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Anti-Nutritional Factor (ANF) Neutralisation Using Enzyme

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Antinutritional factors are compounds that reduces the nutrient digestibility of the plant-based feeds such as phytate, tannins, protease inhibitors, lectins, saponins and non-starch polysaccharides which can impair the health of the fish by reducing protein digestibility, mineral bioavailability and overall feed efficiency. These ANF's can be reduced in the feed ingredients and the feed through enzymatic action by either directly adding purified enzymes to the feeds or by using microbes that secretes these enzymes during the process of fermentation. Use of specific enzymes such as phytase, proteases, xylanases etc reduces the toxic nature of the feed ingredient and increase the potency of the key nutrients, thus increasing the nutritive quality of the feed.

Types of antinutritional factors

General categories of ANF's targeted by enzymes are phytates(phytase), non-starch polysaccharides (xylanase, cellulase, β-glucanase), oligosaccharides(β-galactosidase), protease inhibitors(protease), tannins(tannase) etc. Soybeans, legumes, cereals, oilseed meals are the dominant sources of these ANF's.

Enzyme based strategies to tackle ANF's

Direct enzyme supplementation in feeds: Enzymes such as proteases, phytase, xylanase, tannase etc are generally used to hydrolyse phytate, protein inhibitors and other viscous NSP's when directly added to the feeds or food matrices thus improving mineral and amino acid availability and reduces toxicity and makes them available in more digestible forms.

Microbial enzyme co-fermentation: By combining both the exogenous enzymes together with microbes in the process of fermentation increases the efficiency of ANF degradation. For example, co-fermentation of peanut meal with *Weizmannia coagulans*, *Bacillus subtilis* and protease and hemicellulose has reduced trypsin inhibitors from 0.30 to 0.03% and phytic acid from 1.43 to 0.35% along with boosting protein digestibility and amino acids. Similarly, lactic acid bacterial fermentation of soybeans along with exogenous enzymes such as phytase, α-galactosidase, proteases has significantly reduced tannins from 1.93 to 0.12 mg/g, phytate from 1.16 to 0.04 mg/g and trypsin/ protease inhibitors up to 99%. Solid state fermentation of soybean meal with enzyme rich *Bacillus siamensis* MH03 degraded glycinin, β-conglycinin, trypsin inhibitors and oligosaccharides via secreted protease and α-galactosidase. Fermentation with lactic acid bacteria, *Bacillus* spp., and other microbes activates the endogenous phytase, amylase and glucosidases that degrades ANF's in cereals and legumes. Recently postbiotics which are purified microbial metabolites and enzymes are rising as stable tools for ANF degradation and detoxification in fish food from plants.

Determinants of the rate of degradation efficiency of enzymes

Enzyme/microbe type and spectrum: Combination of several enzymes i.e. multi enzyme strategy works better than single enzymes as in the case of *L. plantarum* has removed up to 90-99% tannins, phytate and protease inhibitors in the presence of endogenous enzymes in

soybeans and mixed microbes and enzymes has cut up to 75% phytic acid and 90% trypsin inhibitors in peanut meal.

Process optimization: The time, dose and moisture play a major role in determining the efficacy of the ANF degradation. In maize gluten feed, controlled *Bacillus subtilis* fermentation degraded up to 48% phytate and 32% WU-arabinoxylans.

Substrate structure and recalcitrance: Presence of lignin, crystalline regions and the particle size plays an important role in the rate of ANF degradation. Saccharification yields of maize shoot fractions showed huge variations based on particle size as the finest and more porous fractions showed highest sugar release compared to coarse fractions.

Synergy with probiotics/fermentation: Enzymes works better when paired with suitable microbes compared to it acting alone against solids. In distillers grains we can observe up to 27% decrease in mannans and 41% decrease in arabinoxylan when probiotics was paired with enzymes, and this process also required less quantity of enzymes compared to direct application of only enzymes.

Efficacy across food/feed ingredients

Soybean meal: Solid state fermentation of soybean meal with *Bacillus siamensis* MH03 has greatly reduced glycinin/β-conglycinin/trypsin inhibitor/oligosaccharides while increasing soluble protein content. Yeast fermentation with added protease also led to 95% reduction in raffinose.

Peanut meal: Co-fermentation with *W. coagulans/ B. subtilis* plus acid protease/hemicellulose reduced trypsin inhibitors from 0.30 to 0.03% and phytic acid from 1.43 to 0.35% while improving protein assimilation.

Cereal grains/legumes: Phytase from *Aspergillus oryzae* has reduced up to 97% reduction in phytic acid content after 24 hours and α-galactosidase producing L. plantarum strains reduced tannins, phytates, trypsin inhibitors significantly during fermentation of soybeans.

By-product valorisation: Fermentation or co-fermentation process of distillers grains/wheat brans has reduced mannan, arabinoxylan and phytic acids contents while increasing protein solubility and nutrient density.

The endogenous enzymes and co-fermentation with exogenous enzyme blends have improved growth rates and feed efficiency in tilapia fingerlings and yellow perch by enhancing nutrient utilization.

Limitation

- As already discussed the enzyme action and co-fermentation depends greatly on various factors such as substrate composition, pH, temperature, enzyme source and purity, dose and treatment time.
- Some ANFs such as phytic acids and tannins are thermally stable so it requires combined physical- enzymatic treatments for optimal removal.
- Cost effectiveness is influenced by enzyme price, substrate specificity and process scalability, in this case microbial fermentation is a cost friendly alternative but it requires strain optimization for maximal enzyme production and activity.

Conclusion

Enzymatic treatments can degrade ANFs whether trough direct supplementation or microbial fermentation up to 40-90% based on various factors but alone with microbial fermentation we can achieve high efficiencies. These strategies can reduce key ANFs thus improves protein and mineral bioavailability and upgrade low value plant materials into higher value foods and feeds, if the optimization of strains and enzyme mixes for specific substrate was done properly.

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