

Dietary interventions for resilient poultry gut health in the AMR era



by **Ajay Bhojar**, *Global Technical Manager, EW Nutrition*

Gut health is critical for profitable poultry production, as the gastrointestinal tract (GIT) plays a dual role in nutrient digestion and absorption while serving as a crucial defense against pathogens. A healthy gut enables efficient feed conversion, robust immune function, and resilience against diseases, reducing reliance on preventive and therapeutic antibiotics. Optimum gut health has become increasingly important in poultry production to combat antimicrobial resistance (AMR), a pressing global challenge threatening animal agriculture and public health.

AMR arises when bacteria develop antibiotic resistance, often due to overuse or misuse in human and animal settings. Predictive models suggest that by 2050, AMR could result in 10 million annual deaths and a 2.0%–3.5% reduction in global gross domestic production, amounting to economic losses between 60 and 100 trillion USD. In poultry, AMR compromises flock health, leading to higher mortality, reduced growth performance, and elevated treatment costs, directly impacting profitability. Additionally, resistant pathogens increase the risk of zoonotic disease transfer, posing serious food safety concerns.

Stricter regulations and rising consumer demand for antibiotic-free poultry products drive a shift toward sustainable, antibiotic-free production systems. However, A lack of understanding about strategies to replace AMU and their effectiveness under field conditions hampers change in farming practices (Afonso et al., 2024). Addressing AMR requires a holistic approach, encompassing enhanced biosecurity, innovative health-promoting strategies, and sustainable management practices. This paper explores practical dietary interventions to support poultry gut health while reducing dependency on antimicrobials, offering solutions for the long-term sustainability of poultry production.

Gut Mediated Immunity in Chickens

The gut is a critical component of the immune system, as it is the first line of defense against pathogens that enter the body through the digestive system. Chickens have a specialized immune system in the gut, known as gut-associated lymphoid tissue (GALT), which helps to identify and respond to potential pathogens. The GALT includes Peyer's patches, clusters of immune cells in the gut wall, and the gut-associated lymphocytes (GALs) found throughout the gut. These immune cells recognize and respond to pathogens that enter the gut.

The gut-mediated immune response in chickens involves several mechanisms, including activating immune cells, producing antibodies, and releasing inflammatory mediators. GALT and GALs play a crucial role in this response by identifying and responding to pathogens and activating other immune cells to help fight off the infection.

The gut microbiome is a diverse community of microorganisms that live in the gut. These microorganisms can significantly impact the immune response. Certain beneficial bacteria, for example, can help stimulate the immune response and protect the gut from pathogens.

Overall, the gut microbiome, GALT, and GALs work together to create an environment hostile to pathogens while supporting the growth and health of beneficial microorganisms.

Key Factors Affecting Poultry Gut Health

The key factors affecting broiler gut health can be summarized as follows:

1. **Early gut development:** Gut-associated immunity responds to early feeding and dietary nutrients and is critical for protecting against exogenous organisms during the first week of life post-hatch.
2. **Feed and Water Quality:** The form, type, and quality of feed provided to broilers can significantly impact their gut health. Consistently available cool and hygienic drinking water is crucial for optimum production performance.
3. **Stressors:** Stressful conditions, such as high environmental temperatures or poor ventilation, can lead to an imbalance in the gut microbiome and an increased risk of disease.
4. **Infections and medications:** Exposure to pathogens or other harmful bacteria can disrupt the gut microbiome and lead to gut health issues. A robust immune system is important for maintaining gut health, as it helps to prevent the overgrowth of harmful bacteria and promote the growth of beneficial bacteria.
5. **Biosecurity:** Keeping the poultry environment clean and free of pathogens is crucial for maintaining gut health, as bacteria and other pathogens can quickly spread and disrupt the gut microbiome.
6. **Management practices:** Best practices, including proper litter management, can help maintain gut health and prevent gut-related issues.

Dietary Interventions for Optimum Gut Health

Gut health means the absence of gastrointestinal disease, the effective digestion and absorption of feed, and a normal and well-established microbiota (Bischoff, 2011). Various dietary measures can be taken to support the healthy functioning of the GIT and host defense. Water and feed safety and quality, feeding

management, the form the feed is provided in (e.g., pellets), the composition of the diet, and the use of various feed additives are all tools that can be used to support health (Smits et al., 2021).

Various gut health-supporting feed additives, including organic acids, probiotics, prebiotics, phytochemicals/essential oils, etc., in combination or alone, have been explored as an alternative to antimicrobials in animal production. There were differences in the impacts of the strategies between and within species; this highlights the absence of a 'one-size-fits-all' solution. Nevertheless, some options seem more promising than others, as their impacts were consistently equivalent or positive when compared with animal performance using antimicrobials (Afonso et al., 2024). Including insoluble fibers, toxin binders, exogenous enzymes, and antioxidants in the feed formulations also play a crucial role in gut health optimization, which goes beyond their primary functions to combat AMR challenges.

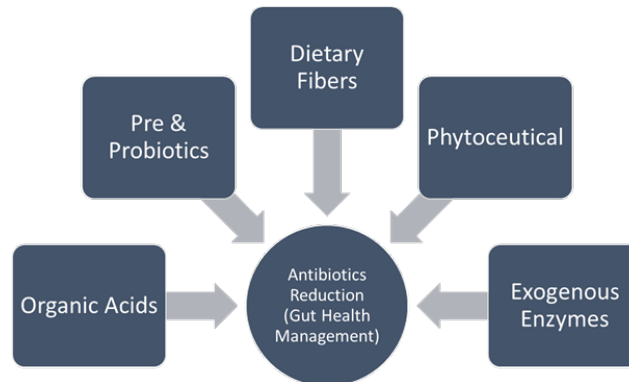


Fig. 1: Multifactorial approach to gut health management in reduced antimicrobial use

Organic Acids

The digestive process extensively includes microbial fermentation, and as a result, organic acids are commonly produced by beneficial bacteria in the crop, intestines, and ceca (Huyghebaert et al., 2010). Organic acids' inclusion in the poultry diet can improve growth performance due to improved gut health, increased endogenous digestive enzyme secretion and activity, and nutrient digestibility. Butyrate is highly bioactive in GIT. It increases the proliferation of enterocytes, promotes mucus secretion, and may have anti-inflammatory properties (Bedford and Gong, 2018; Canani et al., 2011; Hamer et al., 2008). These effects suggest that it supports mucosal barrier function. Butyrate is becoming a commonly used ingredient in diets to promote GIT health.

Including organic acids in the feed can decontaminate feed and potentially reduce enteric pathogens in poultry. Alternately, the formaldehyde treatment of feed is highly effective at a relatively low cost (Jones, 2011; Wales, Allen, and Davies, 2010).

Organic acids like formic and citric acid are also used in poultry drinking water to lower the microbial count by lowering the water's pH and preventing/removing biofilms in the water lines. By ensuring feed and water hygiene, producers can minimize pathogen exposure, enhance bird health, and significantly reduce their reliance on antibiotics.

Probiotics, Postbiotics, Prebiotics and Synbiotics

Probiotics and prebiotics have drawn considerable attention to optimizing gut health in animal feeds. Probiotic supplementation could have the following effects: (1) modification of the intestinal microbiota, (2) stimulation of the immune system, (3) reduction in inflammatory reactions, (4) prevention of pathogen colonization, (5) enhancement of growth performance, (6) alteration of the ileal digestibility and total tract apparent digestibility coefficient, and (7) decrease in ammonia and urea excretion (Jha et al., 2020). Certain Lactobacilli or Enterococci species may be used with newly hatched or newborn animals; single or

multi-strain starter cultures can be used to steer the initial microbiota in a desired direction (Liao and Nyachoti, 2017). Apart from using probiotics in feed and drinking water, probiotic preparations can be sprayed on day-old chicks in the hatchery or immediately after placement in the brooding house. This way, the probiotic strains/beneficial bacteria gain access to the gut at the earliest possible time (early seeding). Postbiotics are bioactive compounds produced by probiotics during fermentation, such as short-chain fatty acids, peptides, and bacterial cell wall components. Unlike live probiotics, postbiotics are stable, safer, and provide consistent health benefits.

Prebiotics like mannan-oligosaccharides (MOS), inulin, and its hydrolysate (fructo-oligosaccharides: FOS) play an important role in modulating intestinal microflora and potential immune response. Prebiotics reduce pathogen colonization in poultry and promote selective stimulation of beneficial bacterial species. Synbiotics are a combination of probiotics and prebiotics. This synergistic approach offers dual benefits by promoting the growth of beneficial bacteria and directly combating pathogens.

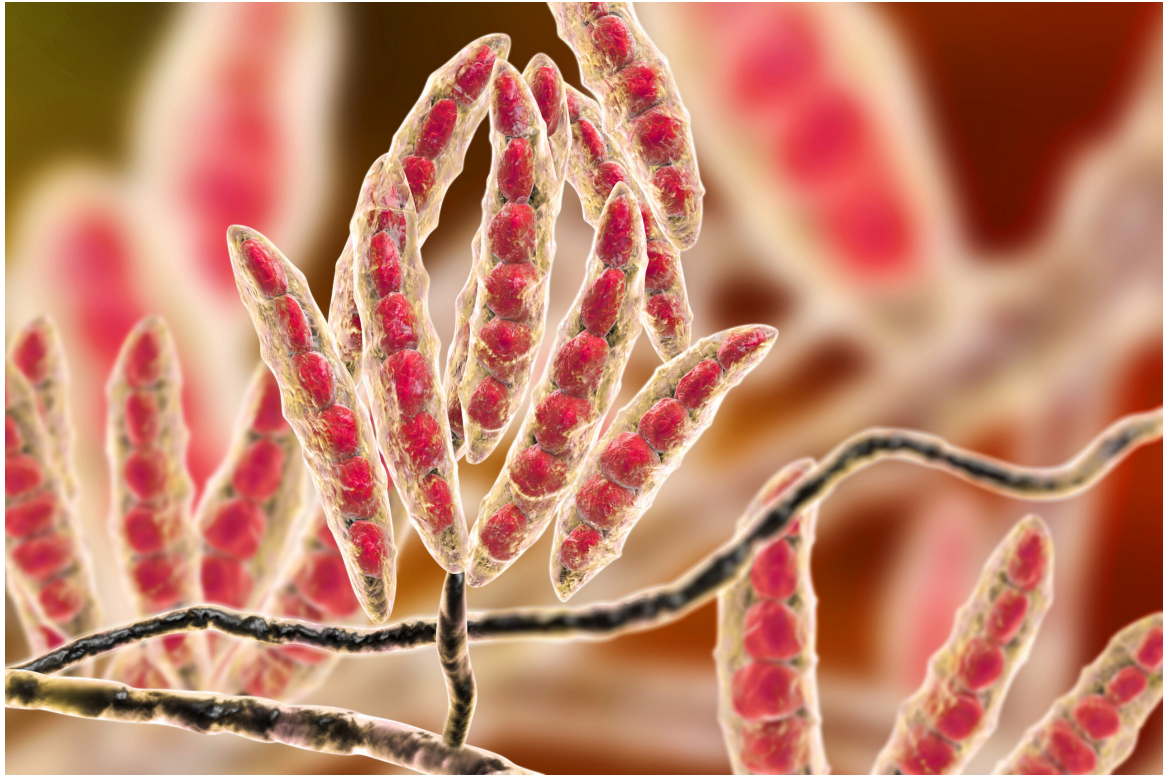
Dietary Fibers (DF)

The water-insoluble fibers are regarded as functional nutrients because of their ability to escape digestion and modulate nutrient digestion. A moderate level of insoluble fiber in poultry diets may increase chyme retention time in the upper part of the GIT, stimulating gizzard development and endogenous enzyme production, improving the digestibility of starch, lipids, and other dietary components (Mateos et al., 2012). The insoluble DF, when used in amounts between 3-5% in the diet, could have beneficial effects on intestinal development and nutrient digestibility.

Dietary fibers influence the development of the gizzard in poultry birds. A well-developed gizzard is a must for good gut health. Jiménez-Moreno & Mateos (2012) noted that coarse fiber particles are selectively retained in the gizzard, ensuring a complete grinding and a well-regulated feed flow. Secretion of digestive juices regulates GIT motility and feed intake. Including insoluble fibers in adequate amounts improves the gizzard function and stimulates HCl production in the proventriculus, thus helping control gut pathogens.

Toxin Risk Management

Mycotoxins may have a detrimental impact on the mucosal barrier function in animals (Akbari et al., 2017; Antonissen et al., 2015; Basso, Gomes and Bracarense, 2013; Pierron, Alassane-Kpembi and Oswald, 2016). Mycotoxins like Aflatoxin B1, Ochratoxin A, and deoxynivalenol (DON) not only suppress immune responses but also induce inflammation and even increase susceptibility to pathogens (Yuhang et al., 2023). To avoid intestinal health problems, poultry producers need to emphasize avoiding levels of mycotoxins in feedstuffs and rancid fats that exceed recommended limits (Murugesan et al., 2015; Grenier and Applegate, 2013).



Fusarium mycotoxin

Bacterial lipopolysaccharides (LPS), also known as [endotoxins](#), are the main components of the outer membrane of all Gram-negative bacteria and are essential for their survival. In stress situations, the intestinal barrier function is impaired, allowing the passage of endotoxins into the bloodstream. When the immune system detects LPS, inflammation sets in and results in adverse changes in gut epithelial structure and functionality. Dietary Intervention to bind these endotoxins in the GIT can help mitigate the negative impact of LPS on animals. Given this, [toxin risk management](#) with an appropriate binding agent able to control both mycotoxins and endotoxins appears to be a promising strategy for reducing their adverse effects. Further, adding antioxidants and mycotoxin binders to feed can reduce the effects of mycotoxins and peroxides and is more necessary in ABF programs (Yegani and Korver, 2008).

Essential oils/Phytomolecules

Essential oils (EOs) are important aromatic components of herbs and spices and are used as natural alternatives for replacing antibiotic growth promoters (AGPs) in poultry feed. The beneficial effects of EOs include appetite stimulation, improvement of enzyme secretion related to food digestion, and immune response activation (Krishan and Narang, 2014)

Essential oils (EOs), raw extracts from plants (flowers, leaves, roots, fruit, etc.), are an unpurified mix of different phytomolecules. The raw extract from Oregano is a mix of various phytomolecules (Terpenoids) like carvacrol, thymol and p-cymene. Whereas the phytomolecules are active ingredients of essential oils or other plant materials. Phytomolecule is clearly defined as one active compound.

These botanicals have received increased attention as possible growth performance enhancers for animals in the last decade via their beneficial influence on lipid metabolism, and antimicrobial and antioxidant properties (Botsoglou et al., 2002), ability to stimulate digestion (Hernandez et al., 2004), immune enhancing activity, and anti-inflammatory potential (Acamovic and Brooker, 2005). Many studies have been reported on supplementing poultry diets with some essential oils that enhanced weight gain, improved carcass quality, and reduced mortality rates (Williams and Losa, 2001). The use of some specific EO blends has been shown to have efficacy towards reducing the colonization and proliferation of *Clostridium perfringens* and controlling coccidia infection and, consequently, may help to reduce necrotic enteritis (Guo et al., 2004; Mitsch et al., 2004; Oviedo-Rondón et al., 2005, 2006a, 2010).



Salmonella

Antimicrobial properties of [phytomolecules](#) hinder the growth of potential pathogens. Thymol, eugenol, and carvacrol are structurally similar and have been proven to exert synergistic or additive antimicrobial effects when combined at lower concentrations (Bassolé and Juliani, 2012). In in-vivo studies, essential oils used either individually or in combination have shown clear growth inhibition of *Clostridium perfringens* and *E. coli* in the hindgut and ameliorated intestinal lesions and weight loss than the challenged control birds (Jamroz et al., 2006; Jerzsele et al., 2012; Mitsch et al., 2004). One well-known mechanism of antibacterial activity is linked to their hydrophobicity, which disrupts the permeability of cell membranes and cell homeostasis with the consequence of loss of cellular components, influx of other substances, or even cell death (Brenes and Roura, 2010; Solórzano-Santos and Miranda-Novales, 2012; Windisch et al., 2008; O'Bryan et al., 2015).

Apart from use in feed, the liquid phytomolecules preparations for drinking water use can prove to be beneficial in preventing and controlling losses during challenging periods of bird's life (feed change, handling, environmental stress, etc.). Liquid preparations can potentially reduce morbidity and mortality in poultry houses and thus the use of therapeutic antibiotics. Barrios et al. (2021) suggested that commercial blends of phytomolecule preparations may ameliorate the impact of Necrotic Enteritis on broilers. Further, they hypothesized that the effects of liquid preparation via drinking water were particularly important in improving overall mortality.

In modern, intensive poultry production, the imminent threat of resistant *Eimeria* looms large, posing a significant challenge to the sustainability of broiler operations. *Eimeria* spp., capable of developing resistance to traditional anticoccidial drugs, has become a pressing global issue for poultry operators. The resistance of *Eimeria* to traditional drugs, coupled with concerns over drug residue, has necessitated a shift towards natural, safe, and effective alternatives. It was found that if a drug to which the parasite has developed resistance is withdrawn from use for some time or combined with another effective drug, the sensitivity to that drug may return (Chapman, 1997).

Several phytochemical compounds, including saponins, tannins, essential oils, flavonoids, alkaloids, and lectins, have been the subject of rigorous study for their anticoccidial properties. Among these, saponins

and tannins in specific plants have emerged as powerful tools in the fight against these resilient protozoa. Botanicals and natural identical compounds are well renowned for their antimicrobial and antiparasitic activity so that they can represent a valuable tool against *Eimeria* (Cobaxin-Cardenas, 2016). The mechanisms of action of these molecules include degradation of the cell wall, cytoplasm damage, ion loss with reduction of proton motive force, and induction of oxidative stress, which leads to inhibition of invasion and impairment of *Eimeria* spp. development (Abbas et al., 2012; Nazzaro et al., 2013). [Natural anticoccidial products](#) may provide a novel approach to controlling coccidiosis while meeting the urgent need for control due to the increasing emergence of drug-resistant parasite strains in commercial poultry production (Allen and Fetterer, 2002).

Role of Feed Enzymes Beyond Feed Cost Reduction

Feed enzymes have traditionally been associated with improving feed efficiency and reducing feed costs by enhancing nutrient digestibility. However, their role can extend well beyond economic benefits, profoundly impacting gut health and supporting reduced antimicrobial use in poultry production. Exogenous enzymes reduce microbial proliferation by reducing the undigestible components of feed, the viscosity of digesta, and the irritation to the gut mucosa that causes inflammation. Enzymes also generate metabolites that promote microbial diversity which help to maintain gut ecosystems that are more stable and more likely to inhibit pathogen proliferation (Bedford, 1995; Kiarie et al., 2013).

High dietary levels of non-starch polysaccharides (NSPs) can increase the viscosity of digesta. This leads to an increase in the retention time of the digesta, slows down the nutrient digestion and absorption rate, and may lead to an undesired increase in bacterial activity in the small intestine (Langhout et al., 2000; Smits et al., 1997). Further the mucosal barrier function may also be adversely affected. To solve this problem, exogenous enzymes, such as arabinoxylanase and/ or β -glucanase, are included in feed to degrade viscous fibre structures (Bedford, 2000). The use of xylanase and β -glucanase may also cause oligosaccharides and sugars to be released, of which certain, for example, arabinoxylan oligosaccharides, may have prebiotic properties (De Maesschalck et al., 2015; Niewold et al., 2012).

[New generation xylanases](#) coming from family GH-10 are known to effectively breakdown both soluble and insoluble arabinoxylans into a good mixture of smaller fractions of arabino-xylo-oligosaccharides (AXOS) and xylo-oligosaccharides (XOS), which exert a prebiotic effect in the GIT. Awati et.al. (2023) observed that a novel GH10 xylanase contributed to positive microbial shift and mitigated the anti-nutritional gut-damaging effects of higher fiber content in the feed. With a substantial understanding of the mode of action and technological development in enzyme technology, nutritionists can reliably consider new-generation xylanases for gut health optimization in their antibiotic reduction strategy.

Conclusions

The challenge of mitigating antimicrobial resistance (AMR) in poultry production necessitates a multidimensional approach, with gut health at its core. Dietary interventions, such as organic acids, probiotics, prebiotics, phytomolecules, toxin binders, and feed enzymes, promote gut resilience, enhance immune responses, and reduce reliance on antimicrobials. These strategies not only support the health and productivity of poultry but also address critical global issues of AMR and food safety.

While no single solution fits all circumstances, integrating these dietary tools with robust biosecurity measures, sound management practices, and continued research on species-specific and field-applicable strategies can pave the way for sustainable, antibiotic-free poultry production. The transition to such systems aligns with regulatory requirements and consumer expectations while contributing to global efforts against AMR.

Ultimately, embracing holistic and innovative dietary strategies ensures a resilient gastrointestinal environment, safeguarding poultry health and productivity while protecting public health and environmental sustainability for future generations.

References: The references can be made available upon request to the author.

How xylanase can enhance swine productivity



By Dr. Ajay Awati, Director of Enzymes, EW Nutrition

The use of by-products and high-fiber ingredients in feed formulations has increased in swine operations. Driven by both economic and sustainability goals, this shift has emphasized the importance of understanding the role of dietary fibers and carbohydrases in swine nutrition and health (Petry & Patience, 2020). These feeds rich in fiber are generally considered to have low nutritional value due to the lower digestive energy or amino acid levels when compared to concentrated feeds with high starch or proteins (Woyengo et al., 2014).

Dietary fiber is vital in pig nutrition, necessitating a baseline inclusion to support regular digestive tract functions (Wenk, 2001). Incorporating fiber into the diets of monogastric animals raises concerns due to its correlation with reduced nutrient utilization and diminished net energy levels (Noblet; Le Goof, 2001). High-fiber diets can present challenges for inclusion in monogastric animals' feeds, especially young

animals, due to their bulky nature and restricted ability to ferment fiber, impacting nutrient uptake based on fiber type, the age of the pig, and diet composition (Bach Knudsen et al., 2012).

Moreover, the apparent ileal digestibility (AID) of nutrients is adversely affected by dietary fiber, attributed to the small intestine's deficiency in endogenous enzymes necessary for breaking down these bonds (Bach Knudsen et al., 2012).

This article aims to demonstrate how enzymatic degradation of arabinoxylans, particularly through xylanase enzymes, can mitigate anti-nutritional effects and enhance the nutritional value of high-fiber swine diets, thereby improving animal health and performance.

Into the World of Arabinoxylans

In plants classified as monocotyledonous, such as cereals, the main non-starch polysaccharides (NSP's) components of the cell wall are arabinoxylans, cellulose and β -glucan (Bach Knudsen, 1997). Arabinoxylans represent a complex group of dietary fibers with significant implications for swine nutrition and health. Their structural heterogeneity can influence physicochemical properties, biological activities, and affect pigs' gut microbiota and immune system. Present in both soluble and insoluble forms, it consists of a backbone of xylose residues with arabinose side chains, playing a crucial role in the nutritional dynamics within swine diets (Mudgil & Barak, 2013).

The fermentability of corn-based dietary fiber is limited by its insoluble fraction and complex branched structure; impacting the digesta transit rate and reducing the digestibility of nutrients (Gutierrez et al., 2013). Supplementing exogenous carbohydrases offers a viable approach to enhance the utilization of fiber that is otherwise difficult to ferment, potentially amplifying its positive effects.

Xylanase's Impact on Fiber and Gut Health

Non-digestible carbohydrates may be fermented by microbial populations along the gastrointestinal tract to synthesize short-chain fatty acids that may be absorbed and metabolized by the pig. Such indigestible carbohydrates consist of specific disaccharides, oligosaccharides, resistant starches, and non-starch polysaccharides. The presence and composition of these indigestible carbohydrates in pig diets vary based on the types of feed ingredients incorporated into their meals (Navarro et al., 2019). Xylanase works on the hydrolysis of the arabinoxylan fraction of NSPs. The NSPs present in the walls of plant cells encapsulate nutrients, making them unavailable for the action of the animal's own digestive enzymes. Moreover, NSPs exhibit a high affinity for water within the gastrointestinal lumen, leading to elevated digesta viscosity. This increased viscosity reduces gastrointestinal motility, facilitating an environment conducive to the proliferation of pathogenic microflora (Choct, 1998). The advantageous outcomes of enzyme supplementation arise from the enzymatic disruption of intact cellular membranes, leading to the release of sequestered nutrients, or are a consequence of modifying the physicochemical properties of non-starch polysaccharides, due to changes in viscosity and water-holding capacity and/or changes in the composition and content of bacteria in the intestine (Bedford, M. R., & Classen, 1992).

Arabinoxylans in Cereal Grains and Their By-products

Factors such as genetics, climate, maturity stage, fertilizer use, and post-harvest storage time influence the proportion of total cell wall polysaccharides in cereal grains. These factors vary across production systems and countries, depending on the availability of feed resources (Paloheimo et al., 2010).

Cereal grains and their by-products, including wheat bran, corn distillers dried grains with solubles (DDGS), and rice husks, serve as significant sources of arabinoxylans. Their incorporation into swine diets is

growing due to economic advantages.

The ethanol industry's growth has increased the availability of distillery by-products. Brazil alone generates an estimated 366 million tons of DDGS annually (USDA, 2017). Among these by-products, distiller-dried grains are prevalent, especially in the U.S. pork industry as feed ingredients.

Corn, wheat, and barley, as staple ingredients in swine feed, exhibit significant variations in their NSP and arabinoxylans content. In grain form, corn contains 4.7% AX with a soluble component of 0.5%, while wheat has a higher arabinoxylans content at 7.3% with 1.8% being soluble. Barley stands out with the highest arabinoxylans content at 8.4%, of which 1.2% is soluble, reflecting its rich fiber composition. The processing into flour results in a reduction of arabinoxylans content across all three cereals, highlighting the impact of processing on dietary fiber availability (Knudsen, 2014).

Rice distillers' by-product is recognized as a valuable protein source, boasting high crude protein levels. Nonetheless, its high fiber content can restrict usage (Huang et al., 2017). Wheat bran is particularly rich in arabinoxylans, enhancing its dietary fiber content. DDGS also contain significant amounts of both soluble and insoluble arabinoxylans, resulting from the corn kernel's residual non-starch polysaccharides (Agyekum & Nyachoti, 2017).

It is essential to understand the specific levels of arabinoxylans in these components to create balanced diets that optimize nutritional benefits while minimizing potential anti-nutritional effects.

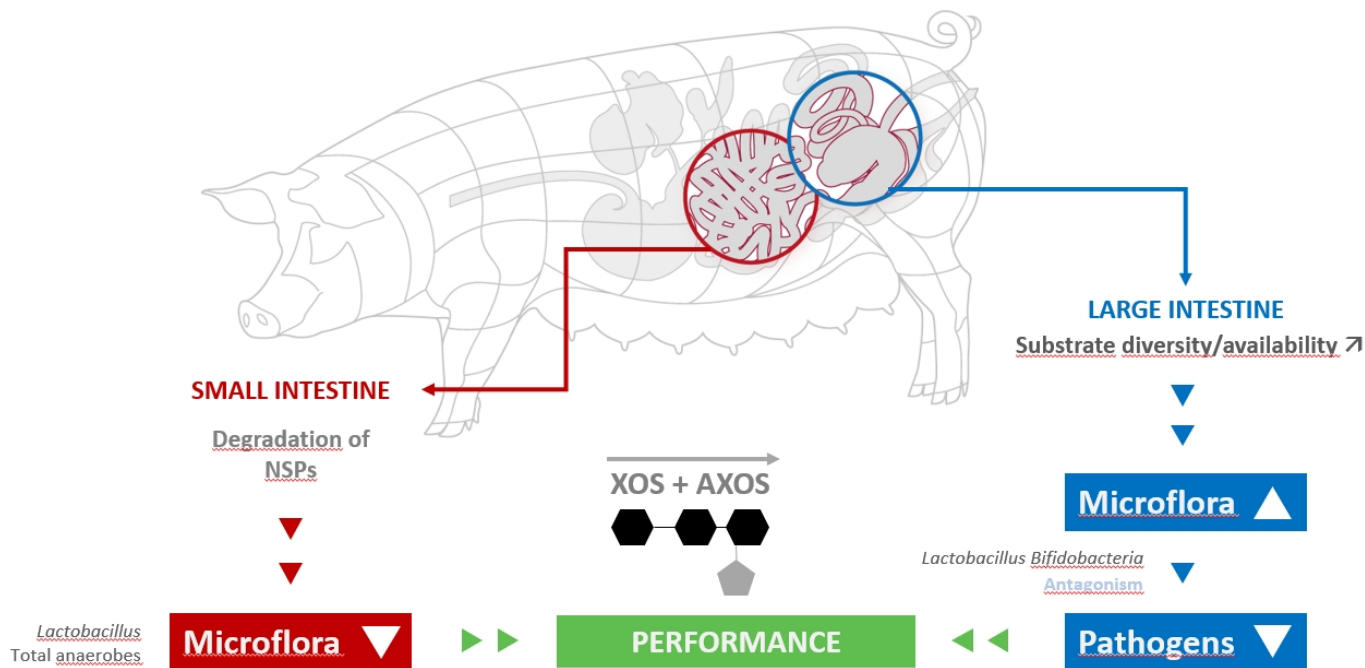
Addressing Arabinoxylan Degradation

Xylanases target specific substrates, necessitating the presence of arabinoxylans for their effective action. The complex structure of arabinoxylans makes them resistant to degradation by the swine's endogenous enzymes, presenting a dual challenge: how to harness the beneficial effects of soluble arabinoxylans while mitigating the negative impacts of their insoluble counterparts.

These enzymes specifically cleave the 1,4- β -D-xylosidic bonds in arabinoxylans, randomly targeting xylose linkages within the xylan structure. Each enzyme type has a unique pattern of degradation (Collins et al., 2005) and GH 10 xylanases specialize in breaking down arabinoxylans with high arabinose substitution into smaller oligosaccharides. These oligosaccharides are valuable for fermentation, serving as energy sources or prebiotics.

Also, this group of enzymes action not only reduces gut viscosity but can lead to enhanced feed efficiency, growth performance, and overall health of swine by improving the digestibility of fibrous components in feed (Lærke et al., 2015). GH 10 xylanases often have optimal activity at pH levels found in the animal gut, and their thermal stability ensures they retain activity under feed processing temperatures. Lei et al. (2016) highlighted the efficacy of xylanase in improving nutrient digestibility and overall feed efficiency. By targeting the arabinoxylans present in swine diets, xylanase enzymes facilitate a more efficient conversion of feed into energy, contributing to improved growth rates and performance metrics.

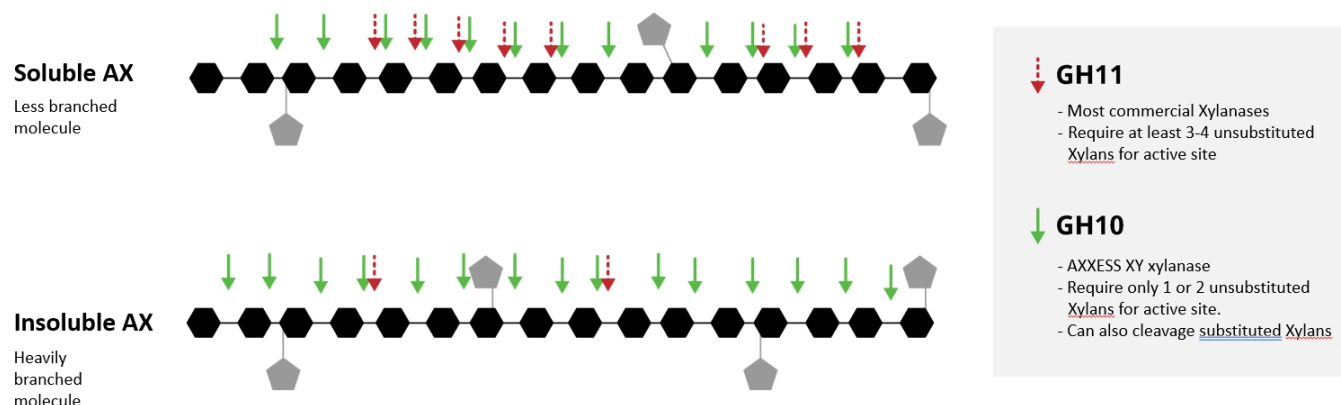
As detailed by Tiwari, Singh, & Jha (2019), arabinoxylans undergo fermentation in the gut, leading to the production of short-chain fatty acids (SCFAs) that beneficially alter the gut microbial ecology. The application of GH 10 xylanases has been highlighted for its potential to significantly enhance the degradation of arabinoxylans, thereby improving the fermentation process and increasing the yield of SCFAs. This enzymatic breakdown facilitates more efficient nutrient absorption and overall better gastrointestinal health, directly influencing swine growth and performance positively.



A study reveals that xylanase supplementation significantly reduces mortality rates in pigs in a dose-dependent manner. With mortality rates dropping from 4.16% in the control group to as low as 2.25% with the highest xylanase dosage, the results highlight xylanase's potential to improve gut health and increase survival rates. This suggests a promising approach for boosting pig well-being and reducing the reliance on enteric antibiotics, marking a significant stride in sustainable animal nutrition practices. (Zier-Rush et al., 2016).

The research conducted by Petry et al. (2020) demonstrated that xylanase increased the digestibility of non-starch polysaccharides, particularly arabinoxylan, in diets high in insoluble corn fiber. This improvement in nutrient absorption highlights xylanase's role in optimizing the use of high-fiber ingredients in swine diets, thereby enhancing animal health and performance. Due to its cost-effectiveness and nutrient profile, xylanase supplementation enhances the nutritional value of DDG in swine diets.

The strategic implementation of xylanase in swine diets represents a promising approach to the challenges posed by high-fiber feed ingredients. By improving the digestibility of arabinoxylans and other complex carbohydrates, xylanase supplementation can mitigate the anti-nutritional effects of insoluble fibers, enhance feed efficiency, and support optimal growth and health outcomes in swine.



Enhancing Swine Productivity with Enzyme Solutions

With the growing incorporation of co-products in swine feed, there arises a crucial need to transform the high fiber content into a beneficial asset for the animals. The strategic incorporation of enzyme solutions, particularly xylanase enzymes, into swine feed formulations emerges as a scientifically supported approach to significantly enhance the digestibility of high-fiber diets. This method effectively addresses the nutritional intricacies posed by arabinoxylans, facilitating improved feed utilization. Moreover, the action of xylanase enzymes extends beyond enhancement of nutrient absorption; it plays a pivotal role in promoting the health and performance of swine. Such targeted nutritional strategies are vital in the context of swine production systems, highlighting the necessity of integrating these enzymatic solutions to achieve optimal animal health, growth, and productivity.

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Overcoming Challenges of Xylanase Inhibitors in Animal Feeds



By Dr. Ajay Awati, Global Director Enzymes, EW Nutrition

In recent years, the scientific understanding of xylanase inhibitors (XIs) and their impact on animal nutrition has grown significantly. Xylanase, a crucial enzyme used to enhance nutrient availability in feed, can face challenges from XIs present in cereal grains. This article explores the evolution of plant protection mechanisms, the economic impact of XIs, and the development of a novel xylanase, Axxess XY, resistant to these inhibitors.

Xylanase inhibitors - an evolutionary protection mechanism of plants

Xylanase inhibitors (XI) are a classic example of the evolutionary development of protection mechanisms by cereal plants against pathogens. Microorganisms, such as fungal pathogens, involve the degradation of xylan as one of the mechanisms in pathogenesis (Choquer et al., 2007). There are also other mechanisms by which microorganism-produced xylanases affect plants.

To protect themselves, plants evolved xylanase inhibitors to prevent the activities of xylanases. XIs are plant cell wall proteins broadly distributed in monocots. There are three classes of XIs with different structures and inhibition specificities (Tundo et al., 2022):

1. Triticum aestivum xylanase inhibitors (TAXI)
2. Xylanase inhibitor proteins (XIP), and
3. Thaumatin-like xylanase inhibitors (TLXI).

Xylanase inhibitors have an economic

impact

In animal nutrition, xylanases are widely used in diets containing cereal grains and other plant materials to achieve a higher availability of nutrients. The inhibitory activity of XIs prevents this positive effect of the enzymes and, therefore, makes them economically relevant. Studies have reported that higher levels of XIs negatively impact broiler performance. For example, in one of the studies, broilers fed with grains of a cultivar with high inhibitory activity showed a 7% lower weight on day 14 than broilers fed with grains of a cultivar with less inhibitory activity (Madsen et al., 2018). Another study by Ponte et al. (2004) also concluded that durum wheat xylanase inhibitors reduced the activity of exogenous xylanase added to the broiler diets.

Xylanase inhibitors can withstand high temperatures

Even though XIs can impact the performance of exogenous xylanase in different ways, only minor attention was paid to the reduction of xylanase's susceptibility to xylanase inhibitors during the xylanase development in the last decades. Firstly, the issue was ignored mainly through the assumption that XIs are denatured or destroyed during pelleting processes. However, Smeets et al. (2014) showed that XIs could sustain significant temperature challenges. They demonstrated that after exposing wheat to pelleting temperatures of 80°C, 85°C, 92°C, and 95°C, the recovery of inhibitory activity was still 99%, 100%, 75%, and 54%, respectively. Furthermore, other studies also confirmed that conditioning feed at 70-90°C for 30 sec followed by pelleting had little effect on the XI activity in the tested feed, showing that xylanase inhibitors are very likely present in most xylanase-supplemented feeds fed to animals.

Do we only have the problem of xylanase inhibitors in wheat?

No. After first reports of the presence of xylanase inhibitors in wheat by Debyser et al. (1997, 1999), XIs were also found in other cereal grains (corn, rice, and sorghum, etc.), and their involvement in xylanase inhibition and plant defense has been established by several reports (Tundo et al., 2022).

In most of the countries outside Europe, exogenous xylanase is used not only in wheat but also in corn-based diets. Besides broiler feeds, also other animal feeds, such as layer or swine feed being part of more mixed-grain diets, are susceptible to the inhibitory activity of XIs. Nowadays, the situation is getting worse with all the raw material prices increasing and nutritionists tending to use other feed ingredients and locally produced cereals. They need a xylanase which is resistant to xylanase inhibitors.

Xylanases' resistance to XIs is crucial - Axxess XY shows it

To prevent xylanases from losing their effect due to the presence of xylanase inhibitors, the resistance of new-generation xylanases to these substances is paramount in the development process, including enzyme discovery and engineering.

In the past 25 years, scientists have learned much about XI-encoding genes and discovered how xylanase inhibitors can block microbial xylanases. Additionally, there has been a significant increase in understanding the structural aspects of the interaction between xylanases and XIs, mainly how xylanase inhibitors interact with specific xylanases from fungi or bacteria and those in the GH10 or GH11 family. With such understanding, a new generation xylanase, Axxess XY, was developed. Besides showing the

essential characteristics of intrinsic thermostability and versatile activity on both soluble and insoluble arabinoxylan, it is resistant to xylanase inhibitors.

Axxess XY takes xylanase application in animal feeds to the next level.

Axxess XY outperforms other xylanases on the market

Recent scientific developments (Fierens, 2007; Flatman et al., 2002; Debyser, 1999; Tundo et al., 2022; Chmelova, 2019) and internal research can be summarized as follows:

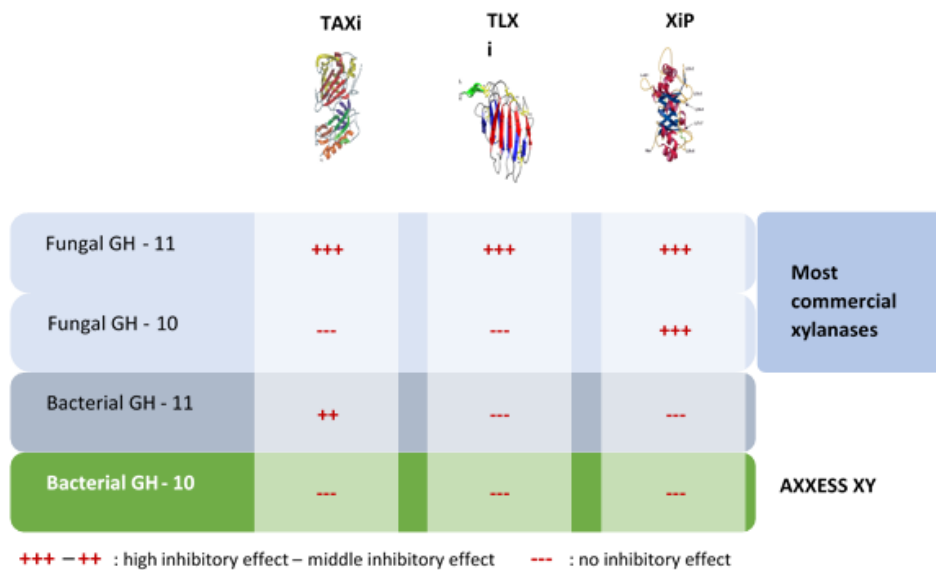


Figure 1: Schematic summary of the susceptibility of different xylanase to xylanase inhibitors from three main groups.

The high resistance to xylanase inhibitors is one of the reasons that a novel xylanase with bacterial origin and from the GH-10 family was chosen to be Axxess XY. EWN innovation, together with research partners, made an interesting benchmark comparison between xylanases that are commercially sold by different global suppliers and Axxess XY. For these trials, all xylanase inhibitors from wheat were extracted. The inhibitors, together with the respective xylanase, were incubated at 40 °C (to mimic birds' body temperature) for 30 mins. Then, the loss of xylanase activity was calculated by analyzing remaining activity after incubation. Results are shown below in Figure 2. There were varying levels of activity loss observed in the different commercially sold xylanases. In some xylanases, the losses were alarmingly high. However, Axxess XY was not inhibited at all.

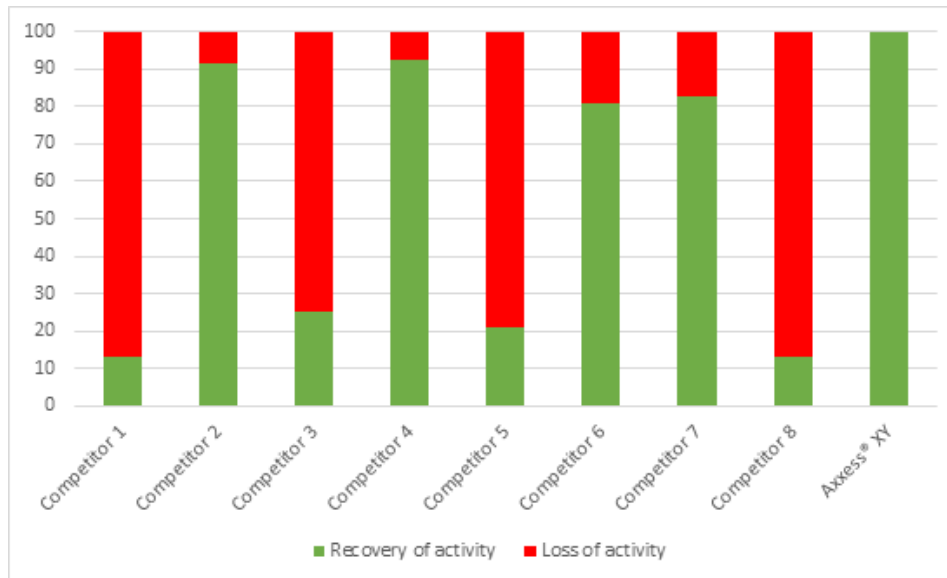


Fig. 2: Extracted total xylanase inhibitors from wheat incubated with the respective xylanase at 40°C for 30 mins. – Loss of activity after incubation with xylanase inhibitors

Conclusion:

Xylanase inhibitors are present in all cereal grains and, unfortunately, heat tolerant (up to 90°C, still 75% of inhibition activity was retained). Regardless of the diets used, there is a possibility that the xylanase used may come across xylanase inhibitors, resulting in a loss of activity. More importantly, this can lead to inconsistent performance.

For effective, consistent, and higher performance of NSP enzyme application, it is a must to use xylanase that is resistant to xylanase inhibitors.

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