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The Benefits of Exogenous Xylanase in Wheat–Soy Based Broiler Chicken Diets, Consisting of Different Soluble Non-Starch Polysaccharides Content

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Abstract: Four wheat-based diets with either low soluble non-starch polysaccharide (NSPs, 13 g/kg) content (low viscosity, LV) or high NSPs content (33.5 g/kg; high viscosity, HV), without and with exogenous xylanase (XYL), were fed to male Ross 308 broiler chickens from 7 to 21 days age. The enzyme was supplemented at 100 FXU/kg diet, and its preparation was based on endo-1,4-beta-xylanase produced by *Aspergillus oryzae*. Each diet was fed to eight pens, with five birds in each pen, following randomisation. Chicks fed XYL had an improved feed efficiency, hepatic coenzyme Q10, cecal butyric acid concentration, nitrogen digestibility ($p < 0.05$) and increased dietary ME ($p < 0.001$). Compared to HV, birds fed LV diets had reduced weight of proventriculus, gizzard and the pancreas and higher blood glutathione peroxidase and dietary ME ($p < 0.05$), but no differences were observed on nutrient digestibility and growth performance variables. This also suggests that birds may tolerate a greater dietary NSPs content; thus, further benefits may be obtained by the application of XYL in low energy wheat-based diets.

Keywords: wheat; non-starch polysaccharides; xylanase; metabolisable energy; caecal fermentation; chickens

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1. Introduction

Exogenous xylanase (XYL) has been routinely used in poultry diets to hydrolyse non-starch polysaccharides (NSP) and improve the feeding quality of fibre-rich diets, including wheat [1]. The beneficial effect of XYL in wheat-based diets is mainly attributed to the reduction of digesta viscosity, improving digestion and absorption of nutrients, dietary energy availability and subsequent growth performance of broilers [2,3]. In layers, XYL has been assessed as improving bone strength and eggshell quality [4]. Some further benefits of XYL may be related with the partial hydrolysation of the cell wall pentosans producing short-chain xylo-oligosaccharides, which can possess prebiotic properties when fermented in the chicken caeca [1]. The prebiotic oligosaccharides are essential to stimulate and establish beneficial microbiota in the gastrointestinal tract (GIT) of broilers, improving pre-caecal digestibility of major nutrients and providing a substrate for caecal production of short-chain fatty acids (SCFA) [5–7]. Caeca fermentation of dietary fibre and the production of SCFA, especially butyrate, have been associated with small intestinal villus development, delays in gastric emptying and improved overall gut health, benefiting feed efficiency [5,6].

The impact of digesta viscosity and XYL on dietary apparent metabolisable energy (AME) and bird performance has been widely studied. Feeding wheat and maize based diets, Kiarie et al. [8] reported that XYL improved growth performance and nitrogen corrected AME (AMEn) independent of diet type. In another study, supplementary XYL improved AMEn and nutrient availability of all wheat samples that was irrespective of differences in pentosan content, although ileal digesta viscosity was not reduced by XYL supplementation [9]. Although wheat diets are associated with superior growth performance than maize, the AMEn was similar in both diets [8]. In agreement, previous reports [10–12] also did not observe relationships between dietary AMEn and the birds' growth performance.

Engberg et al. [13] reported reduced feed intake (FI), increased body weight (BW), no differences in feed conversion ratio (FCR) and reduced ileal digesta viscosity in 42 d old broilers fed XYL supplemented wheat-based diets. After feeding wheat-based diets with and without XYL, Wu et al. [14] found an improvement in weight gain (WG) and FCR, while ileal digesta viscosity was reduced, but no changes in FI in 21 d old broilers supplemented with XYL were observed. Pirgozliev et al. [9], however, did not find an impact of supplementary XYL on FI, WG, feed efficiency and ileal digesta viscosity when feeding wheat-based diets to 21 d old broilers. These studies highlight the issue of inconsistency in XYL studies of broilers. However, in the majority of studies, feeding XYL increased caecal SCFA production in broilers, although there were differences in the individual acid response. Barekatain et al. [15] and Pirgozliev et al. [16] found an increase in butyrate production only; Kiarie et al. [8] meanwhile observed an increase in acetate production, whereas an increase in acetate, butyrate and total SCFA content was reported by Massey O'Neill et al. [5].

In view of the presented inconsistent results, the aim of this study was to investigate the effects of dietary XYL supplementation to wheat-based diets with different NSP contents on broiler performance, including daily FI, WG and FCR, hepatic antioxidant status, nutrient retention coefficients, AMEn and caecal SCFA production.

2. Materials and Methods

2.1. Wheat Samples and Experimental Diets

Two wheat samples were used to produce the experimental diets. These samples were selected based on the relative differences in their soluble NSP (NSPs) content, although the proximate composition was similar (Table 1).

Table 1. Analysed chemical composition of the wheat samples used in the experimental poultry dietary formulations.

Item [1]	LV Wheat [2]	HV Wheat [3]
Dry matter (g/kg)	887	885
Crude protein (g/kg)	118	126
Fat (g/kg)	15.4	9.8
Ash (g/kg)	15.6	17.4
Starch (g/kg)	718	674
NSPt (g/kg)	85.5	106.8
NSPs (g/kg)	13.0	33.5
NSPin (g/kg)	72.5	73.3
Gross energy (MJ/kg)	15.83	15.81

[1] NSPt = total non-starch polysaccharides, NSPs = soluble non-starch polysaccharides, NSPin = insoluble non-starch polysaccharides. [2] LV wheat = low viscosity wheat. [3] HW wheat = high viscosity wheat.

Sample A contained 13 g/kg NSPs, while sample B contained over 33.5 g/kg NSPs. It is expected that diets based on high NSPs wheat will produce high digesta viscosity and that low NSPs wheat will produce low viscosity digesta. Thus, the low NSPs wheat diet was named low viscous (LV), and the high NSPs wheat diet was named high viscous (HV).

Two basal diets were produced with the main component being 670 g/kg of either the LV or HV wheat (Table 2).

Table 2. Composition of broiler chicken diets (g/kg, as-fed) used in the experiment.

Ingredients [1], %	**Control LV [2]**	**Control HV [3]**
Low NSPs wheat	67.00	-
High NSPs wheat	-	67.00
Soybean meal (48% CP)	21.97	21.97
Soybean meal (full fat)	5.00	5.00
Vegetable oil	2.00	2.00
Dicalcium phosphate	1.45	1.45
Limestone	1.25	1.25
Salt	0.27	0.27
Lysine	0.27	0.27
Methionine	0.39	0.39
Vitamin and mineral premix	0.40	0.40
	100	100
Calculated analysis (as fed):		
Crude protein, g/kg	194	202
ME, 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
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

[1] NSPs = NSPs = soluble non-starch polysaccharides, CP = crude protein, ME = metabolisable energy, The vitamin and mineral premix contained vitamins and trace elements to meet the requirements. 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 molybdenum 0.5 mg. [2] LV = low viscosity diet, [3] HV = high viscosity diet.

The diets were formulated to be slightly lower in protein and metabolisable energy, compared to breeders' recommendations for male broilers between 7 and 21 d age (Aviagen Ltd., Edinburgh, UK). Each diet was then split into two equal batches, and one batch of each diet was supplemented with 100 FXU/kg of a commercial xylanase (Ronozyme WX (CT), DSM, Kaiseraugst, 4303, Switzerland) resulting in four diets in total. The enzyme was obtained from Target Feed Ltd. (Whitchurch, SY13 2DX, UK), and its preparation was based on endo-1,4-beta-xylanase produced by *Aspergillus oryzae*. No matrix adjustments were made for differences in protein and dry matter between the wheat samples. All diets were supplied with 5 g/kg of TiO_2 as an indigestible feed marker.

2.2. Birds, Management and Sample Collection

The experiment was conducted at the National Institute of Poultry Husbandry (Harper Adams University, Newport, TF10 8NB, UK) and approved by the Harper Adams University Research Ethics Committee. A total of 180 male Ross 308 broilers were obtained from a commercial hatchery (Cyril Bason Ltd., Craven Arms, SY7 9NG, UK), allocated to a single floor pen and offered a standard wheat-based broiler starter feed formulated to meet Ross 308 nutrient requirements (Aviagen Ltd., Edinburgh, UK). At 7 d age, 160 birds were allocated to 32 raised-floor pens each holding 5 birds. Each of the pens had a solid floor and was equipped with an individual feeder and drinker. At the beginning of the experiment the temperature was maintained at 33 °C and was gradually reduced to 21 °C

at 20 d old (standard rearing temperature). A standard lighting program for broilers was used, decreasing the light–dark ratio from 23 h:1 h from day old to 18 h:6 h at 7 d of age, which was maintained until the end of the study. Each diet was fed to 8 pens following randomisation. Birds and feed were weighed at the start (day 7) and at the end of the study (day 21), and FI, WG and FCR were determined. Excreta were collected for three days from 17 to 21 d age, oven dried at 60 °C, milled and used for determination of dietary AMEn and nutrient retention coefficients. During this period, the solid floor of each pen was replaced with a wire mesh to enable excreta collection. At the end of the study, on day 21, one bird per pen, selected at random, was electrically stunned, and blood was obtained during exsanguination in heparin coated tubes from the jugular vein for hemoglobin (Hb) and glutathione peroxidase (GSH-Px) determination. The organs from the digestive system, including proventriculus and gizzard (PG), duodenum, pancreas, jejunum, ileum, caeca, liver and the spleen were weighed and presented relative to bird body weight. The liver was freeze dried, milled and analysed for antioxidant content. Fresh caecal digesta samples were also collected for determination of volatile fatty acid (VFA) concentration.

2.3. Laboratory Analysis

Dry matter (DM) in feed and excreta samples was determined by drying of samples in a forced draft oven at 105 °C to a constant weight [17]. Crude protein (CP; 6.25 × N) in samples was determined by the combustion method [18] using a LECO FP-528 N (Leco Corp., St. Joseph, 269, MI, USA). Oil (as ether extract) was extracted with diethyl ether by the ether extraction method [19] using a Soxtec system (Foss Ltd., Warrington, UK). The gross energy (GE) value of feed and excreta samples was determined in a bomb calorimeter (model 6200; Parr Instrument Co., Moline, IL, USA) with benzoic acid used as the standard. Non-starch polysaccharides (NSP) and total starch content in wheat samples were determined as previously described [20,21]. Titanium dioxide in feed and excreta, to measure digestibility, was determined using the method published by Short et al. [22]. Blood Hb concentrations were measured by a standard absorbency test, run on a Horiba vet ABC plus analyser (Northampton, NN3 6FL, UK). GSH-Px was determined using a Ransel GPx kit (Randox Laboratories Ltd., Crumlin, UK) that employs the method based on Paglia and Valentine [23]. The concentration of hepatic vitamin E and coenzyme Q10 was determined as previously described by Karadas et al. [24,25]. The VFA concentrations, including acetic acid (AA), butanoic acid (BA), pentanoic acid (PA) and propanoic acid (PRA), in poultry caecal digesta were determined by using an Agilent 5973 N GC/MS equipped with an Agilent 6890 N GC and an Agilent 7683 autosampler.

2.4. Calculations

Dietary DMR coefficient was calculated using the following equation:

$$DMR = \frac{Ti\ Excreta - Ti\ Diet}{Ti\ Excreta}$$

where *Ti Excreta* and *Ti Diet* are the concentrations of *Ti* in the excreta and diet, respectively.

Dietary nutrient retention coefficients, i.e., NR and FR, were calculated using the following equation:

$$Nutrien\ retention = \frac{(Nutr/Ti)Diet - (Nutr/Ti)Excreta}{(Nutr/Ti)Diet}$$

where (*Nutr/Ti) Diet* = ratio of the respective nutrient to titanium in diet, and (*Nutr/Ti*) *Excreta* = ratio of the respective nutrient to titanium in excreta.

The AMEn value of the experimental diets was determined following the method of Hill and Anderson [26].

$$AMEn = GE\ Diet - \frac{(GE\ Excreta \times Ti\ Diet)}{Ti\ Excreta} - 34.39 \times N\ Retained$$

where *AMEn* (MJ/kg) = N-corrected apparent metabolisable energy content of the diet; *GE Diet* and *GE Excreta* (MJ/kg) = GE of the diet and excreta, respectively; *Ti Diet* and *Ti Excreta* (%) = titanium in the diet and excreta, respectively; 34.39 (MJ/kg) = energy value of uric acid; and *N Retained* (g/kg) is the N retained by the birds per kilogram of diet consumed. The retained N was calculated as

$$NRetained = N\ Diet - \frac{N\ Excreta \times Ti\ Diet}{Ti\ Excreta}$$

where *N Diet* and *N Excreta* (%) = N contents of the diet and excreta, respectively.

The relative development of organs was determined as follows:

$$\%\ Organ\ weight = \frac{Organ\ weight}{Body\ weight} \times 100\%$$

where *Organ weight* and *Body weight* are the weight of the organs and each bird, respectively.

2.5. Statistical Analysis

Performance data were analysed using Genstat (18th edition) statistical software (IACR Rothamsted, Harpenden, AL5 2JQ, UK). Comparisons among the studied variables were performed by two-way ANOVA using a 2 × 2 factorial design (dietary NSP × exogenous 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 analysed chemical composition (g/kg) of the two wheat samples is presented in Table 1. The LV wheat had more starch and fat but less CP and NSPs compared to HV wheat. The determined chemical composition of the experimental diets was similar, and the main difference was in the NSPs content (Table 2). Table 3 presents information on growth performance variables, AMEn and nutrient retention coefficients. There were no differences ($p > 0.05$) in final BW, daily FI and WG due to dietary viscosity or XYL supplementation. However, birds fed XYL utilised diets more efficiently, indicated by a lower FCR ($p = 0.015$). The LV diets had higher AMEn ($p = 0.002$) and tended to have a higher AMEn:GE coefficient ($p = 0.079$) compared to HV diets. Dietary NSPs content did not have an impact ($p > 0.05$) on DM and nitrogen retention coefficients. Supplementary XYL increased dietary AMEn, AMEn:GE, DMR ($p < 0.001$) and NR ($p = 0.049$). There were no treatment interactions observed ($p > 0.05$) within the studied variables in Table 3.

Measurements of GIT components at 21 d of age in relation to the experimental diets are shown in Table 4. Feeding LV diets reduced the weight of PG ($p = 0.004$) and the pancreas ($p = 0.014$). There were no interactions ($p > 0.05$) within the studied variables in Table 4.

Table 5 presents the results of the impact of the experimental treatments on bird antioxidant status. Feeding XYL increased the hepatic concentration of coenzyme Q10 ($p = 0.037$) but tended ($p = 0.068$) to reduce blood GSH-Px in birds. However, feeding HV diets reduced GSH-Px ($p = 0.010$) in whole blood. No differences ($p > 0.05$) were detected within the remaining variables studied. There were no interactions ($p > 0.05$).

The VFA contents in cecal digesta are presented in Table 6. Feeding XYL increased ($p = 0.040$) the BA concentration in caecal digesta but there were no further differences ($p > 0.05$) or interactions observed.

Table 3. Effect of dietary viscosity and xylanase supplementation on growth performance, metabolisable energy and nutrient retention coefficients when fed to broiler chickens from 7 to 21 d age.

Item [1] Treatment [2]	BW (g/b 7 d)	BW (g/b 21 d)	FI (g/b/d)	WG (g/b/d)	FCR (g:g)	AMEn (MJ/kg DM)	AME:GE	DMR	NR
VIS									
LV	147	878	68.6	51.6	1.324	13.38	0.734	0.752	0.668
HV	147	875	68.5	52.2	1.337	13.23	0.730	0.748	0.664
SEM	0.9	9.8	0.68	0.66	0.0079	0.034	0.0019	0.0021	0.0051
XYL									
No	148	879	69.3	51.8	1.345	13.17	0.725	0.743	0.659
Yes	146	874	67.9	51.9	1.317	13.44	0.739	0.756	0.673
SEM	0.9	9.8	0.68	0.66	0.0079	0.034	0.0019	0.0021	0.0051
Probabilities									
VIS	0.871	0.857	0.948	0.544	0.252	0.002	0.079	0.220	0.524
XYL	0.184	0.682	0.137	0.896	0.015	<0.001	<0.001	<0.001	0.049
VIS × XYL	0.797	0.281	0.121	0.113	0.529	0.911	0.923	0.858	0.154

[1] BW = body weight, FI = daily feed intake, WG = weight gain, FCR = feed conversion ratio, AMEn = dietary N-corrected apparent metabolisable energy, AMEn:GE = AMEn to gross energy ration, DMR = dry matter, NR = nitrogen retention coefficient. [2] VIS = viscosity, LV = low dietary viscosity, HV = high dietary viscosity, SEM = pooled standard error of means, XYL = exogenous xylanase.

Table 4. Effect of dietary viscosity and xylanase supplementation on gastrointestinal organs as percent (%) from the body weight of 21 d old broiler chicken.

Item [1] Treatment [2]	BW (g)	Spleen	Liver	PG	Pancreas	SI	Caeca
VIS							
LV	1008	0.07	2.35	1.93	0.27	3.43	0.54
HV	973	0.08	2.38	2.13	0.30	3.32	0.53
SEM		0.004	0.064	0.045	0.007	0.078	0.021
XYL							
No	983	0.07	2.34	2.03	0.29	3.42	0.54
Yes	997	0.07	2.39	2.03	0.28	3.33	0.54
SEM		0.004	0.064	0.045	0.007	0.078	0.021
Probabilities							
VIS		0.140	0.783	0.004	0.014	0.350	0.608
XYL		0.512	0.544	0.997	0.450	0.424	0.916
VIS × XYL		0.457	0.809	0.814	0.450	0.485	0.218

[1] BW = body weight of the dissected birds, PG = proventriculus and gizzard. SI = small intestine. [2] VIS = viscosity, LV = low dietary viscosity, HV = high dietary viscosity, SEM = pooled standard error of means, XYL = exogenous xylanase.

Table 5. Effect of dietary viscosity and xylanase supplementation on dry liver weight, hepatic and blood antioxidant status of 21 d old broiler chicken.

Item [1] Treatment [2]	Dry Liver Weight (g)	Vit E (µg/g)	Co Q10 (µg/g)	Hb	GSH-Px (U/g Hb)
VIS					
LV	6.89	84	202	111	173
HV	6.93	81	221	111	155
SEM	0.208	4.5	9.4	2.5	4.8
XYL					
No	6.88	86	197	112	170
Yes	6.93	80	226	109	158
SEM	0.208	4.5	9.4	2.5	4.8

Table 5. *Cont.*

Item [1] Treatment [2]	Dry Liver Weight (g)	Vit E (µg/g)	Co Q10 (µg/g)	Hb	GSH-Px (U/g Hb)
Probabilities					
VIS	0.903	0.652	0.169	0.891	0.010
XYL	0.866	0.325	0.037	0.420	0.068
VIS × XYL	0.363	0.601	0.899	0.121	0.885

[1] Vit E = hepatic concentration of vitamin E, Co Q10 = hepatic concentration of coenzyme Q10, Hb = blood hemoglobin, GSH-Px = blood glutathione peroxidase. [2] VIS = viscosity, LV = low dietary viscosity, HV = high dietary viscosity, SEM = pooled standard error of means, XYL = exogenous xylanase.

Table 6. Effect of dietary viscosity and xylanase supplementation on cecal production of volatile fatty acids of 21 d old broiler chicken.

Item [1] Treatment [2]	VFA Tot (ug/g)	AA (ug/g)	BA (ug/g)	PentA (ug/g)	PrA (ug/g)	AA:BA
VIS						
LV	1148	856	223	10	60	0.249
HV	1180	861	248	10	62	0.280
SEM	95.5	70.1	25.5	0.8	6.0	0.0184
XYL						
No	1082	807	197	10	67	0.243
Yes	1246	909	274	9	55	0.285
SEM	95.5	70.1	25.5	0.8	6.0	0.0184
Probabilities						
VIS	0.816	0.961	0.487	0.916	0.853	0.246
XYL	0.229	0.313	0.040	0.287	0.173	0.114
VIS × XYL	0.489	0.556	0.377	0.788	0.687	0.660

[1] VFA tot = total volatile fatty acids, AA = acetic acid, BA = butanoic acid, PentA = pentanoic acid, PrA = propanoic acid, AA:BA = ratio between AA and BA. [2] VIS = viscosity, LV = low dietary viscosity, HV = high dietary viscosity, SEM = pooled standard error of means, XYL = exogenous xylanase.

4. Discussion

Supplementary XYL can improve nutrient digestibility by hydrolysing dietary NSP into simpler forms that can either be absorbed directly by the host or can easily be digested by brush-border enzymes [1,7]. If some of the hydrolysed NSP fractions leave the upper GIT, they can still provide a suitable substrate for microbial fermentation in the lower GIT [6]. Usually, the observed discrepancies in the enhancement of bird growth performance, digestion and gut fermentation are attributed to variations in dietary fibre, birds' age, study duration and transit rate of digesta in various segments of the broilers' GIT [9,11,27]. In this study, there was no significant difference in growth performance in response to dietary XYL, but birds fed XYL had lower FCR, supporting the better energy and nutrient utilisation in XYL supplemented diets. This improvement in FCR in response to XYL is consistent with previous findings [7,28–31], when birds are fed diets containing fibrous materials. Similar to this study, where AME was slightly lower than breeder recommendations, XYL supplementation has been found effective in improving the FCR of broilers fed a marginally energy-deficient diet [32,33]. The lack of XYL by wheat NSPs interaction suggests that the difference in the NSPs content of the diet of the present study, due to the different wheats, was within the tolerable range of NSPs, such that increased levels of xylanase were not warranted. The better response (2%) in FCR by XYL addition is due to the reduced feed intake (2%), not higher weight gain.

Birds fed the HV wheat diet had reduced AMEn independently of XYL addition. It is widely accepted that the anti-nutritional effect of wheat is mediated by its NSPs constituents that raise the viscosity of gut contents and may modulate the microflora [11]. An increase in intestinal digesta viscosity is associated with enhanced bacterial fermentation and reduced digestion and absorption of nutrients by the host [1]. Dietary XYL can improve growth performance, nutrient availability and bird health via reduced digesta viscosity,

hydrolysing dietary fibres and the generation of prebiotic fibres [34]. The reduced AMEn of the birds fed HV diets is coupled with heavier PG and pancreas weights. In general, if the efficiency of digestion is consistently suboptimal, whether due to ingredient quality, microbial interaction or anti-nutritive factors, the GIT responds by increasing in both size (surface area) and digestive enzyme output [35].

It has previously been discussed that although diet is the main determinant of antioxidant composition in liver, feed supplements/additives other than antioxidants (e.g., phytase and XYL) can affect the efficiency of antioxidant assimilation from the diet and subsequently, their accumulation in the liver [9,24,25]. The mode of action of XYL accounting for the observed increase in hepatic coenzyme Q10 in XYL fed birds in this study is unclear. Coenzyme Q10 is the lipid soluble compound present in endo membranes of cells as well as in the mitochondria [36]. It is involved in the mitochondrial respiratory chain, where it accepts and transports electrons to oxygen and at the same time the proton gradient promotes ATP synthesis. The presence of high concentrations of coenzyme Q10 in all membranes enhances the antioxidant status of these membranes either by direct reaction with free radicals or by regeneration of tocopherols and ascorbate [37]. A protective effect of coenzyme Q10 against lipid peroxidation was shown in fatty acid emulsions. The observed increase in hepatic antioxidants suggests that in addition to enhanced dietary energy and nutrient availability, the improvements seen in commercial poultry fed XYL may also contribute to the improvement of gut health and antioxidant status within the bird.

Increased viscosity of intestinal digesta, i.e., by feeding high NSPs wheat, may result in inefficient mixing of digesta and movement of solutes, with a resultant depression in nutrient digestibility [1,38] and reduced hepatic antioxidant concentration [39]. High digesta viscosity may also provoke more nutrient oxidation in the GIT. This may explain the tendency for a marginally reduced blood GSH-Px level in HV birds. The observed GSH-Px levels were, however, in accordance with other studies [40,41]. A high GSH-Px status usually indicates higher antioxidant status, and conversely, lower GSH-Px would be expected in higher oxidative stress situations [37]. Thus, the reported results further support the view of the negative impact of high digesta viscosity on the antioxidant status of poultry.

Choct et al. [3] found that the concentration of cecal SCFA rises in response to XYL supplementation due to an increase in microbial fermentation. The hindgut microflora diversity can therefore be modulated by dietary NSP composition [42] and is thus intrinsically associated with the concentration of gut SCFA [43,44]. For example, Rodriguez et al. [45] found greater numbers of *Escherichia coli* and *Lactobacilli* in the digesta of broilers fed wheat and barley compared to those fed maize-based diets. These bacteria are predominantly acetic and butyric acid producers [46] and might explain the higher concentrations of these two acids in the ceca digesta observed in this report.

In general, observed differences regarding performance, metabolisable energy and other variables previously discussed between published reports and data in the present study may be due to external experimental validity linked to differences in experimental design [47] including XYL activity, dietary formulations, age of birds, environmental conditions, etc. External experimental validity should be considered just as important as internal validity in future research, which could benefit from more complex experimental designs (e.g., factorial structures) to consider multiple factors (e.g., different modes of action of the enzyme).

5. Conclusions

The present study shows that young broilers can perform similarly with both low and high NSPs wheat diets; however, further enhancements in performance (FCR) and health (hepatic antioxidant status) are possible with XYL supplementation. Besides increasing feed efficiency, XYL improved dietary energy and nutrient availability, hepatic antioxidant status and caecal butyrate production, irrespective of NSPs diet content. This also suggests that

birds may tolerate a greater dietary NSPs content; thus, further benefits may be obtained by the application of XYL in low energy wheat-based diets.

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