

# Unlocking Optimum Poultry Performance: Harnessing the Power of GH10 Xylanase



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Exogenous feed enzymes are increasingly utilized in poultry diets to manage feed costs, mitigate the adverse effects of anti-nutritional factors, and enhance nutrient digestion and bird performance. These enzymes are primarily employed to bolster the availability of nutrients within feed ingredients. Among the various enzymes utilized, those capable of breaking down crude fiber, starch, proteins, and phytates are commonly integrated into animal production systems.

In monogastric animals such as poultry and swine, a notable deficiency exists in the endogenous synthesis of enzymes necessary for the hydrolysis of non-starch polysaccharides (NSPs) like xylan ([McLoughlin et al., 2017](#)). This deficiency often manifests in poultry production as a decline in growth performance, attributed to increased digesta viscosity arising from the prevalence of NSPs in commonly utilized poultry feed ingredients. Without sufficient endogenous enzymes to degrade xylan, NSPs can increase digesta viscosity, encase essential nutrients, and create a barrier to their effective digestion. In response to this issue, monogastric animal producers have implemented exogenous enzymes such as xylanases into the feeds for swine and poultry to degrade xylan to short-chain sugars, thus reducing intestinal viscosity and improving the digestive utilization of nutrients ([Sakata et al., 1995](#); [Aragon et al., 2018](#))

# Understanding Xylanase Enzymes

Xylanase enzymes belong to the class of carbohydrases that specifically target complex polysaccharides, such as xylan, a backbone nonstarch polysaccharide (NSP) prevalent in plant cell walls. These enzymes catalyze the hydrolysis of xylan into smaller, more digestible fragments, such as arabino-xylo-oligosaccharides (AXOs) and xylo-oligosaccharides (XOs), thereby facilitating the breakdown of dietary fiber in poultry diets.

## Mechanism of action

It is generally agreed that the beneficial effects of feed xylanase are primarily due to the reduction in viscosity. Studies have shown that supplementing xylanases to animal feeds reduces digesta viscosity and releases encapsulated nutrients, thus improving the overall feed digestibility and nutrient availability ([Matthiesen et al., 2021](#)). The reduction in digesta viscosity by adding xylanase is achieved by the partial hydrolysis of NSPs in the upper digestive tract, leading to a decrease in digesta viscosity in the small intestine ([Choct & Annison, 1992](#)).

## GH10 vs. GH11 Xylanases

Well-characterized xylanases are mostly grouped into glycoside hydrolase families 10 (GH10) and 11 (GH11) based on their structural characteristics (amino acid composition), mode of xylan degradation, the similarity of catalytic domains, substrate specificities, optimal conditions, thermostability, and practical applications.

## Why are GH10 xylanases more efficient in animal production?

While both GH10 and GH11 xylanases act on the xylan main chain, these two enzyme types have different folds, substrate specificities, and mechanisms of action ([Biely et al., 2016](#)). The GH10 xylanases are more beneficial in animal feed production due to their efficient mechanism of action, broader substrate specificity, and better thermostability, as discussed below.

## GH10 xylanase exhibits broader substrate specificity

Generally, the GH10 xylanases exhibit broader substrate specificity and can hydrolyze various forms of xylan, including soluble and insoluble substrates. On the other hand, GH11 xylanases have a narrower substrate specificity and are primarily active on soluble xylan substrates. GH10 xylanases exhibit higher catalytic versatility and can catalyze the cleavage of the xylan backbone at the nonreducing side of substituted xylose residues, whereas GH11 enzymes require unsubstituted regions of the xylan backbone ([Collins et al., 2005](#); [Chakdar et al., 2016](#)).

As a result, GH10 xylanases generally produce shorter xylo-oligosaccharides than members of the GH11 family ([Collins et al., 2005](#)). Moreover, as shown in Fig.1, the GH10 xylanase can rapidly and effectively break down xylan molecules.

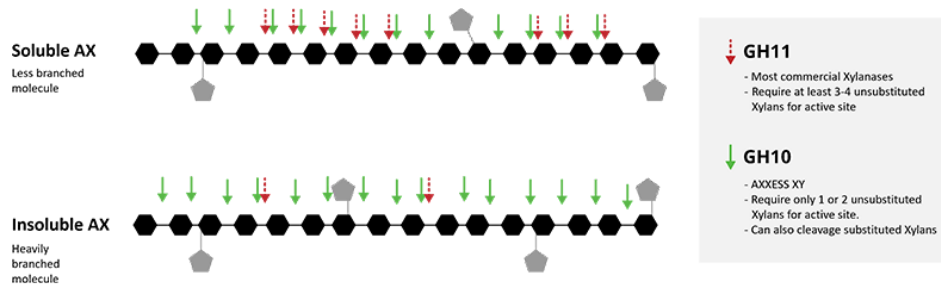


Fig.1.: Activity of a bacterial GH10 xylanase against soluble and insoluble arabinoxylans

## Higher thermostability

Enzymes are proteins, and the protein's primary structure determines their thermostability. The enzyme protein tends to denature at higher than tolerable temperatures, rendering it inactive. An enzyme's high-temperature tolerance ensures its efficacy throughout the pelleted feed manufacturing. This results in consistent enzyme activity in the finished feed, subsequent gut health, and predictable performance benefits.

Xylanases with higher thermostability are more suitable for applications requiring high-temperature processes. An intrinsically heat-stable bacterial xylanase maintains its activity even under high-temperature feed processing conditions, such as pelleting.

A study conducted at the University of Novi Sad, Serbia (Fig. 2), with three pelleting temperatures (85 °C, 90 °C, and 95 °C) and conditioning times of 4 and 6 mins, showed that Axxess XY, an intrinsically thermostable GH10 xylanase, demonstrated more than 85% recovery even at 4 to 6 mins conditioning time and 95 °C temperature.

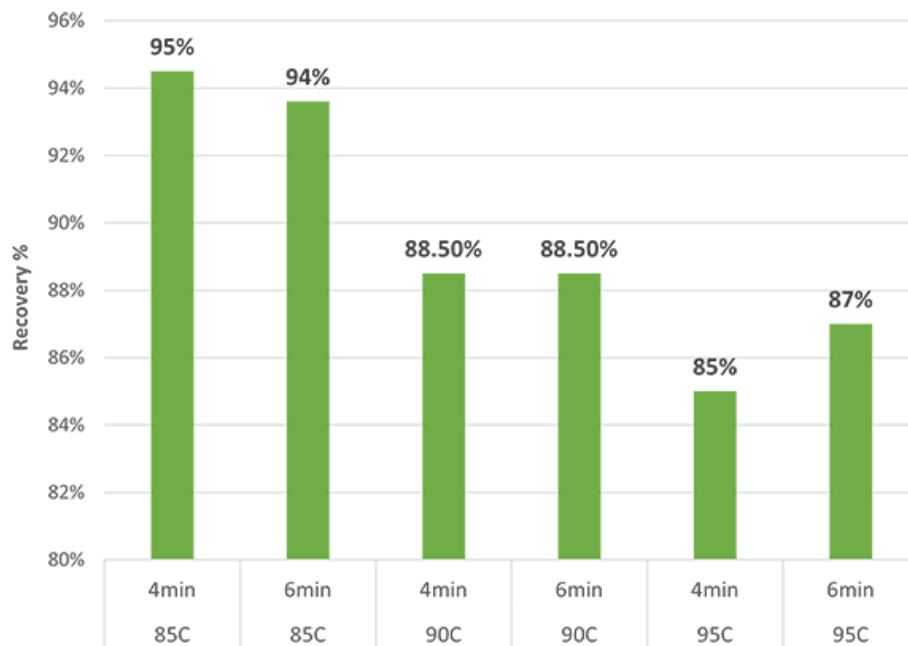


Fig.2: Optimum recovery of Axxess XY at elevated conditioning time and temperatures

Maintaining consistently optimum enzyme activity is crucial for realizing the benefits of enzyme inclusion in feed under challenging feed processing conditions.

# Conclusion

In conclusion, exogenous feed enzymes, including xylanase, have gained widespread recognition for their pivotal role in poultry nutrition. The increasing use of xylanase is attributed to its ability to effectively manage feed costs while incorporating high-fiber ingredients without compromising poultry performance. However, the efficacy of xylanase is based on several factors, including its mode of action, substrate specificity, catalytic efficacy, and thermostability. Selecting the appropriate xylanase enzyme tailored for specific needs is crucial to harnessing its full benefits.

A GH10 xylanase, such as Axxess XY, designed explicitly as a feed enzyme, offers distinct advantages in poultry production. Its efficient mechanism of action, broader substrate specificity, and superior thermostability make it a preferred choice for optimizing animal performance. Notably, Axxess XY exhibits exceptional activity against soluble and insoluble arabinoxylans, thereby enhancing nutrient utilization, promoting gut health, and ultimately elevating overall performance levels in poultry.

Incorporating specialized GH10 Xylanase enzymes like Axxess XY represents a strategic approach to unlocking the nutrients in feedstuffs, ensuring optimal performance, and maximizing profitability in the poultry business.

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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:

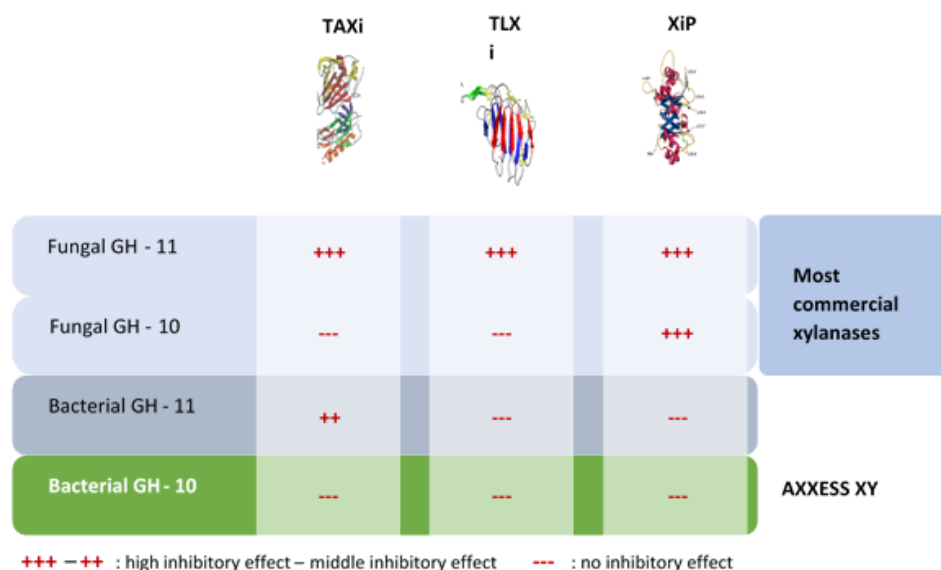


Figure 1: Schematic summary of the susceptibility of different xylanase to xylanase inhibitors from three main groups.

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.

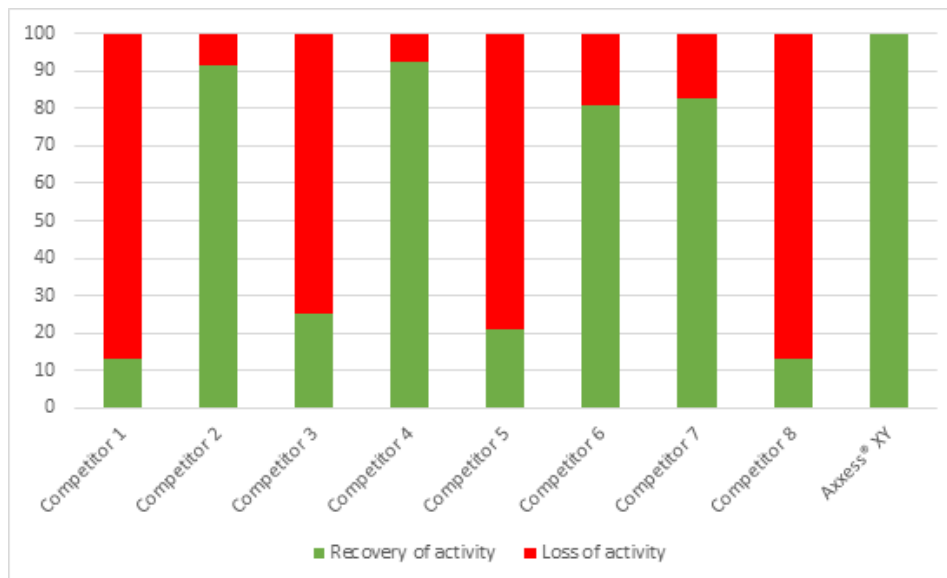


Fig. 2: Extracted total xylanase inhibitors from wheat incubated with the respective xylanase at 40°C for 30 mins. – Loss of activity after incubation with xylanase inhibitors

## 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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## Xylanase solutions for broiler



# feed: Enzyme innovation finally hits the market



By **Dr. Ajay Awati**, Global Category Manager for Gut Health and Nutrition, EW Nutrition, and **Dr. Howard Simmins**, InSci Associates

**After 30 years of stagnating solutions, in-feed xylanase innovation has finally arrived - with a complete focus on the needs of the broiler feed industry.**



It has been over 30 years since xylanase was first introduced in broiler diets in Europe. In the meantime, it has been widely used worldwide with few, if any, major improvements. While the animal feed industry evolved in terms of production landscape, feed processing technologies and [use of various by-products](#), xylanase enzyme technology did not keep pace. In fact, it did not evolve to meet customers' changing needs and provide that much-needed flexibility of diet formulation for a commercial nutritionist. The wait is over: new in-feed xylanase technology is about to revolutionize broiler nutrition.

## Why we need innovative xylanase enzymes for broiler production

Initially, in the 1980s, xylanase was leveraged from industries unrelated to animal production into the feed business. Gut viscosity had been a continuing problem in broiler chickens fed wheat-based diets. It led to an increased risk of enteric disease, generally reducing performance. Xylanase was shown to reduce gut viscosity in wheat-based feed by breaking down soluble arabinoxylans.

As a result, the birds grew as well as if they were fed a low-viscosity corn/soya diet. An additional benefit was lower disease risks from the reduced level of anti-nutritional factors (ANFs) and the multiple negative effects of viscosity in the intestine.

In addition to reducing viscosity, xylanase augments the release in the small intestine of nutrients from previously undigested feedstuffs. The outcome has been the use of an energy matrix value for xylanase, which essentially helps diets through least-cost formulation.

These effects account for the growth of xylanase use in the monogastric feed market. Today, the penetration is above 50%.

## Limitations of existing xylanase solutions

Leveraging xylanases from other industries for viscosity reduction in poultry comes with a couple of distinct limitations:

1. Most broiler diets around the globe are based on a corn-soybean formulation, which contains far higher levels of insoluble arabinoxylans than soluble arabinoxylans. In such cases, viscosity is a relatively minor issue compared to the anti-nutritional effect of insoluble arabinoxylans.
2. The reduction of gut viscosity is less relevant in other poultry sectors, such as laying hens and turkeys.

Commercial xylanases would be required to **break down insoluble NSPs** in which substrate activity may be limited and difficult to predict. Fiber constituents of different cereal grains used in feed are highly variable. By- and co-products derived from cereals contain even more complex fiber components, altered further by the manner of processing that the raw material has undergone.

Additionally, poultry response is highly variable: For an individual bird, the effectiveness of xylanase depends on the enzyme's interaction with feed in the gastrointestinal tract (GIT) of the animal, which varies depending on the species and the animal's age. This may explain why xylanase penetration on the feed market is not as high as that of phytase.

## GH10: the next-level xylanase for feed application

A xylanase for feed is required to provide multiple functionalities, of which four are essential:

1. Capacity to break down soluble and insoluble arabinoxylan across a range of typical feedstuffs
2. Rapid activity at optimal pH in the preferred section of the GIT
3. No inhibition in the presence of xylanase inhibitors
4. Comprehensive feed processing thermostability

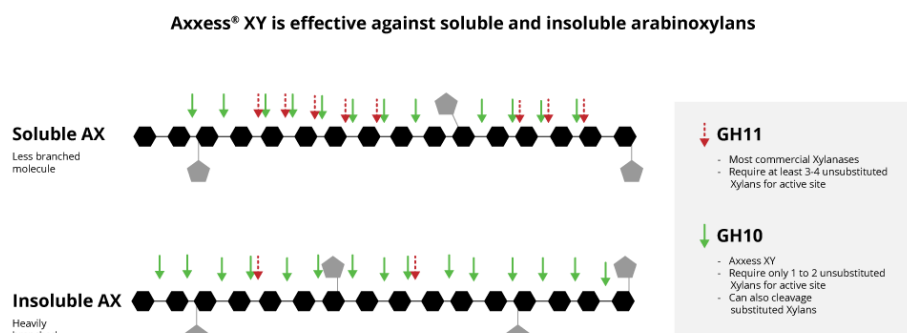
The GH11 family of xylanases commonly used in feed does not offer these aggregated benefits. They successfully reduce soluble NSPs in wheat-based diets, hence lowering the viscosity level in the broiler GIT. However, they are less effective in the presence of insoluble NSPs in which the arabinoxylan backbone is more complex.

## Why GH10 instead of GH11?

The explanation for this can be found in the 3-dimensional structure of the GH11 xylanase. The activity of GH11 xylanases requires 3 or 4 consecutive unsubstituted xylan monomers on the backbone to find an active site. That is why they are hindered by the presence of branches, or side chains, on arabinose backbones. Consequently, they are highly specific, favoring the particularly low-branching wheat backbone.

Xylanases from the GH10 family are entirely different. Although well known, they have not been used in feed yet. The GH10 xylanases require two or fewer consecutive unsubstituted xylan monomers on the backbone to find an active site. Therefore, they can act on xylose residues near branches. This results in both more and shorter xylo-oligomers than found with GH11 xylanases. In simple terms, the GH10 xylanases have a less deep cleft than the GH11 xylanases, providing greater catalytic versatility (Pollet 2010).

Significantly, this potentially allows a broader range of feedstuffs to be incorporated into the complete diet, including co- and by-products, while maintaining performance. Therefore, **with GH10, higher levels of cheaper ingredients may be included, with a significant value proposition of further reducing feed costs.**



## GH10 xylanases generate a range of important prebiotics

As early as 1995, it was proposed that xylanase may affect microbial activity in the gastrointestinal tract through the provision of fermentable oligosaccharides and low molecular weight polysaccharides. These are produced from the hydrolysis of soluble and insoluble arabinoxylans in cereals.

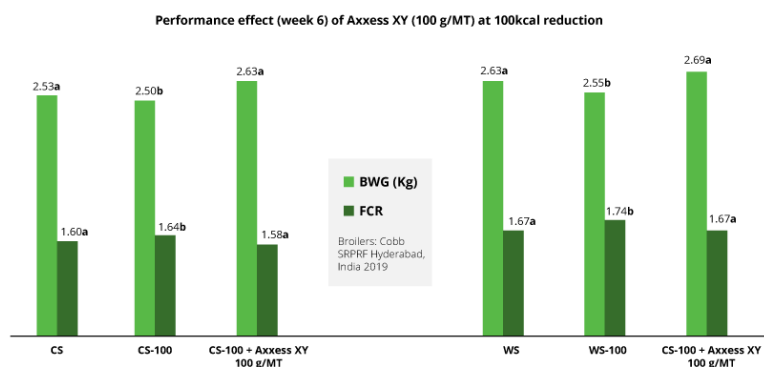
A development of particular interest is that the GH10 xylanases break down the backbone of different fibre components into small xylooligosaccharides (XOS) and arabino-xylooligosaccharides (AXOS). This action, research shows, has value in supporting the selective growth of fibre-degrading bacteria in the large intestine, conferring positive effects on the host's health.

The most well-known probiotic strains belong to bifidobacteria and lactobacilli, which have quite different XOS and AXOS utilization systems. *Bifidobacterium adolescentis* has been shown to consume AXOS and XOS, whereas *Lactobacillus brevis* utilises only XOS. The outcome is that AXOS releases butyrate, the short-chain fatty acid, which can improve the host's gut barrier function, as well as reduce *Salmonella* colonization in broilers. Alongside these health benefits, their presence may improve performance also by reducing FCR. (Courtin et al. 2008; Ribeiro et al. 2018)

As mentioned earlier, the GH10 xylanase requires only two consecutive unsubstituted xylan monomers to cleave the xylan main chain, whereas a GH11 xylanase requires 3 or 4 consecutive unsubstituted xylan monomers. Therefore, the number of potential AXOS and XOS oligomers is higher from the action of the GH10 xylanase. This results in a wider size range of oligomers. The range is valuable as the effect is spread across the large intestine, each oligomer having a different fermentation rate. Consequently, the large intestine's microbial activity becomes saccharolytic, which potentially reduces the undesirable products of proteolytic degradation, such as phenols and cresols.

Prebiotic combinations will vary depending on the substrate available. However, there is more flexibility in breaking down insoluble NSPs across different feedstuffs using GH10 xylanase compared to GH-11 xylanase.

### Efficacy in both corn soy and wheat soy diets (42 days)



## The future of xylanase: Reducing feed costs through flexible formulation

EW Nutrition's GH10-based AXCESS XY xylanase, specifically developed for animal feed, has a wide-ranging activity across typical substrates, both in corn-soy and wheat-soy diets. It also allows for a greater proportion of cheaper ingredients, enabling increased flexibility in feedstuff choices and resulting in more stable feed pricing. The activity of the GH10 xylanase in producing oligomers from the breakdown of the arabinoxylan backbone also indicates that it can produce a greater number and diversity of valuable prebiotics that sustain the growth of fiber-degrading microbiota. Consequently, the metabolism of the large intestine is shifted from proteolytic to saccharolytic, which supports the animal's general health.

The combination of these benefits from using this xylanase results in a bird with a balanced digestive system that is more robust in the face of environmental and health challenges, supporting better performance. Furthermore, **this novel enzyme solution gives nutritionists a reliable tool to reduce feed costs by being flexible in diet formulation and opportunistic in using raw materials while maintaining consistency in animal performance.** Especially in these [times of supply problems and raw material price hikes](#), such advantages are invaluable.

The naturally thermostable AXCESS XY 1000G is the most advanced xylanase yet. It is a GH10 xylanase that delivers what the industry has been asking for: a fiber-degrading enzyme suited for all poultry feed.

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