Harper Adams University

New strategies for enhancing the value of fibre in modern poultry nutrition

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Abstract

This PhD project aimed to investigate the potential prebiotic effects of natural feed fibres, fibre degrading enzymes and their interactions in diets when fed to Ross 308 broiler chickens. Throughout the duration of this project, three live animal experiments were completed. The first study investigated the impact of wheat bran, xylanase (XYL), xylo-oligosaccharides (XOS), and XYL+XOS on the growth performance, energy and nutrient availability, jejunum histomorphometry and caecal production of short-chain fatty acids (SCFA) in chicks. Feeding the XYL+XOS combination was most efficient at degrading dietary fibre and improving bird production performance. The second experiment studied the efficiency of XYL and XYL+XOS supplementation to diets with low (LV), medium (MV) and high (HV) viscosity, on energy and nutrient availability, gastrointestinal tract development and production performance of broiler chickens. Overall, birds fed LV diet had greater feed efficiency compared to the other treatment groups. The N-corrected apparent metabolisable energy (AMEn) increased in the LV diet when supplemented with XYL and XYL+XOS. In addition, fibre and nutrient retention coefficients were greater for HV diet ($P < 0.001$) and coincided with better developed caeca in those birds. The third study involved two different sources of XOS, with 2-6 and 2-9 degrees of polymerisation, fed at two levels (50 and 500 g/t), on AMEn, nutrient availability, ileal and caecal SCFA production and production performance of broilers fed XYL supplemented maize-based diets. Compared to the control diet, feeding XOS improved production performance, AMEn and nutrient availability. Feeding 50 g/t of either XOS sources produced a greater concentration of caecal SCFA but did not modulate production performance. In conclusion, feeding XYL and XOS may improve bird performance and nutrient availability regardless of XOS level fed.

Declaration

I declare that this thesis has been composed entirely by the author. It has not previously been submitted before to qualify for any other academic degree. No part of this thesis has been previously submitted for an academic award by the author and all help given by others has been acknowledged. The information used in the thesis was cited according to the recommendation of HAU Guide to Referencing (2023).

Antonija Šimić

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Published work

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Šimić, A., González-Ortiz, G., Mansbridge, S.C., Rose, S.P., Bedford, M.R., Tukša, M. and Pirgozliev, V. 2022. The effect of xylanase and fermentable xylo- oligosaccharide supplementation on broiler chicken*. 26th World's Poultry Congress. Book of abstracts 2022, Paris, France.*

Šimić, A., González-Ortiz, G., Mansbridge, S.C., Rose, S.P., Bedford, M.R. and Pirgozliev V. 2021. Feeding a combination of xylanase and arabino-xylo-oligosaccharides to young broiler chickens. *2020 British Poultry Abstracts, 16:1, 1-34.*

Šimić, A., González-Ortiz, G., Mansbridge, S.C., Rose, S.P., Bedford, M.R. and Pirgozliev V. 2021. A stimbiotic improving caecal fermentation and growth performance in 35d old broilers. *Virtual International Poultry Scientific Forum, January 25-26, 20, Atlanta, USA.*

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List of Common Abbreviations

AME	Apparent metabolisable energy
AMEn	Nitrogen corrected apparent metabolisable energy
ANOVA	Analysis of variance
BW	Body weight
CD	Crypt depth
CF	Crude fibre
CW	Crypt width
DF	Dietary fibre
DM	Dry matter
DP	Degree of polymerisation
DMR	Coefficient of dry matter retention
FAO	Food and Agriculture Organisation
FI	Feed intake
FR	Fat retention
GIT	Gastrointestinal tract
FCR	Feed conversion ration
NR	Nitrogen retention
NSP	Non-starch polysaccharides
P	Phosphorus
SCFA	Short-chain fatty acid
VFA	Volatile fatty acid
VH	Villus hight
VH:CD	Villus hight to crypt depth ratio
VW	Villus width
WB	Wheat bran
XOS	Xylooligosaccharides
XYL	Xylanase
WB	Wheat bran
WG	Weight gain

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1. Chapter: Literature review

1.1. General introduction

Concerns over food security have increased as a result of the world's population more than tripling between years 1950 and 2020. As the world population is expected to approach 11 billion by the end of the twenty-first century novel approaches will be needed in the future to meet the growing global demand for meat protein (Kim et al., 2019; United Nations, 2023). According to Food and Agriculture Organisation (FAO) poultry production accounted for 37% of the total meat production in 2017, from which chicken accounted for 92% of the world's poultry population (FAO, 2013). In the last sixty years, chicken meat production has increased by 1535%, and it is continuing to expand (Figure 1), exceeding 123.63 million tonnes of produced chicken meat in 2022 (FAOSTAT, 2024). As the market is expanding, there is a bigger emphasis on feed efficiency, aiming to improve the effectiveness of processes in the poultry industry.

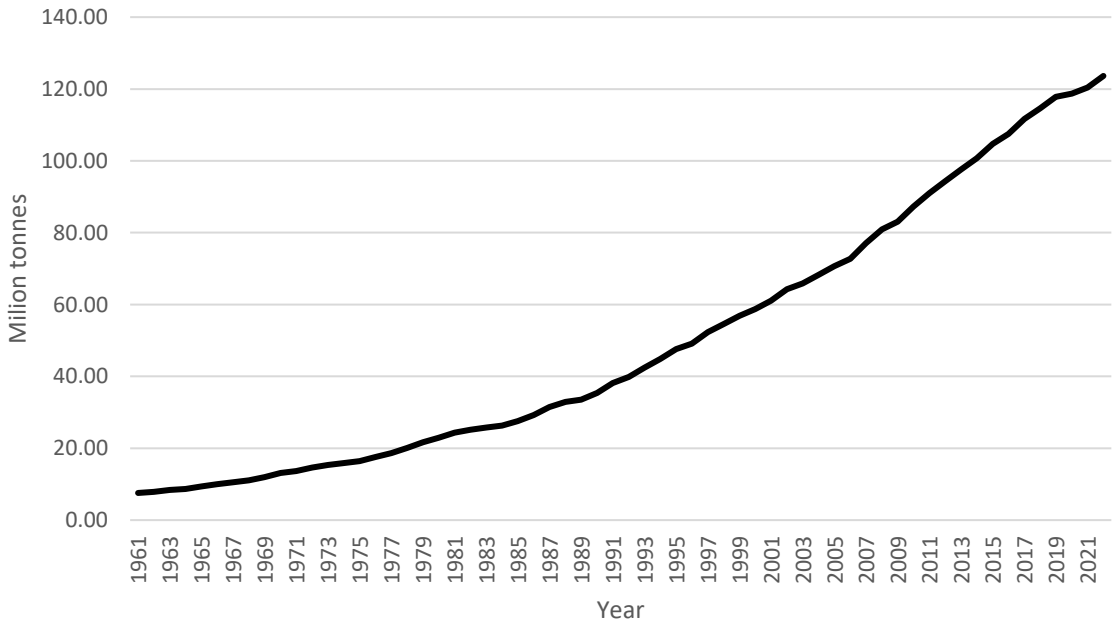

Figure 1: Global chicken meat production from 1961 to 2022.

(Source: FAOSTAT, 2024)

One of the important factors for reaching chicken genetic potential and improving production is poultry nutrition. Havenstein et al. (2003) compared the changes in feed conversion, growth and mortality of typical poultry diets used in 1957 and 2001. The results presented

that the average body weight of Ross 203 at day (d) 42 from the year 1957 reached 2.126g with a feed conversion of 1.9 compared to 2.627g and 1.62 when it was fed with a representative diet from 2001. This indicates how advancements in feed formulation and utilisation of nutrients can have a big production impact. Antibiotics in the EU were allowed as growth promoters in poultry diets until January 2006 (European Commission, 2005) when the need for alternative products for growth promotion arose. Although the application of locally obtainable or alternative cheaper feed ingredients could be beneficial, the use of unconventional ingredients in poultry diets can be restricted due to high undigestible non-starch polysaccharides (NSP) content (Adebiyi et al., 2010). Besides being an energy source, fibre could provide additional value in diets by its effects on digestive and metabolic processes (Iqbal et al., 2019). However, poultry has a monogastric digestive system which lacks the ability to produce necessary enzymes and digest beta-type of linkages in NSP. Based on advancing research in gut microbiota, the observation of NSP as an anti-nutrient in diets is gradually shifting to acknowledge them as growth and health stimulating fibre named - dietary fibre (DF) (Bautil and Courtin, 2019). A better understanding of DF functions in the digestive system could open an opportunity to improve control of animal well-being and production performances.

The following chapter will review current views and some of the available literature on DF supplementation and its importance in animal production, particularly in broilers. The review will try to establish current gaps in knowledge on poultry production while evaluating the impacts of DF supplementation.

1.2. Dietary Fibre definition and properties

Despite the use of fibre in practical nutrition being understood, the definition of fibre is not universally defined. The first time DF was introduced was in 1953 when Hipsley coined it while connecting the positive health effects of the high fibre diet (Hipsley, 1953). At that time the DF was not given enough relevance until Trowell (1974) introduced a wider vision of its significance and specified the concept. He originally defined DF as the remains of plant cell walls in the diet, which are resistant to hydrolysis by the digestive enzymes of humans; and later redefined the definition to include non-digestible plant materials within the cell, such as mucilages and gums (Trowell et al., 1976). Despite the worldwide interest and broad work of research, no organisation uniformly regulates the use of DF worldwide labelling, however, some institutions have their own definitions. Countries use definitions from various organisations, for instance from the AACCI (Cereals & Grains Association), the FNB (Food

and Nutrition Standards), the AOAC International (Association of Analytical Communities), the FSANZ (Food Standards Australia and New Zealand) and the CAC (Codex Alimentarius Commission) (Lunn and Buttriss, 2007).

Before accepting the new CAC definition, fibre were expressed in the United Kingdom as non-starch polysaccharides (NSP) which are only cell wall fibre compartments of plants and include cellulose, hemicelluloses, gums, pectins, beta-glucans and mucilages, but do not include synthetic, non-hydrolysed polymers, or polymers extracted from raw food material by enzymatic, physical or chemical means (Jones, 2014). Along with the United Kingdom, many other countries accepted the CODEX definition of DF, including state members of the European Union, China and Australia (Table 1.1).

The CAC was established in 1963 by FAO and WHO (World Health Organization) with the main mission to develop international food standards, codes of practice and guidelines to ensure fair practises while protecting the health of consumers (Zielinski et al., 2013). After more than 15 years of debating, CAC agreed on a definition of DF in 2009 and according to the following definition, DF is a ‘’carbohydrate polymer with ten or more monomeric units which are not hydrolysed by the endogenous enzymes in the small intestine of humans’’. National authorities can individually decide on whether to include carbohydrates from 3 to 9 monomeric units in the definition. The DF derived from plant origin may include fractions of lignin and/or other compounds associated with polysaccharides in the plant cell walls, however, if extracted and re-introduced into the food they are not included in the definition of the DF. To be defined as DF according to CAC, polymers must fall into one of the following categories: edible carbohydrates naturally occurring in the food as consumed; carbohydrates polymer which has a proven health benefit while being obtained food raw material by physical, enzymatic or chemical means; and synthetic carbohydrate with scientifically proven physiological health benefit.

Table 1.1 Countries that accept CODEX classification

Accepting all monomeric units larger than 3 and CODEX definition	Rejecting less than 10 monomeric units but accepting CODEX definition	Unresolved with proposals	Indeterminate status
• European Union • United Kingdom • Australia • New Zealand • China • Japan • Korea • Chile for labelling	• Chile for health claims • South Africa	• USA • Canada	• African countries (other than South Africa) • India • Brazil • Russia • Switzerland • American countries • Other South • Middle East

(Adapted from Stephen et al., 2017)

Fibre is not made from a chemically, nutritionally, or physically uniform material and due to its complex definition, DF is still evolving depending on the available information (Van Soest et al., 1991). Although many countries accepted the CODEX definition, there is still no worldwide uniform definition. Previous research indicates that a better understanding of DF and the application of DF to reformulate animal diets may benefit animal production systems in terms of intestinal health (Jha and Mishra, 2021), welfare and environmental impact through improved feed efficiency (Bedford et al., 2024).

1.3. Chemistry of dietary fibre

Sugars, oligosaccharides, and polysaccharides are some of the main types of carbohydrates (Table 1.2) (Asp, 2000). The DF is a carbohydrate that is not made from a single chemical entity but from a complex mixture of plant polymers that resist total digestion in the small intestine (Figure 2) (Cruz-Requena et al., 2019). Dietary fibre are frequently divided into subgroups of non-starch polysaccharides (NSP; Monomeric units - MU ≥ 10), resistant

oligosaccharides (MU <10), resistant starch (RS; MU number MU ≥10) and associated non-carbohydrate substances (lignin, waxes and chitins) (Stephen et al., 2017).

Table 1.2 Main carbohydrates classification

Class (DP)	Subgroup	Components	Typical monomers	Digestibility*
Sugars (1–2)	Mono-saccharides	Glucose		+
		Galactose		+.
		Fructose		+
	Disaccharides	Sucrose	Glu, Fru	+
		Lactose	Glu, Gal	+(-)
		Trehalose	Glu	+
Oligosaccharides	Malto-oligo-saccharides	Maltodextrins	Glu	+
	Other oligo-saccharides	α-Galactosides	Gal,Glu	-
		Fructo-oligosaccharides	Fru, Glu	-
Polysaccharides	Starch	Amylose	Glu	+(-)
		Amylopectin	Glu	+(-)
		Modified starch	Glu	+ -
	Non-starch polysaccharides	Cellulose	Glu	-
		Hemicelluloses	Variable -	-
		Pectins	Uronic acids -	-
		Hydrocolloids	Variable	-

DP, degree of polymerisation; *Digestibility in the small intestine,

(Adapted from Asp, 2000; FAO, 1998)

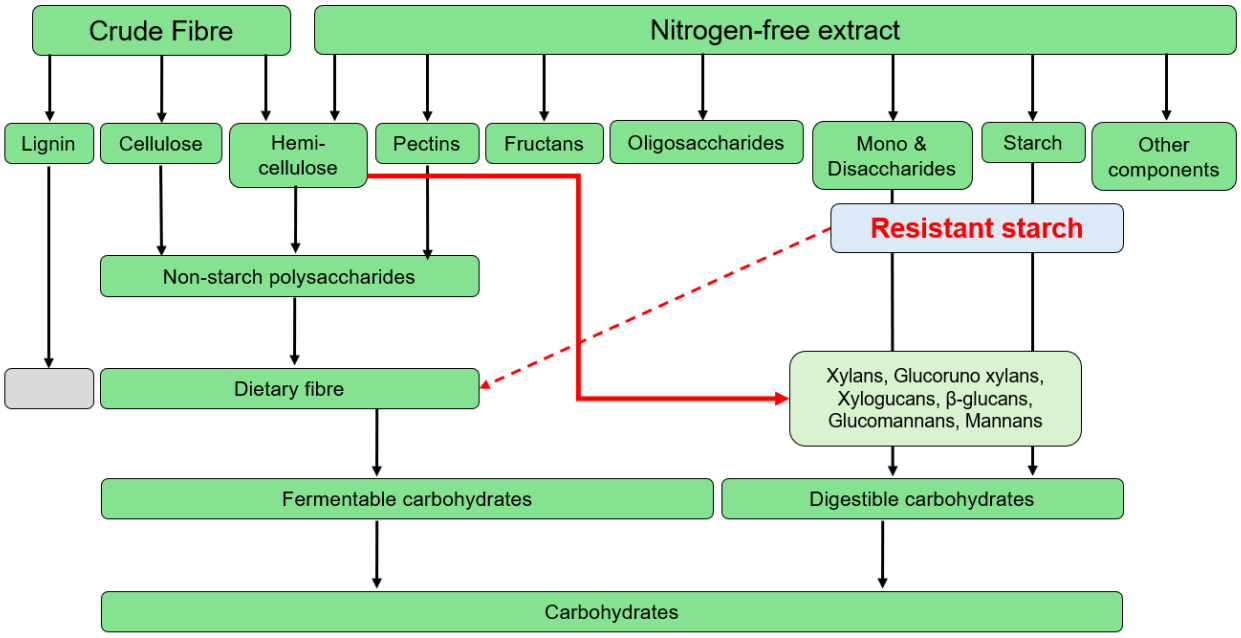

Figure 2: The main components of dietary fibre as part of total carbohydrates

(Adapted from Jha and Mishra, 2021)

The viscosity of NSPs is determined by the structure, solubility, molecular weights and concentration of fibre (Chesson, 2001; Knudsen, 2001). Grain components of the plants commonly include a combination of insoluble and soluble NSPs in variating proportions (Table 1.3), which can also depend on the maturity and phase of grains. By interpenetration of individual polymer chains, water-soluble polysaccharides create a high level of viscosity, forming an entangled network. This occurs only at the point when polymer entanglement begins or above the critical concentrations of polymers (called C* point). When mixed with water, soluble NSPs form dispersions and can increase the viscosity of digesta, causing anti-nutritive effects in monogastric animals (Kumar et al., 2012). High levels of viscosity have been linked with decrease of nutrients absorption and digestion (Ellis et al., 1996; Hung et al., 2022), an increase of feed conversion ratio (FCR) (Jørgensen et al., 1996) and a decrease in average daily body weight gain (WG) (Pourazadi et al., 2020).

Table 1.3 The types and levels of NSP identified in various cereal grains and their by-products (% dry matter)

Cereal		Arabinoxylan	ß-Glucan	Cellulose	Mannose	Galactose	Total
Maize	Soluble	0.1			trace	trace	0.1
	Insoluble	5.1		2.0	0.2	0.6	8.0
Wheat	Soluble	1.8	0.4		trace	0.2	2.4
	Insoluble	6.3	0.4	2.0	trace	0.1	9.0
Wheat bran	Soluble	1.0	0.2		trace	0.1	0.4
	Insoluble	8.8	2.8		0.1	0.6	12.3
Barley	Soluble	0.8	3.6		trace	0.1	4.5
	Insoluble	7.1	0.7	3.9	0.2		12.2
Rye	Soluble	3.4	0.9		0.1	0.1	4.6
	Insoluble	5.5	1.1	1.5	0.2	0.2	8.6
Oatmeal	Soluble	0.1	4.4		trace	0.1	4.5
	Insoluble	0.8	0.3		trace	trace	1.1

(Adapted from Choct,1997 and Englyst, 1989)

Cell wall building blocks of polysaccharides can be categorised as the hexoses (glucose, galactose and mannose), the pentoses (arabinose and xylose), the 6-deoxyhexoses (rhamnose and fucose) and the uronic acids (glucuronic and galacturonic acids or 4-O-methyl ether). The most significant polysaccharides of plant cell walls are cellulose, arabinoxylans, mixed linked (1→3), (1→4) β-d-glucan (β-glucan), arabinogalactans, rhamnogalacturonans, and xyloglucans (Bacic et al., 1988; Selvendran, 1984; Theander et al., 1989).

1.4. Dietary fibre analysis methods

A significant part of the evolution of the DF concept was the evolution of the methods used for its determination. During the last century, the continuous development of DF analytical techniques for characterisation and quantification has been connected with a better understanding of DF (Alyassin and Campbell, 2019). The basis of all DF analysis methods is similar, but the approach differs depending on the equipment used, application, desired end product and the source of fibre (Maphosa and Jideani, 2016). The most common analytical methods used for DF determination can be divided into three categories: non-enzymatic-gravimetric, enzymatic-gravimetric, and enzymatic-chemical methods (Elleuch et al., 2011).

Gravimetric methods are based on the principle of measuring the weight of the remaining undissolved residue after enzymatic or non-enzymatic (chemical) treatment (Alyassin and Campbell, 2019). While gravimetric methods are routinely used for total, insoluble, and soluble DF; the enzymatic-chemical (Englyst or Uppsala) methods are more suitable for use in scientific purposes (De Vries, 2015). Table 1.4 summarises a comparison of different possible advantages and limitations of DF analysis methods, developed over the years. Although there has been an improvement in knowledge and advances in technology over the years, more research is required to further develop more uniform and enhanced methods.

Table 1.4 Advantages and disadvantages of DF analysis methods

Method	Products	Advantages	Disadvantages	References
Nonenzymatic-gravimetric	Hemicellulose, cellulose, lignin	High purity products	Low selectivity Difficult extraction conditions	Mwaikambo, (2006)
Enzymatic-gravimetric	Total dietary fibre, insoluble and soluble fibre, crude fibre	Higher yield than enzymatic-chemical Quick and easy	Some insoluble fibre, lignin and all soluble fibre are lost Residues contain nitrogenous material	Gordon and Okuma, (2002)
Enzymatic-chemical	Hemicellulose, cellulose, total dietary fibre, soluble fibre	Faster and easier than enzymatic-gravimetric	Chemical residues in products Time-consuming	Devinder et al., 2012

(adapted from Cruz-Requena *et al*., 2019)

The first method used for the determination of fibre was nonenzymatic-gravimetric Crude fibre method, developed by 1806 Heinrich Einhof in 1806 (Cruz-Requena et al., 2019; Soest and McQueen, 1973). Crude fibre is residual left after chemical decomposition of fibre by oxidative or hydrolytic treatment (Elleuch et al., 2011). The method can be found misleading as the consecutive treatment could averagely remove about 50-90% of lignin, 80% of the hemicellulose and 30-50% of the cellulose, but despite that Crude fibre method is registered as an official method (AOAC 926.09) (Hell et al., 2014; Norman, 1935; Soest and McQueen, 1973). Afterwards, Van Soest and his co-workers developed the detergent methods which provided a more suitable alternative to fibre analysis (Soest and Wine, 1967; Van Soest et al., 1991; Van Soest, 1963). Detergent methods can be divided into two main classification categories: neutral-detergent (NDF) and acid-detergent fractions (ADF) (Cruz-Requena et al., 2019). The NDF extraction measures the fraction of the fibre that is insoluble in neutral

detergents and isolates cellulose, lignin, and neutral detergent insoluble hemicellulose, while ADF extraction measures cellulose and lignin fraction of the fibre (Van Soest and Wine, 1967). However, it has been reported that starch and protein might contaminate the NDF residue, water-insoluble pectic and water-soluble NSP substances could be lost in the NDF analysis method and the hemicellulose might be left in ADF fraction (Knudsen, 2001). Despite potential loss of specific components and contamination, the development of NDF and ADF analysis could provide significant progress in the reliability and accuracy of fibre analysis compared to crude fibre determination method.

The enzymatic-gravimetric method was developed in the early 1980s and it represents the summation of insoluble and soluble polysaccharides and lignin, considered to be total dietary fibre (TDF) (FAO, 1998). This method applies to foods, plant materials and food ingredients as consumed and is consistent with the 2009 CODEX definition (McCleary, 2000). The main steps in the method include enzymatic treatments for starch and protein removal; precipitation of soluble DF components by aqueous ethanol; isolation and weighing of the DF residue; and correction for protein and ash in the residue (Asp, 2000).

In the enzymatic-chemical method, the first fundamental step is to remove starch. Following the gelatinisation of starch, precipitation or extraction by 80% (v/v) ethanol is used to separate soluble DF polysaccharides from low molecular weight sugars and starch hydrolysis products (Asp, 2001). In Uppsala Method (AOAC 994.13) individual sugar residues are quantified by converting them into alditol acetates and determining them using a gas chromatograph (Theander et al., 1995). The Englyst method has been approved mainly in the UK (Asp, 2001), where dimethyl sulphoxide is used to disperse the starch before amylolysis and NSPs are determined by high performance liquid chromatography (Quigley and Englyst, 1994). Two substantial advantages of this method are the ability to fractionate NSP based on their solubility in water; and the ability to separate the individual sugar composition of DF that gives an idea of the type of polysaccharides present in an ingredient (Choct, 2015).

The method should be carefully considered based on specific goals of DF analysis, as determination analysis can have variations in operational simplicity, loss of fibre components and chemical residue contaminations.

1.5. Value of Dietary Fibre

High DF was initially associated with impaired nutrient utilisation and decreased animal performance. Research trials in previous years reported their negative impact on daily intake, growth performance and digestibility of nutrients (Jørgensen et al., 1996; Sklan et al., 2003). However, experiments performed in the recent past have elaborated the role of fibre in improving broilers performance (González-Alvarado et al., 2010; Jiménez-Moreno et al., 2009). The fibre distinctly has more value than initially thought, moreover, dietary fibre is required to maintain normal physiological functions in the gastrointestinal tract (Tejeda and Kim, 2021). The overall growth and development of GIT depend on diets quantity and quality of fibre (Owusu-Asiedu *et al*., 2006). When DF is fed to birds in low to moderate levels (up to 50 g/kg), it may enhance GIT health, bird performance and nutrient digestibility (Iqbal et al., 2019; Mateos et al., 2012). Animals are benefiting from DF nutritionally in a way that it directly provides energy (Varel and Yen, 1997) and indirectly by stimulating the GIT and immune system (Choct et al., 1996; Jha et al., 2010; Pieper et al., 2008). The effect on immune function from microbial fermentation depends on fibre fermentation and their SCFA, which as a result may help safeguard the digestive system (Niba et al., 2009).
In poultry diets, DF can contribute to the nutritive value as the direct energy source, and indirectly through its effects on metabolic and digestive processes (De Vries, 2015). A better understanding of the characterisation of fibre fractions, physiological effects and fibre degradation in the chicken is needed in future to in order to more precisely predict the nutritional impact of fibre from feed ingredients.

1.6. Poultry nutrition

1.6.1. Poultry diet

When selecting feed ingredients in the diet, it should be taken into consideration the absence of any biological contaminants, physical nature, content, variability and availability of the nutrients (Kleyn, 2013). According to Department for Environment, Food and Rural Affairs (2018) cereal grains wheat, barley, oats and maize make up the largest proportion in poultry diets, with wheat being the most commonly used cereal in the UK. These cereal grains can be used as whole grains or in more refined forms. For example, wheat whole grain includes all parts of the grain kernel: the fibre-rich bran, the endosperm, the nutrient-rich aleurone layer and the nutrient-packed germ (Figure 3) (Lunn and Buttriss, 2007).

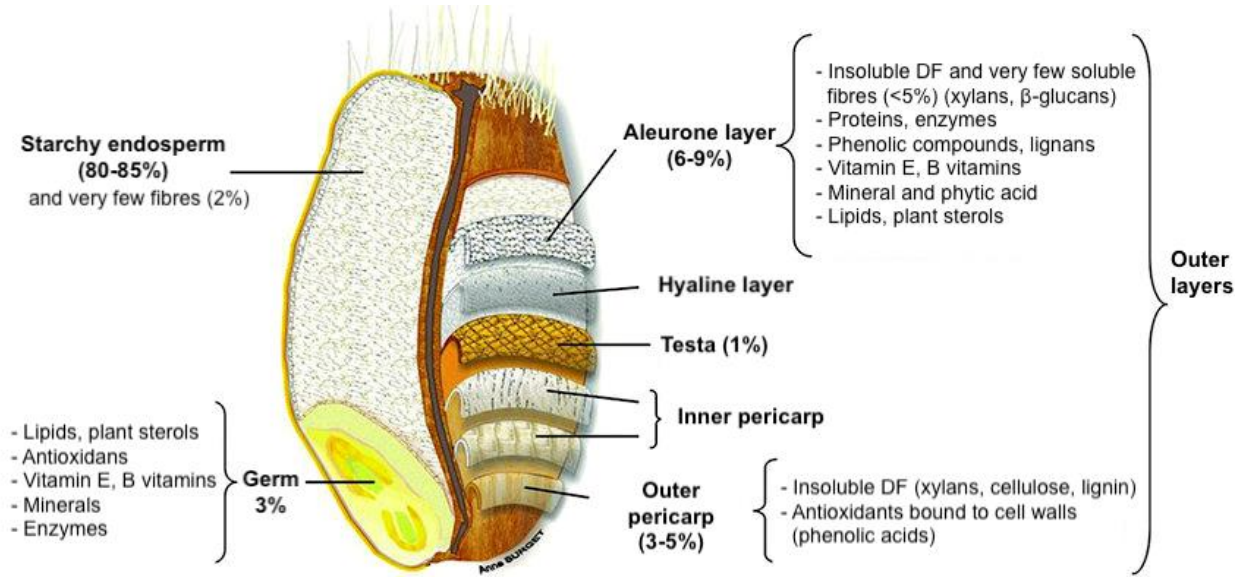

Figure 3: Histological structure of wheat grain

(Source: Brouns et al., 2012; Surget and Barron, 2005)

Apparent metabolisable energy (AME) values of cereals can differ between batches, depending on the chemical composition and physical characteristics of the kernels. Previous research reported variation of wheat AME values up to 5% (Black et al., 2005), which can be attributed as the main reason for the inconsistency discrepancy in broiler performance (Yegani and Korver, 2012). Azhar et al. (2019) assessed the qualitative characteristics, chemical composition, impact on AME and nutrient digestibility of seventeen wheat samples that are available in the UK. Research has confirmed variability amongst these characteristics in wheat samples and their effect on broiler WG, however, no differences were identified in FCR.

The nutrient composition of the wheat grain is presented in Figure 4. The typical wheat grain is made of starch (50-60%), protein (10-15%), fat (2-5%), DF (10-20%); phytochemicals and micronutrients (Lunn and Buttriss, 2007).

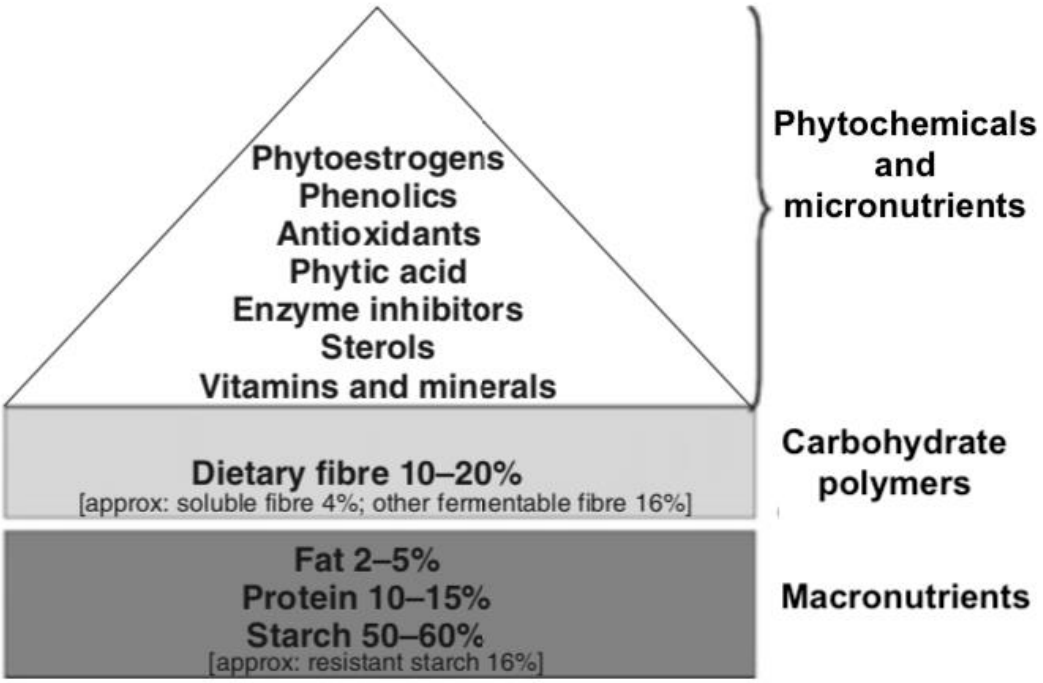

Figure 4: Typical wheat grain nutrient composition

(Source: (Bednar et al., 2001; Lunn and Buttriss, 2007)

Energy is one of the most important components of the diet that makes up from 60% to 70% of the diet cost. While carbohydrate is the primary source of energy, fat is secondary, containing 2.25 times more energy than carbohydrates. Despite this, the amount of fat in the diet should not exceed 5% and most of the energy requirements are utilised from carbohydrates (Kleyn, 2013). As previously stated, using high fibre feedstuffs could be valuable if poor growth performance is avoided. To achieve genetic potential of broilers, poultry nutrition should incorporate an understanding of biochemistry and digestive physiology while taking into consideration the economic influence of the nutrients that are supplied in practice (Titus, 1961).

1.6.2. Form of feed

It is well established that broilers fed pelleted diets outperform those fed with mash diets. Despite a higher cost, the pelleting process is compensated by the effect on the performance, with differences reported in pelleted diets in higher WG by 18.2% and increased FI by 16.3% (Pirgozliev et al., 2016). The main reason for better poultry performance on pelleted diets seems to relate to density of the diet. This theory was

established by Jensen et al. (1962) with experiments observing broiler feed intake behaviour. They concluded that broilers fed with pelleted diets were ingesting the same amount of feed six times faster compared to birds fed with mash diets; resulting in 67% less energy wasted in the process. While total feed intake did not differ among mash and pellet fed groups, birds performed significantly better when fed with pelleted diet. Trials performed in recent years have supported the theory of Jensen et al. (1962), by acknowledging improved efficiency of energy utilisation observed with pelleted diets was due to feeding activity. Furthermore, previous research suggested pelleting had hardly any effect on the classical estimations of digestibility or AME (McKinney and Teeter, 2004; Preston et al., 2000; Skinner-Noble et al., 2005). These conclusions question the commonly held belief that steam-pelleting improves diet digestibility by thermal processing. Moreover, the subsequent publication from Zimonja and Svihus, (2009) showed how the standard pelleting process does not induce meaningful gelatinisation of starch from combinations of temperature, moisture and time. They detected little starch gelatinisation and no difference in the ileal digestibility or degree of gelatinisation of starch among cold or steam pelleted diets. To conclude, it seems that the valuable effect of pelleting relies on the bird's ability to intake feed more quickly, generally resulting in overall greater feed intake, body weight and feed conversion ratio.

1.6.3. Particle size

Particle size is defined as the average diameter of individual particles of feed, expressed as geometric mean diameter (Zaefarian et al., 2019). There are two contradictory theories regarding questioning the optimum fineness of the grind for broilers. The first theory supports fine grinding, based on the idea that it will increase surface area and therefore provide more exposure to digestive enzymes. Another theory advocates coarse grinding, with a hypothesis that it stimulates more functional development of the gizzard, thus improving nutrient utilisation (Aftab et al., 2018). The Amerah et al. (2008) study concluded that the importance of particle size of wheat was more critical in mash than in crumble or pelleted diets. The effect of pelleting has been shown in several studies to even out differences in particle size (Abdollahi et al., 2014; Amerah et al., 2007; Péron et al., 2005; Svihus et al., 2004), however, Nir et al., 1995 noted that even after pelleting the effect of grain particle size is still sustained. Therefore, the beneficial effect of coarse particles on gizzard may still occur after pelleting. A possible reason for the variable effect of pelleting might relate to different cereal bases used and grain hardness, which was confirmed by (Péron et al., 2005). Following researchers concluded that contrasted to birds fed diets with fine particles; pelleting very hard wheat-based diets with coarse particles increased gizzard weight. The reason for that might be

found in the resistance of hard particles to reduce size during the pelleting process. Broilers seem to adjust the development of their GIT depending on the diet, moreover, its functionality is fundamental for performance improvement. Even though pelleting generally shortens the retention of gizzard and trims down particle size, it seems how the beneficial effect may still occur when coarse particles are used.

1.7. The use of non-nutritive feed additives in poultry nutrition

1.7.1. General features

Non-nutritive feed additives can be used in addition to optimal nutrients to ensure nutrients are ingested, digested, absorbed, protected from destruction, transported to the cells or provide better growth by altering the metabolism of chicken (Leeson and Summers, 2001). The list of common additives used in poultry nutrition and their possible action is shown in Table 1.5. In addition to enhancing feed quality and nutrient digestibility, these feed additives protect animals from parasites and illnesses. Through pathogen reduction, oxidation prevention, and gut health promotion, they work as well to improve animal health, productivity, and feed safety.

Table 1.5 List of commonly used non-nutritive feed additives in poultry nutrition and their potential action

Non-nutritive feed additive	Potential action
Pellet binders	Affect firmness and texture of pelleted feeds
Flavouring agents	Improve palatability of feed
Enzymes	Improve digestibility of specific nutrients
Antibiotics	Used at low levels to prevent production of toxins by the intestinal microflora and help protect feeds from microbial destruction
Antifungals	Prevent the growth of harmful moulds and fungi in the feed and digestive system
Anticoccidials	Help prevent coccidiosis, a parasitic disease that affects the intestines
Worming drugs	Protect animals from intestinal parasites (worms)
Antioxidants	Protect nutrients like fat-soluble vitamins and polyunsaturated fatty acids from oxidative damage
Carotenoids	Enhance pigmentation, often used to improve the colour of egg yolks and broiler skin
Probiotics	Beneficial bacteria that positively influence intestinal microflora, promoting gut health
Prebiotics	Provide nutrients for beneficial gut bacteria, reducing the presence of pathogens in the digestive system
Odour and fly control agents	Reduce odours and flies by influencing manure composition

(Adapted from Koyun and Callaway, 2019; Leeson and Summers, 2001; Nair et al., 2019)

1.7.2. Enzymes

Although the use of exogenous enzymes started in poultry diets as early as 1925 (Clickner and Follwell, 1926), their use and distribution were not widely spread until the 1980s (Elwinger et al., 2016). In the 1980s many of the enzymes were created to break down NSP, reduce gut viscosity and improve nutrient absorption. Exogenous enzymes such as xylanase (XYL), protease and β-glucanase have been successful in improving the nutritive value of wheat, rye and barley (Hesselman and Åman, 1986; Pettersson and Åman, 1988). Currently, additional enzymes are being further developed to improve feed formulations by enhancing nutrient absorption (Dittoe et al., 2019). A list of commercial enzymes can be found in Table 1.6. According to Markets and Markets report (2020), the global feed enzymes market size is estimated to account for 1.04 billion British pounds (GBP) in 2020 and is projected to reach 1.52 billion GBP by 2025. More than 70% of the world's enzymes are produced and sold by four key players: BASF, DSM/Novozymes, Addisseo and Danisco Animal Nutrition. Other suppliers include AB Vista, Alltech, Kemin, Chemigen and Novus (Barletta, 2010). Ever since the early use of enzymes, they have become integral to poultry diets, especially from the 1980s onward. Enzymes such as XYL and protease have enhanced nutrient absorption, and the global enzyme market is projected to grow significantly, driven by prominent companies leading the industry.

Table 1.6 The feed enzymes, their target substrates and examples of market products

Enzyme	Target substrate	Target feedstuffs	Market producer: products
Amylase	Starch	Cereal grains, grains legumes	BASF:Fuelzyme® 650 alpha-amylase
Lipases	Lipids	Lipids in feed ingredients	Sukahan: SUKALip Lipase
Phytases	Phytic acid	All plant-derived ingredients	DSM: RONOZYME® ProAct
Protease	Proteins	All plant protein sources	BRI: Versazyme®
α-Galactosidases	Oligosaccharides	Soybean meal, grain legumes	Aumgene Biosciences: Alpha-Galzyme XP
β-Glucanases	β-glucan	Barley, oats and rye	Megazyme: β-Glucan
Xylanases	Arabinoxylans	Wheat, triticale, rye, barley, fibrous plant materials	AB Vista, Econase XT®

(Source: Ravindran, 2013; BASF, Sukahan, DSM, BRI, Aumgene Biosciences, Megaenzyme and AB Vista)

The principal mode of action of enzymes is to enhance the digestibility of dietary components; while indirectly improving production consistency, environment, reducing cost and maintaining gut health (Figure 5). Enzymes achieve this by eliminating the encapsulating effect of the cell walls and improving access of the digestive enzymes to the feed components (Kleyn, 2013). As highly complex structure proteins, their mechanism of action can be influenced by pH, moisture content, temperature, substrate and enzyme concentration. The optimum pH for most enzymes is between 4 to 6; otherwise, they are likely to denature. While moisture is conceivably essential for the mobility of enzymes and solubility of the substrate/enzyme, temperatures of up to 40˚C increase activity and then sharply declines due to loss of structure. In theory reaction rate would increase with a higher concentration of enzymes, however, due to constrains in the digestive tract, there is no linear expansion. When substrate concentration is increased, the rate of reaction increases until a turnover maximum is achieved (Ravindran, 2013).

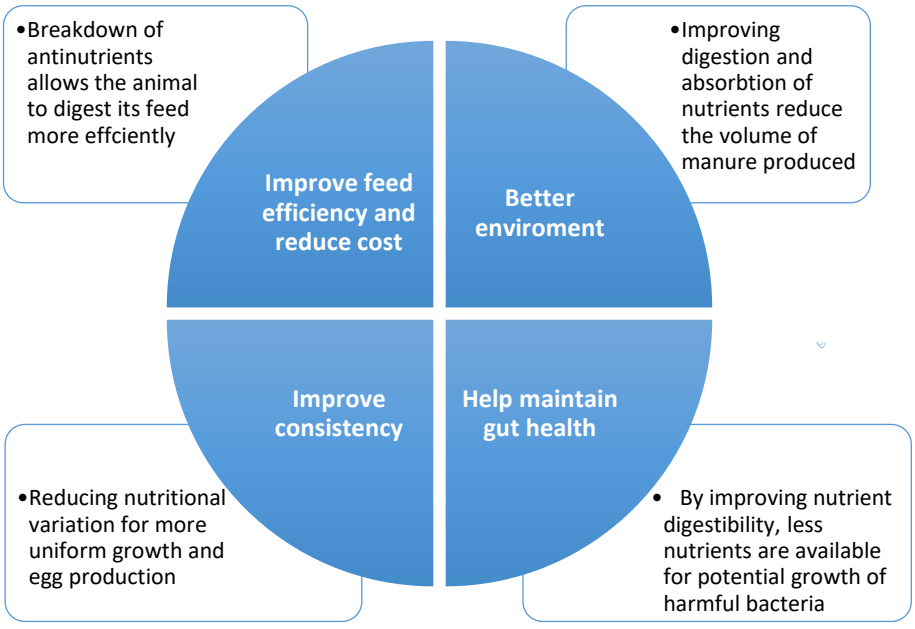

Figure 5: Potential benefits from enzyme supplementation

(Adapted from Barletta, 2010)

1.7.2.1. Xylanase

The use of non-starch polysaccharide degrading enzymes (NSPase) is widely accepted and routinely added to the diets (Gonzalez-Ortiz et al., 2019a). Xylanase is the most commonly used NSPase enzyme, that helps degrade arabinoxylan (AX) - main NSP in cereals (Aftab and Bedford, 2018; Knudsen, 2014). The AX is cut by endo-xylanases by hydrolysing the 1,4-β-D-xylosidic linkage between xylose residues in the backbone in a random manner;

consequently, trimming down their size and delivering their beneficial effects (Mendis et al., 2016).

The outcomes of the diet supplemented with xylanase could depend on age, feed ingredients, the strain and sex of the bird (Bedford and Cowieson, 2012). The beneficial effects of xylanase in poultry and pig digestion are associated with three main mechanisms: (1) reducing digesta viscosity by the breakdown of the soluble AX, thus allowing faster diffusion of digestive enzymes and substrates; (2) interference of the cell wall through the degradation within feedstuff cell walls and the following realise of captured nutrients; and (3) as a result of AX degradation, xylooligosaccharides (XOS) are released in the distal parts of the gastrointestinal tract when xylan breaks down into smaller oligosaccharides (Figure 6), which serve as a signalling molecule among certain beneficial bacteria (Bedford, 2018; González-Ortiz et al., 2019a).

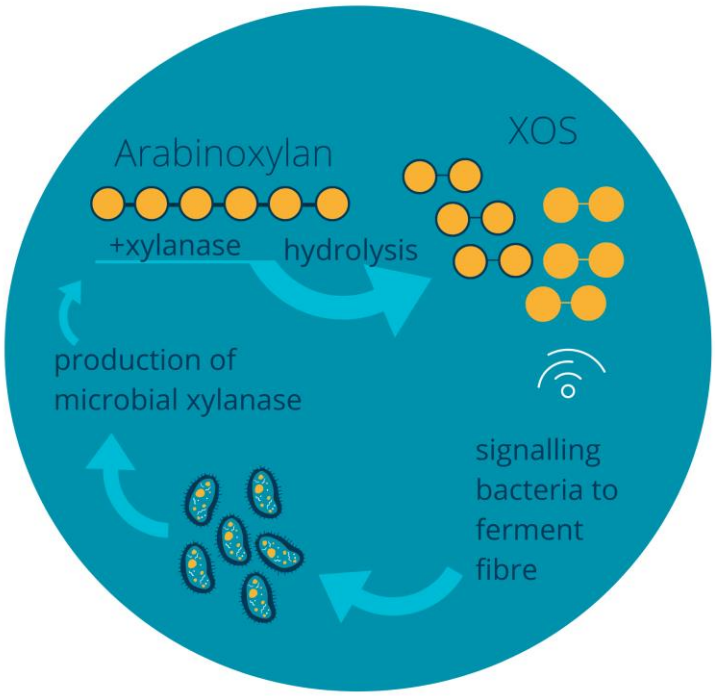

Figure 6: Potential effect of xylanase on hydrolytic arabinoxylan degradation and release of smaller oligosaccharides (XOS, xylooligosaccharides)

(Adapted from Bedford, 2018)

1.7.3. Prebiotics

In 2017 the board of directors of the International Scientific Association for Probiotics and Prebiotics formed an expert panel to form a new definition for prebiotic, described as a

‘’substrate that is selectively utilised by host microorganisms conferring a health benefit’. The hosts’ microbiota is influenced by a variety of substances besides prebiotics. Prebiotics can be distinguished (Figure 7) from other substances by the criterion of their selective utilisation by host microorganisms (Gibson et al., 2017).

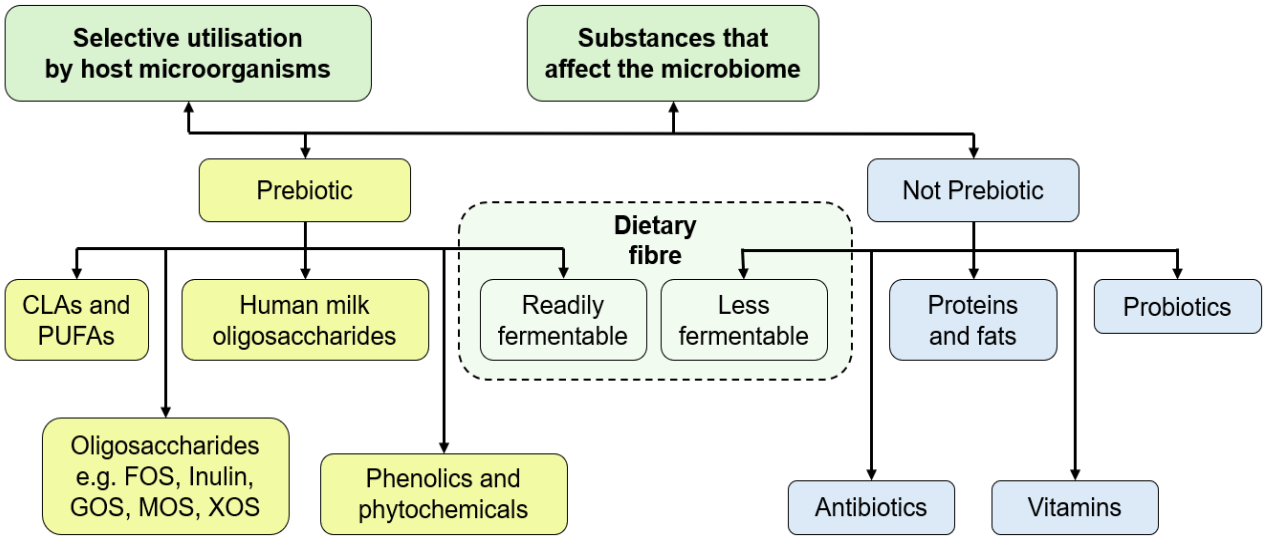

Figure 7: Differentiation of prebiotics using the suggested definition

CLA, conjugated linoleic acid; PUFA, polyunsaturated fatty acid; FOS, fructooligosaccharides; GOS, galactooligosaccharides; MOS, mannanoligosaccharide; XOS, xylooligosaccharide**.**

(Adapted from Gibson et al., 2017)

The FAO report (2007) listed commonly used prebiotics, such as XOS, inulin, fructooligosaccharides, galactooligosaccharides, soy-oligosaccharides, pyrodextrins, isomaltooligosaccharides and lactulose, as well as new emerging prebiotics including pecticoligosaccharides, lactosucrose, the sugar alcohols, gluco-oligosaccharides, levans, resistant starch, xyloosaccharides and soy-oligosaccharides.

1.7.3.1. Xylooligosaccharides

The XOS is oligomer comprised of xylose units (Figure 8) linked by β-(1,4) bonds (Manicardi, et al., 2023). The prebiotic effect of hydrolytic AX degradation, and realise of AXOS and XOS, may be a reason for improving health benefits in the gastrointestinal microbiota. Endo-β 1,4-xylanases split β-xylosidic glycosidic linkages to short-chain XOS or xylans (Jommuengbout et al., 2009). The produced mixture of low molecular hydrolysed oligomers XOS and AXOS could optimise colon function, change or increase the profile of SCFA, stimulate the immune system, increase villus length and increase mineral absorption, therefore possibly resulting in better performance (Kim et al., 2011).

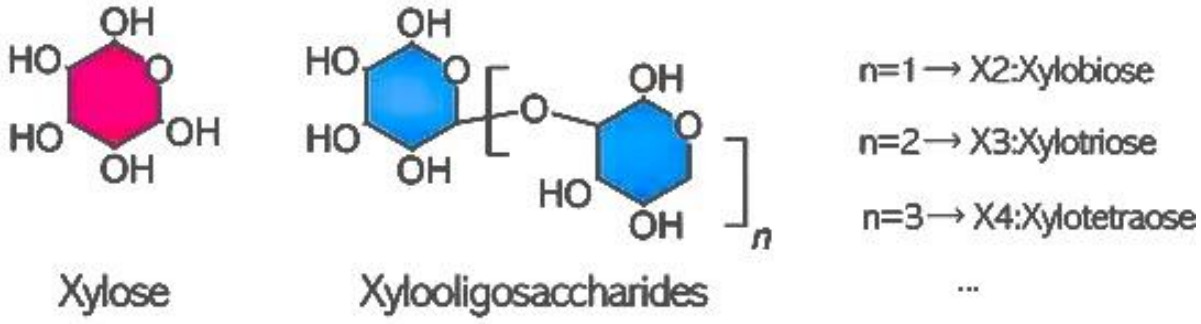

Figure 8: Main structure of XOS

(Adapted from Manicardi et al., 2023)

The effects of XOS on performance and microbiota in broiler chickens on wheat/rye-based diet was examined in the study of De Maesschalck et al. (2015). Results showed how XOS significantly improved FCR and increased villus length in the ileum. At 26 days, a higher concentration of lactobacilli was found in the colon; and in caeca higher number of butyryl-CoA:acetate CoA-transferase. Pourabedin et al. (2015) found that addition of 2000 g/t XOS increased the enhanced the *Lactobacillus* genus relative abundance in the caecum, but the diversity of the microbiota as a whole was not altered.

There has been substantial variation observed in the inclusion rate of XOS. According to previous studies, the incorporation rate of XOS has been recorded at low levels as 2 g/t, (Yuan et al., 2018) 50 g/t (Singh et al., 2021), raising up to 100 g/t, 1000 g/t, 10 000 g/t (Jazi et al., 2019; Ribeiro et al., 2018; Zhou et al., 2021) and even as high as 20 000 g/t (Zhenping et al., 2013). Furthermore, research done with corn-soybean meal base supplemented with

0, 25, 50, 75 or 100 g of XOS per ton of diet demonstrated that the addition of 100 g/t of XOS resulted in lowest FCR (Suo et al., 2015).

Courtin et al. (2008) found a significant improvement in FCR when AXOS was supplemented in a maize-based or a wheat-based diet. Their analysis of caecal content significantly increased level of *bifidobacteria* but not total bacteria. In another experiment, Courtin et al. (2008b) stated how XOS showed an increase of *bifidobacteria* counts in caeca compared to control after one week (108 g−1 vs 103 g−1) and obtained similar results for AXOS supplementation after 2 weeks, indicating probiotic potential. In the study of Morgan et al. (2019) was concluded that method of adding AX and XYL, which can reproduce xylans and XOS, was more effective than natural AXOS generation in the digestive tract. A study by González-Ortiz et al. (2019b) used a combination of xylanase and XOS to determine their influence on the performance of broilers fed with wheat-based diets. Results suggested that the dual combination of XYL and XOS may act synergistically, improving more effectively performance beyond that possible with a carbohydrase alone. Although the mechanism of the beneficial effect is not completely understood, there is a potential to use XOS and XOS as efficacious prebiotic in broiler diets. Future studies could benefit from using high-throughput sequencing techniques to provide a community-wide characterisation of the gut microbiota following prebiotic administration at various levels of taxonomic categorisation.

1.8. New strategies in poultry nutrition

1.8.1. Enzyme and prebiotic effects on arabinoxylan as a substrate for microbiota in the chicken gut

As previously stated, the supplementation of endo-β 1,4-xylanases effectively hydrolyses the xylan backbone of arabinoxylan (AX), generating AXOS, as well as XOS. There are indications xylanase improves the development of caecal microbiome, from beneficial bacteria fermentation of enzyme end products hydrolysis (Masey-O'Neill et al., 2014; Mc Cracken et al., 2006). A study (Morgan et al., 2019) suggested how feeding AXOS results in similar performance effects as supplementing xylanase in diet, which suggested how the substrate for beneficial bacteria could be AXOS generated from AX.

The oligosaccharides demonstrate prebiotic effects, by being fermented in the microbiome into valuable volatile fatty acids (VFA), principally butyrate and acetate and in the caeca and colon (Choct et al., 1999). However, concentration results in GIT may represent only the fraction of total VFA fermented over a certain period and may be influenced by the frequency

of caecal evacuation (Boets et al., 2015). It remains to be established whether it is more efficient to supplement broilers diet with XOS or provide additional AX which will be hydrolysed into XOS through NSPase enzyme supplementations.

The probiotic effects of XOS are correlated to its chemical structure and may depend on the ratio of xylose and arabinose, as well as on the impact of enzymatic treatment on degrees of polymerisation (de Freitas et al., 2019; Morgan et al., 2019). Prebiotic oligosaccharides have polymerisation degree of between 2 and 20 monosaccharides (Hume, 2011). Usually, supplementation with XOS has a DP of 2 – 7 (Fuso et al., 2022). However, research has indicated that a DP of 2 – 5 xylose units may promote the growth of Bifidobacterium and lactic bacteria (Ho et al., 2018; Reddy and Krishnan, 2016).

The DP of XOS used in supplementation are typically 2 – 7 (Fuso et al., 2022), however, studies have shown that a low DP of 2 – 5 xylose units could increase growth of lactic and Bifidobacterium bacteria (Ho et al., 2018; Reddy and Krishnan, 2016).

A study was conducted to examine the effects of using xylanase and XOS and xylanase alone (González-Ortiz et al., 2019b). It was hypothesised that using both xylanase and fermentable oligosaccharide would allow a better response of gut fermentable microbiome stimulation, more so than using xylanase alone. Results showed how combination improved performance growth results, concluding it may have more efficiently reduced viscosity, improved nutrient digestibility and stimulated microbial communities. Using supplementation mixtures may be a feasible strategy, however, thus far there is limited research on this particular subject.

It is worth noting how the microbiome is possibly adapting over time, taking up to 21 days for xylanase to show response (Bedford, 2018; Mendes et al., 2013). Heath and physiological status of the bird, characteristics of diet, level and source of DF can influence the response fibre digestion. With many factors influencing results of trials, more research is needed to elaborate fibre and NSPase efficiency effects.

1.8.2. Assessing bacterial activities in chicken microbiota

In the digestive system of poultry (Figure 8), the small intestine digests proteins, starches, sugars and lipids, while caeca and large intestine digestive systems operate with help of the microbiota’s beneficial bacteria by absorbing parts of remaining undigestible fractions (Kleyn, 2013). Those remaining undigestible fractions are mostly composed of NSP from plant origin,

including cellulose, arabinoxylans, mannans, β- glucans and several related polymer sugars (Knudsen, 1997).

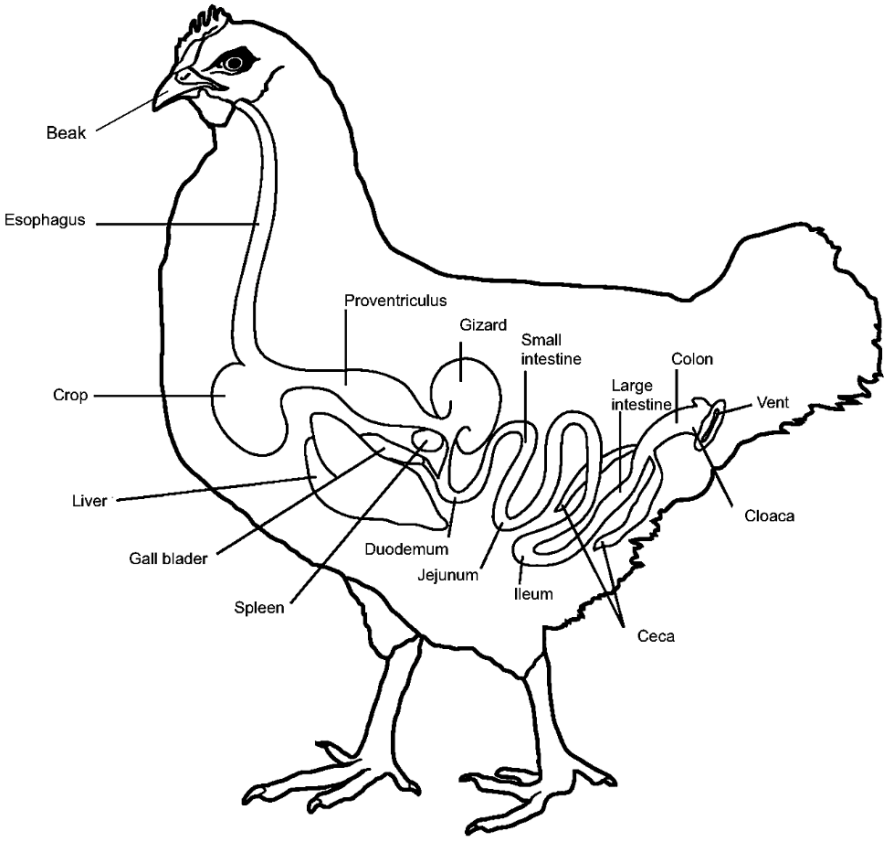

Figure 9: Gastrointestinal tract of a chicken

(Adapted from Clavijo and Flórez, 2018)

The Chicken microbiota contains an enormous number of different species that can be called the microbiome or microbial community (Apajalahti and Rinttilä, 2019; Yadav and Jha, 2019). Within the upper part of the intestinal tract microbial concentration is very low (approximately 10^3 per gram), higher in the ileum (approximately 10^9 per gram) and the highest in caeca (approximately 10^{11} per gram) (Ducatelle et al., 2019). Commonly found bacterial habitats in caeca composition are *Ruminococci, Bacteroides, Clostridia, Streptococci, Enterococci, Lactobacilli* and *E. coli (*Figure 9). It is well established that different types of diet have an immense impact on gut microbiota composition (Shang et al., 2018; Yadav and Jha, 2019; Rodríguez-Lagunas and Pérez-Cano, 2019), moreover, different components of a diet can shape and diversify the composition of the microbiota (Sawicki et al., 2017). Diets with DF appear to impact abundance in microbiota and long-lasting reduction of DF can cause the withdrawal of important microbial taxa (Sonnenburg et al., 2016). The subsequent publication of Mathlouthi et al. (2002) showed how substituted wheat and barley-based diets from the

corn-based diet, increased quantities of coliforms and Lactobacillus alongside other facultative bacteria populations. When water-soluble NSP- rich diets were used, higher production of SCFA was noticed, as well as the increase of transit time and viscosity of digestive content.

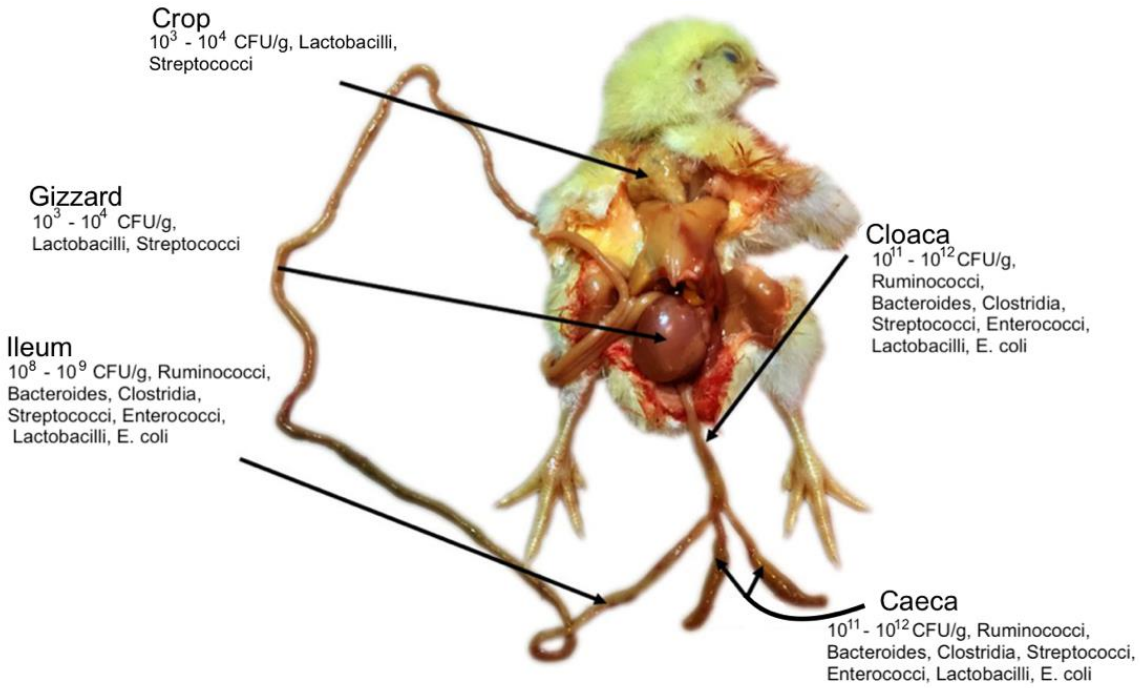

Figure 10: Concentration of the major bacterial habitations in chicken

(Source: Yadav and Jha, 2019)

Gut microbiota extensively interacts with the host, diet, and within themselves, while having a conclusive role in sustaining the normal physiology of host animals. Some of the main roles are supporting the normal development or formation of gut morphology and structure, boosting immune responses, helping to protect from luminal pathogens, along with having an active role in digestion and utilisation of nutrients (Grozina et al., 2023). However, the gut microbiota has also some indirect and direct damaging effects on chickens, for example, production of toxic metabolites from the protein fermentation, decrease of fat digestibility and possibly leading to inadequate growth performance (Jha and Berrocoso, 2015; Rinttilä and Apajalahti, 2013).
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1.8.3. Short-chain fatty acid production

The key broiler metabolic end products of carbohydrate fermentation by intestinal bacteria are SCFA, such as butyrate, acetate, propionate, succinate, and lactate (Bjerrum et al., 2006; Hooper et al., 2002). Whereas in pigs, total energy obtained from SCFA producing

hindgut fermentation may provide up to 30% of the total energy requirements (Bergman, 1990), in poultry there is no robust evidence supporting a specific generated value is being produced. However, a valuable source of energy for a host is converted by lover intestinal bacteria, that otherwise would be permanently lost. In the caeca, more than 90% of total SCFAs are constituted of several volatile fatty acids (VFA; including acetic, valeric, propionic, butyric, isovaleric, isobutyric and 2-methylbutyric acids) (Apajalahti and Rinttilä, 2019).

Butyrate is especially important as it is the preferred energy source for the enterocytes and is known to regulate proliferation within the intestinal mucosa and cellular differentiation, thus increasing intestinal tissue weight (Fukunaga et al., 2003). Production of butyrate is mainly produced by members of *Rose-buria spp*. and *Eubacterium rectale*, which are members of the family *Lachnospiraceae*, as well as *Faecalibacterium prausnitzii*-like organisms of family *Ruminococcaceae* (Bjerrum et al., 2006; Hold et al., 2003; Louis et al., 2010). Together with other SCFA, butyrate contributes to epithelial development that is essential for the maintenance of normal intestinal barrier functions and could prevent the passage of toxic and pro-inflammatory molecules from the external milieu into the submucosa and systemic circulation (Niba et al., 2009). The formation of SCFA in chicken caecum also reduces the pH of the intestinal environment. Lower pH may lead to inhibiting acid-sensitive pathogenic bacteria, such as members of the family *Enterobacteriaceae* (van der Wielen et al., 2000). Lactic acid is the strongest of the common acid produced by GIT bacteria, therefore with a tendency to reduce residual pH more than other SCFA (Belenguer *et al.*, 2007). Apajalahti and Rinttilä (2019) concluded how lactic acid accumulation is not characteristic for caeca fermentation, but ileum fermentation. Even though lactic acid is a significant metabolic intermediate in caeca fermentation, according to subsequent publications, the level of residual lactic acid in healthy birds with good microbiota balance should be up to 5% of total SCFA.

1.9. Conclusions and research gaps

For fast-growing broilers, optimising feed formulation is of a high importance to reach their full genetical potential. Recent research has demonstrated that DF generally enhances production performance, digestive tract physiology and animal welfare. Although increasing DF content in poultry diets reduces their overall energy values, it could positively affect the production of SCFA and improve chicken microbiome. The use of DF in poultry diet formulations is debated among researchers, mainly due to lack of evidence available on the effects of the physicochemical composition of fibre from different types of cereals and their

nutrient effects. Poultry diets high in fibre are regularly supplemented with NSPase enzymes, which help break down plant cell walls, reduce viscosity in the intestinal tract and possible formation of oligomers that have probiotic properties. It is not established would combining enzymes and prebiotic such as XYL and XOS have synergistic effect, providing better performance, nutrient utilisation and changes in the broiler gut. The published studies do not show clear benefits of high DF diets with added exogenous enzymes and prebiotics on microbial colonisation and the host. Therefore, further research is necessary to precisely determine the optimal types and inclusion levels of DF and supplements required to enhance broiler performance while mitigating potential adverse effects.

2. Chapter: General materials and methods

2.1. Ethics statement

The study procedures were approved by Harper Adams University Research Ethics Committee (reference numbers: 0646-201910-PGMPHD, 1356-202011-PGMSC, 0333-202203-PGMPHD) and reported here in accordance with the ARRIVE 2.0 guidelines (Percie du Sert et al., 2020).

2.2. Animal Housing

The Animal Welfare Act of 2006 and UK standards were followed in all experimental techniques, which were authorised by the research ethics committee at Harper Adams University. Hatched day-old Ross 308 chicks were obtained from a commercial hatchery (Cyril Bason Ltd., Craven Arms, UK) in the first and second studies and from Annyalla chicks Ltd (Boston, UK) in the third study. After being weighed upon arrival, the birds were put into floor pens. Each pen had a solid floor with an area of 2.1 m^2 that was covered with clean wood shavings. Birds well-being was monitored throughout the study with regular checks. Any mortality (including euthanasia due to meeting the set humane endpoint) was recorded as it occurred. Birds were located in a thermostatically controlled room with a standard lighting program which decreased the light:dark ratio from 23h:1 hour from day old to 18h:6 hours at 7 d of age which was subsequently maintained until the end of the study. At the start of the experiment, the room temperature was approximately 32 ºC and was gradually reduced to about 20 ºC at 21 d age. On days 0, 21, and 35 the birds and residual feed were weighed; furthermore, feed intake (FI), body weight gain (WG), and FCR were calculated and corrected for mortality. For the performance study, broilers were weighed on a pen weight basis at a day old, 21 d and 35 d age (end of the experiment) and FI was recorded on the same days as the birds were weighed.

At 19 and 32 days old, 4 birds from each pen were selected at random and transferred to one of the raised-floor pens (60 × 60 cm floor area) in the same controlled environment room. Number of raised floor pens corresponded to the number of the floor pens in each experiment, respectively. Each raised floor pen was equipped with metal feeders and 2 nipple drinkers with cups. Feed and water were offered *ad libitum*. The selected birds were kept in the pens for 72 h and excreta were collected twice (every 36 h) from the trays beneath. Spilled feed and feathers were removed from the excreta, which was kept in a freezer (-20°C) before drying at 65°C. Excreta samples were afterwards ground on a 1 mm screen and stored for further analysis. On days 21 and 35, one bird randomly selected from

each pen, was humanely killed by cervical dislocation; where caecal digesta and samples of GIT were collected along with jejunal villus morphometry determination.

2.3. Treatments

All diets were isocaloric and isonitrogenous, and their compositions were in accordance with Aviagen guidelines (Aviagen Ltd, Edinburgh, UK, 2018). Diets were manufactured in Target Feeds, Ltd. in an Alvan blanch horizontal mixer with 0.5 to 2 tonne size and mixing time from 8 to 10 minutes. The pelleted diets were steam-conditioned with all the feed reaching 50 to 60°C for 20 s before pelleting and then pelleted using a pellet press (Paladin 350; Andritz Feed & Biofuel Technologies, Hull, UK), capable of manufacturing approximately 4 tonnes of feed/h. The steam pressure applied was 2 bar, and the pellets were cooled with ambient temperature air in a ventilated counter flow cooler for approximately 12 min. The pellet diameter was 3 mm.

2.4. Laboratory Analysis

2.4.1. NSP analysis

Soluble, insoluble and total NSP was determined by the Englyst *et al.* method (1994). The analysis was done with gas-liquid chromatography by Englyst Carbohydrates Ltd. (Southampton, UK).

2.4.2. Enzyme activity

Using a Quantiplate Kit for Econase XT® provided by Envirologix (AB Vista Laboratories, Innovation & Technology Centre, Ystrad Mynach, UK), dietary enzyme activity was assessed using a product-specific approved ELISA approach. Endo-1,4-xylanase, generated by a strain of *Trichoderma reesei* (CBS 114044), is the active ingredient of Econase XT® and SIGNIS®. The quantity of endo-1,4-xylanase needed to release 1 nmol of xylose from birchwood xylan per second at pH 5.3 and 50 °C is measured in xylanase units (BXU), or BXUs.

2.4.3. Undigestible marker

For the first and second experiments acid insoluble ash (AIA) in feed and excreta was analysed by the Van Keulen and Young method (1977). For the AIA analysis, a dry milled sample weighing (4-5g) was placed in a porcelain crucible and burned for 4 hours at 550°C

in a muffle furnace to determine the quantity of ash. Firstly, the sample was weighted and then boiled for 10 mins in 100 mL of 2 M of Hydrochloric acid at 175˚C. The acidic solution was filtered and washed using hot water and afterwards put in a muffle furnace for 4 hours at 550˚C. Following the determination of the quantity of ash, the crucible's contents were placed in a Kjeldal tube together with 100 ml of 2M HCl and digested for 10 minutes at 175 C. Hot distilled water is used to clean the filter and tube after the hot digest has been filtered through ash-free filter paper. Folding the filter paper and adding the digested sample to the crucible again, the crucible was then heated in a muffle furnace for an additional four hours at 550 °C.

For the third experiment titanium dioxide was used as indigestible marker and the recovery of the marker in feed and excreta has been done at DM Scientific Ltd (Dalton Thirsk, UK) following the method Short et al., 1996.

2.4.4. Gross Energy

The combustion method was used to determine gross energy (GE), with benzoic acid as the standard in a bomb calorimeter (Parr 6200 Instrument Company, Moline, IL, 61,265, United States).

2.4.5. Nitrogen

Crude protein was determined by the combustion method in excreta and feed (AOAC, 2000, method 990.03), using Leco FP-828 (Leco Corp., St. Joseph, MI) with Na_2EDTA as a standard. Samples were weighed in tin foil capsule and placed into loader which transfers them into the furnace where atmospheric gas removed. The aliquot gas in the analyser is carried to a thermal conductivity cell to detect nitrogen.

2.4.6. Neutral detergent fibres

The neutral detergent fibres (NDF) in diets and excreta were analysed following ANKOM Technology (Macedon, New York). Filter bags and samples are weighted, sealed and placed in NDF analyser instrument bag suspender trays. In the instrument was added 1800 mL of neutral detergent solution, 20 g of sodium sulphite and 4.0 mL of alpha amylase in the vessel. The NDF extraction was set for seventy-five minutes, after which the samples were rinsed out the first time with deionised water and 4.0 mL of alpha amylase, followed by two additional rinses with only deionised water. Filter bags with samples were soaked in acetone for 3 - 5 minutes and dried in the oven for 4 hours at 100°C.

2.4.7. Ether extract

Using a Soxtec system (Foss Ltd., Warrington, UK) fat was extracted by the ether extraction method (AOAC 2000; method 945.16) and following the FAO procedure FOSS (2008). Petroleum ether 40-60 was used as a solvent to extract the oil.

2.4.8. Viscosity

For determination of viscosity, contents of jejunal digesta were carefully excised and frozen from one bird from each pen at 35d. Digesta were thawed on ice before being subsampled in a 50 mL plastic centrifuge tube, vortexed for 10 seconds, and then centrifuged with relative centrifugal force of 10,000 g for 10 minutes at 4°C. The supernatant was transferred into a 2-mL sample cup and set in a 40°C warmed water bath (Precision, GCA Corp., College Park, MD) until the temperature of the sample reached the temperature of the water in the water bath. The viscosity of these samples was measured in centipoise (cP) using a cup and cone viscometer (Vibro viscometer, model SV-1A, A&D Instruments Ltd, Oxfordshire, United Kingdom). Deionised water (viscosity 0.66 cP) was used to calibrate the viscometer.

2.4.9. The relative development of GIT

At the end of the starter and finisher phase, one bird from each pen was weighed and humanely killed by cervical dislocation. The relative development of organs was completed according to the procedure described by Amerah et al. (2008). The GIT was cautiously taken out and any digesta was gently removed out by palpation. The empty weight of crop, proventriculus with gizzard, duodenum, jejunum, and caeca were measured for weight and length. The weights of liver and spleen were also noted. Following this, the relative organ was determined comparing the weight of the intestinal organ and weight of the bird (g/kg of bird BW) and presented in %.

2.4.10. SCFAs analysis

One bird from each pen was chosen at random, and its caecal and ileum SCFA were analysed. Digesta samples were collected in Biofreezer tubes for SCFA analyses where sample vial was used for initial digesta collection and homogenisation. From the homogenized digesta, one evenly filled spoonful of sample, which equals approximately of 1 g, was transferred into BioFreeze™ vial containing 9 ml of BioFreeze™ preservation buffer. Spoon with the sample was placed into the vial, cap closed carefully, and vial was shaken vigorously to completely suspend the sample material into the preservation buffer. Analysis was performed by Alimetrics Diagnostics Ltd. (Koskelontie 19B, FIN-02920, Espoo, Finland)

using gas chromatography as described by González-Solé et al. (2022). The SCFA profiles were analysed by gas chromatography (Agilent Technologies, Santa Clara, CA, USA) using pivalic acid (Sigma-Aldrich, St. Louis, MO, USA) as an internal standard. The chromatography procedure which used a glass column packed with 80/120 Carbopack B-DA/4% Carbowax stationary phase, helium as a carrier gas, and a flame ionisation detector. Lactic acid and volatile fatty acids (Acetic acid, propionic acid, isobutyric acid, butyric acid, 2-methylbutyric acid, isovaleric acid and valeric acid) were derivatised to the respective phenyl esters by using phenyl chloroformate reagent. Resulting esters were analysed by Agilent GC-FID. Matrix-matched internal standard calibration with butyric-d7 - and acetic-d3 acids was used in quantitation.

2.4.11. Histomorphometry

Histomorphometry was done in collaboration with Faculty of Veterinary Medicine, Trakia University (Stara Zagora, Bulgaria). Middle jejunum samples from one bird per pen (approximately 2 cm were collected) were fixed in 10% aqueous formaldehyde solution, priorly rinsed with deionised water. Ethanol was used to dehydrate, xylene was used to rinse, and finally paraffin was used to embed them. Serial histological slices of 5 to 7 micron thickness were cut from the waxed tissues using a microtome YD-335A (J.Y.M.A. Ltd., China), and placed on the slides. At intervals of 20 to 30 seconds, the slices were deparaffinized twice in xylene (two cuvettes) for 30 to 60 seconds. They were then placed in a falling alcoholic range (absolute to 70% ethyl alcohol), followed by two to five minutes in water and haematoxylin. Following staining, the preparations were washed with water, stained with eosin, and then kept in distilled water for five to ten minutes until a blue colour was achieved. Following a water rinse, the preparations were cleaned in xylene and dehydrated in an ascending alcohol line. Permanent microscopic preparations were produced following entelan inclusion. The VDN-200M light microscope (LUMENLAB, China) was used to view the preparations, and a digital CMOS camera within the microscope documented the results of the experiment. Villus height and thickness, and crypt depth of jejunum were the morphometric parameters that were analysed. The micro morphometric parameters were determined by selecting 10 intact, precisely vertically orientated crypts and villi from each histological preparation. The height of the villus was represented by the distance from the crypt opening to the tip on the right side of the villus. The distance between the outer surfaces of two adjacent epithelial edges that pass through the intestinal villi's vertical centre was used to determine the villi's width. The depth of the crypt was defined as the depth of the invagination between adjacent villi.

2.4.12. Caecal microbiota composition and diversity

Following humane slaughter, digesta were collected from the caeca. These samples were immediately stored on dry ice (-78 °C) and remained in long-term storage (-80 °C) until they were freeze dried and DNA extraction for high throughput sequencing was completed. Bacterial DNA was isolated from caecal content using the FastDNA™ SPIN Kit for soil (MP Biomedicals, Ohio, USA) according to the manufacturer's instructions. Using NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, United States) the DNA was quantified. Utilising the PrimeSTAR® HS DNA Polymerase kit (TaKaRa, Beijing, China), amplicon sequencing libraries were prepared for the V1-V2 region of the 16S rRNA gene using the technique outlined in Kaewtapee et al., 2017. The isolated DNA as a template bacterial 16S rRNA gene was PCR amplified with primers 27f (5′ CAAGRGTTHGATYMTGGCTCAG 3′) and 338r (5′ TGCTGCCTCCCGTAGGAGT 3′). The 1µL of DNA was added for the first PCR, which carried out in a 20µl reaction containing 0.5µL of each primer and 0.2µL of PrimeSTAR HS DNA polymerase. With a total amount of 50 µL, the second PCR was conducted using 1 µL of the first PCR as a DNA template. Following a three-minute initial denaturation at 95°C, there were fifteen (first PCR) or twenty (second PCR) cycles of denaturation at 98°C for ten seconds, annealing at 55°C for ten seconds, an extension step at 72°C for forty-five seconds, and a final extension at 72°C for two minutes. The amplicons that were obtained were examined using agarose gel electrophoresis, followed by purification and normalisation using the SequalPrep Normalisation Kit (Invitrogen Inc., Carlsbad, CA, USA). Using paired-end sequencing chemistry with 250 base pairs (bp), the samples were sequenced on an Illumina Novaseq 6,000 base station. The Bioconductor workflow (Callahan et al., 2016a) was used to process the raw reads (FASTQ files) in R version 4.3.3 (R Core Team, 2024). Briefly, sequence reads were trimmed, filtered, merged and chimeras removed using DADA2 (Callahan et al., 2016b) Unique sequences were clustered into ASV tables using DADA2 and taxonomy assigned using the Silva SSU taxonomic training data formatted for DADA2 with Silva version 138 (McLaren, 2020) database (Quast et al., 2013). The phyloseq package (McMurdie and Holmes, 2013) was used for downstream processing.

For the microbiota beta diversity analysis, Bray-Curtis distances were tested for significance using Permutational Multivariate Analysis of Variance with the "adonis" function in the "Vegan" version 2.5-7 package (Oksanen et al., 2022) R 4.3.3. (R Core Team, 2024). Alpha diversity measures were calculated with the "estimate_richness" function of the phyloseq package (McMurdie and Holmes, 2013) in R 4.3.3. (R Core Team, 2024) and tested

statistically with pairwise comparisons using the Wilcoxon rank sum test with Holm p value adjustment.

2.5. Calculations

The following equation was used to calculate the mortality corrected FCR:

$$FCRm = \frac{FI\ pen}{pen\ WG\ + dead\ bird\ BW}$$

Where WG represents weight increase per pen and BW is the reported body weight of birds that died or were killed for sampling.

Nutrient retention coefficients (NR) were calculated using the following equation:

$$NR = \frac{(N/marker)Diet - (N/marker)Feces}{(N/marker)Diet}$$

Where (N / marker) Diet = ratio of the respective nutrient to marker (AIA or TiO_2) in diet, and (N / marker) Faeces = ratio of the respective nutrient to marker (AIA or TiO_2) in excreta samples.

Calculations for AMEn were done according to Hill and Anderson (1958) using the formulas below:

Apparent metabolisable energy (AME; MJ/kg):

$$AME = gross\ energy\ in\ feed - \frac{(gross\ energy\ in\ excreta\ \times AIA\ feed)}{AIA\ in\ excreta}$$

Apparent metabolisable energy nitrogen corrected (AMEn; MJ/kg):

$$AMEn = gross\ energy\ in\ feed - \frac{(gross\ energy\ in\ excreta\ \times AIA\ feed)}{AIA\ in\ excreta} - \frac{(34.39\ \times\ N\ retained)}{1000}$$

N retained (g/kg):

$$N\ retained = nitrogen\ in\ feed - \frac{(nitrogen\ in\ excreta\ \times\ AIA\ in\ feed)}{AIA\ in\ excreta}$$

Dry matter retention (DMR):

$$(acid\ insoble\ ash\ in\ digesta - acid\ insouble\ ash\ (AIA)\ in\ feed) \div acid\ insoble\ ash\ (AIA)\ in\ excreta$$

The following equation was used to calculate the dietary nutrient retention coefficients:

$$Nutrient\ retention\ coefficients\ = \frac{N\ in\ \ feed/AIA\ in\ feed - \ N\ in\ excreta\ /\ AIA\ in\ digesta}{nitrogen\ in\ feed\ /AIA\ in\ feed}$$

2.6. Statistical analyses

Statistical analysis was performed using GenStat statistical software (21th edition, Rothamsted, Hertfordshire, UK). Details of statistical analysis were detailed in each chapter, respectively.

3. Chapter: The response of broiler chicken to xylanase and a fermentable xylo-oligosaccharide supplementation on metabolisable energy, nutrient retention, gastrointestinal tract development, and growth performance of Ross 308 broilers fed diets with and without 5 % of wheat bran

3.1. Introduction

Feed accounts for the majority of the economic expenditure of poultry production, accounting for 65 – 70 % of the total cost (Ravindran, 2013). Whilst maize is the most common feed grain used in broiler feeds around the world due to suitable growing conditions (Dei, 2017), wheat is also a preferred base grain in some regions, e.g. UK (AHDB, 2023.). The application of locally obtainable or alternative less expensive feed materials such as industrial by-products is increasing (Dey et al., 2021); however, they contain high levels of indigestible NSP. The poultry digestive system lacks the ability to produce the necessary endogenous enzymes to digest the beta type of linkages in NSP. Broilers must therefore rely on their gut microbiota to hydrolyse and ferment the DF into metabolisable substrates such as SCFA (Bautil et al., 2019). It is well established that DF can negatively impact daily feed intake, growth performance, and digestibility of nutrients (Jørgensen et al., 1996; Sklan et al., 2003). An important antinutritional factor found in maize is AX (Nian et al., 2011). Maize-derived AX are poorly fermented by the endogenous microbiota (Knudsen, 2014) and so NSPase are commonly added to poultry diets (Bautil et al., 2019). Xylanase is a commonly used NSPase enzyme that helps degrade AX (Bach Knudsen, 2014). Endogenous xylanases break down AX by hydrolysing the 1,4-D-glycosidic bond between xylose residues in the backbone, releasing both AXOS and XOS (Broekaert et al., 2011). Benefits of the addition of NSPase in non-ruminant animals are explained by three main modes of action: (1) reducing digesta viscosity by the breakdown of the high molecular weight soluble AX, thus allowing faster diffusion of digestive enzymes and substrates, and improving the rate of nutrient absorption and digestion; (2) interference of/partial disruption of the cell wall through the degradation of critical components holding the feedstuff cell walls together and hence allowing the release of captured nutrients; and (3) the release of XOS in the gastrointestinal tract distal regions as a result of continued xylan degradation into smaller oligosaccharides which act as a signalling molecule for certain beneficial bacteria (Bedford, 2018; González-Ortiz et al., 2019a). Modern poultry diets can therefore reflect these advances in fibre nutrition by exploiting these beneficial functions through selective addition of functional fibbers and NSPase enzymes.

Selective XOS fermentation may result in prebiotic effects, reportedly by modifying the composition and activity of the gut microbiota (Courtin et al., 2008), such that it conifers a gut

health benefit through enhanced intestinal immunity (Ding et al., 2018). The positive effects of XOS supplementation in broilers may be due to direct stimulation of lactate-producing bacteria, with lactate being further fermented to butyrate in the large intestine (De Maesschalck et al., 2015). Supplementing poultry with XOS may therefore increase caecal short-chain fatty acids (SCFA), boost the immune system, increase the population of beneficial bacteria and positively influence the intestinal environment (Ding et al., 2018). Caeca fermentation of dietary fibre and the synthesis of SCFA, particularly butyrate, have been linked to the formation of small intestinal villus, postponed emptying of the digestive tract, and improved gut health, all which could potentially increase feed efficiency (Jha et al., 2019; O'Neill et al., 2012).

3.2. Objective

Main objective of this study was to determinate the effects of supplementing XYL, XOS, and combined XYL and XOS with and without additional DF (wheat bran at 50 g/kg) on growth performance, metabolisable energy, nutrient digestibility, GIT development, SCFA concentrations in caeca, and jejunum histomorphology in broilers. The following general hypotheses will be examined:

1. Added wheat bran will negatively influence WG and FCR, ME, while potentially modulate jejunum histomorphology and enlarge parts of digestive system.
2. Feed enzymes and prebiotic supplements could alleviate the negative effect of additionally added fibre content and increase production performance, and caecal content of SCFA.

3.3. Materials and methods

General materials and methods can be found in chapter three.

3.3.1. Animal housing

One thousand nine hundred and twenty Ross 308 chicks (960 males and 960 females) were obtained from a commercial hatchery (Cyril Bason Ltd, Craven Arms, UK). The broiler chicks were weighed and divided into 96 floor pens, 48 pens with males and 48 with females, with 20 birds in each pen.

3.3.2. Treatments

The composition of the two basal diets is described in Table 3.1. There were two main diet series (Figure 10); the first of the diets contained 54% maize as a positive control (PC), and in the second, 5% of the maize was replaced by wheat bran as a negative control (NC) produced at Research Diet Services B.V. (Wijk Bij Duurstede, Netherlands). The diets were split into four batches: one of them was used as a control, and each of the others were supplemented either with 100 g/tonne of xylanase (Econase XT 25P, AB Vista, Marlborough, UK; 16000 BXU/kg) or 50 g/tonne of XOS (AB Vista, with a degree of polymerisation between 2 and 7) or combination of both xylanase and XOS additives (Signis®, AB Vista, Marlborough, UK). There were 12 replicates per diet, 6 with males and 6 with females. Chickens were fed with the experimental diets in two phases: starter (0 - 21 d) in crumb form and finisher (22 - 35 d) in pellet form with a maize-soybean-based meal. Diatomaceous earth (Multi-Mite®, Wiltshire, UK) was used as an acid insoluble ash (AIA) digestibility marker and was included at 20 g/kg of feed.

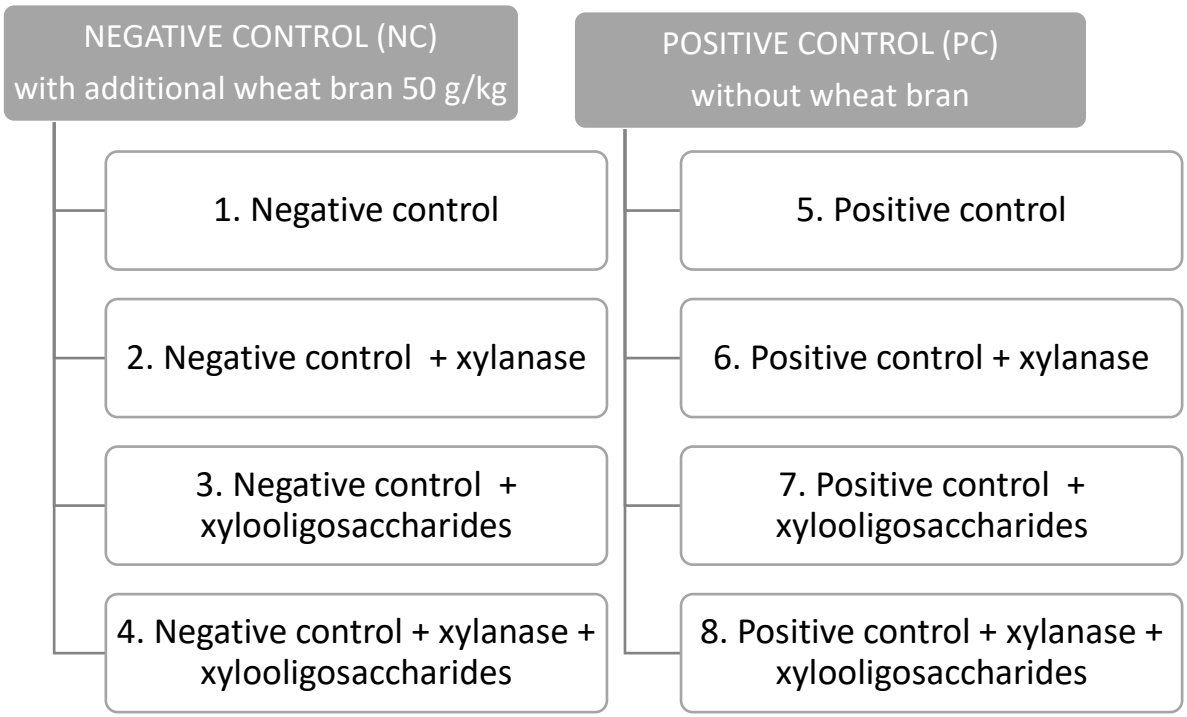

Figure 11: Positive and negative control diets

Table 3.1 Ingredient composition of the experimental diets

Ingredient	Starter PC (g/kg)	Starter NC (g/kg)	Finisher PC (g/kg)	Finisher NC (g/kg)
Maize	538.8	488.8	625.2	575.2
Soybean meal	386.9	386.9	296.8	296.8
Wheat Bran	0.00	50.0	0.00	50.0
Soy oil	23.6	23.6	33.1	33.1
Salt	3.8	3.8	3.3	3.3
DL Methionine	2.7	2.7	1.7	1.7
Lysine HCl	0.6	0.6	0.5	0.5
Limestone	7.5	7.5	7.1	7.1
Mono Dical Phos	11.0	11.0	7.0	7.0
Quantum Blue[1]	0.1	0.1	0.1	0.1
Acid insoluble ash[2]	20.0	20.0	20.0	20.0
Vitamin mineral premix[3]	0.1	0.1	5.0	5.0
Total	1000	1000	1000	1000
Calculated analysis (as-fed basis)				
Crude protein %	22.79	23.21	19.06	19.48
ME (MJ/kg)	12.59	12.28	13.18	12.87
Calcium (%)	0.92	0.92	0.80	0.80
Phosphorus (%)	0.78	0.81	0.66	0.69
Analyzed values (as-fed basis)				
Crude protein (%)	23.0	23.2	19.2	19.6
Crude fat (%)	4.2	4.0	4.8	5.0
Total NSP (%)	8.7	10.3	7.9	9.4
Soluble NSP (%)	1.9	1.4	1.7	1.5
Insoluble NSP (%)	6.8	8.9	6.2	7.9
Main constituents of total NSP				
Arabinose (%)	1.6	2	1.5	1.9
Xylose (%)	1.5	2.2	1.6	2.4
Mannose (%)	0.3	0.3	0.3	0.3
Galactose (%)	1.7	1.7	1.3	1.4
Glucose (%)	2.4	2.9	2.2	2.5

PC, Positive control; NC, Negative control; NSP, Non-starch polysaccharide.

[1] Quantum Blue 5G, AB Vista, Marlborough, UK; 5,000 FTU/g.

[2] Feed grade diatomaceous earth (Multi-Mite®, Wiltshire, UK).

[3] Vitamin mineral premix provided per kg of diet: vitamin A, 10,000 IU; vitamin D3, 2,500 IU; vitamin E, 25 IU; vitamin E, 50 mg; vitamin K_3, 1.5 mg; vitamin B1, 2 mg; vitamin B2, 7.5 mg; vitamin B6, 3.5 mg; vitamin B12, 20 µg; niacin, 35mg; D-pantothenic acid, 12 mg; choline chloride, 460 mg; folic acid, 1.0 mg; biotin, 0.2 mg; iron as iron sulphate, 265 mg; copper as

copper sulphate, 48 mg; manganese as manganese oxide, 140 mg; zinc as zinc sulphate, 165 mg; iodate as potassium iodide, 1.2 mg; and selenium as sodium selenite, 0.33 mg.

3.3.3. Statistical analysis

Calculations and data handling were performed in Excel 2020 (Microsoft Corporation), while GenStat statistical software (21st edition, Rothamsted, Hertfordshire, UK) was used for statistical analyses Maize-based positive control or negative control, and control, XYL, XOS, or combination of XYL and XOS in the diet were used in 2 x 4 factorial arrangement. Data were analysed by two-way ANOVA based on a completely randomized design. At $P < 0.05$, differences were reported as significant. All data were checked for outliers, normality and homogeneity of residuals prior to ANOVA and turkey test was used to determinate the differences between the treatment means.

3.4. Results

3.4.1. Diet analysis

The formulated nutritional profiles of the diets were met (Table 3.1). The enzyme recoveries of phytase and xylanase are presented in Table 3.2 and the activity of phytase in the diets analysed was as expected. Mean value for XYL supplemented diets was 14137.5 BXU/kg ($r = 0.98$). Overall mortality was 4.85% and no differences were observed between the experimental treatments ($P = 0.469$, data not shown). At 21 and 35 days of age, the broilers' mean weights were 906 g and 2080 g, respectively.

Table 3.2 Analysis of phytase and xylanase activity in the experimental diets.

		Expected		Analysed	
Treatments	Wheat bran	Phytase, FTU/kg[1]	Xylanase, BXU/kg[2]	Phytase, FTU/kg	Xylanase, BXU/kg
Starter diet					
PC	No	500	0	705	<2000
PC +XYL	No	500	16000	524	10600
PC + XOS	No	500	0	707	<2000
PC + XYT + XOS	No	500	16000	720	16100
NC	Yes	500	0	529	<2000
NC + XYL	Yes	500	16000	793	10700
NC + XOS	Yes	500	0	510	<2000
NC + XYL + XOS	Yes	500	16000	668	18000
Finisher diet					
PC	No	500	0	645	<2000
PC + XYL	No	500	16000	870	11500
PC + XOS	No	500	0	710	<2000
PC + XYL+ XOS	No	500	16000	767	17300
NC	Yes	500	0	607	<2000
NC + XYL	Yes	500	16000	621	11100
NC + XOS	Yes	500	0	719	<2000
NC + XYL + XOS	Yes	500	16000	652	17800

NC, negative control; PC, positive control: XYT, xylanase; XOS, Xylooligosaccharides

[1] The amount of enzyme necessary to release 1 mmol of inorganic P per minute from sodium phytate, at 37°C and pH 5.5, is defined as one FTU.

[2] The amount of enzyme that generates 1 nmol reducing sugars from birchwood xylan in one second, at 50°C and pH 5.3, is measured as one BXU

3.4.2. Growth performance

The effects of experimental dietary treatments on broiler chicken growth performance are shown in Table 3.3. No significant interactions were observed in any of the performance parameters at any of the measured periods or the overall. The addition of WB did not affect ($P > 0.05$) WG and FCR of younger birds (0 - 21 d); however, it had a negative effect ($F< 0.05$) between 21 - 35 d and over the whole 0 - 35 d period on both parameters. Addition of WB also had an effect on WG in the finisher and overall period ($P = 0.008$ and $P = 0.024$, respectively), where a positive response was observed when combination of XYL and XOS was added to the control diet. Similarly, there was a treatment effect for FCR from 21 - 35 d and 0 - 35 d period, where the supplementation of XOS and XYL resulted in FCR improvements ($P = 0.016$ and $P = 0.014$, respectively). In the overall period from 0 d to 35 d, combination of XOS and XYL supplementation improved FCR from 1.459 to 1.425 ($P = 0.014$).

Table 3.3 The effect of dietary treatments on broiler chicken growth performance fed with and without the addition of 50 g/kg wheat bran.

	Feed intake (g/b/d DM)			Weight gain (g/b/d)			Feed Conversion Ratio (g:g DM)		
	0-21 d	21-35 d	0-35 d	0-21 d	21-35 d	0-35 d	0-21 d	21-35 d	0-35 d
Wheat bran									
No	52.38	123.40	82.77	36.87	87.97	55.45	1.279	1.405	1.421
Yes	52.48	121.39	81.65	36.03	83.14	52.30	1.300	1.445	1.473
SEM	0.663	1.886	0.996	0.769	1.435	0.639	0.0292	0.0149	0.0077
Treatment									
Control	52.67	120.19	81.34	36.78	82.97[b]	52.79[b]	1.274	1.453[a]	1.459[a]
XYL	51.98	122.23	82.32	36.71	83.99[b]	53.58[b]	1.266	1.444[a]	1.455[a]
XOS	52.57	121.48	81.67	36.27	85.31[b]	53.53[b]	1.289	1.418[ab]	1.450[a]
XYL + XOS	52.50	125.68	83.52	36.04	89.96[a]	55.59[a]	1.328	1.387[b]	1.425[b]
SEM	0.937	2.667	1.409	1.088	2.029	0.904	0.0413	0.0210	0.0108
Probabilities									
Wheat bran	0.881	0.492	0.270	0.274	0.002	<.001	0.446	0.010	<.001
Treatment	0.882	0.218	0.436	0.885	0.008	0.024	0.472	0.016	0.014
Wheat bran x Treatment	0.901	0.479	0.456	0.756	0.500	0.542	0.419	0.949	0.364

[a-b] P<0.05; SEM, pooled standard error of means; XYL, xylanase; XOS, xylooligosaccharides.

3.4.3. Metabolisable energy and nutrient retention

There was no effect of treatments on AME and AMEn (Table 3.4). The inclusion of WB had a negative effect on AME and AMEn values at both 21 d ($P < 0.001$; 13.779 vs. 12.963 and 13.208 vs. 12.431, respectively) and 35 d ($P < 0.001$; 14.200 vs. 13.681 and 13.810 vs. 13.182, respectively).

Table 3.4 The effect of dietary treatments on broiler chicken apparent metabolisable energy (AME) and nitrogen corrected apparent metabolisable energy (AMEn) fed with and without the addition of 50 g/kg wheat bran

	AME (DM)		AMEn (DM)	
	21 d	35 d	21 d	35 d
Wheat bran				
No	13.779	14.200	13.208	13.810
Yes	12.963	13.681	12.431	13.182
SEM	0.0593	0.0751	0.1115	0.0714
Treatment				
Control	13.339	13.959	12.788	13.514
Xylanase	13.452	13.986	12.899	13.543
XOS	13.314	13.956	12.766	13.509
XYL + XOS	13.379	13.861	12.825	13.420
SEM	0.0838	0.1063	0.1577	0.1010
Probabilities				
Wheat bran	<0.001	<0.001	<0.001	<0.001
Treatment	0.382	0.666	0.358	0.651
Wheat bran x Treatment	0.976	0.726	0.978	0.754

SEM, pooled standard error of means; XYL, xylanase; XOS, xylooligosaccharides.

There were no interactions (P > 0.05) noted on the NR, DMR, and FR (Table 3.5). The DMR decreased (P < 0.001) when WB was added to the experimental diet both during the starter for 6% and finisher phase for 3%. The negative impact on the retention was also observed in NR (8% decrease) and FR (6% decrease), but only in the starter phase (P < 0.001).

Table 3.5 The effect of dietary treatments on broiler chicken dry matter retention, nitrogen retention, and fat retention fed with and without the addition of 50 g/kg wheat bran.

	Dry matter retention		Nitrogen retention		Fat retention	
	21 d	35 d	21 d	35 d	21 d	35 d
Wheat bran						
No	0.721	0.740	0.654	0.510	0.879	0.952
Yes	0.679	0.719	0.604	0.589	0.831	0.953
SEM	0.0023	0.0038	0.0039	0.007	0.007	0.0008
Treatments						
Control	0.696	0.730	0.628	0.596	0.858	0.952
XYL	0.704	0.732	0.629	0.593	0.857	0.953
XOS	0.699	0.729	0.629	0.599	0.858	0.952
XYL + XOS	0.700	0.725	0.631	0.590	0.846	0.952
SEM	0.0033	0.0053	0.0055	0.0097	0.0103	0.001
Probabilities						
Wheat bran	<0.001	<0.001	<0.001	0.125	<0.001	0.099
Treatments	0.180	0.595	0.963	0.795	0.608	0.964
Wheat bran x Treatments	0.918	0.644	0.360	0.204	0.566	0.486

SEM, pooled standard error of means, XYL, xylanase; XOS, xylooligosaccharides.

There was a significant effect of treatment on NDF digestibility on day 21, where adding combination of XYL and XOS or XYL only supplements significantly improved (P = 0.001) digestibility compared to control diet and diet supplemented with XOS (Table 3.6). The effect of experimental diets intensified by day 35, showing an interaction between wheat bran addition and dietary treatments (P = 0.001). In the control maize-based diets, none of the treatments increased NDF digestibility. When wheat bran was present, the treatments diverged considerably. Even the simple addition of wheat bran to the maize diet elevated 35 d NDF digestibility, while highest NDF digestibility was achieved in birds that were fed wheat bran and combination of XYL and XOS or xylanase supplements (0.2663 and 0.2493, respectively, P = 0.001).

Table 3.6 The effect of dietary treatments on broiler chicken neutral detergent fibre (NDF) digestibility at 21 and 35 d fed with and without the addition of 50 g/kg wheat bran

		NDF digestibility	NDF digestibility
		21 d	35 d
Wheat Bran			
No		0.186	0.162
Yes		0.194	0.232
SEM		0.0056	0.0058
Treatment			
Control		0.171[b]	0.176
Xylanase		0.208[a]	0.209
XOS		0.179[b]	0.180
XYL + XOS		0.201[a]	0.214
SEM		0.0079	0.0082
Wheat bran x Treatment			
Control	No	0.165	0.1532[c]
Control	Yes	0.178	0.1991[b]
XYL	No	0.203	0.1685[c]
XYL	Yes	0.213	0.2493[a]
XOS	No	0.187	0.1642[c]
XOS	Yes	0.172	0.2139[b]
XYL + XOS	No	0.189	0.1609[c]
XYL + XOS	Yes	0.213	0.2663[a]
SEM		0.0112	0.0115
Probabilities			
Wheat bran		0.142	<0.001
Treatments		<0.001	<0.001
Wheat bran x Treatments		0.106	0.001

[a-c] P<0.05; SEM, pooled standard error of means; NDF, neutral detergent fibre; XYL, xylanase; XOS, xylooligosaccharides.

3.4.4. Gastrointestinal tract development and jejunum histomorphometry

The response of dietary treatments on the relative weights of the GIT organs is shown in Table 3.7 and 3.8. At the end of the study, proventriculus and gizzard weight % was subject to an interaction between the addition of wheat bran and treatments ($P < 0.001$), where the heaviest weight percentage with was found with supplementation of WB and XYL (1.740%), followed by the intermediate relative weight in wheat bran and combination of XYL and XOS (1.521%) and all the rest of treatments. Similarly, the addition of wheat bran increased the percentage of relative duodenum weight of 21 d old birds ($P = 0.012$), from 1.041% to 1.142%. No differences were observed in the small intestine, caeca or total GIT ($P > 0.05$).

Table 3.7 The effect of dietary treatments on broiler chicken relative weight (%) of organs and gastrointestinal tract fed with and without the addition of 50 g/kg wheat bran

		Proventriculus and gizzard (%)		Pancreas (%)		Duodenum (%)	
		21 d	35 d	21 d	35 d	21 d	35 d
Wheat bran	Wheat bran						
No		2.542	1.436	0.368	0.218	1.041	0.580
Yes		2.614	1.514	0.361	0.217	1.142	0.562
SEM		0.0737	0.0546	0.0118	0.0066	0.0397	0.0206
Treatments							
Control		2.570	1.412	0.359	0.213	1.132	0.538
XYL		2.611	1.581	0.373	0.226	1.099	0.588
XOS		2.582	1.446	0.353	0.201	1.059	0.577
XYL + XOS		2.550	1.461	0.374	0.220	1.076	0.581
SEM		0.1042	0.0772	0.0166	0.0093	0.0562	0.0292
Wheat bran x treatment							
Control	No	2.492	1.421^{b}	0.362	0.218	1.068	0.586
Control	Yes	2.648	1.403^{b}	0.356	0.209	1.195	0.491
XYL	No	2.492	1.421^{b}	0.360	0.218	1.124	0.572
XYL	Yes	2.648	1.740^{a}	0.387	0.235	1.075	0.605
XOS	No	2.543	1.500^{b}	0.376	0.220	0.995	0.589
XOS	Yes	2.621	1.392^{b}	0.330	0.199	1.123	0.564
XYL + XOS	No	2.463	1.400^{b}	0.376	0.215	0.977	0.571
XYL + XOS	Yes	2.636	1.521^{ab}	0.373	0.226	1.176	0.590
SEM		0.1474	0.1092	0.0235	0.0132	0.0795	0.0413
Probabilities							
Wheat bran		0.334	0.159	0.548	0.930	0.012	0.407
Treatments		0.947	0.162	0.491	0.305	0.599	0.334
Wheat bran x Treatments		0.477	0.047	0.197	0.156	0.160	0.140

SEM, pooled standard error of means; XYL, xylanase; XOS, xylooligosaccharides.

Table 3.8 The effect of dietary treatments on broiler chicken relative weight (%) of organs and gastrointestinal tract fed with and without the addition of 50 g/kg wheat bran

		Small intestine (%)		Caeca (%)		GIT without liver (%)	
		21 d	35 d	21 d	35 d	21 d	35 d
Wheat bran	Wheat bran						
No		0.3684	0.218	0.497	0.3608	7.996	4.629
Yes		0.3613	0.217	0.541	0.3450	8.198	4.636
SEM		0.0118	0.0066	0.0232	0.01554	0.1482	0.0926
Treatments							
Control		0.3586	0.213	0.541	0.3368	8.219	4.503
XYL		0.3735	0.226	0.550	0.3456	8.264	4.820
XOS		0.3532	0.201	0.498	0.3550	7.919	4.589
XYL + XOS		0.3743	0.220	0.486	0.3743	7.986	4.621
SEM		0.0166	0.0093	0.0328	0.02198	0.2095	0.1309
Wheat bran x treatment							
Control	No	0.3615	0.218	0.521	0.3283	8.023	4.622
Control	Yes	0.3557	0.209	0.562	0.3453	8.414	4.384
XYL	No	0.3603	0.218	0.544	0.3443	8.441	4.732
XYL	Yes	0.3867	0.235	0.556	0.3468	8.087	4.907
XOS	No	0.3762	0.220	0.460	0.3915	7.742	4.694
XOS	Yes	0.3303	0.199	0.537	0.3185	8.096	4.483
XYL + XOS	No	0.3758	0.215	0.464	0.3791	7.780	4.470
XYL + XOS	Yes	0.3727	0.226	0.508	0.3695	8.193	4.772
SEM		0.0235	0.0132	0.0464	0.03108	0.2963	0.1851
Probabilities							
Wheat bran		0.548	0.930	0.065	0.316	0.178	0.940
Treatments		0.491	0.305	0.149	0.373	0.274	0.117
Wheat bran x Treatments		0.197	0.156	0.811	0.199	0.204	0.109

SEM, pooled standard error of means; XYL, xylanase; XOS, xylooligosaccharides.

Statistical analysis did not reveal any interaction (P > 0.05) in jejunum histomorphology parameters (Table 3.9, Figure 11).

Table 3.9 The effect of dietary treatments on the jejunum histomorphometry in 35 d old broiler chicken fed with and without the addition of 50 g/kg wheat bran

	Crypt depth (µm)	Crypt width (µm)	Villus height (µm)	Villus width (µm)	Villus height: Crypt depth
Wheat bran					
No	63.07	164.7	108.4	998	15.97
Yes	62.27	160.1	111.4	972	15.73
SEM	1.201	4.42	3.53	47.7	1.175
Treatments					
Control	63.83	162.9	108.5	1055.	16.67
XYL	62.75	160.3	114.7	941	15.11
XOS	61.67	166.1	106.6	959.	15.71
XYL + XOS	62.42	160.4	109.9	987	15.91
SEM	1.698	6.25	4.99	67.5	0.831
Probabilities					
Wheat bran	0.505	0.303	0.406	0.591	0.782
Treatments	0.643	0.758	0.415	0.353	0.616
Wheat bran x Treatments	0.858	0.428	0.097	0.298	0.392

SEM, pooled standard error of means; XYL, xylanase; XOS, xylooligosaccharides.

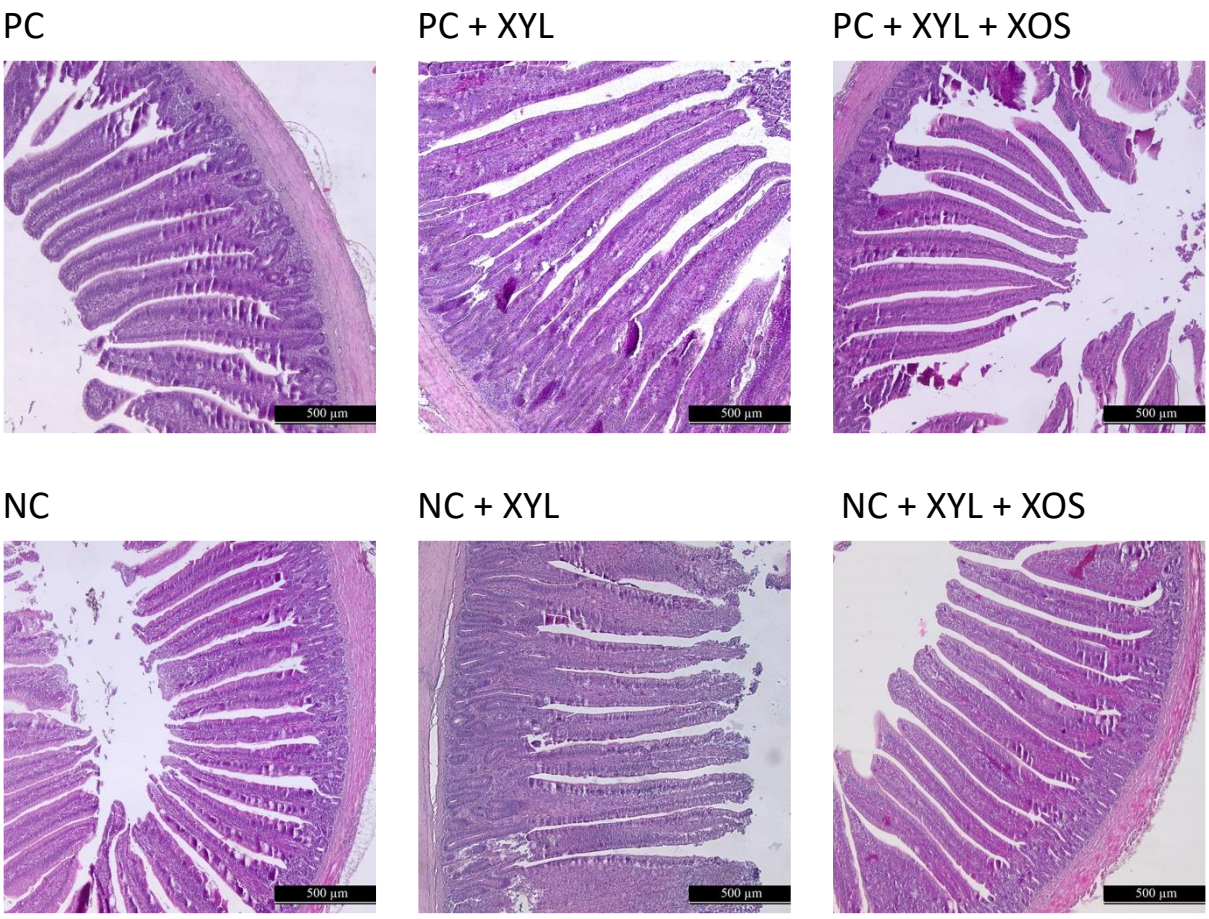

Figure 12: Histological representation of the jejunum villi of broiler chickens;

PC, maize based diet positive control; NC, maize based negative control with addition of 5% wheat bran; XYL, xylanase; XOS, xylooligosaccharides; (x 500).

3.4.5. SCFA production

Significant responses in caecal SCFA were seen only at 35 d (Table 3.10 and 3.11). When wheat bran was included in diet, broilers had a higher content of acetic acid (P = 0.035), valeric acid (P = 0.012), propionic acid (P = 0.018), SCFA (P = 0.013) and VFA (P = 0.046). There was no significant difference between treatments, except in lactic acid (P = 0.013). The highest concentration of lactic acid was noted in xylanase-supplemented birds.

Table 3.10 The effect of dietary treatments on broiler chicken caecal content of SCFA at 21 and 35 d fed with and without the addition of 50 g/kg wheat bran

	Acetic acid (mmol/kg)		BCFAs (mmol/kg)		Butyric acid (mmol/kg)		Lactic acid (mmol/kg)	
	21 d	35 d	21 d	35 d	21 d	35 d	21 d	35 d
Wheat bran								
No	69.7	68.3	1.600	2.35	9.23	9.44	7.71	5.11
Yes	75.2	80.6	1.406	2.44	10.27	10.94	7.34	3.99
SEM	5.18	5.62	0.1543	0.331	1.834	1.506	1.767	1.243
Treatments								
Control	78.1	73.8	1.237	1.98	11.52	8.74	8.79	3.72[b]
XYL	69.9	79.6	1.641	2.43	9.26	10.61	7.50	7.83[a]
XOS	72.2	66.5	1.408	2.38	8.87	9.70	7.74	4.08[b]
XYL + XOS	69.5	78.0	1.725	2.78	9.37	11.71	6.09	2.57[b]
SEM	7.33	7.94	0.2181	0.468	2.594	2.130	2.499	1.758
Probabilities								
Wheat bran	0.294	0.035	0.218	0.788	0.575	0.324	0.834	0.378
Treatments	0.627	0.367	0.122	0.413	0.737	0.552	0.757	0.031
Wheat bran x Treatments	0.145	0.596	0.073	0.483	0.927	0.118	0.644	0.378

[a-b] $P<0.05$; SEM, standard error of means; SCFA, short-chain fatty acids; BCFs, branch-chain fatty acids; XYL, xylanase; XOS, xylooligosaccharides.

Table 3.11 The effect of dietary treatments on broiler chicken caecal content of SCFA at 21 and 35 d fed with and without the addition of 50 g/kg wheat bran

	SCFA (mmol/kg)		Valeric acid (mmol/kg)		VFAs (mmol/kg)		Propionic acid (mmol/kg)	
	21 d	35 d	21 d	35 d	21 d	35 d	21 d	35 d
Wheat bran								
No	95.3	92.6	0.788	0.994	87.6	92.6	7.49	10.54
Yes	101.4	113.1	0.784	1.231	94.1	109.4	6.84	14.38
SEM	7.77	7.86	0.1084	0.0895	7.16	8.13	1.029	1.556
Treatments								
Control	106.7	102.8	0.739	1.098	97.9	99.4	6.44	13.87
XYL	95.7	107.1	0.732	1.276	88.2	108.1	6.91	14.57
XOS	98.3	93.3	0.888	0.973	90.6	90.1	7.99	8.74
XYL + XOS	92.8	108.3	0.784	1.103	86.7	106.4	7.30	12.65
SEM	10.99	11.11	0.1532	0.1266	10.12	11.50	1.455	2.201
Probabilities								
Wheat bran	0.435	0.013	0.965	0.012	0.369	0.046	0.531	0.018
Treatments	0.623	0.529	0.725	0.143	0.697	0.400	0.750	0.053
Wheat bran x Treatments	0.180	0.599	0.205	0.809	0.168	0.536	0.250	0.385

SEM, pooled standard error of means; SCFA, short-chain fatty acids; VFAs, volatile fatty acids; XYL, xylanase; XOS, xylooligosaccharides.

3.5. Discussion

3.5.1. Effect on bird growth performance, metabolisable energy and nutrient retention

Birds remained healthy during the study with low unexplained mortality of 4,85%, that did not relate to diets. It should be noted that the BW of the birds was 8 to 9% lower than Ross 308 broiler target weight. Despite mortality rate being less than 5%, it was the highest compared to the second (Chapter 4) trial's 4.7% and the third trial's (Chapter 5) 3.33%, suggesting that the broilers may have been of lower quality. The chicks in the first experiment arrived late in the afternoon, which differentiated from the following two trials, and the birds may have been impacted by the longer travel time. Furthermore, the stocking density may have affected the performance considering there were 20 birds in the pen in the first trial compared to 15 in the second and 10 in the third. However, this was not considered to be detrimental to the experimental objectives. There was no response in bird performance from experimental treatments in the starter phase; however, that changed as the birds got older. Similarly, the greater response in older birds was also found by Bedford and Morgan (1996). The microbiome of broilers develops slowly over time, resulting in performance responses that are greater over time (Ribeiro et al., 2018).

The additional fibre from the control diet that included wheat bran negatively impacted WG and FCR. The negative impact was significantly reduced when combination of XYL and XOS was added to the wheat bran control diet, providing broilers in the overall period of 0 – 35 d with an improvement of 5.3% in WG and 2.33% in FCR. González-Ortiz et al. (2021) also found the addition of XYL and XOS combination had a higher impact on WG in broilers fed with a 0.21 MJ AME reduction, compared to diets with 3% reduction in amino acid content or positive control that met nutrient recommendations. In that study (González-Ortiz et al., 2021), there was no interaction on the FCR, regardless of the energy reduction or amino acid density; however, combination of XYL and XOS improved FCR as in the current study. Although in the current study the addition of XYL and XOS individually did not significantly impact performance, the data showed a numerical improvement of WG and FCR in the finisher and overall periods. A recent study by Singh et al. (2021) did not find a significant improvement of FI and FCR in broilers fed maize-soybean meal diet supplemented with XYL and XOS. Similarly, in a study by Nian et al. (2011), numerical improvement of FCR was observed in broilers fed a maize-soybean-based diet supplemented with XYL; however, there was no significant response in weight gain. While the effect of XYL in a wheat-based diet is well established (Bedford and Schulze, 1998; Engberg et al., 2004; Whiting et al., 2017), the response in a maize-based diet could be less due to lower amount of soluble NSP and lower gut viscosity. As there is less of amount of high molecular weight soluble AX in maize based

diets compared to wheat diets, activating mechanism of reducing digesta viscosity might not be as pronounced in this trial. Plausibly, with use of cereals higher in NSP and AX, such as wheat, rye and barley, it would be possible to demonstrate more significant impact of XYL and XOS in reduction of viscosity. Maize has 1 g/kg of water-soluble NSP (predominantly arabinoxylan), whereas wheat has 24 g/kg (Choct, 1997). The higher amount of soluble NSP in wheat compared to maize diets likely indicates the greater potential for an effect of xylanase addition in wheat vs. maize-based diets.

The improved performance noted in supplemented diets was not fully reflected in the energy and retention coefficients. The AME, AMEn, DMR, NT, and FR values were not influenced by treatment supplementation but were negatively affected by the addition of wheat bran. The lack of response in nutrient and energy utilisation has previously been reported with supplementation of XYL (Nian et al., 2011; Pirgozliev et al., 2015), XOS (Li et al., 2017), and combination of XYL and XOS (González-Ortiz et al., 2021b), indicating that the digestibility determined may not correlate with the performance improvements reported. The digestibility of NDF was increased at day 21 d by supplementation of XYL and combination of XYL and XOS. The effect progressed at day 35 where the interaction showed the highest digestibility in diets supplemented with xylanase, or combination of XYL and XOS, and the addition of wheat bran. The percentage of digestibility increase in the interaction was 62.75% for xylanase and 73.86% for combination of XYL and XOS compared to the control, potentially by breaking open cell walls of grains and releasing encapsulated nutrients, thus increasing the diffusion of nutrients, and enabling the host better nutrient utilisation (Bedford, 2018).

3.5.2. Effect on gastrointestinal tract development and jejunum histomorphometry

There was an interaction observed for the proventriculus and gizzard between fibre and treatments at the end of the finisher phase, where feeding broilers with higher fibre content and xylanase resulted in higher relative weight. Except for the effects observed in proventriculus and gizzard, the treatments did not have another effect on the development of the GIT of broiler chickens. Similar results were reported by Engberg et al. (2004), Esmaeilipour et al., (2011); González-Ortiz et al. (2019) and Singh et al., (2021). At the end of the starter phase, the addition of wheat bran affected the duodenum by increasing its weight. In the study by Wu et al. (2004) xylanase supplementation increased ileal villus height in whole wheat-based diet. On the contrary, the study by Singh et al. (2021b) reported that XYL and XOS did not change villus height or crypt depth ratio ($P > 0.05$) in maize-soybean meal based diet, indicating the effect of XYL on this ratio may not have been

significantly higher due to the lack of high viscosity in maize-SBM-based diets. The lack of changes influenced by the experimental diets on histomorphometry results is not unusual considering enhanced performance and production is not always linked to jejunal morphometry in poultry (Pirgozliev et al., 2010).

3.5.3. Effect on SCFA production

Higher caeca content of acetic acid, propionic acid, valeric acid, total VFA and SCFA, when wheat bran was included in diets at 35 d suggests how dietary fibre may act as a substrate for the microbial populations. As in Józefiak et al. (2007) study, XYL increased lactic acid concentrations in the caeca. The elevated levels of lactate can promote the growth of lactate-utilising bacteria in the caeca. Through the methylglyoxal pathway, or other fermentation processes, these bacteria which include species of Lactobacillus and Bifidobacterium can convert lactate to acetyl-CoA (Duncan et al., 2004). Despite not being significant, diets fed with combination of XYL and XOS at the end of the experiment resulted in numerically higher SCFA content in caeca compared to control (102.8 vs. 108.3 mmol/kg), similarly as in the study by Dale et al. (2020). It remains unclear whether the observed concentration changes were as a direct result in a modification of the microbiota. However, it supports the hypothesis that the poultry microbiome can potentially adapt over time as a result of added supplementations and by increasing fermentation in caeca to improve performance. In some studies there was no effect of supplements on caecal concentrations on any of the SCFA measured in broilers or turkeys (Engberg et al., 2004; González-Ortiz et al., 2020). In contrast, in Singh et al. (2021), supplemental XYL and XOS in the maize-soybean meal-based diet resulted in an increase of acetate production in caeca on day 42. The XYL also increased the caecal concentration of the total SCFA ($P < 0.01$); however, the increase in SCFA did not result in better FCR. Jozefiak et al. (2004) found that enzyme supplementation significantly increases the butyrate concentration in comparison with un-supplemented groups, but the authors did not find a relationship with the WG of the birds. A potential explanation for the contradictions in SCFA measurements could be explained by their volatile concentrations which are dependent upon production and absorption rates at the exact point in time of measurement. Although oligosaccharides that are added in the diet or produced *in situ* may not be enough to contribute a significantly higher proportion of SCFA production in the caeca of broilers it has been hypothesized that they could act as a singling molecule which would stimulate microbial adaptation to degrade dietary fibre sources (Bedford, 2018).

3.6. Conclusion

In summary, the results showed the expected reduced performance in the finisher phase and the overall study period, attributable to the addition of wheat bran in terms of reduced determined metabolisable energy, nutrient availability, caeca SCFA content and growth performance. With the exception of NDF digestibility, there were no interactions between treatment and wheat bran for any measure of nutrient digestibility. Improved digestibility of the NDF was observed in xylanase and combination of XYL and XOS supplemented diets with wheat bran addition compared with all other treatments suggesting a benefit is derived from combining the two. The performance of each of the maize-based diets was not fully reflected in nutrient retention coefficients. Although the treatment with combination of XYL and XOS did result in the best performance, no treatment effect was observed for AME, AMEn, DMR, NR, or FR and there was no evidence of negative interactions, suggesting the benefits of the combination of XYL and XOS are derived from effects unrelated to changes in nutrient digestibility. Moreover, advances in performance may not always be dependent on changes in microbial diversity or the development of mucosal absorptive surfaces. The present study indicates that a combination of XYL and XOS could result in better performance compared to supplementation of each component individually. These results support the theory that the addition of XYL and XOS could provide benefits in terms of fibre degradation, weight gain, and feed efficiency, especially in diets with enhanced fibre content.

4. Chapter: Evaluation of the impact of Xylanase and XOS on metabolisable energy, nutrient retention, gastrointestinal tract development, and growth performance of Ross 308 male broilers fed diets with different levels of viscosity from 0 to 35 days of age

4.1. Introduction

While the demand for poultry meat is continually rising (FAOSTAT, 2024), the ban on antibiotics and potential fluctuations in cereal availability may influence the increase fibre inclusion in poultry diets (Dey et al., 2021). Although the high fibrous cereals can be more affordable, the inclusion of those often increases the NSP. The raise of NSP and particularly their soluble component, which are known to raise digesta viscosity and encapsulate nutrients, impacts nutrient absorption and digestion as well as litter quality (Choct et al., 1996; Nguyen et al., 2021).

Supplementary XYL has been routinely used in poultry diets to hydrolyse NSP, break down the arabinoxylan backbone and release the nutrients, thus improving the feeding value of fibre-rich viscous diets (Bedford, 2018). Previous research suggested that adding XOS along with XYL may be more beneficial for broilers WG, FCR and fibre degradation than adding XYL alone (Šimić et al., 2023). In a study by 09/04/2025 20:09:00 viscosity in different types of grains was analysed in the presence of XYL and β-glucanase alone or in combination. The results showed that combining both enzymes significantly reduced viscosity in diet digesta samples of wheat, triticale, rye, barley, oat, and pea. This study confirmed that different feedstuffs and their variations in NSP and arabinoxylan composition can have an impact on the viscosity. Arabinoxylans are primary NSP of wheat (Bonnin et al., 1998) and rye (Knudsen and Lærke, 2010), but can also be found in maize, barley and oats (He et al., 2021). The increase of NSP in diet has shown to impact the size GIT development (Banfield et al., 2002). In study by Smulikowska et al. (2002) high viscous rye based diet has negatively impacted development of GIT and motility, however, these effects were mitigated by XYL supplementation indicating the use of NSP degrading enzymes could be a beneficial strategy for enhancing the value of fibre in broiler diet.

4.2. Objective

The objective of this chapter is to investigate the effects of different dietary viscosity levels on broiler growth performance with the addition of XYL and a combination of XYL and XOS, given that chapter three has shown that supplementing only XOS did not have as effective

results. In particular, FI, WG, FCR, ME, nutrient digestibility, and GIT development will be taken into consideration. The following general hypotheses will be examined:

1. High viscous diets will have a negative impact on bird performance, reduce nutrient digestibility and metabolisable energy; and have enlarging impact on GIT.
2. That negative impact of high viscous diets will be overcomed by the addition of XYL and a combination of XYL and XOS, by significantly improving bird growth performance, nutrient digestibility and apparent metabolisable energy.

4.3. Materials and methods

General materials and methods can be found in chapter two.

4.3.1. Animal housing

A total of eight-hundred-and-ten birds day-old male Ross-308 broilers were used in this experiment. Broilers were obtained from a local hatchery (Cyril Bason Ltd, Craven Arms, UK). After the arrival, the birds were weighed and divided into fifty-four floor pens, each with fifteen birds, and handled as described in section 3.1.

4.3.2. Treatments

The experimental diets were formulated to contain three different levels of NSP's, i.e. different viscosity (Table 4.1 and Table 4.2). The viscous diet was wheat and soyabean meal based, with addition of barley, oats and rye. The intermediate viscous diet was wheat, maize and soyabean meal based; and the non-viscous diet was maize and soyabean-meal based diet. Each basal diet with three levels of viscosity were split in three batches, one being control and others either supplemented with XYL or a combination of XYL and XOS. The treatment diets were formulated to contain 100 g/tonne of XYL (Econase XT 25P, AB Vista, Marlborough, UK; 160,000 BXU/kg) or combination of XYL and XOS (AB Vista, Marlborough, UK; with XOS degree of polymerisation between 2 and 7; 160,000 BXU/kg). A total of 9 dietary treatments were offered and each was fed to 6 pens following randomisation. The experimental diets were fed to chickens in two phases: starter (0-21 d) in crumb form and finisher (22-35 d) in pellet form, with a diet made by Target Feeds (Witchurch, Shropshire, UK). Diatomaceous earth (Multi-Mite®, Wiltshire, UK) was added at 20 g/kg of feed as an acid insoluble ash (AIA) as indigestible marker.

Table 4.1 Ingredient composition of the experimental diets

Ingredient	Starter Viscous	Starter Intermediate	Starter Non-Viscous	Finisher Viscous	Grower Intermediate	Grower Non-Viscous
Wheat	373.9	250	0	437.4	400	0
Maize	0	328.4	566.7	0	296.2	677.6
Barley	50	0	0	100	0	0
Oats	50	0	0	50	0	0
Soybean meal	327	344.3	361.8	206.4	220.9	248.8
Rye	100	0	0	100	0	0
Soya oil	46.1	24.7	19.8	57.5	34.3	26.5
Salt	3.8	3.9	3.8	3.4	3.5	3.4
Limestone	5.6	5.5	5	6.5	6.5	5.6
Dic-Phos, 18%P	12.7	13.3	14	6.9	7.4	8.5
L-Tryptophan	0	0	0	0	0	0
Lysine HCl	1.9	1.6	1.2	2.7	2.5	1.8
DL-Methionine	2.6	2.6	2.6	2.2	2.2	2.1
Threonine	0.7	0.4	0.1	1.1	0.9	0.4
Valine	0.5	0.3	0	0.7	0.5	0
Quantum Blue[1]	0.1	0.1	0.1	0.1	0.1	0.1
Acid insoluble ash[2]	20	20	20	20	20	20
Vitamin mineral premix[3]	5	5	5	5	5	5
TOTAL	1000	1000	1000	1000	1000	1000

[1] Quantum Blue 5G, AB Vista, Marlborough, UK; 5,000 FTU/g.

[2] Feed grade diatomaceous earth (Multi-Mite®, Wiltshire, UK).

[3] Vitamin mineral premix provided per kg of diet: vitamin A, 10,000 IU; vitamin D3, 2,500 IU; vitamin E, 25 IU; vitamin E, 50 mg; vitamin K_3, 1.5 mg; vitamin B1, 2 mg; vitamin B2, 7.5 mg; vitamin B6, 3.5 mg; vitamin B12, 20 µg; niacin, 35mg; D-pantothenic acid, 12 mg; choline chloride, 460 mg; folic acid, 1.0 mg; biotin, 0.2 mg; iron as iron sulphate, 265 mg; copper as copper sulphate, 48 mg; manganese as manganese oxide, 140 mg; zinc as zinc sulphate, 165 mg; iodate as potassium iodide, 1.2 mg; and selenium as sodium selenite, 0.33 mg.

Table 4.2 Ingredient composition of the experimental diets

Ingredient	Starter Viscous	Starter Intermediate	Starter Non-Viscous	Finisher Viscous	Grower Intermediate	Grower Non-Viscous
Calculated analysis (as-fed basis)						
Crude protein (g/kg)	225	225.6	226.6	180	180	181.4
ME (MJ/kg)	12.34	12.34	12.34	12.97	12.97	12.97
DM g/kg	860.5	857.9	857.4	860.2	857.4	856.5
Calcium g/kg	9	9	9	7.6	7.6	7.6
Phosphorus g/kg	7.7	7.8	8.1	6.1	6.2	6.6
Analysed values (as-fed basis)						
Viscosity (cP)	15.1	8.13	6.6	18	12	7.3
Crude protein (g/kg)	218.6	219.2	223.5	182.6	179.0	175.6
Gross energy (MJ/kg)	16.9	15.9	16.3	17.1	16.4	16.6
Total NSP (%)	11.2	8.6	9.3	10.5	8.6	8.7
Soluble NSP (%)	3.3	2.1	1.9	3.4	2.4	2.2
Insoluble NSP (%)	7.9	6.5	7.5	7.1	6.1	6.5
Main constituents of total NSP						
Arabinose (%)	2.1	1.9	1.8	2.1	1.84	1.78
Xylose (%)	2.7	1.9	1.6	3.0	2.2	1.9
Mannose (%)	0.3	0.2	0.3	0.2	0.2	0.2
Galactose (%)	1.7	1.6	1.9	1.3	1.3	1.5
Glucose (%)	3.2	2.3	2.5	3.1	2.3	2.5

4.3.3. Statistical analysis

Calculations and data handling were performed in Excel 2020 (Microsoft Corporation) and GenStat statistical software (21st edition, Rothamsted, Hertfordshire, UK) was used for statistical analyses. Viscous, intermediate viscous and non-viscous diets; and control, xylanase, or combination of XYL and XOS in the diet were used in a 3 x 3 factorial arrangement. The data were analysed using two-way ANOVA based on a completely randomised design. Differences were reported as significant at $P < 0.05$. To evaluate significant differences between the means, Fisher's protected least significant difference test was used. Prior to ANOVA, all data were evaluated for outliers, normality, and residual homogeneity.

4.4. Results

4.4.1. Diet analysis

The diets were formulated to meet breeders' recommendations (Aviagen Ltd, UK; Table 4.1 and Table 4.2). The *in vitro* analyses of diets viscosity showed levels of viscosity in starter and finisher diet for low (6.6 and 7.3 cP, respectively), intermediate viscous (8.13 and 12 cP, respectively) and viscous diets (15.1 and 18 cP, respectively). Table 4.3 shows the expected and the determined activity of dietary PHY and XYL. The mean result for XYL supplemented diets was 15325 BXU/kg and was similar to intended 16000 BXU/kg. The Viscous control diet in the finisher phase indicated unusually higher activity of XYL, with activity of XYL reaching 6910 BXU/kg. The mean activity of dietary PHY was 737 FTU/kg and was slightly higher than the expected 500 FTU/kg diet.

Table 4.3 Analysis of phytase and xylanase activity in the experimental diets

	Expected		Analysed	
Treatments	**Phytase, FTU/kg[1]**	**Xylanase, BXU/kg[2]**	**Phytase, FTU/kg**	**Xylanase, BXU/kg**
Starter diet				
Viscous	500	0	808	~3280
Viscous + XYL	500	16000	816	15500
Viscous + XYL+ XOS	500	16000	708	14700
Intermediate	500	0	1040	<2000
Intermediate + XYL	500	16000	744	14000
Intermediate + XYL+ XOS	500	16000	586	15500
Non-Viscous	500	0	753	<2000
Non-Viscous + xylanase	500	16000	600	11800
Non-Viscous + XYL+ XOS	500	16000	926	17600
Finisher diet				
Viscous	500	0	608	6910
Viscous + XYL	500	16000	566	16200
Viscous + XYL+ XOS	500	16000	521	17800
Intermediate	500	0	722	~2720
Intermediate + XYL	500	16000	740	13100
Intermediate + XYL+ XOS	500	16000	660	17500
Non-Viscous	500	0	802	~2500
Non-Viscous + XYL	500	16000	761	12700
Non-Viscous + XYL+ XOS	500	16000	911	17500

XYL, Xylanase; XOS, Xylooligosaccharides;

[1] The amount of enzyme necessary to release 1 mmol of inorganic P per minute from sodium phytate, at 37°C and pH 5.5, is defined as one FTU.

[2] The amount of enzyme that generates 1 nmol reducing sugars from birchwood xylan in one second, at 50°C and pH 5.3, is measured as one BXU.

4.4.2. Growth performance

The results on bird growth performance are presented in Table 4.4. There were not many differences observed between birds' growth performance fed the experimented diets. Birds fed non-viscous diet had FI lower than those fed diet with intermediate viscosity ($P = 0.021$), but the FI of birds fed the vicious diet did not differ ($P > 0.05$) from non-viscous or intermediate viscosity diet fed broilers. There was a diet x supplementation interaction for WG ($P = 0.039$), where XYL supplementation brought some inconsistent changes during starter period, increasing WG of birds fed non-viscous diet compared to control, and decreasing WG of birds fed intermediate diet compared to XYL + XOS diet. Interaction in WG was also observed for the finisher period ($P = 0.043$) as birds fed XYL + XOS non-viscous diet had lower WG than the control, and those fed XYL had higher WG than the control diet. No differences were observed ($P > 0.05$) for the overall WG during study. Birds fed non-viscous diets utilised feed more efficiently having lower FCR for starter ($P = 0.008$) and finisher ($P = 0.004$) periods, respectively.

Table 4.4 Effect of experimental diets on feed intake (FI), weight gain (WG) and mortality corrected feed conversion ratio (FCR) in 21 and 35d old broilers

	Treatment	FI 0-21d (g/b/d)	FI 22-35d (g/b/d)	FI 0-35d (g/b/d)	WG 0-21d (g/b/d)	WG 21-35d (g/b/d)	WG 0-35d (g/b/d)	FCR 0-21d	FCR 21-35d	FCR 0-35d
Treatment										
Control		64.86	172.0	100.41	38.28	101.9	58.14	1.348	1.647	1.532
XYL		65.73	176.5	102.85	38.90	105.6	59.94	1.355	1.637	1.533
XYL + XOS		65.50	176.1	102.26	37.96	102.3	58.02	1.368	1.672	1.556
Viscosity										
Non-Viscous		64.49^{b}	175.4	101.52	38.95	104.1	59.40	1.328^{b}	1.633	1.517^{b}
Intermediate		66.38^{a}	177.0	103.00	38.29	103.4	58.58	1.374^{a}	1.662	1.553^{a}
Viscous		65.21^{ab}	172.1	101.00	37.90	102.2	58.12	1.369^{a}	1.660	1.551^{a}
SEM		0.462	2.20	0.937	0.412	2.32	1.000	0.0010	0.0129	0.0082
Treatment x Viscosity										
Non-Viscous	Control	63.65	177.8	101.82	37.98^{bc}	110.9^{a}	61.06	1.334	1.589	1.501
	XYL	65.68	175.6	102.41	40.22^{a}	102.4^{abc}	59.67	1.319	1.645	1.517
	XYL + XOS	64.15	172.9	100.34	38.66^{abc}	99.1^{bc}	57.47	1.330	1.665	1.533
Intermediate	Control	65.70	169.6	99.47	37.87^{bc}	95.7^{c}	55.44	1.354	1.688	1.550
	XYL	66.32	177.8	105.86	39.52^{ab}	110.5^{ab}	62.15	1.356	1.622	1.527
	XYL + XOS	67.13	177.8	103.67	37.47^{c}	104.1^{abc}	58.16	1.414	1.678	1.583
Viscous	Control	65.23	168.5	99.95	38.99^{abc}	99.0^{bc}	57.92	1.357	1.665	1.545
	XYL	65.18	170.2	102.77	36.97^{c}	104.0^{abc}	58.02	1.391	1.643	1.554
	XYL + XOS	65.21	177.6	102.77	37.74^{bc}	103.7^{abc}	58.42	1.359	1.673	1.554
SEM		0.800	3.81	1.623	0.713	4.02	1.732	0.0173	0.0224	0.0142
Significance										
Treatment		0.393	0.283	0.171	0.267	0.461	0.323	0.382	0.160	0.066
Viscosity		0.021	0.292	0.305	0.200	0.842	0.659	0.003	0.209	0.004
Treatment x Viscosity		0.529	0.103	0.158	0.039	0.043	0.134	0.088	0.099	0.328

$^{a\text{-}c}$ $P < 0.05$; SEM, pooled standard error of means; XYL, xylanase; XOS, xylooligosaccharides.

4.4.3. Metabolisable energy and nutrient retention

There was treatment x viscosity interaction ($P < 0.001$) for AME and AMEn for both starter and finisher diets determined at 21 and 35d, respectively (Table 4.5). It seems that AME and AMEn of non-viscous starter diet were increased with XYL and XYL + XOS supplementation, although in intermediate diet it led to energy reduction and only XYL + XOS supplementation increase metabolisable energy in viscous diet. For the finisher diets however, metabolisable energy in non-viscous diet were increased with XYL and XYL + XOS supplementation, but their supplementation led to a decrease in metabolisable energy of intermediate and viscous diets. However, supplements did not change ($P > 0.05$) NR and DMR coefficients in starter or finisher diets (Table 4.6).

Table 4.5 Effect of the experimental diets on the apparent metabolisable energy (AME) and nitrogen-corrected metabolisable energy (AMEn) of 21d and 35d old broilers

	Treatment	AME 21d (MJ/kg)	AMEn 21d (MJ/kg)	AME 35d (MJ/kg)	AMEn 35d (MJ/kg)
Treatment					
Control		12.953[a]	12.448[a]	13.972[a]	13.550[a]
XYL		12.916[a]	12.429[a]	13.876[b]	13.464[b]
XYL + XOS		12.854[b]	12.371[b]	13.739[c]	13.344[c]
Viscosity					
Non-Viscous		13.013[b]	12.469[b]	13.602[c]	13.207[c]
Intermediate		12.415[c]	12.003[c]	13.837[b]	13.433[b]
Viscous		13.296[a]	12.777[a]	14.148[a]	13.718[a]
SEM		0.0924	0.0614	0.0317	0.0295
Treatment x Viscosity					
Non-Viscous	Control	12.648[c]	12.127[c]	13.145[f]	12.777[f]
	XYL	13.240[b]	12.683[b]	13.797[cd]	13.382[cd]
	XYL + XOS	13.152[b]	12.597[b]	13.865[c]	13.463[c]
Intermediate	Control	13.187[b]	12.697[b]	14.240[b]	13.808[b]
	XYL	12.270[d]	11.878[c]	13.677[ed]	13.287[ed]
	XYL + XOS	11.788[e]	11.433[d]	13.593[e]	13.203[e]
Viscous	Control	13.025[b]	12.522[b]	14.532[a]	14.065[a]
	XYL	13.238[b]	12.725[b]	14.153[b]	13.725[b]
	XYL + XOS	13.623[a]	13.083[a]	13.758[cd]	13.365[cd]
SEM		0.1600	0.1064	0.0548	0.0510
Significance					
Treatment		0.562	0.655	<0.001	<0.001
Viscosity		<0.001	<0.001	<0.001	<0.001
Treatment x Viscosity		<0.001	<0.001	<0.001	<0.001

[a-f] $P < 0.05$; SEM, pooled standard error of means; XYL, xylanase; XOS, xylooligosaccharides.

Table 4.6 Effect of the experimental diets on the dry matter retention (DMR) and nitrogen retention (NR) in 21d and 35d old broilers

	Treatment	DMR 21d	DMR 35d	NR 21d	NR 35d
Treatment					
Control		0.743	0.788	0.702	0.708
XYL		0.749	0.783	0.667	0.687
XYL + XOS		0.749	0.791	0.672	0.693
SEM		0.0050	0.0040	0.0135	0.0110
Viscosity					
Non-Viscous		0.752	0.786	0.686	0.695
Intermediate		0.749	0.788	0.668	0.693
Viscous		0.740	0.788	0.686	0.701
SEM		0.0050	0.0040	0.0135	0.0110
Treatment x Viscosity					
Non-Viscous	Control	0.740	0.782	0.722	0.708
	XYL	0.763	0.787	0.660	0.683
	XYL + XOS	0.753	0.788	0.677	0.693
Intermediate	Control	0.757	0.793	0.665	0.682
	XYL	0.748	0.783	0.680	0.692
	XYL + XOS	0.743	0.787	0.680	0.705
Viscous	Control	0.733	0.788	0.718	0.735
	XYL	0.735	0.780	0.680	0.687
	XYL + XOS	0.752	0.797	0.660	0.680
SEM		0.00872	0.00698	0.0235	0.0190
Significance					
Treatment		0.640	0.448	0.158	0.380
Viscosity		0.210	0.876	0.567	0.876
Treatment x Viscosity		0.180	0.600	0.497	0.345

SEM, pooled standard error of means; XYL, xylanase; XOS, xylooligosaccharides.

The response of experimental diets on NDF digestibly is shown in Table 4.7. At day 21 supplemented diets had significantly higher NDF digestibility compared to the control (P = 0.039). Viscosity has impacted NDF digestibility both at 21 and 35 d (P = 0.001), where compared to non-viscous diet, viscous diet had increase of 42% in starter phase and 43% in finisher phase.

Table 4.7 Effect of the experimental diets on the neutral detergent fibre (NDF) retention in 21d and 35d old broilers

	NDF 21d	NDF 35d
Treatment		
Control	0.127[b]	0.209
XYL	0.171[a]	0.186
XYL + XOS	0.180[a]	0.213
SEM	0.0213	0.0211
Viscosity		
Non-Viscous	0.118[b]	0.146[c]
Intermediate	0.157[b]	0.204[b]
Viscous	0.204[a]	0.257[a]
SEM	0.0213	0.0211
Significance		
Treatment	0.039	0.404
Viscosity	0.001	<.001
Treatment x Viscosity	0.944	0.121

[a-c] $P < 0.05$; SEM, pooled standard error of means; XYL, xylanase; XOS, xylooligosaccharides.

The results of the NSP digestibility at 35 d analysis revealed no significant interaction between the experimental diets (Table 4.8). However, viscosity consistently impacted total, insoluble and soluble NSP digestibility ($P < 0.001$), where viscous diet had highest digestibility, followed by the intermediate and lastly non-viscous diet.

Table 4.8 Effect of experimental diets on total, insoluble and soluble non-starch polysaccharide (NSP) digestibility in 35d old broilers

	Total NSP	Insoluble NSP	Soluble NSP
Treatment			
Control	0.3642	0.3444	0.411
XYL	0.3207	0.3076	0.378
XYL + XOS	0.3429	0.3339	0.361
SEM	0.01496	0.01569	0.0228
Viscosity			
Non-Viscous	0.2769[b]	0.2797[b]	0.301[b]
Intermediate	0.3146[b]	0.3049[b]	0.339[b]
Viscous	0.4364[a]	0.4013[a]	0.511[a]
SEM	0.01496	0.01569	0.0228
Significance			
Treatment	0.134	0.244	0.304
Viscosity	<0.001	<0.001	<0.001
Treatment x Viscosity	0.674	0.694	0.198

[a-c] $P < 0.05$; SEM, pooled standard error of means; XYL, xylanase; XOS, xylooligosaccharides.

4.4.4. Ileal viscosity

Ileal digesta viscosity analysis identified significance in different treatment and different viscosity levels at 35 d (Table 4.9). Feeding the viscous diet produced the highest ileal digesta viscosity ($P < 0.001$) and it was reduced when XYL was added to the diet ($P = 0.049$).

Table 4.9 Effect of experimental diets on cP of ileal digesta viscosity in 35d old broilers

	Ileal digesta viscosity (cP)
Treatment	
Control	4.52[a]
XYL	3.76[b]
XYL + XOS	4.02[ab]
SEM	0.31
Viscosity	
Non-Viscous	3.35[b]
Intermediate	3.69[b]
Viscous	5.27[a]
SEM	0.31
Significance	
Treatment	0.049
Viscosity	<.001
Treatment x Viscosity	0.882

[a-c] $P < 0.05$; SEM, pooled standard error of means; cP, centipoise; XYL, xylanase; XOS, xylooligosaccharides.

4.4.5. Gastrointestinal tract development

The responses of dietary treatments on the relative weights of the GIT organs are presented in Tables 4.10 and 4.11. At 21 d the % of weights of small intestine increased as the inclusion of NSP increased, raising from 3.035% in low viscous diet, 3.189% in intermediate viscous diet to 3.364% in viscous diet ($P < 0.001$). At the end of the study the heaviest caecal weight % was found in viscous diets ($P < 0.001$). No differences were observed in the proventriculus and gizzard, pancreas or total gastrointestinal tract ($P > 0.05$).

Table 4.10 Effect of experimental diets on the Gastrointestinal tract development on relative organ weights (% of live body weight) of broilers at the 21 d and 35 d of age

	Treatment	Proventriculus and Gizzard		Pancreas	
		21 d	35 d	21 d	35 d
Treatment					
Control		2.385	1.439	0.2894	0.1612
XYL		2.448	1.421	0.3129	0.1669
XYL + XOS		2.413	1.555	0.3135	0.1769
SEM		0.0723	0.0532	0.01020	0.00829
Viscosity					
Non-Viscous		2.416	1.480	0.3102	0.1708
Intermediate		2.407	1.424	0.3191	0.1607
Viscous		2.423	1.511	0.2866	0.1735
SEM		0.0723	0.0532	0.01020	0.00829
Treatment x Viscosity					
Non-Viscous	Control	2.334	1.478	0.2965	0.1556
	XYL	2.462	1.549	0.3079	0.1924
	XYL + XOS	2.451	1.414	0.3261	0.1642
Intermediate	Control	2.417	1.340	0.3125	0.1622
	XYL	2.304	1.324	0.3266	0.1515
	XYL + XOS	2.502	1.607	0.3182	0.1683
Viscous	Control	2.404	1.500	0.2592	0.1658
	XYL	2.577	1.391	0.3042	0.1566
	XYL + XOS	2.287	1.643	0.2963	0.1980
SEM		0.1252	0.0921	0.01766	0.01436
Significance					
Treatmant		0.830	0.170	0.176	0.410
Viscosity		0.989	0.503	0.078	0.521
Treatment x Viscosity		0.388	0.153	0.769	0.155

SEM, pooled standard error of means; XYL, xylanase; XOS, xylooligosaccharides.

Treatment		% Small intestine		% Caeca		% Gastrointestinal tract (no liver)	
		21 d	35 d	21 d	35 d	21 d	35 d
Treatment							
Control		3.160	2.167	0.518	0.425	6.358	4.305
XYL		3.169	2.240	0.510	0.397	6.509	4.338
XYL + XOS		3.259	2.131	0.462	0.401	6.533	4.386
Viscosity							
Non-Viscous		3.035[c]	2.148	0.501	0.364[b]	6.333	4.283
Intermediate		3.189[b]	2.133	0.479	0.393[b]	6.478	4.225
Viscous		3.364[a]	2.258	0.510	0.467[a]	6.588	4.520
SEM		0.0583	0.0481	0.0251	0.0171	0.1132	0.0929
Treatment x							
Non-Viscous	Control	2.885	2.210	0.506	0.402	6.091	4.355
	XYL	3.106	2.245	0.539	0.358	6.488	4.452
	XYL + XOS	3.113	1.988	0.457	0.331	6.420	4.043
Intermediate	Control	3.122	1.996	0.489	0.395	6.424	4.014
	XYL	3.218	2.225	0.492	0.376	6.408	4.195
	XYL + XOS	3.226	2.178	0.458	0.407	6.602	4.468
Viscous	Control	3.471	2.295	0.559	0.478	6.558	4.547
	Xylanase XYL	3.185	2.250	0.500	0.457	6.631	4.367
	XYL + XOS	3.437	2.228	0.471	0.464	6.576	4.647
SEM		0.1010	0.0834	0.0435	0.0297	0.1961	0.1609
Significance							
Treatment		0.424	0.278	0.245	0.450	0.502	0.827
Viscosity		0.001	0.148	0.680	<.001	0.289	0.071
Treatment x		0.158	0.136	0.840	0.708	0.805	0.089

Table 4.11 Effect of experimental diets on the Gastrointestinal tract development on relative organ weights (% of live body weight) of broilers at the 21 d and 35 d of age

[a-c] $P < 0.05$; SEM, pooled standard error of means; XYL, xylanase; XOS, xylooligosaccharides.

4.5. Discussion

4.5.1. Bird growth performance and effect on AME

The enzyme recovery of xylanase did not reach our original expectations in our trial. Despite careful planning and implementation of the study, the achieved recovery rate of XYL in the finisher viscous diet was above the anticipated level. The contamination has likely occurred in the process of diet mixing at the mill. Although it is significantly less than the standard XYL dose of 16 000 BXU/kg, it is possible that 6910 BXU/kg XYL activity could have affected the results in the viscous finisher diet.

Low unexplained mortality of 4.7 %, which had no correlation to diets, indicated that the birds were in good health throughout the trial. The birds performed better than expected compared to Aviagen's performance objectives (2020), with the average weight of the bird at 35 d being 2556 g compared to the estimated 2441 g. Overall, the FCR was also improved in the current experiment, averaging 1.349 compared to the targeted 1.399. When compared to the results of the initial study (Chapter 3), a significant enhancement was observed, as the previous study reported an average FCR of 1.688 and a corresponding bird weight of 2080 g.

It is possible that high performance in the control group was the reason why there were no differences between the dietary treatments ($P > 0.05$) in overall (0- 35 d) performance, as the bird's potential was reached regardless of them. Due to the increased WG in the current study, birds had higher FI compared to the Aviagen objectives (101.84 vs 91.74 g/b/d).

The impact of digesta viscosity and XYL on growth performance, dietary energy, nutrient availability and GIT development has been widely studied (Bedford, 2018; Bedford and Classen, 1993). Supplementary XYL has been routinely used in poultry diets to hydrolyse NSP and improve the feeding value of fibre-rich viscous diets (Bedford, 2018). Arabinoxylans, a soluble fraction of NSP, are prevalent in grains such as wheat and rye and are directly related to the composition of the carbohydrate fraction. These compounds could contribute to increase of viscosity in intestinal lumen (Smits and Annison, 1996; He et al., 2021). Three different diets were formulated for the current experiment to include different amounts of NSPs, which could affect bird's intestinal viscosity. It was expected that maize based low viscous diet, would not adversely affect bird performance, while a gradual increase in NSP levels would correspond to a rise in intestinal viscosity. It was hypothesised that broilers would experience greater difficulty in digesting the intermediate-viscose diet, which contained a combination of maize and wheat. The viscose diet, incorporating wheat, rye, oats, and barley, was expected to have the most pronounced negative impact on digestion and, consequently, broiler performance.

The FI was increased in intermediate viscous diets compared to non-viscous diets. By extending the amount of time that nutrients spend in contact with absorptive cells and digestive enzymes, lower FI slows down the rate at which feed passes through the digestive tract, improving nutrient digestibility (Washburn, 1991). Fibrous feeds could increase bulk and as the DF increases, so does the FI until the gastrointestinal capacity is reached causing intake to plateau, which was confirmed by Jørgensen et al. (1996) study. However, certain odours, colours and textures may contribute to reduced feed intake (Kleyn, 2013) and it is possible that birds fed with a maize-based diet ate less due to it being less palatable than a combination of maize and wheat diets. Many factors can affect feed intake, such as temperature, amino acid content and type of cereals. Similarly as Gheisari et al. (2018) reported, maize-based diet had significantly lower FI compared to the diet with a combination of maize and wheat. The FI not being different from 22 - 35 and 0 - 35 d can indicate that the digestive tract became more developed in the finisher phase and therefore the FI has not been influenced by the diet. Despite statistical significance in FI, it could be considered of little importance as it did not corelate with other performance parameters in starter phase nor did it continue in finisher phase or in overall period.

The WG and FCR were impacted negatively by the additional NSP from the diets that had higher levels of viscosity compared to the maize based diet. The highest WG in starter phase was found in non-viscous maize based diets supplemented with XYL. Over the years XYL has been added routinely to wheat-based diet, however, it was not the case for maize-based diets due to inconsistent results (Kim et al., 2022). This experimental result supports the hypothesis that XYL can be beneficial even in maize based diets (Cowieson, 2005). Similarly as in Pourazadi et al. (2020) study, in the finisher phase the WG was reduced in higher fibre diets, decreasing for 13.7% in intermediate viscous diet and 10.7% in the viscous diet. In intermediate viscous diet, the negative effect of higher NSP was significantly reduced in diets that had added XYL. In the present study, birds fed non-viscous diet had approximately 2 - 3% lower FCR, i.e. better feed efficiency, at 21 and 35d age, compared to the birds fed intermediate and viscous diets. The effect of increased FCR found in higher NSP content in diets found in the starter and overall period was in accordance with the published data (Jørgensen et al., 1996). Similar results have been reported in a study by Nian et al. (2011), where broilers fed a diet based on maize and soybean meal supplemented with xylanase showed numerical improvements in FCR but no significant difference in weight gain. While xylanase and XOS alone did not significantly affect performance in the current study, the inclusion of XYL resulted in a numerical improvement of WG in all phases and FCR during the finisher phase.

Exogenous xylanases, as well as a combination of XOS and XYL, supplementation in broiler diets has been shown to impact the AME and AMEn (Pirgozliev et al., 2023). Research has shown that the addition of XYL and XOS can increase AME and AMEn, indicating enhanced energy utilisation by the broilers (Kiarie et al., 2014; Šimić et al., 2023). At the end of starter phase, AME and AMEn in non-viscous diet control differed from XYL and combination of XYL and XOS with an increase in supplemented diets. While supplementation at 21 d did not improve the AME and AMEn in diets that were maize and wheat-based, the highest improvement through all treatments was a result of a combination of XYL and XOS supplementation in wheat-based diet with added rye, oats and barley, both in AME and AMEn. Similarly, Gorenz et al. (2022) and Vasanthakumari et al. (2023) showed the xylanase has improved the AME and AMEn. At day 21, there was significant improvement at 35 d to maize-based control when supplements were added to the diet for metabolisable energy. Correspondingly to 21 d, at 35 d there was no improvement in the intermediate diets when supplements were added. Unusually, the viscous diets control had the highest AMEn and AME at 35 d. A potential explanation for this could be the effect of contamination of XYL in a viscous finisher diet. It is likely that high levels (6910 BXU/kg) that were found recovered in XYL activity could have positively affected the AME and AMEn. However, the AME and AMEn results were not supported by the performance and digestibility results.

The retention coefficients did not fully reflect the improved performance observed in supplemented diets. The addition of supplements and different levels of viscosity in diet had no effect on DMR and NR. Previous research on the supplementation of XYL (Nian et al., 2011; Pirgozliev et al., 2015) and the combination of XYL and XOS (González-Ortiz et al., 2021a) has shown a lack of responses in nutrient utilisation, indicating performance results may not be always correlating with digestibility results.

Birds fed with higher fibre levels in diets had higher NDF digestibility both in starter phase and finisher phase. In finisher phase there is clear difference between the level of DF, with non-viscous diet having lowest NDF digestibility, followed by intermediate viscous diet and highest level in viscous diet. The higher NDF digestibility in high viscosity diets may be the result of increased dietary fibre slowing down the passage rate or mean transit time, which promotes DF fermentation (Langhout, 1998). In starter phase birds fed with addition of supplements significantly differ from the control diet. Similarly, as in Kiarie et al. (2014) and Petry et al. (2021) studies, xylanase and XOS addition has increased the NDF digestibility.

Dietary content and the presence of certain enzymes have shown in previous research to have a major impact on the broilers ability to digest NSPs. Increased digestibility of NSPs, especially in diets based on wheat, could be achieved by adding NSP-degrading enzymes such as xylanase, subsequently improving broiler growth performance, as well as nitrogen retention (Godbout et al., 2024). Contrarily, while adding enzyme supplements usually increases digestibility, intake of high amounts of fibre might decrease the absorption of nutrients, emphasising the necessity for well-balanced dietary formulations. A moderate amount of soluble NSP has been linked to improved nutrient utilisation as opposed to low or high levels (Nguyen et al. 2022). While incorporation of NSP should be wisely managed, the increased NDF and NSP digestibility coefficients of high-viscous diets in this experiment may be due to the fact that they contained more fibre.

4.5.2. Effect on ileal viscosity

It is well known that the soluble NSPs raise the viscosity of the digesta (Smits and Annison, 1996), which was confirmed in this trial by viscosity and NSP diet analysis, as well as NSP digestibility in excreta and ileal viscosity. As the diet inclusion rates of cereals with higher rate of fibres has risen, specifically the soluble NSP fraction, the viscosity subsequently increases. The FCR performance results were in line with the lower digesta viscosity at 35 d age, for non-viscous diet compared to viscous one, but there was no difference between the viscosity of non-viscous and intermediate diets. The rise of ileal viscosity in diets that are higher in fibre has been well documented (Konieczka and Smulikowska, 2018; Hung et al., 2020; Nguyen et al., 2021). In Langhout et al. (2000), the highly methylated citrus pectin was included in a maize base diet at a dosage level of 30 g/kg as a source of NSP. The digesta's viscosity in the small intestine was increased when birds were fed the diet containing higher level of NSPs. It is well established that xylanase releases the contained nutrients by breaking down the arabinoxylan backbone. As a result, broken-down arabinoxylans reduce the viscosity of the digesta, allowing it to mix properly and enable better nutrient absorption (Bedford and Schulze, 1998; Amerah et al., 2008). Similarly as in Matthiesen et al., 2021 and Hong et al., 2024, the negative effects of high viscosity have been successfully decreased when xylanase was added to the diet, as predicted by previous *in vitro* research by Bedford and Classen (1993). It indicates that use of NSP enzymes can be an effective tactic to decrease the negative effect of increased ileal digestibility.

4.5.3. Effect on Gastrointestinal tract development

A higher viscosity was linked to different weights of the digestive organs; diets rich in soluble fibre, for example can cause the gizzard and small intestine to be heavier (Tejeda and Kim, 2021). The caeca could have important part in chicken's digestion for nutritional fermentation and the absorption of fermentation products (Svihus et al., 2013). Research done by Dorado-Montenegro et al. (2024) hypothesised that enhanced fermentation processes may promote development of the caeca, with diets higher in AX positively corelating with higher relative weight of caeca, as well as caeca length.

In a study done by Pirgozliev et al., 2023 comparing wheat-based diets of low soluble non-starch polysaccharide (NSPs, 13 g/kg) content (low viscosity) and high NSPs content (33.5 g/kg; high viscosity), low viscosity diet had reduced weight of proventriculus and gizzard. However, another study comparing diets with different viscosities did not find any appreciable variations in GIT development, indicating that additional variables might possibly affect broiler performance (Rezaei et al., 2011; Saki, 2005). González-Ortiz et al. (2019) also noted that, with the exception of the crop, which was smaller in birds given XYL supplements, XYL had no effect on the relative weights of any intestinal sections. The authors hypothesised that XYL would enhance performance without having a noticeable impact on the intestines of broilers, which this experiment supported.

4.6. Conclusion

High fibre diets, specifically those higher with soluble NSP, resulted in increased viscosity, which had impact on performance, nutrient digestibility, ileal viscosity and changes in parts of GIT. While in starter phase supplementing XYL has been shown to be the most successful approach at improving WG in non-viscous and intermediate viscous diets, in finisher phase that was the case for intermediate diets. Overall, lower FCR in diets with intermediate and viscous diets was supported by lower ileal digesta in broilers. The increased fibre content in high-viscosity diets may have contributed to its higher NDF and NSP digestibility coefficients, which was supported by the increased size of caeca at 35 d. The supplementation of XYL and XOS improved ME gradually in non-viscous diets only and no effects of feed additives were observed in this experiment in NR and DMR. The observed inconsistencies in studied variables regarding XOS and XYL may be due to viscous finisher control having higher level of XYL recovery than expected. Increasing amount and different types of fibre can influence performance and digestion and overall GIT tract and using XOS and XYL can potentially help

to eradicate part of the negative traits. Further supporting the lack of response to XYL and XOS in this study is an observation that the birds outperformed the ROSS 308 objective, indicating that they may have reached their genetic potential for growth.

5. Chapter: Evaluation of Xylanase and XOS impact on growth performance, metabolisable energy, nutrient retention, caecal ileal volatile fatty acids production, and caecal 16s ribosomal ribonucleic acid gene sequencing of Ross 308 male broilers fed diets with corn-based diets from 0 to 35 days of age

5.1. Introduction

The XYL is a hydrolytic enzyme, used widely in poultry diets, that target the polysaccharides xylan and liberate XOS from arabinoxylans (Morgan et al., 2020). It has been hypothesised that oligosaccharide generation in gut is not as effective as when added directly in broiler diets (Morgan et al., 2019). Several in vitro and in vivo studies have demonstrated that the fermentation of XOS produces SCFA (Broekaert et al., 2011; Scott et al., 2014) and modulate chickens gut microbiome by helping to increase beneficial bacteria in caeca and perhaps even in ileum (Bedford et al., 2024; Ding et al., 2018). In addition to improving the gut microbiome, XOS has been shown to enhance growth performance, improve immune function and boost endocrine metabolism (Zhenping et al., 2013). In published literature the inclusion rate of XOS has reportedly included at low levels as 2 g/t (Yuan et al., 2018) and 50 g/t (Amit K. Singh et al., 2021), 100 g/t , 1000 g/t, 10 000 g/t (Jazi et al., 2019; Ribeiro et al., 2018; Zhou et al., 2021) and as high as 20 000 g/t (Zhenping et al., 2013). Besides substantial range of inclusion rates used in trials, there is lack of understanding how poultry utilise XOS that varies in DP. The prebiotic effects of XOS are correlated to its chemical structure (de Freitas et al., 2019), with DP being regarded as one of the most impactful factor on the functional properties of the molecule (Singh et al., 2015). The DP of XOS used in supplementation are typically 2 – 7 (Fuso et al., 2022), however, studies have shown that a low DP (2 – 5 xylose units) could increase growth of lactic and Bifidobacterium bacteria (Ho et al., 2018; Reddy and Krishnan, 2016).

The use of molecular diagnostic techniques has grown in popularity recently and one of ways to determinate microbial composition of GIT is through analysing the 16s ribosomal RNA gene in bacteria (Crnčević et al., 2022). The broilers GIT hosts a variety of bacterial strains, with *Firmicutes* being the dominant phylum and representing up to 96.8% of all bacteria found in the caecum (Al-Marzooqi, 2024). Findings from 22 independent commercial broiler rearing farms suggest that in extensive farms *Bacteroidetes* predominately were found in microbiota of broilers, while in intensive farms *Firmicutes* dominated (Marcolla et al., 2023). There are many factors that could influence gut microbiota diversity such as gender, genetics, environment, as well as diet (Haag and Siegmund, 2015), and more studies are necessary to identify the functions of XYL and XOS in performance

response, improvement of fermentation metabolites in broilers and modulation of caecal microbiota.

5.2. Objective

The aim of this chapter is to assess the role of using two different sources of XOS, with 2-6 and 2-9 degrees of polymerisation, at two levels, 50 and 500 g/t, on growth performance, AME, AMEn, nutrient availability, ileal and caecal SCFA production and caecal microbiome variables of male broilers fed XYL supplemented maize-based diets.
The following general hypothesis was examined:

1. The use of XOS with 2-9 degrees of polymerisations will have less impact on broilers performance and gut changes compared to birds fed with shorter DP of 2-6.
2. Higher dose of 500 g/T XOS might influence positively digestibility and gut health parameters in comparison to lower dose of 50 g/T.

5.3. Materials and methods

General materials and methods can be found in chapter three.

5.3.1. Animal housing

This study used five hundred and forty day-old male Ross-308 broilers. Broilers were supplied from a nearby hatchery (Cyril Bason Ltd, Craven Arms, UK). Following arrival, the birds were weighed and divided into fifty-four floor pens, each containing ten birds, and handled as described in section 3.1.

5.3.2. Treatments

A maize and soybean-meal basal feed was formulated to meet the required Aviagen Ltd. (Edinburgh, UK) nutritional standards (Table 5.1).

Table 5.1 Ingredient composition of the experimental diets

Ingredient	Starter diet (g/kg)	Finisher diet (g/kg)
Maize	538	602.1
Soybean meal	355.4	283
Wheat Bran	49.8	49.8
Soya oil	21.4	34.2
Salt	3.6	3.6
Limestone	10.8	8.8
Monocal Phos	6.9	4.6
Lysine HCl	1.1	1.3
DL-Methionine	2.6	2.3
Threonine	0.3	0.3
Valine	0.1	0.1
Quantum Blue[1]	0.1	0.1
Vitamin & Mineral premix[2]	5.0	5.0
TiO2 marker	5.0	5.0
TOTAL	1000	1000
Calculated analysis (as-fed basis)		
Crude protein (%)	22.89	19.90
ME (MJ/kg)	12.28	12.91
DM (%)	87.50	87.53
Calcium (%)	0.90	0.76
Phosphorus (%)	0.78	0.69

[1] Quantum Blue 5G, AB Vista, Marlborough, UK; 5,000 FTU/g.

[2] Vitamin mineral premix provided per kg of diet: vitamin A, 10,000 IU; vitamin D3, 2,500 IU; vitamin E, 25 IU; vitamin E, 50 mg; vitamin K_3, 1.5 mg; vitamin B1, 2 mg; vitamin B2, 7.5 mg; vitamin B6, 3.5 mg; vitamin B12, 20 µg; niacin, 35mg; D-pantothenic acid, 12 mg; choline chloride, 460 mg; folic acid, 1.0 mg; biotin, 0.2 mg; iron as iron sulphate, 265 mg; copper as copper sulphate, 48 mg; manganese as manganese oxide, 140 mg; zinc as zinc sulphate, 165 mg; iodate as potassium iodide, 1.2 mg; and selenium as sodium selenite, 0.33 mg.

The treatment diets included 100 g/t of XYL (Econase XT 25P, AB Vista, Marlborough, UK; 16 000 BXU/kg) or a combination of XYL and XOS (AB Vista, Marlborough, UK) with degrees

of polymerisation ranging from 2 to 6 and 2 to 9 and dose of 50 or 500 g/t (Table 5.2). The experimental diets were fed to chickens in two phases: starter (0-21 d) in crumb form and finisher (22-35 d) in pellet form, with a maize-soybean-based meal made by Target Feeds (Whitchurch, Shropshire, UK). Titanium dioxide (5 g/kg) was added as an inert marker in the meal.

Table 5.2 Overview of dietary treatments

Diet	0	1	2	3	4	5
Pytase (FTU/kg)	500	500	500	500	500	500
Xylanase (BXU/kg)	0	16000	16000	16000	16000	16000
XOS DP 2 - 6 g/t	0	0	50	0	500	0
XOS DP 2 - 9 g/t	0	0	0	50	0	500
Number of replicates	9	9	9	9	9	9

DP; degree of polymerisation.

5.3.3. Statistical analysis

Data handling and calculations were performed in Excel 2020 (Microsoft Corporation) and GenStat statistical software (21st edition, Rothamsted, Hertfordshire, UK) was used for statistical analyses. The data was analysed as One-way ANNOVA and comparisons among the studied variables were performed by Duncan's multiple range test. Contrast technique was also used to compare directly control and all XOS supplemented diets; diets based on XOS with different degrees of polymerisation; diet based on XOS with different inclusion level. 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).

5.4. Results

5.4.1. Diet analysis

The diets formulated nutritional characteristics were satisfied (Table 5.1). Table 5.3 shows the enzyme recoveries of phytase and XYL, and the activity of phytase in the diets studied was as expected or higher. The mean result for diets supplemented with XYL was 1578 BXU/kg. The control diet in starter phase indicated unusually higher activity of XYL, with activity of XYL reaching 6110 BXU/kg. Due to higher activity in the basal control, its results were not presented and included in statistical
analysis.

Table 5.3 Analysis of phytase and xylanase activity in the experimental diets

Treatments	Expected		Analysed	
	Phytase, FTU/kg[1]	Xylanase, BXU/kg[2]	Phytase, FTU/kg[1]	Xylanase, BXU/kg[2]
Starter diet				
Control	500	0	844	6110
Control + XYL	500	16000	757	12800
Control + XYL + 2 - 6 DP XOS 50g/t	500	16000	679	15300
Control + XYL + 2 - 9 DP XOS 50g/t	500	16000	1010	17300
Control + XYL + 2 - 6 DP XOS 500g/t	500	16000	561	13700
Control + XYL + 2 - 9 DP XOS 500g/t	500	16000	744	14500
Finisher diet				
Control	500	0	825	<2000
Control + XYL	500	16000	674	12300
Control + XYL + 2 - 6 DP XOS 50g/t	500	16000	772	16400
Control + XYL + 2 - 9 DP XOS 50g/t	500	16000	818	15800
Control + XYL + 2 - 6 DP XOS 500g/t	500	16000	964	15000
Control + XYL + 2 - 9 DP XOS 500g/t	500	16000	819	17600

DP; degree of polymerisation.

[1] The amount of enzyme necessary to release 1 mmol of inorganic P per minute from sodium phytate, at 37C and pH 5.5, is defined as one FTU.

[2] The amount of enzyme that generates 1 nmol reducing sugars from birchwood xylan in one second, at 50°C and pH 5.3, is measured as one BXU.

5.4.2. Growth performance

There were no interactions (P > 0.05) in FI, WG or FCR (Table 5.4). The average overall feed intake was 105.73 (g/b/d), the weight gain was 68.50(g/b/d) and FCR 1.516 (g/b/d). When comparing the XOS supplemented diets to the XYL control, the contrast comparison revealed that there was an increase of WG from 21-35 d. The contrast comparison showed that in the overall period from 0 to 35 d diets that had added XOS compared to xylanase-only diets had improved WG (P = 0.035, 69.16 vs 65.85 g/b/d) and FCR (P = 0.017, 1.527 vs 1.564 g/b/d).

Table 5.4 Effect of dietary treatments on feed intake (FI), weight gain (WG) and mortality corrected feed conversion ratio (FCR) in 21 and 35d old broilers

	DP	Level (g/T)	FI (g/b/d) 0-21 d	FI (g/b/d) 21-35 d	FI (g/b/d) 0-35 d	WG (g/b/d) 0-21 d	WG (g/b/d) 21-35 d	WG (g/b/d) 0-35 d	FCR (g/b/d) 0-21 d	FCR (g/b/d) 21-35 d	FCR (g/b/d) 0-35 d
Diets											
Control	-	-	62.08	162.4	104.31	47.48	97.2	65.85	1.379	1.637	1.564
XOS	2 - 6	50	63.35	168.9	106.95	48.54	105.6	69.96	1.355	1.601	1.523
XOS	2 - 9	50	63.18	161.4	103.94	48.44	99.4	67.28	1.358	1.630	1.542
XOS	2 - 6	500	61.94	168.7	107.84	47.94	105.7	69.66	1.351	1.600	1.526
XOS	2 - 9	500	62.30	167.9	105.61	48.37	106.2	69.73	1.333	1.599	1.516
SEM			0.838	2.98	1.310	0.753	2.89	1.322	0.0148	0.0174	0.0133
P value			0.665	0.225	0.187	0.846	0.095	0.124	0.316	0.359	0.106
Contrasts											
Control vs XOS treatments			0.514	0.200	0.233	0.320	0.035	0.031	0.082	0.145	0.017
DP			0.914	0.174	0.053	0.828	0.330	0.331	0.592	0.415	0.753
Level			0.181	0.298	0.334	0.662	0.239	0.422	0.345	0.367	0.398

[a-c] $P < 0.05$; XOS, xylooligosaccharides; DP; degree of polymerisation, SEM, pooled standard error of means; Contrasts, preplanned contrast tests; Treatments, control diet vs other four diets; DP, 2 - 6 vs 2 - 9 degrees of polymerisation; Level, 50 vs 500 g/t.

The results on metabolisable energy and nutrient retention coefficients are presented in Table 5.5. The Control diet had lower AME (P < 0.001), AMEn (P < 0.001), DMR (P = 0.001) and NR (P = 0.009) compared to XOS supplemented diets. This comparison contrast test confirmed the higher overall values in XOS supplemented diets compared to the control (P < 0.001). No differences were observed for NDF digestibility (P > 0.05).

Table 5.5 The effect of dietary treatments broiler chicken apparent metabolisable energy (AME), nitrogen corrected apparent metabolisable energy (AMEn), dry matter retention, nitrogen retention and neutral detergent fibre (NDF) digestibility at 35d

	DP	Level (g/t)	AME (MJ/kg) 35 d	AMEn (MJ/kg) 35 d	Dry Matter Retention 35 d	Nitrogen Retention 35 d	NDF digestibility 35 d
Diets							
Control	-	-	13.39[b]	12.97[b]	0.768[b]	0.675[b]	0.231
XOS	2-6	50	13.63[a]	13.19[a]	0.782[b]	0.706[a]	0.238
XOS	2-9	50	13.74[a]	13.31[a]	0.791[b]	0.720[a]	0.277
XOS	2-6	500	13.69[a]	13.24[a]	0.789[b]	0.718[a]	0.294
XOS	2-9	500	13.66[a]	13.22[a]	0.786[b]	0.716[a]	0.264
SEM			0.056	0.051	0.0037	0.0093	0.0215
P value			< 0.001	< 0.001	0.001	0.009	0.222
Contrasts							
Control vs XOS treatments			< 0.001	< 0.001	< 0.001	< 0.001	0.131
DP			0.426	0.359	0.509	0.511	0.841
Level			0.814	0.674	0.842	0.690	0.319

[a-c] *P* < 0.05; XOS, xylooligosaccharides; DP; degree of polymerisation, SEM, pooled standard error of means; Contrasts, preplanned contrast tests; Treatments, control diet vs other four diets; DP, 2 - 6 vs 2 - 9 degrees of polymerisation; Level, 50 vs 500 g/t.

5.4.3. SCFA production

The results on SCFA concentration are presented in Table 5.6. The mean acetic acid concentration was 82.8 mmol/kg with no difference (P > 0.05) between experimental diets. However, birds fed 50 g/tonne of XOS had higher (P = 0.042) acetic acid concentration in caeca compared to those fed 500 g/tonne, 88.9 vs 76.6 mmol/kg, respectively. There were differences (P = 0.003) in caecal butyric acid concentration between diets, as the lowest was from birds fed diet higher levels of 500 g/T XOS with 2 - 9 DP and the highest was from diet with lower inclusion rate of 50 g/T and DP 2 - 9. Similar to acetic acid, the contrast comparison showed that birds fed lower levels of XOS had higher (P < 0.001) caecal butyric acids

concentration, 19.6 vs 12.9 mmol/kg, respectively. There was no difference in caecal lactic acid concentration ($P = 0.344$), although lower inclusion levels led to lower ($P = 0.045$) caecal lactic acid concentration in contrast comparison test, 1.8 vs 2.6 mmol/kg, respectively. Feeding different diets did not lead to differences in propionic acid concentration ($P > 0.05$). Valeric acid in caeca was higher ($P = 0.019$) in birds fed lower levels of XOS, 1.5 vs 1.3. The sum of caecal SCFA and VFA was higher ($P = 0.021$) in birds fed 50 g/ton XOS than in 500 g/ton, 120.5 vs 102.5 mmol/kg ($P = 0.021$) and 118.6 vs 99.9 mmol/kg ($P = 0.016$), respectively.

No interactions were observed between the SCFA ($P > 0.05$) broiler chicken ileal content at 35 d (Table 5.7).

Table 5.6 The effect of dietary treatments on broiler chicken caecal content of Short-chain fatty acids (SCFA) at 35d

	DP	Level (g/t)	Acetic acid (mmol/kg)	Propionic acid (mmol/kg)	Butyric acid (mmol/kg)	Valeric acid (mmol/kg)	Lactic acid (mmol/kg)	SCFA (mmol/kg)	VFAs (mmol/kg)
Diets									
Control	-	-	83.0	6.6	17.8[ab]	1.5	2.2	113.1	110.9
XOS	2 - 6	50	82.1	6.4	17.6[ab]	1.4	2.0	111.5	109.5
XOS	2 - 9	50	95.6	6.4	21.7[a]	1.6	1.7	129.4	127.7
XOS	2 - 6	500	78.3	6.3	14.2[bc]	1.4	2.6	105.3	102.8
XOS	2 - 9	500	74.8	7.5	11.7[c]	1.2	2.6	99.6	97.0
SEM			5.88	0.74	1.72	0.10	0.36	7.49	7.43
P value			0.147	0.790	0.003	0.061	0.344	0.083	0.064
Contrasts									
Control vs XOS treatments			0.968	0.975	0.431	0.560	0.974	0.848	0.846
DP			0.402	0.416	0.646	0.996	0.743	0.421	0.408
Level			0.042	0.539	< 0.001	0.019	0.045	0.021	0.016

[a-c] $P < 0.05$; XOS, xylooligosaccharides; DP; degree of polymerisation, SEM, pooled standard error of means; Contrasts, replanned contrast tests; Treatments, control diet vs other four diets; DP, 2 - 6 vs 2 - 9 degrees of polymerisation; Level, 50 vs 500 g/t.

Table 5.7 The effect of dietary treatments on broiler chicken ileal content of Short-chain fatty acids (SCFA) at 35d

	DP	Level (g/t)	Acetic acid (mmol/kg)	Propionic acid (mmol/kg)	Butyric acid (mmol/kg)	Isobutyric acid (mmol/kg)	Lactic acid (mmol/kg)	SCFA (mmol/kg)	VFAs (mmol/kg)
Diets									
Control	-	-	6.35	0.012	0.046	0.008	47.0	57.7	6.41
XOS	2 - 6	50	6.59	0.014	0.031	0.013	20.9	27.8	6.68
XOS	2 - 9	50	6.41	0.047	0.043	0.008	36.8	43.3	6.53
XOS	2 - 6	500	6.04	0.004	0.039	0.006	37.3	43.4	6.11
XOS	2 - 9	500	6.35	0.021	0.046	0.010	56.3	75.7	6.44
SEM			0.665	0.0115	0.0048	0.0038	11.01	12.17	0.677
P value			0.985	0.111	0.203	0.585	0.239	0.088	0.983
Contrasts									
Control vs XOS treatments			1.000	0.484	0.270	0.722	0.463	0.461	0.970
DP			0.921	0.350	0.069	0.874	0.120	0.057	0.897
Level			0.648	0.131	0.268	0.428	0.110	0.056	0.630

XOS, xylooligosaccharides; DP; degree of polymerisation, SEM, pooled standard error of means; Contrasts, replanned contrast tests; Treatments, control diet vs the other four diets; DP, 2 - 6 vs 2 - 9 degrees of polymerisation; Level, 50 vs 500 g/t.

5.4.4. Caecal 16s ribosomal ribonucleic acid gene sequencing

The relative abundance of bacterial species phylogenetically was annotated to operational taxonomic units (OTUs) in 35-day-old broiler caecal samples among the treatment groups (Figure 12). *Firmicutes* accounted for over 75% of the total community overall and were the most prevalent phylum across all treatments. *Bacteroidete*s were second most abundant in phylum, followed by *Proteobacteria* in third.

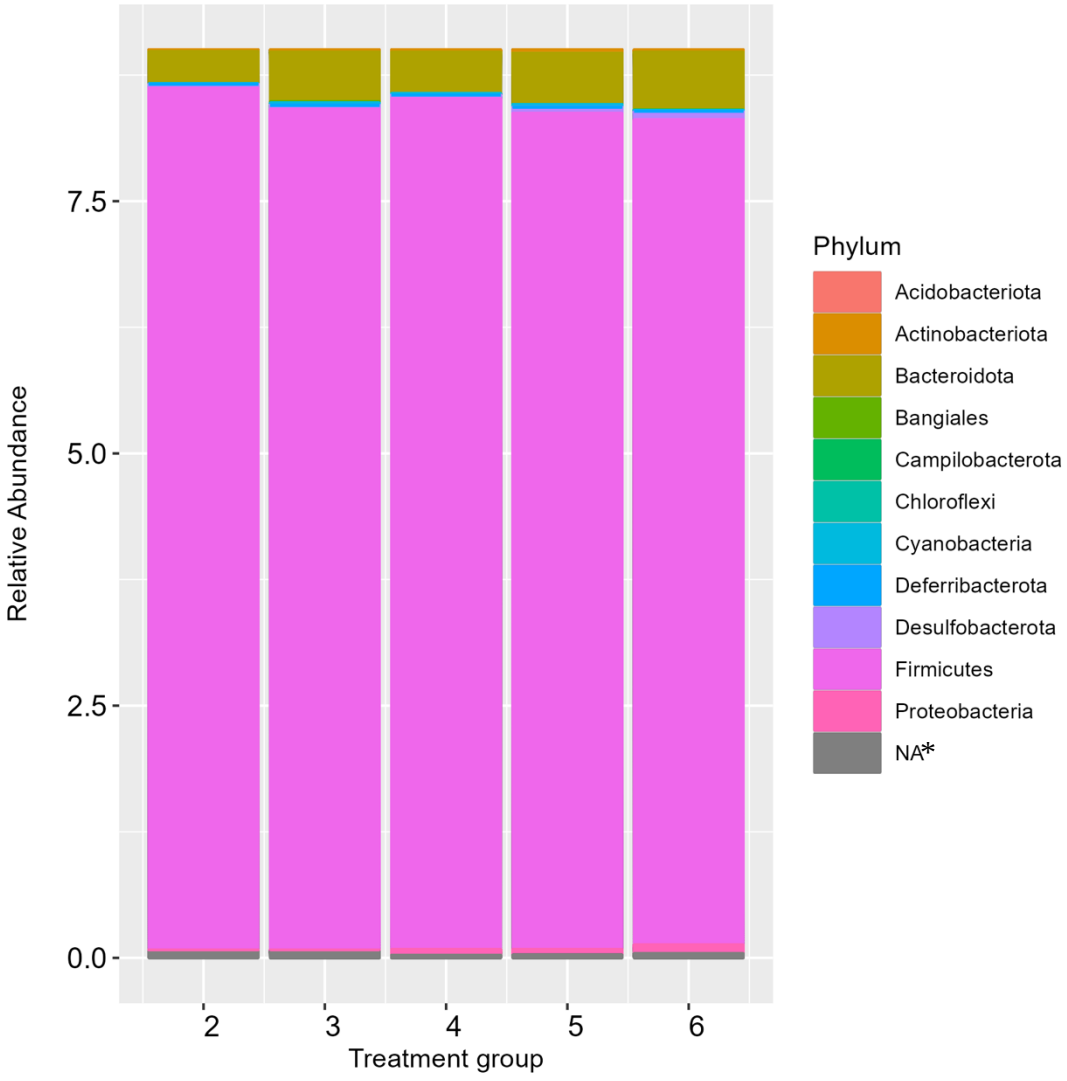

Figure 13: The effect of xylanase (XYL) and xylooligosaccharides (XOS) with different degrees of polymerisation (DP) and inclusion levels on relative abundance of bacterial taxa annotated to OTUs at the phylum level as identified from 35 d old broiler caecal samples among main groups of treatments

(Treatment 2 = XYL; 3 = XYL + 50 g/t XOS 2 - 6 DP; 4 = XYL + 50 g/t XOS 2 - 9 DP, 5 = XYL + 500 g/t XOS 2 - 6 DP, 6 = XYL + 500 g/t XOS 2 - 9 DP). * NA = unclassified at the phylum level.

There was no significant difference (all p>0.10) due to treatment on any alpha diversity measure (Chao1, Shannon, Simpson, Fisher index; Figure 13).

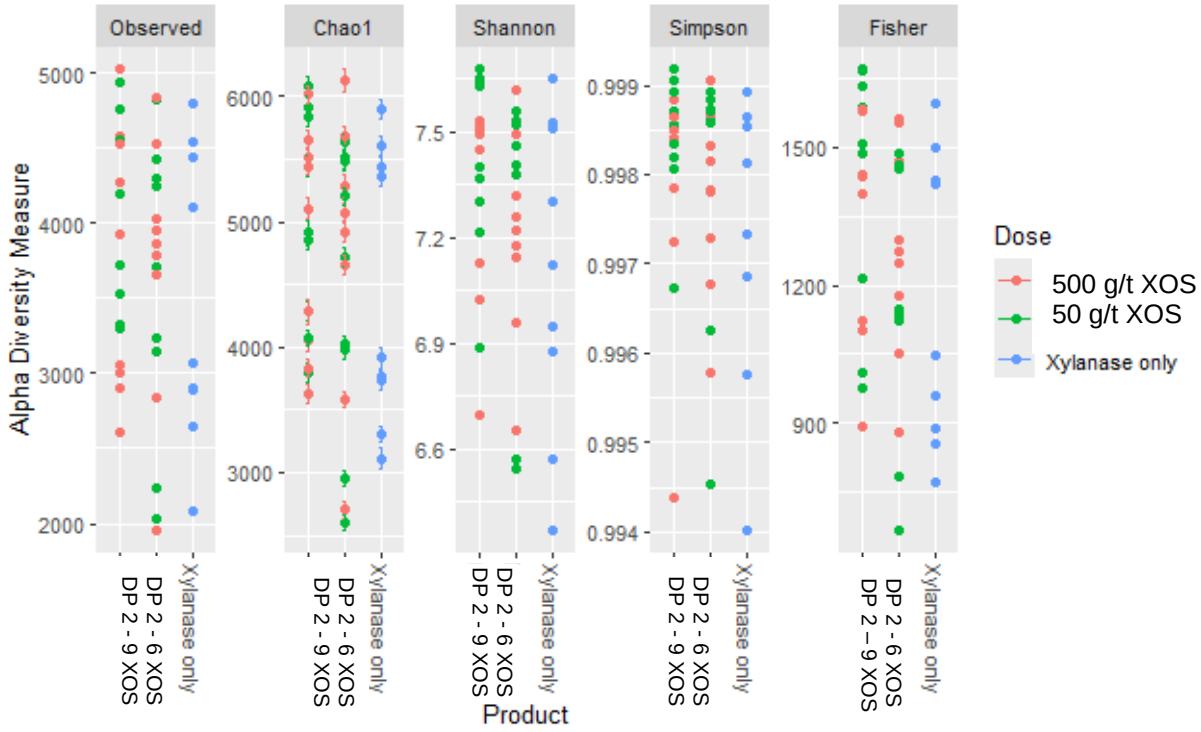

Figure 14: The effect of xylanase (XYL) and xylooligosaccharides (XOS) with different degrees of polymerisation (DP) and inclusion levels on Alpha diversity (Chao1, Shannon, Simpson and Fisher index) as identified from 35 d old broiler caecal samples among main groups of treatments

There was no significant difference (all p>0.05) due to treatment on Bray-Curtis beta diversity measure (Figure 14).

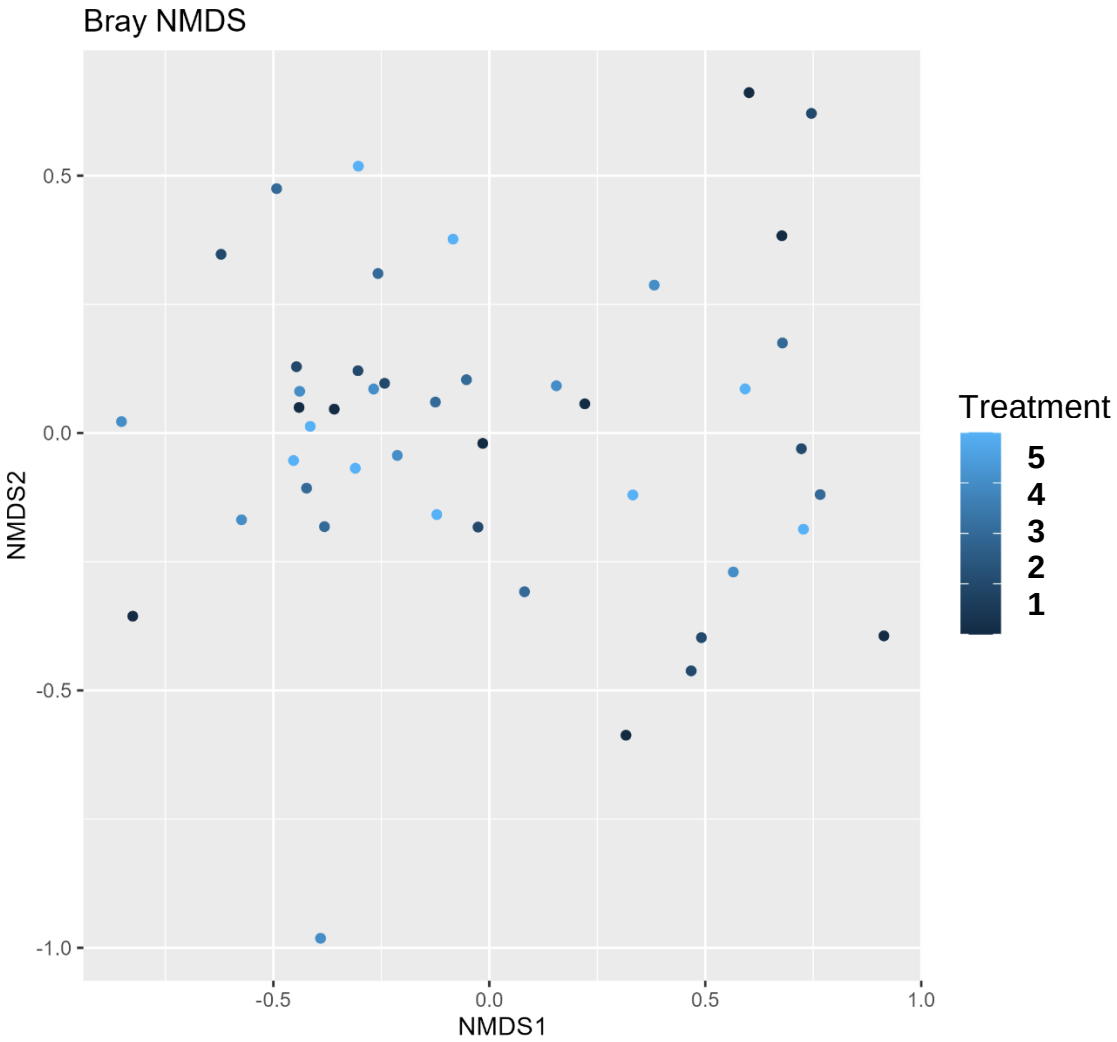

Figure 15: The effect of xylanase (XYL) and xylooligosaccharides (XOS) with different degrees of polymerisation (DP) and inclusion levels on the beta diversity (Bray-Curtis) of broilers caecal samples taken at 35 d.

Treatment 1 = XYL; 2 = XYL + 50 g/t XOS 2 - 6 DP; 3 = XYL + 50 g/t XOS 2 - 9 DP, 4 = XYL + 500 g/t XOS 2 - 6 DP, 5 = XYL + 500 g/t XOS 2 - 9 DP.

5.5. Discussion

5.5.1. Effect on bird growth performance, metabolisable energy and nutrient retention

The recovery of the xylanase enzyme did not meet our initial expectations during our experiment, with higher increased rate of control in the starter diet than expected. Most likely, the contamination happened during the mill's diet mixing procedure. Despite the fact it is far lower than the recommended XYL dose of 16,000 BXU, the results might have been compromised by the 6110 BXU/kg XYL activity. Due to contamination, the first diet that was

designed as control without any additives was not used to compare with the other treatments.

The overall mortality was 3.33% and no differences were observed between the experimental treatments in any of the phases ($P > 0.05$), indicating good health of the birds throughout the trial. The birds performed better than expected when compared to Aviagen's performance objectives (2022), with the average weight of the bird at 35 d 2468 g compared to the estimated 2441 g. Overall, the FCR was increased in the current experiment, averaging 1.516 compared to the targeted 1.390. Due to the improved WG in the current study, birds had higher FI compared to the Aviagen objectives (105.73 vs 91.74 g/b/d). Similarly as in study Pirgozliev et al. (2023) and Šimić et al. (2023) there were no interactions or significant differences or in FI amongst the treatments. However, in contrast comparison of the xylanase-only control, the addition of XOS supplementation improved WG in finisher and overall period. Potential explanation for it would be improved overall FCR in birds fed with XOS addition.

In study done by Afzal et al. (2022) there was no statistical differences between positive control and negative control that had 0.42 MJ and 5% amino acid reduction in diet supplemented XYL and XOS at 100 g/T, in FI and WG finisher phase. In FCR treatment with XOS and XYL showed non-significant differences compared to positive and negative control. Its research suggested that there is potential to reduce energy and amino acid contents of standard recommended diet while adding XYL and XOS without affecting the performance of broilers.

Lin et al. (2023) did not find any differences from 0 to 15 d in performance parameters, or when broilers were challenged with *Eimeria* from d 15 to 21 d maize based diet, however the research studied the effects of XYL and XOS alone. In contrast , research done by De Maesschalck et al. (2015) using 2000 g/t of XOS with 2 - 7 DP improved FCR in wheat-rye based diets. In study by Craig et al. (2020) XOS was supplemented at rate 250 g/t and 1000 g/t, but there was no difference between higher and lower dose in FI, WG and FC, however results indicated XOS or XYL supplemented diets had improved FCR results and decreased FI, while WG remained unchanged, suggesting that using lower dose of XOS could be still efficient enough to improve performance parameters.

The performance results were in line with AME, AMEn, DMR and NR results, consistently showing improvement with XOS supplemented diets compared to the XYL only diets. Previous research with xylanase and XOS has similarly found that supplementation made an improvement on ME and nutrient retention parameters (Dimitrova, 2020). There was no statistical difference in performance between treatments, however, numerically in starter,

finisher and overall period, combination of XOS and XYL, followed by xylanase-only diets, had the improved results in WG and FCR.
In contrast, Craig et al. (2020) and Lin et al. (2022) did not find an effect of improved nutrient digestibility of XOS or XYL supplemented diets. In a previous study, treatments XYL and XOS with DP 2 – 7 did not influence ME, DMR, NR or FR, however it increased NDF digestibility (Šimić et al., 2023). The potential reason for that is that the experiment design had included maize based positive control and negative control, which had 5% of WB included in the diet at the expense of maize, and might have made bigger difference in the broiler digestion by having higher fibre levels substrates.

Limited research has been done so far with different DP XOS, similarly as in Singh et al., (2022) there was no effect on the broilers growth performance parameters, however there was microbial shift in caeca. The dose response was in line with published studies, indicating lowering the dose would not impact positive response provided by additional XOS supplementation (Craig et al., 2020; Ribeiro et al., 2018). Results support the theory that supplementing lower dose of 50 g/T XOS regardless of difference in DP to broilers altered metabolisable energy and nutrient retention, which improved FCR and increased WG in the overall period.

5.5.2. Effect of XOS on SCFA and rRNA microbiome

It is hypothesised that the ability of enzymes to hydrolyse carbohydrates releases more prebiotic oligosaccharides and consequently stimulates the hindgut carbohydrate fermentation (Lin et al., 2023). Oligosaccharides may have an improving gastrointestinal health impact on chickens through increasing the production of SCFA, enhancement of immunity, and increasing the proliferation of beneficial bacteria in the caeca (Morgan, 2023; Singh et al., 2022). Highest levels of butyric acid were found with a diet that had inclusion rate of 50 g/t XOS and DP of 2 - 9 compared to the birds fed diets with higher inclusion rate of 500 g/t and DP of 2 - 9. Higher concentration rates of acetic, propionic, butyric, valeric, SCFA and VFA acid in diets with 50 g/t XOS indicate that a lower dose would be enough to make positive shift in broilers microbiome. Results from trials using XOS with different inclusion rates report inconsistent results of SCFA production. For example, in a study with high and low XOS (250 and 1000 g/t) or XYL (16 000 and 32 000 BXU/kg) supplementation, when control was compared to all other treatment containing additive, irrespective of type or level, control had lower levels of SCFA, butyric and acetic acid at d 28 (Amit K. Singh et al., 2021). In contrast, Lin et al. (2022) with use of 500 g/t and 1000 g/t XOS in maize based diet did not find any difference in SCFA production.

There was increase of lactic acid concentration in higher level of XOS inclusion, although the results did not corelate with the 16s RNA analysis, where there was no increase in lactic acid bacteria. Although it was of statistical significance, it likely was not of biological significance as it was not consistent with any other parameter in performance or nutrient digestibility. The increase of acetate during XOS supplementation rate of 2000 g/t has previously been reported by Pourabedin et al. (2015), as well as relative abundance of *Lactobacillus* genus in caeca, while the overall microbiota alpha and beta diversity remained unmodified. This experiment did not find any differences between the treatment groups in ileal SCFA contents, similarly as in Davies et al. (2024) trial with XOS DP varying from 2 - 3, 2 - 6 to 4 - 6.

One experiment supplementing XOS in 150 g/t, 300 g/t or 450 g/t in maize based diet resulted in higher relative abundance of *Alistipes* ($P < 0.001$), the short-chain fatty acid-producing genera (Rao et al., 2024). The improved caecal microbial diversity was also supported by improvement in growth performance, promoted intestinal health by enhancing intestinal barrier function and positive effects on immunity with the concluded recommended dose being the lowest inclusion rate used, the 150g/t. Dietary supplementation of XOS has been assessed in De Maesschalck et al. (2015) when it was concluded that XOS fermentation increases butyrate production by stimulating butyrate-producing bacteria through cross-feeding interactions.

Animal production and health are strongly impacted by the overall composition of the intestinal flora. While an imbalance in intestinal flora can result in adverse effects like diarrhoea and malabsorption of nutrients, a stable intestinal flora has certain beneficial impacts on the digestion and utilisation of nutrients (Rao et al., 2024). The relative abundance of bacterial species phylogenetically annotated to OTUs in 35-day-old broiler caecal samples outlined *Firmicutes* as the most common phylum in all treatments, accounting for more than 75% of the entire community, followed by *Bacteroidetes. Firmicutes* being the most common phylum is in line with previous research (Al-Marzooqi, 2024; Rao et al., 2024).

There were no differences in the alpha and beta diversity of microbiota between treatments ($P > 0.05$). Similar results were found in Singh et al. (2021) where 2 levels of XYL (8 000 and 16 000 BXU/kg) and 2 levels of XOS (50 g/t and 10 g/t) added to the maize based diet, but the positive dietary effects seen in performance may not always translate in microbiota diversity. Lin et al. (2023) concluded Eimeria spp. challenge significantly ($P < 0.01$) decreases the microbial richness and diversity, however the richness and diversities of the microbial profile were not influenced by the addition of the 500 g/t XOS, nor the XYL alone or combination of XYL and protease. It is possible that the lack of responses in trial was due to the trial lasting only 21d, potentially not allowing broiler microbiome to developed completely.

While in Pourabedin et al. (2015) the total microbiota diversity did not change, it was observed that addition of 2000 g/t of XOS improved the relative abundance of the Lactobacillus genus in the caecum.
In contrast, research by Zhou et al. (2021) demonstrated increase in caecal bacterial richness has been demonstrated by an alpha diversity study in birds fed 200 g/t of XOS. The inconsistency in previously reported research could be explained by small animal numbers used in particular research, big variation of XOS inclusion rate used and or from the various physiochemical characteristics and structures of the XOS used. Additionally, considering 16S rRNA sequencing depends on reference genome availability, it may not be possible to fully annotate all ASVs and it could impact the accuracy of functional predictions (Marcolla et al., 2023). Another potential explanation for the lack of responses in the current trial could be found in methodology used when preparing the samples for 16s RNA microbiome analysis. Due to transporting limitations, after samples were taken, they were immediately stored on dry ice (-78 °C) and remained in long-term storage (-80 °C), but they had to be freeze dried for sample transportation and DNA extraction for high throughput sequencing analysis, which potentially could affect quality (Weißbecker et al., 2017).
Although it was hypothesised that lowering the DP of XOS would be more beneficial for gut microbiome, this experiment did not get confirm that, indicating that the difference between DP 2 - 6 and 2 - 9 may not be large enough to show a response. Comparison of the lower dose of 50 g/t to 500 g/t suggest that lowering dose would be efficient, however, the effects of xylanase and xylo-oligosaccharides affecting the intestinal microbiota require further investigation.

5.6. Conclusion

In conclusion, the addition of XOS to xylanase supplemented diets has shown to be efficient at improving production parameters. In overall period there was increase of WG (P = 0.035, 69.16 vs 65.85 g/b/d) and improvement of FCR (P = 0.017, 1.527 vs 1.564 g/b/d), while the FI did not differ amongst the treatment diets. The performance improvements could partially be attributed to improvements with ME and digestibility results, with XOS supplemented diets increasing AME, AMEn, DMR and NR at 35 d. In this study lower inclusion levels (50 g/t) compared to higher inclusion rate (500 g/t) had shown to result in caecal higher levels of acetic (P = 0.033, 19.6 vs 12.9 mmol/kg), butyric (P = 0.003, 21.7 vs 11.7 mmol/kg), valeric acid (P = 0.019, 1.5 vs 1.3. mmol/kg), as well as SCFA (P = 0.021, 120.5 vs 102.5 mmol/kg) and VFA (P = 0.016, 118.6 vs 99.9 mmol/kg). The response of dietary treatments was not identified in ileal SCFA content, nor caecal difference in relative abundance of bacterial

species, alpha or beta diversity. Overall, in addition to XYL supplementation, the level of 50 g/t XOS may be incorporated for optimal growth, digestibility and SCFA production response in maize based diets.

6. Chapter: General discussion and conclusions on the strategies of using xylanase and xylooligosaccharides in broiler chicken diets

6.1. Introduction

As the global demand for poultry meat continues to rise, strategies to enhance fibre utilisation of poor-quality feed materials in poultry nutrition are becoming more important. The increase in the amount of fibre included in poultry diets may be driven by the EU antibiotic growth promoter ban in 2006 and possible changes in the availability of cereals on the market (Dey et al., 2021). Even though high-fibre cereals can be lower in cost, adding them in significant quantities increases the NSP content of the diet. To hydrolyse NSP, degrade the arabinoxylans, and make available the associated energy, supplemental XYL has been utilised in chicken diets on a regular basis (Bedford, 2018). A new strategy for using XOS along with XYL in diets containing additional DF has emerged as a possible solution for efficient improvements in performance and gut health. However, further direct evidence of the benefits of this strategy is required. The objective of the thesis was therefore to explore the relationship between broiler performance, nutrient digestibility, metabolisable energy, GIT development and microbiome shifts in broilers fed diets containing XYL, XOS, different levels of DF and the interaction of these factors.

6.2. Effect of XYL and/or XOS on broiler production performance, nutrient and metabolisable energy

In all three studies, overall mortality of the birds was under 5% and no differences or corelations were observed between the experimental treatments ($P > 0.05$). While in the first experiment the BW of the birds was 8 to 9% lower than the Ross 308 broiler target weight, in the second and third studies birds outperformed the Aviagen objectives (Aviagen, 2022). In the second experiment, WG was higher by 4.5% than the Ross 308 target, with an average improvement of FCR of 0.05 points. Similarly, in the third study the average weight of the bird at 35 d was increased by 1.1% above target. Across the three studies, the majority of the performance differences were identified in WG and FCR parameters, however, across most feeding phases the FI differences were not significant. In the first experiment (Chapter 3) where XYL, XOS and the combination of XYL and XOS were assessed, it was concluded that the biggest improvements of WG and FCR was in diet containing both XYL and XOS, while the additional DF from added WB negatively influenced WG and FCR. Following confirmation that XYL and the combination of XYL and XOS improved performance compared to XOS supplementation alone in fibre enriched diets, the same combinations

were then tested to see how they would interact with changing dietary viscosity in the second experiment (Chapter 4). The gastrointestinal transit time and nutrient absorption rates of broilers are significantly influenced by the viscosity of their feed, as increased viscosity can slow down the transit time of digesta, leading to reduced nutrient absorption and utilisation (Józefiak et al., 2007; Lázaro et al., 2003). This was confirmed in the second experiment, where increased digesta viscosity impaired nutrient utilisation and growth performance in birds, with higher viscosity diets resulting in a higher feed conversion ratio (FCR), indicating decreased feed efficiency. Enzyme supplementation has been previously shown to accelerate digestive transit and reduce intestinal viscosity, however, the enzyme supplementation had only limited effect on the performance parameters with XYL increasing WG in non-viscous diet. In the third experiment (Chapter 5) the research focused on the level and DP of XOS. When compared to the XYL only control, XOS overall improved WG and FCR. One treatment had to be disregarded in this study due to contamination of XYL detected in the starter phase control diet during enzyme recovery analysis. This limitation prevented a comparison between XYL negative and XYL containing diets.
As expected, nutrient retention and ME were negatively impacted by the inclusion of additional fibre in the first experiment, whilst in the second experiment a positive impact on ME was noted in the starter phase high-viscos diet containing XYL and XOS only. The NDF was increased by the addition of WB in the first trial at the expense of maize content in the diet. In the second (Chapter 4) and third (Chapter 5) studies, birds outperformed the Ross 308 objectives for WG. In studies where birds are performing at their genetic potential there is less scope for production performance improvements resulting from dietary enzyme additive supplementation. Among the key components of improving broiler growth performance is developing balanced and nutritionally adequate diets. Increased levels of DF are normally associated with a reduction in feed efficiency, i.e. increased FCR and a reduced WG, likely due to higher levels of NSP which is also linked to lower digestibility of nutrients, AME and AMEn (Jha and Mishra, 2021, Johnston et al 2003). Even though there were variabilities in the outcomes, according to the findings of the studies done for this thesis, broiler performance can benefit from the use of XYL and XOS.

6.3. Effect of XYL and/or XOS on histomorphometry and GIT development

Jejunum histomorphology parameters did not show any differences amongst the treatments and very little impact on GIT development was observed in the first study (Chapter 3). The addition of WB increased the percentage of relative duodenum weight of 21 d old birds from 1.04% to 1.14%. Feeding broilers with a higher fibre content diet and XYL resulted in higher

relative weight of proventriculus and gizzard, at 35 d age. However, no further effects on the GIT were observed. Similarly, in the second experiment (Chapter 4) birds were affected by NSP levels, with the highest NSP levels in the high viscous diet influencing the weight of the small intestine at d 21 and caeca at d 35. An increase of small intestine, duodenum, proventriculus and gizzard weights suggests enhanced activity of digestive enzymes due to the higher presence of fermentable fibres. This could have also stimulated microbial activity in the caeca thereby resulting in heavier relative weight. Moreover, this could imply that birds might be able to handle higher dietary NSP contents. Over the studies done with XYL and XOS it may be concluded that supplementation with these improved production performance but may not always translate to changes in GIT development and histomorphometry.

6.4. Effect of XYL and/or XOS on the SCFA content and 16s RNA analysis

The development of the small intestinal villus, postponed intestinal emptying, and enhanced gut health have all been associated with the caeca fermentation of DF and the production of SCFA, mainly butyrate in the caeca (Jha et al., 2019; O'Neill et al., 2012). The SCFA have a major impact on poultry metabolic systems and intestine health maintenance. Butyrate is particularly significant SCFA, as it is the enterocytes preferred energy source and is known to control intestinal mucosal proliferation and cellular differentiation, which increases the weight of intestinal tissue (Fukunaga et al., 2003). In addition to improving epithelial cell function and lowering inflammation, SCFA have been reported to modify lipid metabolism in the liver through complex hormonal and signalling pathways, consequently enhancing gut health and integrity. Due to their dual function, SCFAs are important for preserving both gut health and systemic metabolic efficiency, which benefits the general physiological health and growth performance of broiler chickens. In chapter 3 it was shown that the addition of DF from WB increased the production of acetic acid, valeric acid, propionic acid, SCFA and VFA. The highest concentration of lactic acid was noted in birds fed XYL. It is likely that the addition of WB provided a substrate for microbes producing SCFA, which resulted in their increased concentrations. In chapter 5, results indicated that higher concentrations of the SCFA, VFA, butyric, valeric, propionic, and acetic acids were found in diets containing 50 g/t of XOS. Findings indicated that a smaller dosage of 50 g/t compared to 500 g/t of XOS would be sufficient to beneficially impact the microbiome of broilers. At 35 days, there were no interactions in the SCFA ileal content, however, it is well established that the majority of fibre fermentation is in the caeca, which could explain the lack of interaction.
No interactions were observed between the SCFA of ileal content at 35d. Changes in the caeca microflora composition due to dietary differences in the quantity of fibre can be an indication

that DF components have reached the caeca (Svihus et al., 2013). In chapter 5 it was found that *Firmicutes* were the most common phylum in all treatments, making up more than 75% of the overall community. There was no significant difference due to treatment on any alpha diversity or beta diversity measures. A potential explanation for the lack of responses in the third study (Chapter 5) could be due to the methodology used when preparing the samples for 16s RNA microbiome analysis. Due to transporting limitations, after samples were taken, they were immediately stored on dry ice (-78 °C) and were freeze dried after long-term storage at -80 °C, which could have potentially affected sample quality. Overall, the results suggest that adding WB as a substrate for increased microbial fermentation and using lower dose of XOS could increase the levels of caecal SCFA.

6.5. General conclusions and practical recommendations

This thesis evaluated the strategies for using XYL enzyme and XOS prebiotic to enhance the value of broiler diets that have additional fibre levels. The findings suggest that adding XOS to a XYL supplemented diet can enhance the growth performance in broilers by modulating nutrient digestibility and ME availability, which could potentially be attributed to the increase of SCFA and alteration of microbial composition. It was confirmed that including WB at 5% in a maize based diet could negatively affect production performance, energy and nutrient availability similarly to high-viscous diets based on wheat, barley, oats, and rye. The responses observed in studies may be limited by birds meeting their genetic potential for production performance, thereby resulting in inconsistencies in response to XYL and XOS. It was observed that improvement in production performance did not consistently translate into measurable changes in bird physiology and microbiome diversity. Recommendations for XYL and XOS supplementation based on these results would be feeding 16 000 BXU XYL and 50 g/t XOS in broiler diets.

6.6. Areas for further research

This thesis has identified several areas for further research:

1. Would a lower dose of XOS be more suitable for improving gut health and performance in broiler diets? Parameters for gut health to could be tested, such as intestinal morphology parameters, microbial diversity, gut permeability and microbial diversity to assess the minimum XOS level. What is the lowest inclusion rate that would still provide efficiency and beneficial effects that are associated with XOS

supplementation? What are the related advantages for immune system regulation, digestion, and general health, and how do varying doses impact the composition and activity of the gut microbiota? What effects does a lower dose of XOS have on the broiler's resistance to immunological challenges and gut-related diseases like Salmonella or coccidiosis? Research might evaluate the financial impact of utilising lower XOS dosages at various production scales, taking into account both direct expenses (such cost of XOS) and indirect expenses (increased feed efficiency, mortality, and a decrease in illness incidence).

2. What would be the optimum level of DF added to broiler diet for them to still grow to their genetic potential? This would involve taking into account the effects of various fibre sources as well as their fermentability and solubility. Could we further increase the level of DF and what would be its performance efficacy? Would the diet supplementation of XYL and XOS improve negative aspects associated with feeding broilers high fibre diets?
   How would diets that are high in DF affect the sensory qualities and nutritional profile of broiler meat? What effects does increased DF have on the flavour, texture, fat content, and other nutritional qualities of meat? Would raising the amount of fibre in chicken meat change consumers preferences?

3. As the importance of sustainability in animal agriculture grows, it's critical to consider how feeding practices, such as the use of high-fibre diets or alternative prebiotic supplements, affect the environmental impact of raising chickens. Would use of different dietary components in broiler diets affect environmental factors, sustainability of sourcing different types of supplements and cereals that are higher in DF? What would be the variance in carbon footprint of those diets when compared to conventional poultry diets? What wider effects implementing high fibre diets and using prebiotic supplements might have on food security and sustainable farming methods? Additionally, studies might look into whether implementing high fibre diets requires changing farm management techniques, which could either reduce or increase their environmental impact.

LIST OF REFERECES

Abdollahi, M.R., Ravindran, V., Svihus, B., 2014. Influence of feed form on growth performance, ileal nutrient digestibility, and energy utilisation in broiler starters fed a sorghum-based diet. Livestock Science 165, 80–86. https://doi.org/10.1016/j.livsci.2014.04.002

Adebiyi, O.A., Ologhobo, A.D., Adu, O.A., Olasehinde, T.O., 2010. Evaluation of the nutritional potentials of physicaly treated cowpea seed hulls in poultry feed. Emirates Journal of Food and Agriculture 232–239. https://doi.org/10.9755/ejfa.v22i3.4893

Aftab, U., Bedford, M.R., 2018. The use of NSP enzymes in poultry nutrition: myths and realities. World's Poultry Science Journal 74, 277–286. https://doi.org/10.1017/S0043933918000272

Aftab, U., Bedford, M.R., Creswell, D., 2018. Prospects of improving efficiency of feed utilisation in broiler. World's Poultry Science Journal 74, 427–442. https://doi.org/10.1017/S0043933918000466

Afzal, S.A., U.Farooq, M.F.Khalid, S.L.Butt, G.Abbas, 2022. Effect of xylo-oligosaccharide and xylanase supplementation on growth performance, carcass characteristics, nutrient digestibility, and intestinal histology of broilers. Pakistan Journal of Science 74. https://doi.org/10.57041/pjs.v74i4.800

AHDB (not dated). GB animal feed production. URL https://ahdb.org.uk/cereals-oilseeds/cereal-use-in-gb-animal-feed-production (accessed 5.16.23).

Al-Marzooqi, W., 2024. Profiling of bacterial community's dynamics inhibiting the intestinal tract of broiler chickens growing in naturally ventilated house. Ger. J. Vet. Res. 4, 139–150. https://doi.org/10.51585/gjvr.2024.2.0091

Alyassin, M., Campbell, G. m., 2019. Chapter 15 Challenges and constraints in analysis of oligosaccharides and other fibre components, in: The Value of Fibre. Wageningen Academic Publishers, pp. 257–277. https://doi.org/10.3920/978-90-8686-893-3_15

Amerah, A.M., Ravindran, V., Lentle, R.G., Thomas, D.G., 2008. Influence of particle size and xylanase supplementation on the performance, energy utilisation, digestive tract parameters and digesta viscosity of broiler starters. British Poultry Science 49, 455–462. https://doi.org/10.1080/00071660802251749

Amerah, A.M., Ravindran, V., Lentle, R.G., Thomas, D.G., 2007. Influence of Feed Particle Size and Feed Form on the Performance, Energy Utilization, Digestive Tract Development, and Digesta Parameters of Broiler Starters. Poultry Science 86, 2615–2623. https://doi.org/10.3382/ps.2007-00212

Apajalahti, J., Rinttilä, T., 2019. Assessing the complex ecology of intestinal microbiome, in: The Value of Fibre. Brill. https://doi.org/10.3920/978-90-8686-893-3_16

Asp, N.G., 2000. Development of Dietary Fibre Methodology, in: Advanced Dietary Fibre Technology. pp. 77–88.

Aviagen. 2022. Ross 308 Broiler Performance Objectives

Azhar, M.R., Rose, S.P., Mackenzie, A.M., Mansbridge, S.C., Bedford, M.R., Lovegrove, A., Pirgozliev, V.R., 2019. Wheat sample affects growth performance and the apparent metabolisable energy value for broiler chickens. British Poultry Science 60, 457–466. https://doi.org/10.1080/00071668.2019.1605152

Bacic, A., Harris, P.J., Stone, B.A., 1988. The Biochemistry of Plants: Carbohydrates.

Banfield, M.J., Kwakkel, R.P., Forbes, J.M., 2002. Effects of wheat structure and viscosity on coccidiosis in broiler chickens. Animal Feed Science and Technology 98, 37–48. https://doi.org/10.1016/S0377-8401(02)00032-9

Barletta, A., 2010. Introduction: current market and expected developments. | Enzymes in Farm Animal Nutrition 1–12.

Bautil, A., Courtin, C. m., 2019. Chapter 1 Fibres making up wheat cell walls in the context of broiler diets, in: The Value of Fibre. Wageningen Academic Publishers, pp. 17–46. https://doi.org/10.3920/978-90-8686-893-3_1

Bautil, A., Verspreet, J., Buyse, J., Goos, P., Bedford, M.R., Courtin, C.M., 2019. Adaptation of the microbiome towards fibre digestion: effects of age and dietary ingredients, in: González-Ortiz, G., Bedford, M.R., Bach Knudsen, K.E., Courtin, C.M., Classen, H.L. (Eds.), The value of fibre. Wageningen Academic Publishers, The Netherlands, pp. 199–216. https://doi.org/10.3920/978-90-8686-893-3_12

Bedford, M., Morgan, A., 1996. The use of enzymes in poultry diets. Worlds Poultry Science Journal 52, 61–68. https://doi.org/10.1079/WPS19960007

Bedford, M.R., 2018. The evolution and application of enzymes in the animal feed industry: the role of data interpretation. British Poultry Science 59, 486–493. https://doi.org/10.1080/00071668.2018.1484074

Bedford, M.R., 2002. The Foundation of Conducting Feed Enzyme Research and the Challenge of Explaining the Results1. Journal of Applied Poultry Research 11, 464–470. https://doi.org/10.1093/japr/11.4.464

Bedford, M.R., Classen, H.L., 1993. An In Vitro Assay for Prediction of Broiler Intestinal Viscosity and Growth When Fed Rye-Based Diets in the Presence of Exogenous Enzymes. Poultry Science 72, 137–143. https://doi.org/10.3382/ps.0720137

Bedford, M.R., Cowieson, A.J., 2012. Exogenous enzymes and their effects on intestinal microbiology - ScienceDirect. Animal Feed Science and Technology 76–85.

Bedford, M.R., Schulze, H., 1998. Exogenous enzymes for pigs and poultry. Nutrition Research Reviews 11, 91–114. https://doi.org/10.1079/NRR19980007

Bedford, M.R., Svihus, B., Cowieson, A.J., 2024. Dietary fibre effects and the interplay with exogenous carbohydrases in poultry nutrition. Animal Nutrition 16, 231–240. https://doi.org/10.1016/j.aninu.2023.09.007

Bednar, G.E., Patil, A.R., Murray, S.M., Grieshop, C.M., Merchen, N.R., Fahey, G.C., 2001. Starch and Fiber Fractions in Selected Food and Feed Ingredients Affect Their Small Intestinal Digestibility and Fermentability and Their Large Bowel Fermentability In Vitro in a Canine Mode. The Journal of Nutrition 131, 276–286. https://doi.org/10.1093/jn/131.2.276

Bergman, E.N., 1990. Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. Physiological Reviews 70, 567–590. https://doi.org/10.1152/physrev.1990.70.2.567

Bjerrum, L., Engberg, R.M., Leser, T.D., Jensen, B.B., Finster, K., Pedersen, K., 2006. Microbial community composition of the ileum and cecum of broiler chickens as revealed by molecular and culture-based techniques. Poultry Science 85, 1151–1164. https://doi.org/10.1093/ps/85.7.1151

Black, J., Hughes, R., Nielsen, S., Tredrea, A., Macalpine, R., van Barneveld, R., 2005. The energy value of cereal grains, particularly wheat and sorghum, for poultry.

Boets, E., Deroover, L., Houben, E., Vermeulen, K., Gomand, S.V., Delcour, J.A., Verbeke, K., 2015. Quantification of in Vivo Colonic Short Chain Fatty Acid Production from Inulin. Nutrients 7, 8916–8929. https://doi.org/10.3390/nu7115440

Bonnin, E., Le Goff, A., Saulnier, L., Chaurand, M., Thibault, J.-F., 1998. Preliminary Characterisation of Endogenous Wheat Arabinoxylan-degrading Enzymic Extracts. Journal of Cereal Science 28, 53–62. https://doi.org/10.1006/jcrs.1997.0174

Broekaert, W.F., Courtin, C.M., Verbeke, K., Van de Wiele, T., Verstraete, W., Delcour, J.A., 2011. Prebiotic and Other Health-Related Effects of Cereal-Derived Arabinoxylans, Arabinoxylan-Oligosaccharides, and Xylooligosaccharides. Critical Reviews in Food Science and Nutrition 51, 178–194. https://doi.org/10.1080/10408390903044768

Brouns, F., Hemery, Y., Price, R., Anson, N.M., 2012. Wheat Aleurone: Separation, Composition, Health Aspects, and Potential Food Use. Critical Reviews in Food Science and Nutrition 52, 553–568. https://doi.org/10.1080/10408398.2011.589540

Callahan, Benjamin J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods 13, 581–583. https://doi.org/10.1038/nmeth.3869

Callahan, Ben J., Sankaran, K., Fukuyama, J.A., McMurdie, P.J., Holmes, S.P., 2016. Bioconductor Workflow for Microbiome Data Analysis: from raw reads to community analyses. F1000Res 5, 1492. https://doi.org/10.12688/f1000research.8986.2

Chesson, A., 2001. Non-starch polysaccharide degrading enzymes in poultry diets: influence of ingredients on the selection of activities. World's Poultry Science Journal 57, 251–263. https://doi.org/10.1079/WPS20010018

Choct, M., 2015. Fibre-Chemistry and functions in poultry nutrition, in: LII Simposio Científico de Avicultura, Málaga. pp. 113–119.

Choct, M., 1997. Feed Non-Starch Polysaccharides: Chemical Structures and Nutritional Significance. Feed Milling Int. 191, 13–26.

Choct, M., Hughes, R.J., Bedford, M.R., 1999. Effects of a xylanase on individual bird variation, starch digestion throughout the intestine, and ileal and caecal volatile fatty acid production in chickens fed wheat. British Poultry Science 40, 419–422. https://doi.org/10.1080/00071669987548

Choct, M., Hughes, R.J., Wang, J., Bedford, M.R., Morgan, A.J., Annison, G., 1996. Increased small intestinal fermentation is partly responsible for the anti-nutritive activity of non-starch polysaccharides in chickens. British Poultry Science 37, 609–621. https://doi.org/10.1080/00071669608417891

Clavijo, V., Flórez, M.J.V., 2018. The gastrointestinal microbiome and its association with the control of pathogens in broiler chicken production: A review. Poultry Science 97, 1006–1021. https://doi.org/10.3382/ps/pex359

Clickner, F.H., Follwell, E.H., 1926. Application of "Protozyme" (Aspergillus orizae) to Poultry Feeding.*. Poultry Science 5, 241–247. https://doi.org/10.3382/ps.0050241

Courtin, C.M., Broekaert, W.F., Swennen, K., Lescroart, O., Onagbesan, O., Buyse, J., Decuypere, E., Van de Wiele, T., Marzorati, M., Verstraete, W., Huyghebaert, G., Delcour, J.A., 2008. Dietary Inclusion of Wheat Bran Arabinoxylooligosaccharides Induces Beneficial Nutritional Effects in Chickens. Cereal Chemistry 85, 607–613. https://doi.org/10.1094/CCHEM-85-5-0607

Cowieson, A.J., 2005. Factors that affect the nutritional value of maize for broilers. Animal Feed Science and Technology 119, 293–305. https://doi.org/10.1016/j.anifeedsci.2004.12.017

Craig, A.D., Khattak, F., Hastie, P., Bedford, M.R., Olukosi, O.A., 2020. Xylanase and xylo-oligosaccharide prebiotic improve the growth performance and concentration of potentially prebiotic oligosaccharides in the ileum of broiler chickens. British Poultry Science 61, 70–78. https://doi.org/10.1080/00071668.2019.1673318

Crnčević, N., Hukić, M., Deumić, S., Selimagić, A., Dozić, A., Gavrankapetanović, I., Klepo, D., Avdić, M., 2022. Gastrointestinal Tract Microbiome Effect and Role in Disease Development. Diseases 10, 45. https://doi.org/10.3390/diseases10030045

Cruz-Requena, M., Escobedo-García, S., Salas-Tovar, J.A., Mora-Cura, Y., Chávez-González, M.L., Castillo-Reyes, F., Flores-Gallegos, A.C., Rodríguez-Herrera, R.,

2019. Chapter 1 - Definitions and Regulatory Perspectives of Dietary Fibers, in: Galanakis, C.M. (Ed.), Dietary Fiber: Properties, Recovery, and Applications. Academic Press, pp. 1–25. https://doi.org/10.1016/B978-0-12-816495-2.00001-0

Dale, T., Hannay, I., Bedford, M.R., Tucker, G.A., Brameld, J.M., Parr, T., 2020. The effects of exogenous xylanase supplementation on the in vivo generation of xylooligosaccharides and monosaccharides in broilers fed a wheat-based diet. British Poultry Science 61, 471–481. https://doi.org/10.1080/00071668.2020.1751805

Davies, C., González-Ortiz, G., Rinttilä, T., Apajalahti, J., Alyassin, M., Bedford, M.R., 2024. Stimbiotic supplementation and xylose-rich carbohydrates modulate broiler's capacity to ferment fibre. Front. Microbiol. 14. https://doi.org/10.3389/fmicb.2023.1301727

de Freitas, C., Carmona, E., Brienzo, M., 2019. Xylooligosaccharides production process from lignocellulosic biomass and bioactive effects. Bioactive Carbohydrates and Dietary Fibre 18, 100184. https://doi.org/10.1016/j.bcdf.2019.100184

De Maesschalck, C., Eeckhaut, V., Maertens, L., De Lange, L., Marchal, L., Nezer, C., De Baere, S., Croubels, S., Daube, G., Dewulf, J., Haesebrouck, F., Ducatelle, R., Taminau, B., Van Immerseel, F., 2015. Effects of Xylo-Oligosaccharides on Broiler Chicken Performance and Microbiota. Applied and Environmental Microbiology 81, 5880–5888. https://doi.org/10.1128/AEM.01616-15

De Vries, S., 2015. Fiber in poultry nutrition: bonus or burden? Presented at the In: 20th European Symposium on Poultry Nutrition 24-27 August 2015, Prague, Czech Republic.

Dei, H., 2017. Assessment of Maize (Zea mays) as Feed Resource for Poultry. https://doi.org/10.5772/65363

Dey, D., Richter, J.K., Ek, P., Gu, B.-J., Ganjyal, G.M., 2021. Utilization of Food Processing By-products in Extrusion Processing: A Review. Frontiers in Sustainable Food Systems 4, 304. https://doi.org/10.3389/fsufs.2020.603751

Dimitrova, K.P., 2020. The use of exogenous xylanase and dietary fibre for modern broiler chicken production. Harper Adams University.

Ding, X.M., Li, D.D., Bai, S.P., Wang, J.P., Zeng, Q.F., Su, Z.W., Xuan, Y., Zhang, K.Y., 2018. Effect of dietary xylooligosaccharides on intestinal characteristics, gut microbiota, cecal short-chain fatty acids, and plasma immune parameters of laying hens. Poultry Science 97, 874–881. https://doi.org/10.3382/ps/pex372

Dittoe, D., Ricke, S.C., Kiess, A., 2019. Commercial poultry production and gut function: a historical perspective, in: Improving Gut Health in Poultry. Burleigh Dodds Science Publishing.

Dorado-Montenegro, S., Habibi, M.F., Gerrits, W.J.J., de Vries, S., 2024. Effect of adding soluble viscous fibers to diets containing coarse and finely ground insoluble fibers on digesta transit behavior and nutrient digestibility in broiler chickens. Poultry Science 103, 103487. https://doi.org/10.1016/j.psj.2024.103487

Ducatelle, R., Eeckhaut, V., Goossens, E., Haesebrouck, F., Van Immerseel, F., 2019. Chapter 11 Steering broiler intestinal microbiota through nutrition for improved health, in: The Value of Fibre. Wageningen Academic Publishers, pp. 193–198. https://doi.org/10.3920/978-90-8686-893-3_11

Duncan, S.H., Louis, P., Flint, H.J., 2004. Lactate-Utilizing Bacteria, Isolated from Human Feces, That Produce Butyrate as a Major Fermentation Product. Applied and Environmental Microbiology 70, 5810–5817. https://doi.org/10.1128/AEM.70.10.5810-5817.2004

DSM. Not dated. *DSM in Animal Nutrition & Health.* [Online]. DSM Nutritional Products Ltd. Available from: DSM https://www.dsm.com/markets/anh/en_US/products/products-feedenzymes/products-feed-proteaseronozymeproact.html [Accessed 2 August 2020].

Elleuch, M., Bedigian, D., Roiseux, O., Besbes, S., Blecker, C., Attia, H., 2011. Dietary fibre and fibre-rich by-products of food processing: Characterisation, technological functionality and commercial applications: A review. Food Chemistry 124, 411–421. https://doi.org/10.1016/j.foodchem.2010.06.077

Ellis, P.R., Rayment, P., Wang, Q.I., 1996. A physico-chemical perspective of plant polysaccharides in relation to glucose absorption, insulin secretion and the entero-insular axis. Proceedings of the Nutrition Society 55, 881–898. https://doi.org/10.1079/PNS19960086

Elwinger, K., Fisher, C., Jeroch, H., Sauveur, B., Tiller, H., Whitehead, C.C., 2016. A brief history of poultry nutrition over the last hundred years. World's Poultry Science Journal 72, 701–720. https://doi.org/10.1017/S004393391600074X

Engberg, R.M., Hedemann, M.S., Steenfeldt, S., Jensen, B.B., 2004. Influence of Whole Wheat and Xylanase on Broiler Performance and Microbial Composition and Activity in the Digestive Tract. Poultry Science 83, 925–938.

Englyst, H., 1989. Classification and measurement of plant polysaccharides. Animal Feed Science and Technology, Dietary Fibre in Monogastric Nutrition 23, 27–42.

Esmaeilipour, O., Shivazad, M., Moravej, H., Aminzadeh, S., Rezaian, M., van Krimpen, M.M., 2011. Effects of xylanase and citric acid on the performance, nutrient retention,

and characteristics of gastrointestinal tract of broilers fed low-phosphorus wheat-based diets. Poultry Science 90, 1975–1982. https://doi.org/10.3382/ps.2010-01264

European Commission, 2005. null [WWW Document]. Ban on antibiotics as growth promoters in animal feed enters into effect. URL https://ec.europa.eu/commission/presscorner/detail/en/IP_05_1687 (accessed 9.22.24).

FAO, 2013. Poultry development review.

FAO, 1998. Carbohydrates in human nutrition

Food and Agriculture Organization of the United Nations. 2007. *FAO Technical Meeting on Prebiotics, September 15–16*. [Online]. Available from: https://edisciplinas.usp.br/pluginfile.php/1800450/mod_resource/content/1/FAO%20-%20Prebiotics_Tech_Meeting_Report.pdf (accessed 1.7.2020).

Food and Agriculture Organization. Not dated. *Poultry species* [Online]. Available from: http://www.fao.org/poultry-production-products/production/poultry-species/en/ (accessed 17.2.2020).

FAOSTAT, 2024. URL https://www.fao.org/faostat/en/#data/QCL/visualize (accessed 9.6.24).

Fukunaga, T., Sasaki, M., Araki, Y., Okamoto, T., Yasuoka, T., Tsujikawa, T., Fujiyama, Y., Bamba, T., 2003. Effects of the Soluble Fibre Pectin on Intestinal Cell Proliferation, Fecal Short Chain Fatty Acid Production and Microbial Population. Digestion 67, 42–49. https://doi.org/10.1159/000069705

Fuso, A., Rosso, F., Rosso, G., Risso, D., Manera, I., Caligiani, A., 2022. Production of xylo-oligosaccharides (XOS) of tailored degree of polymerization from acetylated xylans through modelling of enzymatic hydrolysis. Food Research International 162, 112019. https://doi.org/10.1016/j.foodres.2022.112019

Gheisari, P. Ghayour Najafabadi, H. Khosravinia, 2018. Energy utilisation and growth performance of broilers receiving maize or wheat based diets supplemented with bacterial or fungal originated xylanase. Europ.Poult.Sci. 82. https://doi.org/10.1399/eps.2018.223

Gibson, G.R., Hutkins, R., Sanders, M.E., Prescott, S., Reimer, R.A., Salminen, S.J., Scott, K., Stanton, C., Swanson, K.S., Cani, P.D., Verbeke, K., Reid, G., 2017. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nature Reviews Gastroenterology & Hepatology.

Godbout, A., Edwards, J., Kuterna, L., Létourneau-Montminy, M.-P., 2024. Effect of a multiactivity non-starch polysaccharide degrading enzyme on broiler growth performance and nitrogen utilization: a meta-analysis of own trial data. Animal - Open Space 3, 100065. https://doi.org/10.1016/j.anopes.2024.100065

González-Alvarado, J.M., Jiménez-Moreno, E., González-Sánchez, D., Lázaro, R., Mateos, G.G., 2010. Effect of inclusion of oat hulls and sugar beet pulp in the diet on productive performance and digestive traits of broilers from 1 to 42 days of age. Animal Feed Science and Technology 162, 37–46. https://doi.org/10.1016/j.anifeedsci.2010.08.010

González-Ortiz, G, dos Santos, T.T., Vienola, K., Vartiainen, S., Apajalahti, J., Bedford, M.R., 2019a. Response of broiler chickens to xylanase and butyrate supplementation. Poultry Science 98, 3914–3925. https://doi.org/10.3382/ps/pez113

González-Ortiz, G., Gomes, G.A., dos Santos, T.T., Bedford, M.R., 2019. New strategies influencing gut functionality and animal performance, in: González-Ortiz, G., Bedford, M.R., Bach Knudsen, K.E., Courtin, C.M., Classen, H.L. (Eds.), The value of fibre. Wageningen Academic Publishers, The Netherlands, pp. 233–254. https://doi.org/10.3920/978-90-8686-893-3_14

González-Ortiz, G., Lee, S.A., Vienola, K., Raatikainen, K., Jurgens, G., Apajalahti, J., Bedford, M.R., 2021a. Interaction between xylanase and a proton pump inhibitor on broiler chicken performance and gut function. Animal Nutrition. https://doi.org/10.1016/j.aninu.2021.06.005

González-Ortiz, G., Olukosi, O.A., Jurgens, G., Apajalahti, J., Bedford, M.R., 2020. Short-chain fatty acids and ceca microbiota profiles in broilers and turkeys in response to diets supplemented with phytase at varying concentrations, with or without xylanase. Poultry Science 99, 2068–2077.

González-Ortiz, G, Rousseau, X., Garcia, M., Sanchez, J., Bedford, M.R., 2019b. Xylanase alone or in combination with xylo-oligosaccharides in broiler chickens performance, in: Proceedings of the 13th Journées de La Researche Avicole et Palmipedes a Foie Gras. Presented at the Proceedings of the 13th Journées de la Researche Avicole et Palmipedes a Foie Gras, Tours, France, pp. 518–523.

González-Ortiz, G., Tedeschi dos Santos, T., Bedford, M., 2021b. Evaluation of xylanase and a fermentable xylo-oligosaccharide on performance and ileal digestibility of broiler chickens fed energy and amino acid deficient diets. Animal Nutrition 7, 488–495. https://doi.org/10.1016/j.aninu.2020.07.008

González-Solé, F., Solà-Oriol, D., Ramayo-Caldas, Y., Rodriguez-Prado, M., González Ortiz, G., Bedford, M.R., Pérez, J.F., 2022. Supplementation of xylo-oligosaccharides to suckling piglets promotes the growth of fiber-degrading gut bacterial populations

during the lactation and nursery periods. Scientific Reports 12, 11594. https://doi.org/10.1038/s41598-022-15963-4

Gordon, D.T., Okuma, K., 2002. Determination of Total Dietary Fiber in Selected Foods Containing Resistant Maltodextrin by Enzymatic-Gravimetric Method and Liquid Chromatography: Collaborative Study. Journal of AOAC International 435-444.

Gorenz, B., Iseri, V., Rubach, J., Dilger, R.N., 2022. Xylanase supplementation of pelleted wheat-based diets increases growth efficiency and apparent metabolizable energy and decreases viscosity of intestinal contents in broilers. Poultry Science 101, 102220. https://doi.org/10.1016/j.psj.2022.102220

Grozina, A.A., Ilina, L.A., Laptev, G.Y., Yildirim, E.A., Ponomareva, E.S., Filippova, V.A., Tyurina, D.G., Fisinin, V.I., Kochish, I.I., Griffin, D.K., Surai, P.F., Romanov, M.N., 2023. Probiotics as an alternative to antibiotics in modulating the intestinal microbiota and performance of broiler chickens. Journal of Applied Microbiology 134. https://doi.org/10.1093/jambio/lxad213

Haag, L.-M., Siegmund, B., 2015. Intestinal Microbiota and the Innate Immune System – A Crosstalk in Crohn's Disease Pathogenesis. Front. Immunol. 6. https://doi.org/10.3389/fimmu.2015.00489

Havenstein, G., Ferket, P., Qureshi, M., 2003. Growth, livability, and feed conversion of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets. Poultry Science 82, 1500–1508. https://doi.org/10.1093/ps/82.10.1500

He, H.-J., Qiao, J., Liu, Y., Guo, Q., Ou, X., Wang, X., 2021. Isolation, Structural, Functional, and Bioactive Properties of Cereal Arabinoxylan─A Critical Review. J. Agric. Food Chem. 69, 15437–15457. https://doi.org/10.1021/acs.jafc.1c04506

Hell, J., Kneifel, W., Rosenau, T., Böhmdorfer, S., 2014. Analytical techniques for the elucidation of wheat bran constituents and their structural features with emphasis on dietary fiber – A review. Trends in Food Science & Technology 35, 102–113. https://doi.org/10.1016/j.tifs.2013.10.012

Hesselman, K., Åman, P., 1986. The effect of β-glucanase on the utilization of starch and nitrogen by broiler chickens fed on barley of low- or high-viscosity. Animal Feed Science and Technology 15, 83–93. https://doi.org/10.1016/0377-8401(86)90015-5

Hill, F.W., Anderson, D.L., 1958. Comparison of metabolizable energy and productive energy determinations with growing chicks. Journal of Nutrition 64, 587–603.

Hipsley, E.H., 1953. Dietary "Fibre" and Pregnancy Toxaemia. Br Med J 2, 420–422. https://doi.org/10.1136/bmj.2.4833.420

Ho, A.L., Kosik, O., Lovegrove, A., Charalampopoulos, D., Rastall, R.A., 2018. In vitro fermentability of xylo-oligosaccharide and xylo-olysaccharide fractions with different

molecular weights by human faecal bacteria. Carbohydrate Polymers 179, 50–58. https://doi.org/10.1016/j.carbpol.2017.08.077

Hold, G.L., Schwiertz, A., Aminov, R.I., Blaut, M., Flint, H.J., 2003. Oligonucleotide Probes That Detect Quantitatively Significant Groups of Butyrate-Producing Bacteria in Human Feces. Applied and Environmental Microbiology 69, 4320–4324. https://doi.org/10.1128/AEM.69.7.4320-4324.2003

Hong, J.S., Yu, M., Nawarathne, S.R., Oketch, E.O., Yun, H., Jayasena, D., Heo, J.M., 2024. Efficacy and matrix value of xylanase in broiler fed an energy-deficient corn-wheat-soybean diet. Journal of Animal Science and Technology. https://doi.org/10.5187/jast.2024.e39

Hooper, L.V., Midtvedt, T., Gordon, J.I., 2002. How host-microbial interactions shape the nutrient environment of the mammalian intestine. Annu Rev Nutr 22, 283–307. https://doi.org/10.1146/annurev.nutr.22.011602.092259

Hume, M.E., 2011. Historic perspective: Prebiotics, probiotics, and other alternatives to antibiotics. Poultry Science 90, 2663–2669. https://doi.org/10.3382/ps.2010-01030

Hung, Y.-T., Zhu, J., Shurson, G.C., Saqui-Salces, M., Urriola, P.E., 2020. 156 Influence of viscosity and fiber on nutrient digestibility and intestinal physiology in growing pigs. Journal of Animal Science 98, 67–68. https://doi.org/10.1093/jas/skaa054.121

Hung, Y.-T., Zhu, J., Shurson, G.C., Urriola, P.E., Saqui-Salces, M., 2022. Decreased nutrient digestibility due to viscosity is independent of the amount of dietary fibre fed to growing pigs. British Journal of Nutrition 127, 177–187. https://doi.org/10.1017/S0007114521000866

Iqbal, A., Qudoos, A., Çetingül, İ.S., Shah, S.R.A., Bayram, İ., 2019. Retraction: Importance of Dietary Fiber in Poultry Nutrition. JASP 2, 21–29.

Jazi, V., Mohebodini, H., Ashayerizadeh, A., Shabani, A., Barekatain, R., 2019. Fermented soybean meal ameliorates Salmonella Typhimurium infection in young broiler chickens. Poultry Science 98, 5648–5660. https://doi.org/10.3382/ps/pez338

Jensen, L.S., Merrill, L.H., Reddy, C.V., McGinnis, J., 1962. Observations on Eating Patterns and Rate of Food Passage of Birds Fed Pelleted and Unpelleted Diets1. Poultry Science 41, 1414–1419. https://doi.org/10.3382/ps.0411414

Jha, R., Berrocoso, J.D., 2015. Review: Dietary fiber utilization and its effects on physiological functions and gut health of swine. animal 9, 1441–1452. https://doi.org/10.1017/S1751731115000919

Jha, R., Fouhse, J.M., Tiwari, U.P., Li, L., Willing, B.P., 2019. Dietary Fiber and Intestinal Health of Monogastric Animals. Frontiers in Veterinary Science 6.

Jha, R., Mishra, P., 2021. Dietary fiber in poultry nutrition and their effects on nutrient utilization, performance, gut health, and on the environment: a review. Journal of

Animal Science and Biotechnology 12, 51. https://doi.org/10.1186/s40104-021-00576-0

Jha, R., Rossnagel, B., Pieper, R., Kessel, A.V., Leterme, P., 2010. Barley and oat cultivars with diverse carbohydrate composition alter ileal and total tract nutrient digestibility and fermentation metabolites in weaned piglets. animal 4, 724–731. https://doi.org/10.1017/S1751731109991510

Jiménez-Moreno, E., González-Alvarado, J.M., de Coca-Sinova, A., Lázaro, R., Mateos, G.G., 2009. Effects of source of fibre on the development and pH of the gastrointestinal tract of broilers. Animal Feed Science and Technology 154, 93–101. https://doi.org/10.1016/j.anifeedsci.2009.06.020

Jommuengbout, P., Pinitglang, S., Kyu, K.L., Ratanakhanokchai, K., 2009. Substrate Binding Site of Family 11 Xylanase from Bacillus Firmus K-1 by Molecular Docking. Bioscience, Biotechnology, and Biochemistry 73, 833–839. https://doi.org/10.1271/bbb.80731

Jørgensen, H., Zhao, X.-Q., Eggum, B.O., 1996. The influence of dietary fibre and environmental temoperature on the development of the gastrointestinal tract, digestibility, degree of fermentation in the hind-gut and energy metabolism in pigs. British Journal of Nutrition 75, 365–378. https://doi.org/10.1079/BJN19960140

Jozefiak, D., Rutkowski, A., Fratczak, M., Boros, D., 2004. The effect of dietary fibre fractions from different cereals and microbial enzyme supplementation on performance, ileal viscosity and short-chain fatty acid concentrations in the caeca of broiler chickens. Journal of Animal and Feed Sciences 13, 487–496.

Józefiak, D., Rutkowski, A., Jensen, B.B., Engberg, R.M., 2007. Effects of dietary inclusion of triticale, rye and wheat and xylanase supplementation on growth performance of broiler chickens and fermentation in the gastrointestinal tract. Animal Feed Science and Technology 132, 79–93. https://doi.org/10.1016/j.anifeedsci.2006.03.011

Kaewtapee, C., Burbach, K., Tomforde, G., Hartinger, T., Camarinha-Silva, A., Heinritz, S., Seifert, J., Wiltafsky, M., Mosenthin, R., Rosenfelder-Kuon, P., 2017. Effect of Bacillus subtilis and Bacillus licheniformis supplementation in diets with low- and high-protein content on ileal crude protein and amino acid digestibility and intestinal microbiota composition of growing pigs. J Animal Sci Biotechnol 8, 37. https://doi.org/10.1186/s40104-017-0168-2

Kiarie, E., Romero, L.F., Ravindran, V., 2014. Growth performance, nutrient utilization, and digesta characteristics in broiler chickens fed corn or wheat diets without or with supplemental xylanase1. Poultry Science 93, 1186–1196. https://doi.org/10.3382/ps.2013-03715

Kim, E., Moss, A.F., Morgan, N.K., Gharib-Naseri, K., Ader, P., Choct, M., 2022. Non-starch polysaccharide-degrading enzymes may improve performance when included in wheat- but not maize-based diets fed to broiler chickens under subclinical necrotic enteritis challenge. Animal Nutrition 10, 54–67. https://doi.org/10.1016/j.aninu.2022.01.009

Kim, G.-B., Seo, Y.M., Kim, C.H., Paik, I.K., 2011. Effect of dietary prebiotic supplementation on the performance, intestinal microflora, and immune response of broilers. Poultry Science 90, 75–82. https://doi.org/10.3382/ps.2010-00732

Kim, S.W., Less, J.F., Wang, L., Yan, T., Kiron, V., Kaushik, S.J., Lei, X.G., 2019. Meeting Global Feed Protein Demand: Challenge, Opportunity, and Strategy. Annu. Rev. Anim. Biosci. 7, 221–243. https://doi.org/10.1146/annurev-animal-030117-014838

Kleyn, R., 2013. Chicken Nutrition: A guide for nutritionists and poultry professionals. Context.

Knudsen, K.E., 2001. The nutritional significance of “dietary fibre” analysis. Animal Feed Science and Technology, The Role of Dietary Fibre in Pig Production 90, 3–20. https://doi.org/10.1016/S0377-8401(01)00193-6

Knudsen, K.E.B., 2014. Fiber and nonstarch polysaccharide content and variation in common crops used in broiler diets. Poultry Science 93, 2380–2393. https://doi.org/10.3382/ps.2014-03902

Knudsen, K.E.B., 1997. Carbohydrate and lignin contents of plant materials used in animal feeding. Animal Feed Science and Technology 67, 319–338. https://doi.org/10.1016/S0377-8401(97)00009-6

Knudsen, K.E.B., Lærke, H.N., 2010. REVIEW: Rye Arabinoxylans: Molecular Structure, Physicochemical Properties and Physiological Effects in the Gastrointestinal Tract. Cereal Chemistry 87, 353–362. https://doi.org/10.1094/CCHEM-87-4-0353

Konieczka, P., Smulikowska, S., 2018. Viscosity negatively affects the nutritional value of blue lupin seeds for broilers. Animal 12, 1144–1153. https://doi.org/10.1017/S1751731117002622

Koyun, O.Y., Callaway, T.R., 2019. Controlling pathogens in the poultry gut, in: Improving Gut Health in Poultry. Burleigh Dodds Science Publishing.

Kumar, V., Sinha, A.K., Makkar, H.P.S., de Boeck, G., Becker, K., 2012. Dietary Roles of Non-Starch Polysachharides in Human Nutrition: A Review. Critical Reviews in Food Science and Nutrition 52, 899–935. https://doi.org/10.1080/10408398.2010.512671

Langhout, D.J., 1998. The Role of the Intestinal Flora as Affected by Non-Starch Polysaccharides in Broiler Chicks.

Langhout, D.J., Schutte, J.B., Jong, J. de, Sloetjes, H., Verstegen, W.A., Tamminga, S., 2000. Effect of viscosity on digestion of nutrients in conventional and germ-free

chicks. British Journal of Nutrition 83, 533–540. https://doi.org/10.1017/S0007114500000672

Lázaro, R., Garcia, M., Medel, P., Mateos, G. 2003. Influence of enzymes on performance and digestive parameters of broilers fed rye-based diets. Poultry science, 82 1, 132-40. https://doi.org/10.1093/PS/82.1.132.

Leeson, S., Summers, J.D., 2001. Nutrition of the Chicken. University Books.

Li, D.D., Ding, X.M., Zhang, K.Y., Bai, S.P., Wang, J.P., Zeng, Q.F., Su, Z.W., Kang, L., 2017. Effects of dietary xylooligosaccharides on the performance, egg quality, nutrient digestibility and plasma parameters of laying hens. Animal Feed Science and Technology 225, 20–26. https://doi.org/10.1016/j.anifeedsci.2016.12.010

Lin, Y., Lourenco, J.M., Olukosi, O.A., 2023. Effects of xylanase, protease, and xylo-oligosaccharides on growth performance, nutrient utilization, short chain fatty acids, and microbiota in *Eimeria*-challenged broiler chickens fed high fiber diet. Animal Nutrition 15, 430–442. https://doi.org/10.1016/j.aninu.2023.08.009

Lin, Y., Teng, P.-Y., Olukosi, O.A., 2022. The effects of xylo-oligosaccharides on regulating growth performance, nutrient utilization, gene expression of tight junctions, nutrient transporters, and cecal short chain fatty acids profile in *Eimeria*-challenged broiler chickens. Poultry Science 101, 102125. https://doi.org/10.1016/j.psj.2022.102125

Louis, P., Young, P., Holtrop, G., Flint, H.J., 2010. Diversity of human colonic butyrate-producing bacteria revealed by analysis of the butyryl-CoA:acetate CoA-transferase gene. Environmental Microbiology 12, 304–314. https://doi.org/10.1111/j.1462-2920.2009.02066.x

Lunn, J., Buttriss, J.L., 2007. Carbohydrates and dietary fibre. Nutrition Bulletin 32, 21–64. https://doi.org/10.1111/j.1467-3010.2007.00616.x

Manicardi, T., Baioni e Silva, G., Longati, A. A., Paiva, T. D., Souza, J. P. M., Pádua, T. F., Furlan, F. F., Giordano, R. L. C., Giordano, R. C., & Milessi, T. S., 2023. Xylooligosaccharides: A Bibliometric Analysis and Current Advances of This Bioactive Food Chemical as a Potential Product in Biorefineries' Portfolios. Foods, 12(16), 3007. https://doi.org/10.3390/foods12163007

Maphosa, Y., Jideani, V.A., 2016. Dietary fiber extraction for human nutrition—A review. Food Reviews International 32, 98–115. https://doi.org/10.1080/87559129.2015.1057840

Marcolla, C.S., Ju, T., Lantz, H.L., Willing, B.P., 2023. Investigating the cecal microbiota of broilers raised in extensive and intensive production systems. Microbiology Spectrum 11, e02352-23. https://doi.org/10.1128/spectrum.02352-23

Masey-O'Neill, H.V., Singh, M., Cowieson, A.J., 2014. Effects of exogenous xylanase on performance, nutrient digestibility, volatile fatty acid production and digestive tract

thermal profiles of broilers fed on wheat- or maize-based diet. British Poultry Science 55, 351–359. https://doi.org/10.1080/00071668.2014.898836

Mateos, G.G., Jiménez-Moreno, E., Serrano, M.P., Lázaro, R.P., 2012. Poultry response to high levels of dietary fiber sources varying in physical and chemical characteristics1. Journal of Applied Poultry Research 21, 156–174. https://doi.org/10.3382/japr.2011-00477

Mathlouthi, N., Lallès, J.P., Lepercq, P., Juste, C., Larbier, M., 2002. Xylanase and β-glucanase supplementation improve conjugated bile acid fraction in intestinal contents and increase villus size of small intestine wall in broiler chickens fed a rye-based diet1. Journal of Animal Science 80, 2773–2779. https://doi.org/10.2527/2002.80112773x

Mátis, G., Mackei, M., Boomsma, B., Fébel, H., Nadolna, K., Szymański, Ł., Edwards, J., Neogrády, Z., Kozłowski, K. 2022. Dietary Protected Butyrate Supplementation of Broilers Modulates Intestinal Tight Junction Proteins and Stimulates Endogenous Production of Short Chain Fatty Acids in the Caecum. Animals, MDPI, 12. https://doi.org/10.3390/ani12151940.

Matthiesen, C.F., Pettersson, D., Smith, A., Pedersen, N.R., Storm, Adam.C., 2021. Exogenous xylanase improves broiler production efficiency by increasing proximal small intestine digestion of crude protein and starch in wheat-based diets of various viscosities. Animal Feed Science and Technology 272, 114739. https://doi.org/10.1016/j.anifeedsci.2020.114739

Mc Cracken, K., Murphy, T., Bedford, M., Apajalahti, J., 2006. Chicken caecal microflora correlates with ME:GE using wheat-based diets.

McCleary, B.V., 2000. Measurement of Dietary Fibre Components: The Importance of Enzyme Purity, Activity and Specificity, in: Advanced Dietary Fibre Technology. John Wiley & Sons, Ltd, pp. 89–105. https://doi.org/10.1002/9780470999615.ch8

McKinney, L.J., Teeter, R.G., 2004. Predicting Effective Caloric Value of Nonnutritive Factors: I. Pellet Quality and II. Prediction of Consequential Formulation Dead Zones. Poultry Science 83, 1165–1174. https://doi.org/10.1093/ps/83.7.1065

McLaren, M.R., 2020. Silva SSU taxonomic training data formatted for DADA2 (Silva version 138). https://doi.org/10.5281/zenodo.3986799

McMurdie, P.J., Holmes, S., 2013. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLOS ONE 8, e61217. https://doi.org/10.1371/journal.pone.0061217

Mendes, A.R., Ribeiro, T., Correia, B.A., Bule, P., Maçãs, B., Falcão, L., Freire, J.P.B., Ferreira, L.M.A., Fontes, C.M.G.A., Lordelo, M.M., 2013. Low doses of exogenous

xylanase improve the nutritive value of triticale-based diets for broilers. Journal of Applied Poultry Research 22, 92–99. https://doi.org/10.3382/japr.2012-00610

Mendis, M., Leclerc, E., Simsek, S., 2016. Arabinoxylans, gut microbiota and immunity. Carbohydrate Polymers 139, 159–166. https://doi.org/10.1016/j.carbpol.2015.11.068

Morgan, N.K., 2023. Advances in prebiotics for poultry: role of the caeca and oligosaccharides. Anim. Prod. Sci. 63, 1911–1925. https://doi.org/10.1071/AN23011

Morgan, N.K., Keerqin, C., Wallace, A., Wu, S.-B., Choct, M., 2019. Effect of arabinoxylo-oligosaccharides and arabinoxylans on net energy and nutrient utilization in broilers. Animal Nutrition 5, 56–62. https://doi.org/10.1016/j.aninu.2018.05.001

Morgan, N.K., Wallace, A., Bedford, M.R., Hawking, K.L., Rodrigues, I., Hilliar, M., Choct, M., 2020. In vitro versus in situ evaluation of xylan hydrolysis into xylo-oligosaccharides in broiler chicken gastrointestinal tract. Carbohydrate Polymers 230, 115645. https://doi.org/10.1016/j.carbpol.2019.115645

Markets and Markets. 2020. *Feed Enzymes Market.* [Online]. Markets and Markets Research Private Ltd. Available from: https://www.marketsandmarkets.com/Market-Reports/feed-enzyme-market-1157.html (1.7.2020).

Megazyme. Not dated. *β-Glucan (Barley; Low Viscosity).* [Online]. Megazyme Ltd. Available from: https://www.megazyme.com/beta-glucan-barley-low-viscosity (accessed 2.8.2020).

Mwaikambo, L., 2006. Review of the history, properties and application of plant fibres. African Journal of Science and Technology 7, 120–133.

Nair, D.V.T., Dewi, G., Kollanoor-Johny, A., 2019. The role of essential oils and other botanicals in optimizing gut function in poultry, in: Improving Gut Health in Poultry. Burleigh Dodds Science Publishing.

Nguyen, H.T., Bedford, M.R., Wu, S.-B., Morgan, N.K., 2021. Soluble non-starch polysaccharide modulates broiler gastrointestinal tract environment. Poultry Science 100, 101183. https://doi.org/10.1016/j.psj.2021.101183

Nian, F., Guo, Y.M., Ru, Y., Peron, A., Li, F., 2011. Effect of Xylanase Supplementation on the Net Energy for Production, Performance and Gut Microflora of Broilers Fed Corn/Soy-based Diet. Asian-Australasian Journal of Animal Sciences 24. https://doi.org/10.5713/ajas.2011.10441

Niba, A.T., Beal, J.D., Kudi, A.C., Brooks, P.H., 2009. Bacterial fermentation in the gastrointestinal tract of non-ruminants: Influence of fermented feeds and fermentable

carbohydrates. Trop Anim Health Prod 41, 1393. https://doi.org/10.1007/s11250-009-9327-6

Nir, I., Hillel, R., Ptichi, I., Shefet, G., 1995. Effect of Particle Size on Performance.: 3. Grinding Pelleting Interactions. Poultry Science 74, 771–783. https://doi.org/10.3382/ps.0740771

Norman, A.G., 1935. The Composition of Crude Fibre. The Journal of Agricultural Science 25, 529–540. https://doi.org/10.1017/S002185960001964X

Oksanen, J., Simpson, G., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P., hara, R., Solymos, P., STEVENS, H., Szöcs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., De Cáceres, M., Durand, S., Weedon, J., 2022. Vegan community ecology package version 2.6-2 April 2022.

O'Neill, H.V.M., Mathis, G., Lumpkins, B.S., Bedford, M.R., 2012. The effect of reduced calorie diets, with and without fat, and the use of xylanase on performance characteristics of broilers between 0 and 42 days. Poultry Science 91, 1356–1360. https://doi.org/10.3382/ps.2011-01867

Percie du Sert, N., Hurst, V., Ahluwalia, A., Alam, S., Avey, M.T., Baker, M., Browne, W.J., Clark, A., Cuthill, I.C., Dirnagl, U., Emerson, M., Garner, P., Holgate, S.T., Howells, D.W., Karp, N.A., Lazic, S.E., Lidster, K., MacCallum, C.J., Macleod, M., Pearl, E.J., Petersen, O.H., Rawle, F., Reynolds, P., Rooney, K., Sena, E.S., Silberberg, S.D., Steckler, T., Würbel, H., 2020. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. PLoS Biol 18, e3000410. https://doi.org/10.1371/journal.pbio.3000410

Péron, A., Bastianelli, D., Oury, F.-X., Gomez, J., Carré, B., 2005. Effects of food deprivation and particle size of ground wheat on digestibility of food components in broilers fed on a pelleted diet. British Poultry Science 46, 223–230. https://doi.org/10.1080/00071660500066142

Petry, A.L., Huntley, N.F., Bedford, M.R., Patience, J.F., 2021. The influence of xylanase on the fermentability, digestibility, and physicochemical properties of insoluble corn-based fiber along the gastrointestinal tract of growing pigs. Journal of Animal Science 99, skab159. https://doi.org/10.1093/jas/skab159

Pettersson, D., Åman, P., 1988. Effects of enzyme supplementation of diets based on wheat, rye or triticale on their productive value for broiler chickens. Animal Feed Science and Technology 20, 313–324. https://doi.org/10.1016/0377-8401(88)90005-3

Pieper, R., Jha, R., Rossnagel, B., Van Kessel, A.G., Souffrant, W.B., Leterme, P., 2008. Effect of barley and oat cultivars with different carbohydrate compositions on the intestinal bacterial communities in weaned piglets. FEMS Microbiology Ecology 66, 556–566. https://doi.org/10.1111/j.1574-6941.2008.00605.x

Pirgozliev, V., Bravo, D., Mirza, M.W., Rose, S.P., 2015. Growth performance and endogenous losses of broilers fed wheat-based diets with and without essential oils and xylanase supplementation. Poultry Science 94, 1227–1232. https://doi.org/10.3382/ps/peu017

Pirgozliev, V., Mirza, M.W., Rose, S.P., 2016. Does the effect of pelleting depend on the wheat sample when fed to chickens? animal 10, 571–577. https://doi.org/10.1017/S1751731115002311

Pirgozliev, V.R., Mansbridge, S.C., Whiting, I.M., Abdulla, J.M., Rose, S.P., Kljak, K., Johnson, A., Drijfhout, F., Atanasov, A.G., 2023. The Benefits of Exogenous Xylanase in Wheat–Soy Based Broiler Chicken Diets, Consisting of Different Soluble Non-Starch Polysaccharides Content. Poultry 2, 123–133. https://doi.org/10.3390/poultry2020012

Pirgozliev, V.R., Rose, S.P., Bedford, M.R., 2010. The effect of amylose:amylopectin ratio in dietary starch on growth performance and gut morphology in broiler chickens. Archiv für Geflügelkunde 74, 21–29.

Pourabedin, M., Guan, L., Zhao, X., 2015. Xylo-oligosaccharides and virginiamycin differentially modulate gut microbial composition in chickens. Microbiome 3, 15. https://doi.org/10.1186/s40168-015-0079-4

Pourazadi, Z., Salari, S., Tabandeh, M.R., Abdollahi, M.R., 2020. Effect of particle size of insoluble fibre on growth performance, apparent ileal digestibility and caecal microbial population in broiler chickens fed barley-containing diets. British Poultry Science.

Preston, C.M., McCracken, K.J., McAllister, A., 2000. Effect of diet form and enzyme supplementation on growth, efficiency and energy utilisation of wheat-based diets for broilers. British Poultry Science 41, 324–331. https://doi.org/10.1080/713654933

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Research 41, D590–D596. https://doi.org/10.1093/nar/gks1219

Quigley, M.E., Englyst, H.N., 1994. Determination of the uronic acid constituents of non-starch polysaccharides by high-performance liquid chromatography with pulsed amperometric detection. Analyst 119, 1511–1518. https://doi.org/10.1039/AN9941901511

R Core Team, 2024. _R: A Language and Environment for Statistical Computing_. R Foundation for Statistical Computing, Vienna, Austria.

Rao, Z., Li, Y., Yang, X., Guo, Y., Zhang, W., Wang, Z., 2024. Diet xylo-oligosaccharide supplementation improves growth performance, immune function, and intestinal

health of broilers. Animal Nutrition 17, 165–176. https://doi.org/10.1016/j.aninu.2024.01.004

Ravindran, V., 2013. Poultry feed availability and nutrition in developing countries. Poultry Development Review 60–63.

Razdan, A., Pettersson, D., 1996. Hypolipidaemic, gastrointestinal and related responses of broiler chickens to chitosans of different viscosity. Br J Nutr 76, 387–397. https://doi.org/10.1079/BJN19960044

Reddy, S.S., Krishnan, C., 2016. Production of high-pure xylooligosaccharides from sugarcane bagasse using crude β-xylosidase-free xylanase of *Bacillus subtilis* KCX006 and their bifidogenic function. LWT 65, 237–245. https://doi.org/10.1016/j.lwt.2015.08.013

Rezaei, M., Karimi Torshizi, M.A., Rouzbehan, Y., 2011. The influence of different levels of micronized insoluble fiber on broiler performance and litter moisture. Poultry Science 90, 2008–2012. https://doi.org/10.3382/ps.2011-01352

Ribeiro, T., Cardoso, V., Ferreira, L.M.A., Lordelo, M.M.S., Coelho, E., Moreira, A.S.P., Domingues, M.R.M., Coimbra, M.A., Bedford, M.R., Fontes, C.M.G.A., 2018. Xylo-oligosaccharides display a prebiotic activity when used to supplement wheat or corn-based diets for broilers. Poultry Science 97, 4330–4341. https://doi.org/10.3382/ps/pey336

Rinttilä, T., Apajalahti, J., 2013. Intestinal microbiota and metabolites—Implications for broiler chicken health and performance1. Journal of Applied Poultry Research 22, 647–658. https://doi.org/10.3382/japr.2013-00742

Rodríguez-Lagunas, M. j., Pérez-Cano, F. j., 2019. Chapter 17 Fibre and fibre breakdown products as microbial and immune defence modulators, in: The Value of Fibre. Wageningen Academic Publishers, pp. 297–311. https://doi.org/10.3920/978-90-8686-893-3_17

Saki, A.A., 2005. Effect of Wheat and Barley Viscosity on Broiler Performance in Hamadan Province. https://doi.org/10.3923/ijps.2005.7.10

Sawicki, C.M., Livingston, K.A., Obin, M., Roberts, S.B., Chung, M., McKeown, N.M., 2017. Dietary Fiber and the Human Gut Microbiota: Application of Evidence Mapping Methodology. Nutrients 9, 125. https://doi.org/10.3390/nu9020125

Schliep, K.P., 2011. phangorn: phylogenetic analysis in R. Bioinformatics 27, 592–593. https://doi.org/10.1093/bioinformatics/btq706

Scott, K.P., Martin, J.C., Duncan, S.H., Flint, H.J., 2014. Prebiotic stimulation of human colonic butyrate-producing bacteria and bifidobacteria, in vitro. FEMS Microbiology Ecology 87, 30–40. https://doi.org/10.1111/1574-6941.12186

Selvendran, R.R., 1984. The plant cell wall as a source of dietary fiber: chemistry and structure. The American Journal of Clinical Nutrition 39, 320–337. https://doi.org/10.1093/ajcn/39.2.320

Shang, Y., Kumar, S., Oakley, B., Kim, W.K., 2018. Chicken Gut Microbiota: Importance and Detection Technology. Front. Vet. Sci. 5. https://doi.org/10.3389/fvets.2018.00254

Short, F.J., Gorton, P., Wiseman, J., Boorman, K.N., 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Animal Feed Science and Technology 59, 215–221. https://doi.org/10.1016/0377-8401(95)00916-7

Šimić, A., González-Ortiz, G., Mansbridge, S.C., Rose, S.P., Bedford, M.R., Yovchev, D., Pirgozliev, V.R., 2023. Broiler chicken response to xylanase and fermentable xylooligosaccharide supplementation. Poultry Science 102, 103000. https://doi.org/10.1016/j.psj.2023.103000

Simon, O., 1998. The mode of action of NSP hydrolysing enzymes in the gastrointestinal tract. Journal of Animal and Feed Sciences 7, 115–123. https://doi.org/10.22358/jafs/69959/1998

Singh, A. K., Mandal, R.K., Bedford, M.R., Jha, R., 2021a. Xylanase improves growth performance, enhances cecal short-chain fatty acids production, and increases the relative abundance of fiber fermenting cecal microbiota in broilers. Animal Feed Science and Technology 277, 114956. https://doi.org/10.1016/j.anifeedsci.2021.114956

Singh, Amit K., Mishra, B., Bedford, M.R., Jha, R., 2021. Effects of supplemental xylanase and xylooligosaccharides on production performance and gut health variables of broiler chickens. Journal of Animal Science and Biotechnology 12, 98. https://doi.org/10.1186/s40104-021-00617-8

Singh, A. K., Mishra, B., Bedford, M.R., Jha, R., 2021b. Effects of supplemental xylanase and xylooligosaccharides on production performance and gut health variables of broiler chickens. Journal of Animal Science and Biotechnology 12, 98. https://doi.org/10.1186/s40104-021-00617-8

Singh, A.K., Tiwari, U.P., Mishra, B., Jha, R., 2022. Effects of in ovo delivered xylo- and mannan- oligosaccharides on growth performance, intestinal immunity, cecal short-chain fatty acids, and cecal microbiota of broilers. Journal of Animal Science and Biotechnology 13, 13. https://doi.org/10.1186/s40104-021-00666-z

Singh, R.D., Banerjee, J., Arora, A., 2015. Prebiotic potential of oligosaccharides: A focus on xylan derived oligosaccharides. Bioactive Carbohydrates and Dietary Fibre 5, 19–30. https://doi.org/10.1016/j.bcdf.2014.11.003

Skinner-Noble, D.O., McKinney, L.J., Teeter, R.G., 2005. Predicting effective caloric value of nonnutritive factors: III. Feed form affects broiler performance by modifying behavior patterns1. Poultry Science 84, 403–411. https://doi.org/10.1093/ps/84.3.403

Sklan, D., Smirnov, A., Plavnik, I., 2003. The effect of dietary fibre on the small intestines and apparent digestion in the turkey. British Poultry Science 44, 735–740. https://doi.org/10.1080/00071660310001643750

Smits, C.H.M., Annison, G., 1996. Non-starch plant polysaccharides in broiler nutrition – towards a physiologically valid approach to their determination. World's Poultry Science Journal 52, 203–221. https://doi.org/10.1079/WPS19960016

Smulikowska, S., Mieczkowska, A., Nguyen, C.V., Bąbelewska, M., 2002. The influence of digesta viscosity on the development of the stomach, on in vitro small intestinal motility and on digestion of nutrients in broiler chickens. J. Anim. Feed Sci. 11, 683–694. https://doi.org/10.22358/jafs/67925/2002

Sonnenburg, E.D., Smits, S.A., Tikhonov, M., Higginbottom, S.K., Wingreen, N.S., Sonnenburg, J.L., 2016. Diet-induced extinctions in the gut microbiota compound over generations. Nature 529, 212–215. https://doi.org/10.1038/nature16504

Stephen, A.M., Champ, M.M.-J., Cloran, S.J., Fleith, M., Lieshout, L. van, Mejborn, H., Burley, V.J., 2017. Dietary fibre in Europe: current state of knowledge on definitions, sources, recommendations, intakes and relationships to health. Nutrition Research Reviews 30, 149–190. https://doi.org/10.1017/S095442241700004X

Surget, A., Barron, C., 2005. Histologie du grain de blé. Ind. céréales 3–7.

Sukahan. Not dated. *Industrial Bio-Enzymes.* [Online]. Shandong Sukahan Bio-Technology Co.,Ltd. Available from: http://www.sukahan.com/en/proItem.aspx?id=179 [Accessed 2 August 2020].

Svihus, B., Choct, M., Classen, H.L., 2013. Function and nutritional roles of the avian caeca: a review. World's Poultry Science Journal 69, 249–264. https://doi.org/10.1017/S0043933913000287

Svihus, B., Juvik, E., Hetland, H., Krogdahl, Å., 2004. Causes for improvement in nutritive value of broiler chicken diets with whole wheat instead of ground wheat. British Poultry Science 45, 55–60. https://doi.org/10.1080/00071660410001668860

Tejeda, O.J., Kim, W.K., 2021. Effects of fiber type, particle size, and inclusion level on the growth performance, digestive organ growth, intestinal morphology, intestinal viscosity, and gene expression of broilers. Poultry Science 100, 101397. https://doi.org/10.1016/j.psj.2021.101397

Theander, O., Åman, P., Westerlund, E., Andersson, R., Pettersson, D., 1995. Total Dietary Fiber Determined as Neutral Sugar Residues, Uronic Acid Residues, and Klason Lignin (The Uppsala Method): Collaborative Study. Journal of AOAC INTERNATIONAL 78, 1030–1044. https://doi.org/10.1093/jaoac/78.4.1030

Theander, O., Westerlund, E., Åman, P., Graham, H., 1989. Plant cell walls and monogastric diets. Animal Feed Science and Technology, Dietary Fibre in Monogastric Nutrition 23, 205–225. https://doi.org/10.1016/0377-8401(89)90098-9

Titus, H.W., 1961. Scientific feeding of chickens.

Trowell, H., 1974. Definitions of fibre 303.

Trowell, H., Southgate, D.T., Wolever, T.S., Leeds, A., Gassull, M., Jenkins, D.A., 1976. Dietary Fibre redefined. The Lancet 307, 967. https://doi.org/10.1016/S0140-6736(76)92750-1

United Nations, 2023. Global trends in population growth, in: Global Population Growth and Sustainable Development. United Nations, pp. 22–28.

van der Wielen, P.W.J.J., Biesterveld, S., Notermans, S., Hofstra, H., Urlings, B.A.P., van Knapen, F., 2000. Role of Volatile Fatty Acids in Development of the Cecal Microflora in Broiler Chickens during Growth. Applied and Environmental Microbiology 66, 2536–2540. https://doi.org/10.1128/AEM.66.6.2536-2540.2000

Van Keulen, J., Young, B.A., 1977. Evaluation of Acid-Insoluble Ash as a Natural Marker in Ruminant Digestibility Studies. Journal of Animal Science 44, 282–287. https://doi.org/10.2527/jas1977.442282x

Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. Journal of Dairy Science 74, 3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Van Soest, P.J., Wine, R.H., 1967. Use of Detergents in the Analysis of Fibrous Feeds. IV. Determination of Plant Cell-Wall Constituents. Journal of Association of Official Analytical Chemists 50, 50–55. https://doi.org/10.1093/jaoac/50.1.50

Van Soest, P.J.V., 1963. Use of Detergents in the Analysis of Fibrous Feeds. II. A Rapid Method for the Determination of Fiber and Lignin. Journal of Association of Official Agricultural Chemists 46, 829–835. https://doi.org/10.1093/jaoac/46.5.829

Van Soest, P.J.V., McQueen, R.W., 1973. The chemistry and estimation of fibre. Proceedings of the Nutrition Society 32, 123–130. https://doi.org/10.1079/PNS19730029

Varel, V.H., Yen, J.T., 1997. Microbial perspective on fiber utilization by swine1. Journal of Animal Science 75, 2715–2722. https://doi.org/10.2527/1997.75102715x

Walker, M., Cooper, J., 2023. Dietary fibre [WWW Document]. Dietary fibre. Institute of Food Science and Technology. URL https://www.ifst.org/resources/information-statements/dietary-fibre (accessed 9.21.24).

Washburn, K.W., 1991. Efficiency of Feed Utilization and Rate of Feed Passage Through the Digestive System1. Poultry Science 70, 447–452. https://doi.org/10.3382/ps.0700447

Weißbecker, C., Buscot, F., Wubet, T., 2017. Preservation of nucleic acids by freeze-drying for next generation sequencing analyses of soil microbial communities. Journal of Plant Ecology 10, 81–90. https://doi.org/10.1093/jpe/rtw042

Whiting, I.M., Pirgozliev, V., Rose, S.P., Wilson, J., Amerah, A.M., Ivanova, S.G., Staykova, G.P., Oluwatosin, O.O., Oso, A.O., 2016. Nutrient availability of different batches of wheat distillers dried grains with solubles with and without exogenous enzymes for broiler chickens. Poultry Science 96, 574–580. https://doi.org/10.3382/ps/pew262

Wu, Y.B., Ravindran, V., Thomas, D.G., Birtles, M.J., Hendriks, W.H., 2004. Influence of method of whole wheat inclusion and xylanase supplementation on the performance, apparent metabolisable energy, digestive tract measurements and gut morphology of broilers. British Poultry Science 45, 385–394. https://doi.org/10.1080/00071660410001730888

Yadav, S., Jha, R., 2019. Strategies to modulate the intestinal microbiota and their effects on nutrient utilization, performance, and health of poultry. J Animal Sci Biotechnol 10, 2. https://doi.org/10.1186/s40104-018-0310-9

Yang, Y., Iji, P.A., Kocher, A., Mikkelsen, L.L., Choct, M., 2008. Effects of Xylanase on Growth and Gut Development of Broiler Chickens Given a Wheat-based Diet. Asian-Australasian Journal of Animal Sciences 21, 1659–1664.

Yegani, M., Korver, D.R., 2012. Review: Prediction of variation in energetic value of wheat for poultry. Can. J. Anim. Sci. 92, 261–273. https://doi.org/10.4141/cjas2011-114

Yuan, L., Li, W., Huo, Q., Du, C., Wang, Z., Yi, B., Wang, M., 2018. Effects of xylo-oligosaccharide and flavomycin on the immune function of broiler chickens. PeerJ 6, e4435. https://doi.org/10.7717/peerj.4435

Zaefarian, F., Abdollahi, M. r., Ravindran, V., 2019. Chapter 13 Influence of feed processing on the gastrointestinal tract development and gizzard physiology in broilers, in: The Value of Fibre. Wageningen Academic Publishers, pp. 217–231. https://doi.org/10.3920/978-90-8686-893-3_13

Zhang, J., Sun, Y., Zhao, L., Chen, T., Fan, M., Jiao, H., Zhao, J., Wang, X., Li, F., Li, H., Lin, H. 2019. SCFAs-Induced GLP-1 Secretion Links the Regulation of Gut Microbiome on Hepatic Lipogenesis in Chickens. Frontiers in Microbiology, 10. https://doi.org/10.3389/fmicb.2019.02176.

Zhenping, S., Wenting, L., Ruikui, Y., Jia, L., Honghong, L., Wei, S., Zhongmie, W., Jingpan, L., Zhe, S., Yuling, Q., 2013. Effect of a straw-derived xylooligosaccharide on broiler growth performance, endocrine metabolism, and immune response. Canadian Journal of Veterinary Research 77, 105–109.

Zhou, J., Wu, S., Qi, G., Fu, Y., Wang, W., Zhang, H., Wang, J., 2021. Dietary supplemental xylooligosaccharide modulates nutrient digestibility, intestinal morphology, and gut microbiota in laying hens. Animal Nutrition 7, 152–162. https://doi.org/10.1016/j.aninu.2020.05.010

Zielinski, G., Devries, J., Craig, S., Bridges, A., 2013. Dietary Fiber Methods in Codex Alimentarius : Current Status and Ongoing Discussions. Cereal Foods World 58, 148–152. https://doi.org/10.1094/CFW-58-3-0401

Zimonja, O., Svihus, B., 2009. Effects of processing of wheat or oats starch on physical pellet quality and nutritional value for broilers. Animal Feed Science and Technology 149, 287–297. https://doi.org/10.1016/j.anifeedsci.2008.06.010