Overcoming Challenges of Xylanase Inhibitors in Animal Feeds

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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 (Madesen 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:

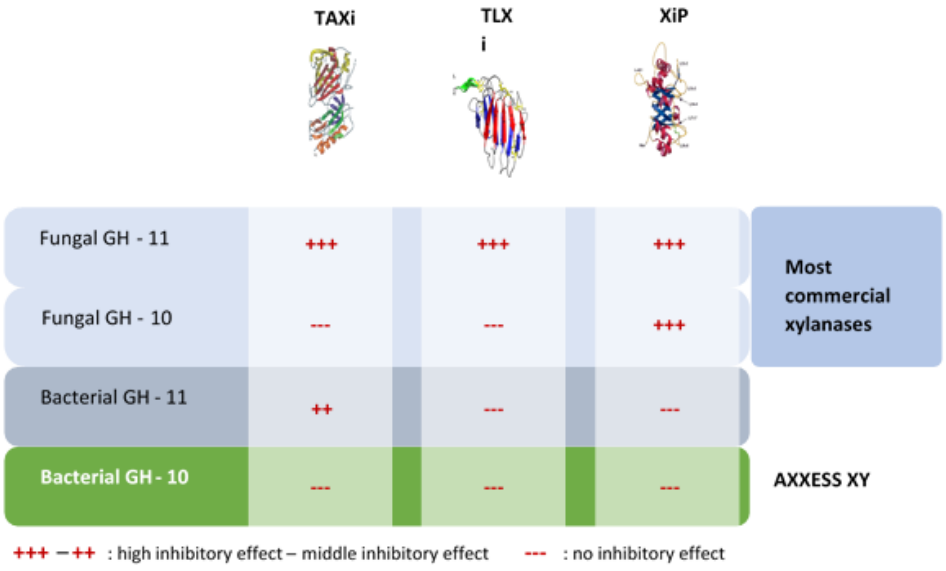

Figure 1: Schematic summary of the susceptibility of different xylanase to xylanase inhibitors from three main groups.

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 ^{0}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.

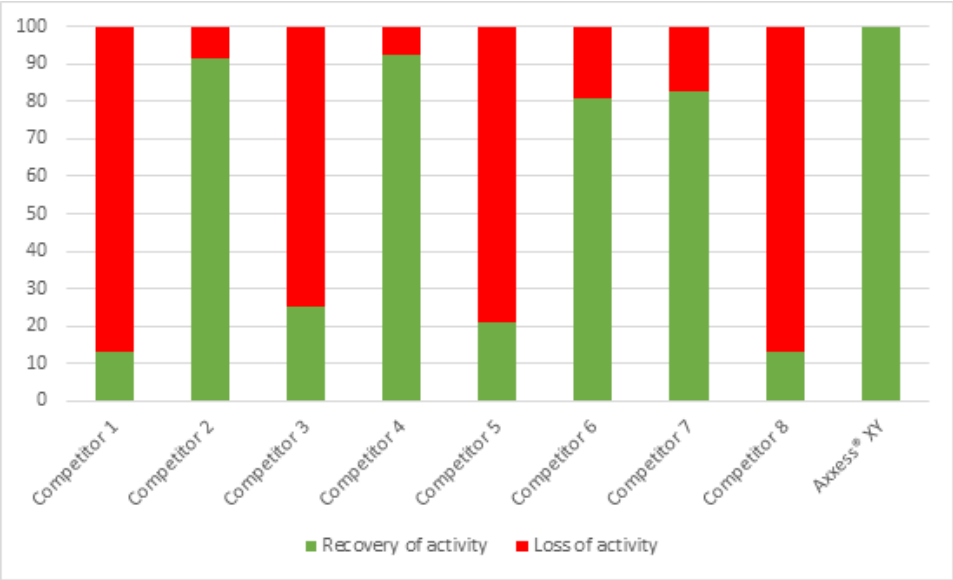

Fig. 2: Extracted total xylanase inhibitors from wheat incubated with the respective xylanase at 40°C for 30 mins. – Loss of activity after incubation with xylanase inhibitors

Conclusion:

Xylanase inhibitors are present in all cereal grains and, unfortunately, heat tolerant (up to 90 ^{0}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.

Literature:

Chmelová, Daniela, Dominika Škulcová, and Miroslav Ondrejovic. “Microbial Xylanases and Their Inhibition by Specific Proteins in Cereals.” *KVASNY PRUMYSL* 65, no. 4 (2019). https://doi.org/10.18832/kp2019.65.127. LINK

Choquer, Mathias, Elisabeth Fournier, Caroline Kunz, Caroline Levis, Jean-Marc Pradier, Adeline Simon, and Muriel Viaud. “*Botrytis Cinerea*Virulence Factors: New Insights into a Necrotrophic and Polyphageous Pathogen.” *FEMS Microbiology Letters* 277, no. 1 (2007): 1–10. https://doi.org/10.1111/j.1574-6968.2007.00930.x. LINK

Debyser, W, WJ Peumans, EJM Van Damme, and JA Delcour. “Triticum Aestivum Xylanase Inhibitor (Taxi), a New Class of Enzyme Inhibitor Affecting Breadmaking Performance.” *Journal of Cereal Science* 30, no. 1 (1999): 39–43. https://doi.org/10.1006/jcrs.1999.0272. LINK