



**Opinion of the**  
**Scientific Committee on Animal Nutrition**  
**on the assessment of efficacy of enzymes**

**(adopted on 27 April 2000)**

**1. TERMS OF REFERENCE**

The Scientific Committee for Animal Nutrition is requested to give an opinion on the following questions:

- (1) What is the most appropriate way to assess the efficacy of enzymes used in animal nutrition in order to improve animal production?
- (2) When an enzyme product contains several different declared enzyme activities, what data are necessary to justify the presence of each active component of the additive?

**2. BACKGROUND**

As part of the assessment process, the amended guidelines (Council Directive 87/153/EEC) require companies to demonstrate the efficacy of enzymatic preparation in each target species in terms of animal production with appropriate data. According to Article 2 of Directive 70/524/EEC one definition of an additive is a product intended:

"to improve animal production, in particular by affecting the gastro-intestinal flora or the digestibility of feedingstuffs;....".

The legislation services of the Commission have indicated that "animal production" should be interpreted as "animal performance", expressed in terms of an improvement in the efficiency of nutrient utilisation, or animal growth, or in the quality and yield of animal products or improved animal welfare.

The guidelines also require that when a enzyme preparation used as a feed additive contains two or more enzyme activities, then the presence of each active component of the mixture should be justified in terms of their contribution to the overall efficacy of the product.

### 3. INTRODUCTION

- 3.1. It is recognised that a proportion of the nutrient content of most feeds is not digested and absorbed. For this reason, some potential feedingstuffs are not considered for high quality diets because of relatively poor nutrient availability. This is generally the result of the presence of high levels of non-starch polysaccharides (fibre) and/or presence of anti-nutritional fractions. Major improvements in nutrition rely on obtaining maximum nutrient utilisation from feedingstuffs. Exogenous enzymes, added to the feed or used during feedingstuff processing, have the potential to improve nutrient utilisation/feed efficiency, to reduce the pollution associated with manure and to increase the use of low cost, lower nutritional quality feed ingredients.
- 3.2. Enzymes are proteins produced by the cells of living organisms with the specific function of catalysing chemical reactions. Enzymes increase the rate at which reactions approach equilibrium. Rate is defined as the change in the amount (moles, grams) of starting materials or products per unit time. Enzymes often have high specificity and their effect is usually substrate specific. Individual enzymes can be selected with activity against the substrates of interest in feed or feed ingredients, and with the physical properties necessary to survive exposure to hostile environments during feed processing and passage through the digestive tract.
- 3.3. With the improvement in knowledge of the composition and structure of plant feedstuffs, enzymes capable of hydrolysing their constituents have been identified and produced on a commercial scale. Currently these derive from bacterial and fungal sources.
- 3.4. Non-starch polysaccharides (NSPs) are present in virtually all feed ingredients of plant origin. NSPs are poorly utilised and can, when solubilised, exert adverse effects on digestion. Exogenous enzymes can contribute to a better utilisation of these NSPs and as a consequence reduce any antinutritional effects. Non-starch polysaccharides degrading enzymes have been widely used for this purpose in cereal-based diets for poultry and, to a lesser extent, for pigs and other species.
- 3.5. Phosphorus is an essential nutrient for all animals. Plants contain phosphorus in a varying amounts bound to phytic acid. Depending on the phytase content of these plants, phytate-bound phosphorus is not fully available to monogastrics animals. Therefore the physiological requirements of these animals has to be met by the addition of inorganic phosphate to the diet. Exogenous phytases are currently used to increase the availability of phytate-bound phosphorus and consequently decrease the need for adding inorganic phosphorus, leading to a reduction in environmental pollution.
- 3.6. An extensive search for novel catalytic activities or physical properties and the ability to express these in “production” strains has increased dramatically the number of single activity enzyme preparations available for commercial development. It is likely that some of these novel constructs will find application in animal feeds for the better utilisation of nutrients or the destruction of substances with undesirable properties.

#### 4. DEMONSTRATION OF THE EFFICACY OF ENZYME PREPARATIONS.

- 4.1. The use of enzymes in animal feeding is now widespread. Assessing their efficacy, however, presents a number of difficulties because of the multiplicity of enzyme preparations with similar activities but from different sources on the market and the mixing of various enzyme preparations in different proportions to create novel products.
- 4.2. For the purpose of efficacy assessment, SCAN recognises two different categories of enzyme preparations according to the type of blend used
  - (1) One or more declared activity(ies) originating from the same strain and the same fermentation, or
  - (2) Several declared activities originating from different strains or the same strain grown under different conditions (being a mixture of different fermentation products).
- 4.3. The efficacy of enzyme products described by 4.2 (1) should be fully assessed (see 4.5 and 4.6). For enzymes consisting of a blend of the products of two or more fermentations as described by 4.2 (2), two approaches are possible. When the efficacy of each fermentation product contributing to the blend has been separately assessed (i.e. activity level, target animal category), then the efficacy of the blend can be assumed. If, however, the efficacy of any component is unknown, or cannot be directly assessed, then the blended enzyme product should be subject to the same assessment as for the category described by 4.2 (1).
- 4.4. Enzymes may be used as technological additives. For such use, efficacy should be demonstrated with the appropriate test.
- 4.5. Addition of an enzyme preparation as zootechnical feed additive is justified by the better use of the diet by the animal. This should lead to improved performance expressed in terms of weight gain, improved feed to gain ratio, or any other parameters which might provide benefit to the animal, the animal product or the environment.

Digestion is a complex process modified by feed intake, by passage rate, degradation rate and extent, fluidity of the intestinal contents and finally rate and extent of absorption of the end products. This justifies the fact that *in vitro* studies can only provide an indication of efficacy and cannot substitute for *in vivo* trials. Therefore the SCAN is of the opinion that the efficacy of enzymes must be determined through the response of the host animal.

#### 4.6. Animal response can be shown by more than one type of experiment:

##### 4.6.1. Animal performance studies

The activity of the enzyme can be demonstrated by measuring different parameters of animal production. This method could be used for the demonstration of the efficacy of enzymes acting either by improving the utilisation of nutrients or by the inactivation of undesired potentially harmful substances.

##### 4.6.2. Digestion / balance studies

For some enzymes digestion / balance studies are necessary in addition to the animal performance studies. These experiments are conducted for a short period of time using appropriate number and type of animals. This type of studies should be introduced as part of the evaluation if the company claims for nutritional and/or environmental benefits. It is an essential trial to support certain type of claims (e.g. phytases).

##### 4.6.3. Other studies

In the case of claims not covered above, additional studies would be needed.

#### 4.7. Mixture of activities

SCAN considers that the present requirement to justify the presence of each enzyme activity in a final product containing several enzymatic activities on the basis of animal trials is neither practical nor theoretically justified.

### 5. RECOMMENDATIONS

#### 5.1. On the way to assess the efficacy of enzymes

5.1.1. Experiments used to demonstrate the efficacy should be conducted according to the requirements of Council Directive 87/153/EEC.

5.1.2. In addition, for each claimed effect and each target animal category, at least three experiments should be provided with significant results ( $P < 0.05$ ). In the case of ruminants, where the homogeneity of animals is not so easy to obtain, it would be acceptable to use a level of probability of  $P < 0.10$ .

5.1.3. For consistency, experiments should be conducted and described in a manner where reproducibility and repeatability can be achieved and data consolidated. The reproducibility of the effect and the independence of efficacy assessment should be identified. Therefore animal studies should be carried out in at least two different locations, preferably by independent organisations, following similar protocols. Experimental conditions should reflect the current European production/feeding systems.

5.1.4. The duration of the performance studies must be representative of the recommended time of use. As examples, it is suggested for the following species :

- Calves (only milk replacer) Total feeding period until slaughter
- Calves (for fattening) A minimum of 6 weeks
- Calves (for rearing) A minimum of 6 weeks
- Piglets Until weaning (creep feed) or, From weaning to 25 kg or, According to local custom
- Pigs for fattening Growing and fattening period until slaughter
- Rabbits for fattening From weaning until slaughter
- Chicken for fattening Day 1 to a minimum of 35 days (until slaughter)
- Laying hens A minimum of 6 months; if total is claimed, then the total laying period
- Turkeys for fattening From day 1 to a minimum of 12 weeks (until slaughter)
- Poultry for reproduction Total rearing period

If feeds for particular feeding purposes are concerned, the experimental duration may be adjusted to the respective recommended feeding time.

5.1.5. Analysis of declared enzymatic activities at feed level must be provided.

5.1.6. A single UNIT to express the enzymatic activity should be used throughout, following where possible IUB/IUPAC recommendations. (e.g. one unit of activity releases 1 $\mu$ mole product (reducing group equivalent in the case of ill-defined polysaccharide substrates) per minute under the stated conditions).

5.2. On the justification of the presence a mixture of several enzymatic activities

Any data that directly or indirectly support the presence of individual enzyme activities in a mixture should be accepted. However, SCAN does recommend that any data submitted as justification should be specific to the product and not simply based on the generality of scientific data available in the literature. The evaluation of the efficacy of blended products should be done in the same manner as for products derived from a single fermentation source.

The proposed way to assess efficacy of enzymes is outlined in the annexed decision tree.

## 6. LITERATURE

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Decision tree to assess the efficacy of enzymes in animal nutrition

