
Yes Yes	872^{a}	152	220	4.39	42.5
SEM	28.1	8.1	3.7	0.140	1.81
Wheat IN XYL					
LSW No No	936	158	179^{a}	5.44^{b}	43.8^{abc}
HSW No No	983	179	221^{cd}	4.53^{a}	40.6^{ab}
LSW Yes No	885	144	212^{cd}	4.35^{a}	47.9^{cd}
HSW Yes No	868	149	227^{d}	4.42^{a}	37.2^{a}
LSW No Yes	714	181	198^{b}	3.99^{a}	46.7^{bcd}
HSW No Yes	965	189	190^{ab}	5.60^{b}	49.2^{cd}
LSW Yes Yes	883	141	209^{c}	4.50^{a}	38.4^{a}
HSW Yes Yes	861	164	226^{d}	4.01^{a}	51.5^{d}
SEM	39.7	11.5	5.2	0.198	2.56
Probabilities					
Wheat	0.027	0.083	<0.001	0.620	0.826
IN	0.369	0.001	<0.001	<0.001	0.471
XYL	0.031	0.169	0.308	0.257	0.028
Wheat × IN	0.004	0.935	0.953	0.053	0.660
Wheat × XYL	0.083	0.908	0.003	0.001	<0.001
XYL × IN	0.044	0.524	0.623	0.850	0.368
Wheat × XYL × IN	0.069	0.353	<0.001	<0.001	0.016

[1]VH = villus height; VW = villus width; CD = crypt depth; VH:CD = ratio between VH and CD; JEH = jejunal epithelium height. [2]LSW = wheat with low soluble non-starch polysaccharides content; HSW = wheat with high soluble non-starch polysaccharides content; SEM = pooled standard errors of mean; IN = inulin; XYL = exogenous xylanase; Probabilities = differences are statistically significant when $p < 0.05$; [a–c]Values within a column not sharing the same superscripts differ significantly.

Šimić et al. (2023) did not find changes in AMEn and nutrient retention coefficients. Xylanase supplemented diets had an increased AMEn and overall DMR independently of dietary NSP contents. This is in accord with changes observed in bird growth

Table 6. Effect of wheat soluble non-starch polysaccharides content, inulin and xylanase supplementation on caecal concentration of volatile fatty acids of 21 d old female Ross 308 broiler chicken.

Item1 Treatment2	VFA tot [ug/g]	AA [ug/g]	BA [ug/g]	PA [ug/g]	BA: AA
Wheat					
LSW	888	738	122	29	0.181
HSW	927	765	135	27	0.212
SEM	62.7	57.9	7.6	2.4	0.0157
IN					
No	862	720	114	28	0.189
Yes	953	782	144	28	0.204
SEM	62.7	57.9	7.6	3.4	0.0157
XYL					
No	982	835	116	32	0.153
Yes	832	667	141	24	0.240
SEM	62.7	57.9	7.6	2.4	0.0157
Probabilities					
Wheat	0.668	0.744	0.217	0.562	0.173
IN	0.307	0.457	0.008	0.920	0.529
XYL	0.097	0.046	0.023	0.030	<0.001
Wheat × IN	0.720	0.636	0.611	0.638	0.508
Wheat × XYL	0.374	0.485	0.117	0.161	0.698
XYL × IN	0.913	0.924	0.142	0.622	0.277
Wheat × XYL × IN	0.612	0.484	0.256	0.948	0.230

[1]VFA tot = total volatile fatty acids, AA = acetic acid, BA = butyric acid, PA = propionic acid, AA:BA = ratio between AA and BA. [2]LSW = wheat with low soluble non-starch polysaccharides content; HSW = wheat with high soluble non-starch polysaccharides content; SEM = pooled standard errors of mean; IN = inulin; XYL = exogenous xylanase; Probabilities = differences are statistically significant when $p < 0.05$.

performance and suggests that the difference in the soluble NSP content of the diets, due to the different wheats in this study, was within the tolerable range of NSP. Although there is an aspiration to formulate diets based on NSP fractions (Morgan et al. 2018; Nguyen et al. 2022), there are currently limitations on recognising and standardising target levels. For example, Morgan et al. (2018) reported dietary soluble NSP content ranging between 5.3 – 14.6 g/kg, but no relationship with FCR was observed. Feeding up to 12.75 g/kg soluble NSP in diets, Nguyen et al. (2022) also did not find an effect on bird production performance up to 35 days. In our study, the diet based on LSW had 18.7 g/kg soluble NSP and there was 32.5 g/kg in the HSW. Thus, the tolerable level of soluble NSP may be greater than previous studies have investigated.

Research by Kiarie et al. (2014) and Pirgozliev et al. (2016) also reported that XYL improved growth performance and AMEn independent of dietary soluble NSP contents. Feeding XYL supplemented diets also coupled with reduced pancreas weights, further supporting the view that enzyme supplementation leads to a reduction in the relative weight of the digestive tract compartments, leading to an increase in carcass yield (Abdulla et al. 2016).

Dietary IN altered the fermentation patterns of caecal content as evidenced by the significant increase in the concentration of BA in caecal digesta, which agrees with other reports (Rehman et al. 2007; Rebolé et al. 2010) that also found the same positive effect of dietary IN caecal fermentation. Although feeding XYL increased caecal VFA concentration in broilers in most of reports, there were differences in the individual acid response. Pirgozliev et al. (2023) found an increase in butyrate concentration only; Kiarie et al. (2014) meanwhile observed an increase in acetate concentration; Šimić et al. (2023)

Table 7. Effect of wheat soluble non-starch polysaccharides content, inulin and xylanase supplementation on dry liver weight, hepatic and blood antioxidant status of 21 d old female Ross 308 broiler chicken.

Item1 Treatment2	Liver dry [g]	Vit E [µg/g]	Co Q10 [µg/g]	TC [µg/g]	GSH-Px [U/g HB]
Wheat					
Low	5.51	39.2	194	1.29	197
High	6.15	34.5	184	1.26	215
SEM	0.288	3.78	7.5	0.053	9.1
IN					
No	5.81	37.9	185	1.25	190
Yes	5.85	35.8	194	1.30	223
SEM	0.288	3.78	7.5	0.053	9.1
XYL					
No	5.53	36.4	177	1.30	212
Yes	6.13	37.3	201	1.25	201
SEM	0.288	3.78	7.5	0.053	9.1
Probabilities					
Wheat	0.125	0.380	0.373	0.639	0.165
IN	0.929	0.683	0.377	0.505	0.012
XYL	0.149	0.876	0.027	0.547	0.380
Wheat × IN	0.459	0.888	0.437	0.487	0.163
Wheat × XYL	0.517	0.179	0.375	0.693	0.339
XYL × IN	0.322	0.984	0.967	0.447	0.095
Wheat × XYL × IN	0.452	0.554	0.222	0.798	0.196

[1]Vit E = hepatic concentration of vitamin E; Co Q_{10} = hepatic concentration of coenzyme Q_{10}; TC = hepatic total carotenoids; GSH-Px = blood glutathione peroxidase. [2]LSW = wheat with low soluble non-starch polysaccharides content; HSW = wheat with high soluble non-starch polysaccharides content; SEM = pooled standard errors of mean; IN = inulin; XYL = exogenous xylanase; Probabilities = differences are statistically significant when $p < 0.05$.

observed an increase in lactate concentration, whereas an increase in acetate, butyrate and total VFA concentration was reported by Masey O'Neill et al. (2014). In the reported study feeding XYL increased the concentration of BA, which agrees with many of the reports. It is believed that prebiotics can induce changes in the intestinal microflora by selective stimulation of health promoting bacteria, such as *Bifidobacteria* and *Lactobacilli*, which will improve intestinal microbial balance through a reduction in the establishment of pathogenic species in the intestine and ultimately to enhance host health (Patterson and Burkholder 2003). Rebolé et al. (2010) and Rodrigues et al. (2011), found greater numbers of *Bifidobacteria* and *Lactobacilli* in the caecal digesta of broilers fed IN or NSP degrading enzymes, respectively. Although microbial count was not determined in the reported study, it can be speculated that feeding IN or XYL increased the proliferation of *Bifidobacteria* and *Lactobacilli* in the caeca, which led to an increase concentration of BA.

Rehman et al. (2007) observed a significant increase in caecal weight in IN fed broilers that coincided with high butyrate but not total SCFA production. Ortiz et al. (2009), on the other hand, did not observe differences in morphological measurements of the GIT, including the caeca, when feeding IN to broilers. Šimić et al. (2023) did not find differences in caecal weight and SCFA production when comparing control with diet containing feeding fermentable xylooligosaccharides or XYL in broiler chickens. Feeding high fibre diet increased caecal weight and content in broilers but there were no differences in BA concentration and even less SCFA were produced in the heavier caeca (Jozefiak et al. 2006). The addition of NSP degrading enzyme did not bring difference in caecal weight and in butyrate production (Jozefiak et al. 2006). Pirgozliev et al. (2023) also reported an increase of caecal BA concentration in broilers fed XYL, but

no difference was observed in caecal weight. Thus, the enlarged caeca in birds fed IN & XYL diet without changes in VFA in the reported study is not a surprise.

Changes in the caecal fermentation, i.e. increased butyric acid concentration, of chickens can alter the mucosal structure and increase nutrient absorptive capacity (Ahsan et al. 2016). It is assumed that large surface area covered with long healthy villi and shallow crypts will provide optimal functioning of the digestive tract (Rebolé et al. 2010). However, studies carried out on the effects of dietary IN on villus morphometry of chickens show inconsistent results. Rehman et al. (2007) reported that adding 10 g/kg IN to a maize- and wheat-based diet resulted in longer jejunal villi and Xu et al. (2003), using FOS, observed an increase in the jejunal villus length of broilers fed a maize-based diet. Rebolé et al. (2010) reported an increase in the ratio of VH to CD in the broilers fed IN at a level of 10 g/kg of diet, that was due to numerically only greater VH, and numerically only lower CD compared with the control birds. Feeding NSP degrading enzymes did not affect VH, but reduced CD and the ratio between VH and CD (Rebolé et al. 2010). Šimić et al. (2023) found no changes in jejunum villus morphometry when feeding XYL or XOS to 35 d old broilers. Awad et al. (2011) showed reduced VH, VW, CD and VH:CD ratio when feeding dried chicory ground pulp to broiler chickens. Research by Li et al. (2018) also found a reduction in VH and VH:CD ratio in broilers fed IN. In the reported study feeding IN reduced VW in agreement with Awad et al. (2011). There were numerous two- and three-way interactions regarding the rest of villus morphometry variables studied in the reported experiment but overall feeding IN or XYL did not improve jejunal villus morphometry.

Shang et al. (2018) reported an increased GSH-Px in blood serum of IN fed laying hens, which agrees with this study findings. Kleniewska et al. (2016) also demonstrated that dietary IN increases GSH-Px in human plasma. Feeding IN to broilers also increased the antioxidant capacity of thigh meat (Untea et al. 2023). Glutathione peroxidase is a significant part of the major enzyme defence mechanism against the damaging effects of reactive oxygen species (Kleniewska et al. 2016). In agreement with previous research (Pirgozliev et al. 2023) dietary XYL improved hepatic coenzyme Q_{10} concentration. Although diet is the main determinant of antioxidant content in liver, seems exogenous XYL can also impact the efficiency of antioxidant absorption from the diet and subsequently, their accumulation in the liver. Thus, suggesting that both, IN and XYL, can serve as feed enhancers that potentially may promote antioxidant status of birds and help poultry to cope with various stress factors during production.

Overall, dietary XYL supplementation, rather than wheat type, drove most physiological responses in broilers. Xylanase consistently increased FI, WG and feed efficiency, supported by greater AMEn and nutrient retention coefficients. It also reduced pancreatic weight and modified caecal fermentation by lowering AA and PA while increasing BA concentration. Dietary IN had milder effects, slightly enhancing growth and elevating blood GSH-Px and caecal BA concentration. Wheat NSP content had minimal influence on performance or nutrient utilisation. Intestinal morphology showed limited shifts, though XYL × IN occasionally increased villus-to-crypt ratios. Overall, XYL exerted the most notable benefits across traits.

5. Conclusion

The present study demonstrates that young broilers can achieve comparable growth performance when fed either low- or high-fibre wheat-based diets. However, growth can be further improved with XYL supplementation. Beyond enhancing growth, XYL also increased dietary metabolisable energy, nutrient availability, hepatic antioxidant status, and caecal butyrate concentration regardless of the dietary NSP content. This suggests that broilers may tolerate higher levels of dietary NSPs, potentially allowing further benefits from XYL use in low-energy wheat-based diets. Similarly, dietary IN increased blood antioxidant status and caecal butyrate concentration, independent of NSP levels. Therefore, both IN and XYL appear to be promising feed enhancers that may improve the birds' antioxidant status and support good gut health through microbiome modulation.

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ORCID

Vasil Radoslavov Pirgozliev http://orcid.org/0000-0002-4213-7609
Isobel Margaret Whiting http://orcid.org/0000-0003-2160-3583
Stephen Charles Mansbridge http://orcid.org/0000-0003-4246-9782
Kristina Kljak http://orcid.org/0000-0003-2655-897X
Heloisa Helena de Carvalho Mello http://orcid.org/0000-0002-0312-7424
José Henrique Stringhini http://orcid.org/0000-0002-3710-6963
Falko Drijfhout http://orcid.org/0000-0002-3210-8038
David Yovchev http://orcid.org/0000-0003-4357-0858
Atanas Georgiev Atanasov http://orcid.org/0000-0003-2545-0967
Zhi Yang http://orcid.org/0000-0001-6636-4606
Stephen Paul Rose http://orcid.org/0000-0001-6459-597X

Conceptualization

VRP, SPR; data curation: IMW, SCM, AGA, ZY; formal analysis: KK, EAJ, FD and DY; funding acquisition: VRP; investigation: VRP, IMW, AGA and SCM; methodology: KK, EAJ, FD, DY, JHS and HHM; project administration: VRP and IMW; resources: VRP and IMW; software: JHS, HHM; supervision: VRP; validation: SCM and SPR; roles/writing – original draft: VRP, IMW, SPR, SCM and AGA; writing – review and editing: SCM, KK, EAJ, DY, FD, JHS, HHM, ZY and VRP. All authors have read and agreed to the published version of the manuscript.

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