

Critical evaluation of enzyme products

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Over the past years feed enzymes have gained more acceptance in the poultry sector. Nutritionists and feedmill managers have become more educated on the modes of action and properties of enzymes, their application and cost benefits. This leads the industry to apply more carbohydrate enzymes in addition to phytase.

While non-starch polysaccharides (NSP) enzymes have been used widely in wheat diets, of recent interest is the development of using NSP enzymes for corn and/or oilseed meals, i.e. corn – soybean meal diets. These enzymes come as:

- A single enzyme concentrating on specific substrate or sugar complexes within a substrate for example, a xylanase.
- A cocktail of enzymes defined as different enzymes produced by different organisms and blended together.
- A natural mixture of enzymes defined as a multiple enzyme product which is produced by the same organism.

With numerous enzyme products available on the market, a selection is often a very challenging process for most practical nutritionists.

Despite assured efficacy or response by enzyme supplier, the actual benefits or returns may vary considerably depending on the enzyme products used, diet formulation, nutritional level, feed processing conditions and animal species as well as their growth stages.

Table 1. Activities spectrum present in a natural enzyme combination (moll/min/mg protein, in Rovabio Excel). Geraert et al 2003.

Enzyme	Activity	Enzyme	Activity
Xylanases			
Endo-1,4-β-xylanase	30,000	Pectinases	
α-Arabinofuranosidase	50	Pectinase	1,000
β-Xylosidase	100	Polygalacturonase	1,000
Feruloyl esterase	<10	Pectin me-esterase	80
Endo-1,5 α-arabinanase	150	Proteases	
β-glucanases			
Endo-1,3(4)-β-glucanase	40,000	Aspartic protease	15
β-1,3-Glucanase (laminarinase)	1,500	Metallo protease	15
Endo-1,4-β-glucanase	20,000	Others	
Cellulohydrolase	200	Endo-1,4-β-mannanase	200
β-Glucosidase	2000	β-Mannosidase	20
		Rhamnogalacturonase	
		α-Galactosidase	

Evaluating or validating various enzyme products has been a difficult task for many nutritionists. This article discusses various aspects on how to evaluate NSP enzyme products under practical conditions.

Pre-screening products

In order to validate an enzyme product for use in an operation, two areas of evaluation should be considered very carefully in order to shortlist enzyme products and to make effective enzyme selection.

The first step is to pre-screen enzyme products from various manufacturers in order to shorten the list of suppliers, followed by the second step, practical evaluation to validate efficacy and economic returns for a given operation. The following questions are advisable for pre-screening or shortlisting enzyme products.

● What organism used?

Today there are numerous species or strains of micro-organisms that may be employed to produce commercial enzymes, both genetically modified organisms (GMO) and naturally selected organisms may be used for fermentation. A GMO organism usually produces one or two enzyme activities with relatively high productivity, but its enzyme spectrum is usually narrow and does not provide meaningful auxiliary activities to cleave branches attached to the main backbones of those targeted NSPs, whilst naturally

	GE	ME	Energy Dig. (%)	Δ ME	Faecal DM (%)
Control	4740	3460 ^a	73.0	-	67.8 ^a
Rovabio AP	4770	3578 ^b	75.0	107	69.1 ^{ab}
Enzyme HC	4805	3574 ^b	74.4	105	70.7 ^b
Enzyme VP	4750	3557 ^b	74.9	91	70.4 ^b
Enzyme G2	4759	3515 ^{ab}	73.9	51	69.4 ^{ab}
Enzyme SF	4768	3490 ^{ab}	73.2	30	68.9 ^{ab}
Enzyme VG	4761	3465 ^a	72.8	7	68.4 ^a
Enzyme AV	4763	3472 ^a	72.9	15	68.6 ^a

Note: Values in the same column not bearing the same alphabets indicate $P < 0.05$.

Table 2. Comparison of various enzyme products on AME uplift for three week old broiler fed on corn/SBM diet (kcal/kg in DM, N=12).

selected organisms, having survived and evolved on numerous challenges by specific indigestible components, can thrive and produce a wide spectrum of activities with synergistic functions to break down targeted substrates such as hemicellulose and NSPs.

Table 1 lists an example of a naturally evolved organism *Penicillium funiculosum*, its fermentation prod-

adjusted to eliminate batch to batch variations.

Moreover, liquid enzyme product becomes possible.

● Enzyme definition.

An enzyme product for feed use should describe each enzyme activity in detail, not simply by general group name, such as xylanase, glucanase or protease.

The example given in Table 1 has

Enzyme	Initial wt (g)	Final wt (g)	Weight gain (g)	Feed intake (g)	FCR
Control	154.7	344.5	189.7	427.6	2.254 ^b
Enzyme A	154.8	349.0	194.2	428.8	2.212 ^{ab}
Enzyme B	155.1	343.1	188.0	423.4	2.255 ^b
Enzyme C	154.6	343.9	189.4	427.5	2.270 ^b
Rovabio AP	154.7	357.4	202.6	430.5	2.134 ^a
LSD(P= 0.05)	0.73	14.05	14.11	19.72	0.0998

¹Means without common superscript differ significantly ($P < 0.05$).

Table 3. Comparison of four enzymes through a short term bio-assay with broilers (Adisseo internal report).

ucts contained 18 enzyme activities with main activities of NSP hydrolysis plus some very useful auxiliary activities.

● Fermentation technology.

Solid state fermentation is a traditional process to produce enzymes by inoculating a selected organism to the moist substrates then incubated under suitable conditions. After fermentation it is usually unable to isolate enzymes from its fermentation substrates thus enzymes are mixed together with fermentation residues.

Batch to batch variation may be substantial. On the other hand, submerged or liquid fermentation is more advanced and enzymes can be purified by ultra-filtration and enzyme activity levels can be

enzyme activities from fungus *Penicillium funiculosum* and determined by three laboratories following the identical methodologies.

The enzymes are described on molecular level and bond cleavages are thus defined.

● Cocktail or natural combination product.

Cocktail enzymes are usually referred to as blends of several enzymes from different origins. Examples are amylase, protease and xylanase, produced by separate organisms and supplied by different manufacturers. By nature, enzymes of heterogeneous origin may not possess the same enzymatic properties such as their optimum pH and

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temperature in spite of some overlapping. Upon blending the cocktail may not possess the required compatibility or could even conflict if mixed with water for a liquid spray, in that some protease enzymes may break down xylanase or other enzymes in the same matrix.

On the other hand, single fermentation enzyme, since all activities are from the same organism, the various enzymes are fully compatible even after diluting in water for post-pelleting spray or administering through drinking water.

If compatibility is a concern, the product must be handled properly to prevent this enzyme cannibalism, for example, a protease with a xylanase or phytase must be kept separate until final application to the feed. This is not a concern for the naturally derived enzyme mixture.

● Liquid enzyme form and proper dosing.

All enzymes are heat labile and will not withstand pelleting above 85°C and certainly not expansion or extrusion. Thus, there must be a liquid form available for post-pelleting. Liquid spray operation involves considerable capital expenditure for the spray equipment itself plus feedmill modifications to accommodate the spray system. If an enzyme product is a cocktail made up of a protease, xylanase and phytase, the storage and spray system will be more sophisticated because the protease needs to be kept separate from the others to avoid denaturing.

Concerns on enzyme selection include whether the supplier has a reliable post-pelleting spray system, spray accuracy, experience in installation and maintenance after installation.

Liquid enzyme products require specific care on freight and storage. Upon arrival, some liquid enzymes need to be stored in a low temperature warehouse while the other enzyme liquids can be stored at normal warehouse temperature up to three months. This can bring about substantial differences in a selection of enzyme for commercial application.

● Quality assurance.

For the purpose of quality control in a feedmill, there are two major hurdles for enzyme analysis. One is lack of internationally recognised analytical method for various enzymes, and the other is that the level of sophistication in enzyme assay goes beyond the analytical capability of a laboratory designed for a feed company.

One solution is to select enzyme products from reputable manufacturers, and also to check whether the enzyme products in question possess a valid registration from well recognised authority such as European Union or FDA.

A product carrying such registration indicates it has passed very strict evaluation process and its

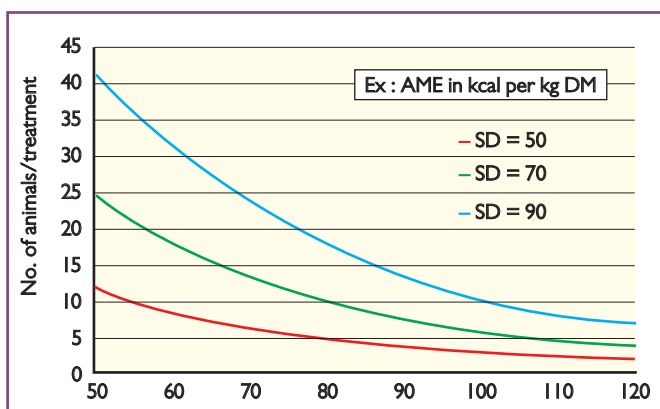


Fig. 1. Differences that can be measured depending on expected SD.

composition, efficacy and safety are thus well ensured.

Evaluating enzymes

The primary benefits of NSP enzymes are to gain extra energy and some digestible amino acids from a given diet, with a magnitude of response ranging between 0-3% in terms of feed conversion and weight gain. As enzymes are nutritional additives, many feed companies or farms require their in-house nutritionists to conduct animal tests in order to validate the benefits of a given enzyme. Notwithstanding such trials have a nature of minor response plus numerous variations hence results obtained are often inconsistent and make it very difficult for the nutritionist to draw a conclusion on whether the product should be accepted for commercial use.

It must be pointed out that an efficacy test aimed at picking up minor responses is a great challenge and cannot be easily obtained through simple routine animal trial. They must be handled by experts, through specific evaluation process and methodology.

● In vitro digestion test.

This test involves in vitro incubation. A given enzyme is mixed with targeted feed ingredients or a compound feed, adjusted to a low pH to simulate stomach and incubated for 2-3 hours. Enzyme efficacy can be measured by digestibility change after filtration. It is a rapid test with good reproducibility, which can examine a large amount of samples in a short time period. Although it has rarely been used for enzyme selection, with appropriate fine tuning, this method can effectively differentiate and rank enzyme products with minimum cost.

● In vivo metabolic test.

This will involve live birds and can yield accurate and reliable results if the methodology is set up correctly. A procedure adapted by Adisseo Animal Research Centre, and some official Research Center worldwide, is shown inset right. This is the European Reference Method with ad libitum feeding and total excreta

collection for one week. It is important to run the test with a minimum 12 replicates and repeated tests are required on various diets and animal species in order to minimise errors and establish proper and valid responses, such as apparent metabolisable energy and digestible amino acids.

These values are to be assigned to the enzyme products as matrix value for diet formulation. With decades of experience Adisseo AME facility can obtain AME results with sensitivity up to 30kcal/kg diet.

Unfortunately some enzyme products provide matrix value based on simple performance tests, which tends to over-claim the response of benefits of the enzyme products. An example of a comparison study is given in Table 2 following the EU reference method, in which broilers were individually caged and fed on corn soybean meal diets. The results indicated the AME uplifts obtained are highly variable among enzyme products, ranging from ME 7kcal/kg (Enzyme VG) to 107kcal/kg (Rovabio AP).

● Short term bio-assay.

Short term bio-assay will involve live birds and semi-purified diets to target specific feed ingredients and results can be reliable. An example is given in Table 3. Birds were Hybro male feather sexed from a hatchery in the Philippines, diets were commercial feed for the first seven days followed by semi-purified diets for 10 days. Test diet contained 14% crude protein, ME 3,200 kcal/kg containing soybean meal, dextrose, corn starch, soy oil, vitamins and minerals. Four enzyme products were compared, namely Rovabio and three unnamed enzyme products, eight replicates per enzyme and 10 birds per pen.

This was a bioassay with a broiler aged from day 7-17, where the only soybean meal was the protein source.

The objective was to compare the value of each enzyme in terms of enhancing protein digestibility of soybean meal. Results show that Rovabio AP improved weight gain by 6.8% and feed conversion by 5.3% (P<0.05) and the other three competitive enzymes failed to improve bird performance.

Performance tests

Many feed companies or farms evaluate enzymes or other nutritional additives through their own animal testing facility. Below are typical designs of such tests.

● Example A, down-spec + Enzyme.

Treatment 1, control.

Treatment 2, down-spec ME 100 kcal/kg + enzyme.

● Example B, adding a negative control.

Treatment 1, positive control.

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In vivo metabolic test

Experimental design:

Two treatments x 12 replicates x one male broiler per cage.

Feed treatment:

A control diet without added enzyme is divided into two equal portions, one portion as control and the other will be fortified with the enzyme in question. Feed quantity requested per treatment is 25kg, following testing procedure:

● From an initial stock of one day old chicks, after individual weighing, 34 birds (24 experimental plus 10 replacement per two treatments) are selected at day 11. They are randomly allocated into treatments in order to form homogenous lots.

● From day 0-11 chicks are reared on floor pens with standard broiler starter diet.

● On day 12 weighing, selection and transfer to individual cages.

● From day 12-19 adaptation period, feeding with the experimental diet.

● At day 19 after one night fasting, all the birds are weighed and collection trays are installed below the cages. The birds are fed with the experimental diets.

● From day 19-22 excreta are collected, weighed and freeze-dried.

● At day 22 Weigh the birds and feed refusals – end of the test.

Measurements:

● Gross energy of the diets and excreta.

● Dry matter content of the diet samples.

● Feed intake and live weight of each bird.

● Control of enzyme activity in the tested diet.

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Treatment 2, negative control.
down-spec ME 100kcal/kg.
Treatment 3, treatment 2 +
enzyme.

The number of replicates per treatment is usually from three to six, mixed sexes. Test starts from day old chicks to marketing weight. Performances usually turn out to be of 'no statistical differences' among the treatments, which leads to a conclusion that the enzyme product can provide AME 100 kcal/kg diet.

In Example B, some tests found no differences even between the positive and the negative controls. This is a clear sign of execution errors, mainly because of inaccurate diet formulation mixed with inaccurate trial execution.

From a statistical point of view, it is relatively easy to reach 'no statistical differences' instead of attempting to detect 'there is a true difference'. This is because all errors under practical trial conditions will inevitably increase variances within a treatment that will out-weigh the 'actual difference' that may exist between the treatments.

There are two key points when setting up a trial:

- What is the variability of the parameter measured (RSD- residual standard deviation)?

- What scope of difference is expected with the treatment (D)?

When the above two questions are clear, one can decide the number of animals per treatment following the formula below.

$$N = \frac{RSD^2}{D^2} \times (\alpha 5\% + (1-\beta) 90\%)$$

Where α is the level of significance and β represents the power of the test. Some statistical software is able to simulate when the trial design is a complex.

Fig. 1 illustrates the relationship among RSD, expected differences and the required number of replicates. When the test parameter has a small scope of difference with a high RSD, a higher number of repli-

	Live wt (kg)	FCR	Mort. adj. FCR	Mortality (%)	Breast meat (% of carcase)	\$ income/kg dry carcase
Trial I, Ross 308, 12 replicate/trt, Apr. to May						
Regular ME	2.587 ^{ab}	2.041 ^b	1.963 ^{ab}	8.64	24.69	2.719
+ Enzyme	2.630 ^a	1.988 ^a	1.936 ^a	7.73	25.22	2.777
Lower ME (-66)	2.567 ^b	2.059 ^b	1.989 ^b	7.58	24.82	2.723
+ Enzyme	2.622 ^{ab}	2.032 ^b	1.973 ^{ab}	10.76	25.44	2.788
Trial II, Ross x Arbor Acres, 12 replicates/trt, Jul. to Sept.						
Regular AME	2.505 ^a	2.101 ^b	2.036 ^b	5.97	22.59 ^b	1.974 ^b
+ Enzyme	2.546 ^a	2.095 ^b	2.010 ^{bc}	6.67	23.31 ^a	1.992 ^a
Lower ME (-66)	2.444 ^b	2.191 ^a	2.089 ^a	8.47	22.66 ^b	1.880 ^b
+ Enzyme	2.526 ^a	2.063 ^b	1.995 ^c	5.83	23.57 ^a	2.026 ^a

Means without common superscript differ significantly (P< 0.05). The Rovabio AP was assumed to add \$2.101/t to feed cost in this trial.

Table 4. Typical design of growth trial to examine energy and enzyme responses in broiler. (Hooge, 2002).

cate is required to detect the true difference.

Another key criterion is the test diets should be similar to those being fed commercially, for example, no very low energy or available phosphorus diets, as nutrient responses can differ. Ingredient make-up of the test diets is also important for changes in major feed ingredients can affect diet digestibility, independent of the enzyme product's effect.

Finally, the number of studies and consistency of response must be evaluated simultaneously. A large number of trials with inconsistent responses are of concern as well as just a few trials showing only positive responses. Consistency of response is a major challenge for enzyme products developed for corn-soybean meal diets.

Growth performance study

An example of sensitive growth performance study is described in Table 4. This design consisted of two consecutive studies with two control diets (regular and lower ME) in USA.

Trial I was conducted with a total of 2,880 Ross x Ross 308 straight run broiler chicks in four treatment groups with 12 replicate pens (60 chicks each) per treatment to 49 days of age. Initial stocking density was moderately heavy at 0.0622 M2 per chick. Feeds were steam pel-

leted and fed as crumbles in starter period or pellets in grower and finisher periods. The four feed treatments were:

- Regular AME control.
- Lower AME control (-66 kcal/kg).
- Regular AME + enzyme.
- Lower AME + enzyme.

Calculated AME levels of regular energy feeds ranged from 3,030 to 3,251 kcal/kg and of lower energy feeds ranged from 2,964 to 3,185 kcal/kg. The feed ingredient costs went from \$136.98 to 19.88 per ton for regular AME basal diets and from \$133.32 to 116.74 per ton for lower AME basal diets. The live broiler value was assumed to be \$1.168/kg.

The same trial design was repeated as Trial 2 utilising Ross x Arbor Acres broiler chickens in a 49 day test, in litter pens. The trial design, dietary energy levels and feed ingredient costs were the same as in the first trial.

The live broiler value was assumed to be \$0.9259/kg.

The overall results demonstrated that adding the enzyme to broiler feeds tended to improve body weight, feed conversion ratio, breast meat yield, but mortality was unaffected.

Supplementation of the enzyme numerically improved net profit (income per kg dry carcass - feed ingredient cost) regardless of energy level. In general, the enzymes

enhanced the energy utilisation of poultry feeds decreasing the feed required per unit of body weight.

Summary

The nutrient benefits assigned to enzyme products have a significant effect on the worth or value of the enzyme product in an operation's feeding programme. The more nutrient benefits or the greater the magnitude of the benefit, the more the product will be worth on paper.

It is imperative that proper and valid nutrient benefits are validated based on sound trial data, consistency of response and especially in vivo metabolic tests by accurate methods. Nutritional benefits obtained from simple growth trial are often misleading in that such trial tests are designed to reach no statistical difference.

There are other benefits to enzymes that feed formulation cannot account for such as improved gut health leading to drier litter or manure, cleaner eggs, better pigmentation due to more efficient fat digestion and improved uniformity.

Over time the birds, and ultimately industry will validate feed enzyme products and their use.

To an extent this has already been accomplished with phytase enzymes and is underway for NSPase and other enzymes for corn-soybean meal diets. ■