Innovative technique reveals how enzymes degrade NSP structure of oilseed feed

by Inge Knap, Global Category Manager Enzymes, DSM Nutritional Products, Switzerland.

Livestock farmers worldwide are under increasing pressure to deliver enhanced performance in combination with improved animal welfare at economical prices.

Feed ingredients that can increase the bioavailability of the nutrients in the feed therefore have an important role to play in protecting livestock farmers' tight operating margins.

Dr Ninfa Rangel Pedersen of the DSM-Novozymes Global Feed Alliance is currently leading a team that has developed an innovative way of visualising how enzymes degrade the non-starch polysaccharides (NSP) structure of oilseed-based feed meals, facilitating improved uptake of the nutritional content of the feed.

With recent estimates indicating that feed enzymes are currently saving the global feed market some US$ 3-5 billion per year, Dr Pedersen's findings have significant implications for the global feed industry.

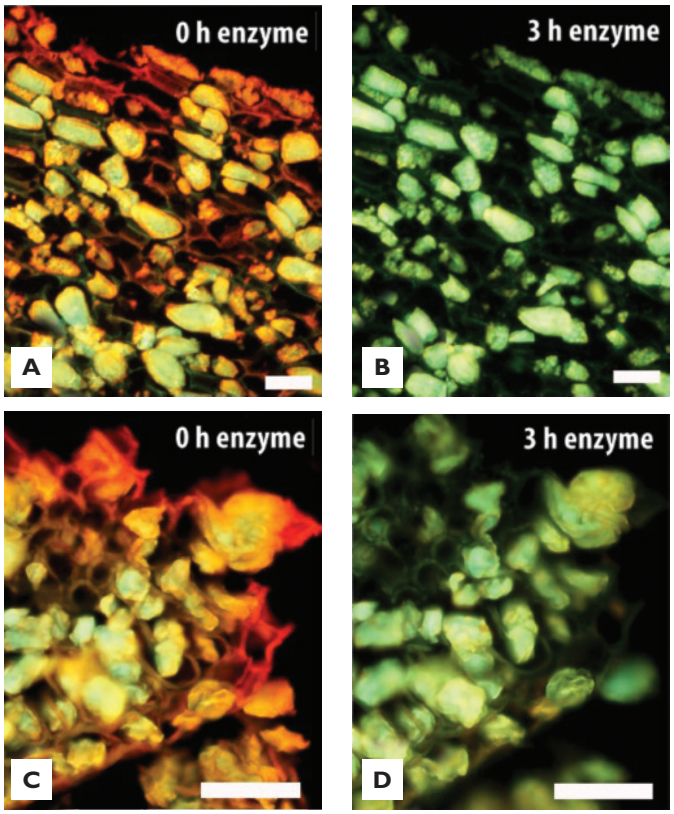

Fig. 1. Pictures taken using a fluorescent microscope. A and B show deparaffinated thick sections of intact whole soybean seed tissue after 0 hours and three hours of enzyme treatment. C and D show deparaffinated thick sections of soybean meal after 0 hours and three hours of enzyme treatment (Bar = 100µm).

Vegetable protein source

Since 2001, EU legislation has limited the use of meat and bone meal as a protein supplement in animal production, boosting the market for soybean meal (SBM) and other plant protein-based feed ingredients.

SBM, produced by the industrial extraction of oil from soybeans, is high in good quality protein, meaning it dominates the global feed market for vegetable protein meals.

However, monogastric animals are unable to digest up to 50% of the SBM they eat because the feed ingredients contain indigestible components that interfere with the digestive process and/or the animal lacks specific enzymes that break down the key components of the feed.

If feed is not efficiently digested by the animals, this constitutes a cost to both the producer and the environment.

Plant protein sources such as soybean also contain a variety of anti-nutritional factors, including serine protease inhibitors and lectins, which have toxic properties in monogastric species.

Like canola (also known as rapeseed), sunflower and palm kernel, soybean contains NSPs.

These may influence the digestion of the feed and the absorption of nutrients, since monogastric animals do not have endogenous enzymes that can break down the complex wall lattices of plant cells.

Perhaps the most important factor influencing the nutritive value of oilseed meals is therefore the indigestible acidic and neutral NSPs that originate from the plant cell walls.

Anti-nutritive effects

To improve digestion and overcome the anti-nutritive effects of NSPs, enzymes can be added to SBM.

Take for example DSM's Ronozyme VP, an NSP-degrading feed additive that contains a unique blend of hemicellulases and pectinases obtained by fermentation of a wild type organism (Aspergillus aculeatus), available in both liquid and coated thermostable (CT) form.

It is the impact of Ronozyme VP on the NSPs present in soybean that is the subject of an ongoing research project conducted by the DSM-Novozymes Global Feed Alliance and Copenhagen University, Denmark.

Led by Dr Pedersen, this project is studying the action of the enzymes developed by the Global Feed Alliance on various cereals and protein sources using viscosity measurements, NSP analyses, fluorescent microscopy and labelling techniques (both antibody and histochemical staining).

More nutrient value

Dr Pedersen and her team have developed a new in vitro technique for visualising how feed enzymes can release more nutrient value from the feed which has also been shown to boost livestock performance in invivo studies.

This innovative new technique is generating considerable interest as it gives the feed industry a better understanding through a series of striking images as to how the feed enzymes operate.

Dr Pedersen explains: "The structure and composition of the cell walls of the most commonly used cereals – such as wheat, corn and barley are relatively well understood.

"The cell wall structure of protein sources such as soy, rape seed and sunflower is much more complex, however, and we may not yet have a full understanding of it. An appreciation of these more complex structures is the precondition for learning how to unlock more nutritional value from them."

In essence, the cell walls of these plants consist of pectin, cellulose and hemicellulose (xyloglucans and mannans). Their structures differ in their detail, as does their pectin content, which is 6% in SBM, 9% in rape seed meal and 5% in sunflower meal.

"It is well known that the NSP content of oilseed-based feed meals has the effect of binding water, which increases viscosity in the intestine and reduces the absorption of nutrients. It also causes wet litter problems," Dr Pedersen continues.

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"Considerable attention has been given to the arabinoxylans and beta-glucans in cereals and to the viscosity issues that they can create in broiler production, but very little attention has so far been accorded to the pectin and hemicellulose content of protein sources and the impact they may have on bird performance and litter quality."

If the NSP-degrading enzymes are to be used in turn to greatest effect, it is therefore essential to develop a good understanding of the primary NSPs. This requires a better grasp of the chemical structure of the various plant cell walls.

The traditional view has been that the original oil-extracting process is so harsh that it totally degrades the structure of the protein cell walls.

As a consequence, it has been assumed that further enzymatic degradation is not a prerequisite for releasing more nutrient from the protein sources. However, the work of Dr Pedersen shows something different.

Using colour dyes

Dr Pedersen's innovative approach involves the use of dyes to help visualise in colour, the pectin solubilisation after enzyme treatment.

When looking more closely at a slice of soybean compared to SBM, the dyes help to show that the cell walls are still present in the soybean meal and that the plant cells still contain protein inside the cell wall structure.

As previously stated, monogastric animals do not produce NSP-degrading enzymes within their intestines. Therefore it could be expected that the protein held within the intact plant cell walls of the SBM would be less accessible to the animal's intestinal proteases, thus reducing the absorption of key nutrients.

Dr Pedersen's pictures are taken using a fluorescent microscope. In Fig. 1, the intense yellow-orange colour is due to the affinity of the histochemical dye Coriphosphine O for acidic polysaccharides, which in this case highlights the pectins present in the cell wall structure of soy.

Alcian blue, a dye with high affinity for pectic substances was also used to support data shown with Coriphosphine O.

If the exact same sample is incubated with a multicomponent enzyme, in this case using Ronozyme VP, the yellow-orange colour disappears within three hours at 39°C, and re-staining with Coriphosphine O does not colour the sample orange again, indicating that the pectin has been degraded.

We already know the cell wall structure is composed of more than just pectin, so another technique is needed to visualise the hemicellulases, in particular the xyloglucans and mannans.

To do this, an antibody-binding technique is used, putting the fibre structure under scrutiny to highlight the antibodies that are visualised as a red signal in the sample.

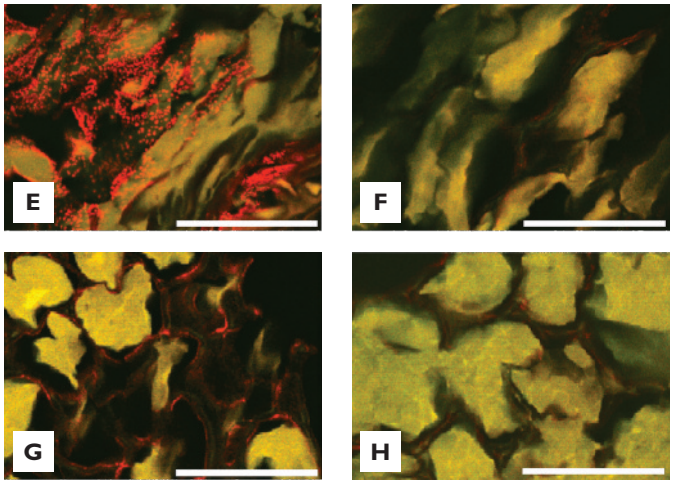

Fig. 2. Soybean meal coloured with antibodies against mannan and xyloglucans and then after three hours of enzyme incubation with Ronozyme VP at 39°C. E shows SBM labelled with antibodies against mannan, and F shows the same after enzyme treatment. G shows SBM labelled with antibodies against xyloglucan, while H shows the same after enzyme treatment (Bar = 50µm).

Fig. 2 shows SBM coloured with antibodies against mannan and xyloglucans and the SBM after three hours of enzyme incubation with Ronozyme VP at 39°C.

Picture E shows SBM labelled with antibodies against mannan, and picture F shows the same after enzyme treatment. Picture G shows SBM labelled with antibodies against xyloglucan, while picture H shows the same after enzyme treatment.

This clearly demonstrates how these hemicellulose fibres are part of the cell wall of SBM and that it is possible to degrade them, using commercial feed enzymes. It also proves that more than one enzyme is required to degrade the cell wall structure of protein sources.

Improving performance

Dr Pedersen and her team, which also includes PhD student Jonas Ravn, are the first researchers to have used a combination of dietary fibre analysis, viscosity measurements and immunochemical microscopy to elucidate the solubilisation and degradation of pectins and hemicellulases in the soybean cell wall using a commercially available multi-enzyme product.

Dr Pedersen concludes: "Single commercial enzymes are not capable of degrading the complex cell wall matrix of SBM. A complex blend of multiple carbohydrases is required to depolymerise the NSPs present in a diet containing SBM due to the diversity and complexity of the cell wall components.

"Successful degradation may lead to improvements in body weight gain, feed conversion efficiency and nutrient digestibility. The DSM-Novozymes/Copenhagen University team hope that our further work in this area will continue to generate findings that offer interesting prospects for the global feed industry". ■

References are available on request from the author

Over the past couple of years, Dr Pedersen has been active at many conferences around the world, presenting her team's work on the visualization techniques that show how plant cell walls are degraded by NSP enzymes.

She has published several accounts of her research, the most recent being "Enzymatic Solubilization and Degradation of Soybean Fiber Demonstrated by Viscosity, Fiber Analysis and Microscopy", which was co-authored by Jonas Laukkonen Ravn, Helle Juel Martens and Dan Pettersson, and has been published online in the Journal of Agricultural Science on 15 August 2015 **(Vol. 7, No. 9; 2015 ISSN 1916-9752 E-ISSN 1916-9760)**.