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Future of feed enzymes: Orientation and perspectives

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SUMMARY - The use of enzymes as a dietary supplement has had a dramatic effect on the utilization of certain feedstuffs in animal husbandry, particularly in poultry and especially with those diets that contain cereals such as barley and wheat. Future research and development will continue to be supported on an ever increasing level by industry in an ever widening field. Some of the future areas of emphasis will be: (i) improvements in the quality and efficacy of current enzymes that are available on the market with regard to cost, thermal stability, resistance to digestion and enhanced activity in the target section of the gastrointestinal tract; (ii) an expanded range of use of enzymes in the diets of poultry and domestic livestock including classes of poultry other than chickens, pigs, fish, and exotic animals such as alligators and turtles; (iii) an expanded availability of different enzymes such as lipases, proteases, amylases, etc. as produced by the biotechnology industry; (iv) alternate sources of genetically engineered enzymes that have been selected and/or designed for the particular target substrate and animal. Included are enzymes produced in microorganisms, plant seeds and the animal itself by recombinant DNA technology; (v) an expanded number of feedstuffs that respond to enzyme treatments; (vi) the development and standardization of procedures to evaluate different enzyme products; (vii) further research into the mode by which enzymes produce their beneficial effects; (viii) development of models to predict response to enzymes in any class of livestock and with any feedstuff so as to facilitate cost-benefits studies; (ix) greater emphasis on other benefits of enzymes such as their effect on reducing pollution, partitioning of nutrients and altering the endocrine response and health status of the animal. Enzyme research will not only continue at a brisk pace but will undoubtedly be accelerated with many benefits being achieved. This exciting field of research will be a focus of animal nutrition research and development in the future.

Key words: Enzyme supplementation, enzyme assays, site of action, alternate sources, synergism, nutrient interactions, modelling, target, other species, substracts, physiological changes.

RESUME - *"Futur des enzymes alimentaires : Orientation et perspectives". L'utilisation d'enzymes comme supplément dans le régime a eu un effet important sur l'utilisation de certains aliments en production animale, particulièrement en aviculture et spécialement en ce qui concerne les régimes qui contiennent des céréales telles que l'orge et le blé. La recherche et le développement futurs continueront d'être menés par l'industrie à des niveaux de plus en plus poussés et dans un champ élargi. Certains des domaines futurs seront : (i) les améliorations de la qualité et de l'efficacité des enzymes qui sont actuellement disponibles sur le marché concernant leur coût, stabilité thermique, résistance à la digestion et activité augmentée dans la section cible du tractus gastrointestinal ; (ii) un spectre élargi d'utilisation des enzymes dans les régimes pour volailles et animaux domestiques, y compris des productions autres que les poulets, comme les porcins, poissons, et animaux exotiques comme les alligators et les tortues ; (iii) une disponibilité augmentée des différentes enzymes telles que les lipases, protéases, amylases, etc, en provenance de l'industrie biotechnologique ; (iv) des sources alternatives d'enzymes produites par génie génétique ayant été sélectionnées et/ou mises au point en ciblant un substrat et un groupe animal particuliers. Ici sont comprises des enzymes produites dans des microorganismes, des graines de plantes et les animaux eux-mêmes par la technologie de l'ADN recombinant ; (v) un nombre grandissant d'aliments répondant aux traitements avec enzymes ; (vi) le développement et la standardisation des procédures d'évaluation des différents produits contenant des enzymes ; (vii) une recherche ultérieure concernant le mode selon lequel les enzymes produisent leurs effets bénéfiques ; (viii) mise au point de modèles pour prédire la réponse aux enzymes chez n'importe quelle classe d'animaux domestiques et avec n'importe quel aliment afin de faciliter les études coût-avantage et (ix) mettre l'accent sur d'autres avantages des enzymes, tels que leur effet vers une réduction de la pollution, le fractionnement des nutriments, la modification de la réponse endocrinienne et l'état de santé des animaux. La recherche en matière d'enzymes non seulement continuera à un*

rythme rapide, mais sera sans aucun doute accélérée et de grands avantages seront acquis. Ce domaine passionnant d'investigation sera le centre d'attention de la recherche et développement en nutrition animale à l'avenir.

Mots-clés : *Supplémentation enzymatiqué, essais enzymatiques, site d'action, sources alternes, synergie, interactions des éléments nutritifs, modélisation, cible, autres espèces, substrats, changement physiologique.*

Introduction

Enzymes as additives to animal feedstuff have had a great impact on the livestock industry. They have not only improved the utilization of diets containing cereals such as barley, wheat, rye and oats, especially for poultry, but have had a positive impact on the quality of the environment through reduced output of excreta and pollutants such as phosphate and nitrogen including ammonia. Enzyme supplementation of cereal-based diets has also been instrumental in producing more uniform performance values in poultry as it tends to be more efficacious with wheat and barley having low metabolizable energy (AME) values compared to those that have higher values. The cereals with low AME values often have a high content of growth inhibiting viscous, water-soluble nonstarch polysaccharides (WSNSP), as a result the response to enzyme treatment is greater than that obtained for cereals with a lower concentration of WSNSP. The net effect of enzymes addition to cereal-based diets has, therefore, not only increased their AME values but have also produced more uniform values. Enzymes have also been shown to decrease the size of the gastrointestinal tract which in addition to increasing the partitioning of nutrients into edible tissue may affect the availability of nutrient due to altered microbial fermentation which in turn may affect the health status of the animal.

Most of the research with enzymes as feed additives has been with poultry. Nevertheless, studies with pigs, especially the young pig, have demonstrated a positive response to enzymes. The recent appearance on the market of recombinant enzymes, especially phytase, should further accelerate the use of enzymes in the feed industry. Some recent reviews on the use of enzymes in the feed industry have been published (Annison and Choct, 1991; Bedford, 1995; Jeroch *et al.*, 1995; Marquardt, 1996).

Although enzymes have proven to be highly beneficial, the use of enzymes is still in its infancy. Also many problems need to be solved before their full potential is reached. Future areas of research that should be considered are presented in this paper.

Areas requiring additional research

Improved enzyme assays

Currently there is no standard procedures to assess the quality of commercial enzyme products nor has a satisfactory assay been developed for the monitoring of the amount of enzyme that is present in the diet. Part of the problem is that many different assays are used to monitor for enzyme activity and within a given assay, assays condition are usually different. Also enzymes, once added to a feedstuff, tends to be present at low concentrations with some being bound tightly to the feedstuff (Bedford, 1993).

Some of the commonly used enzyme assays include measurements for liberated reducing sugars following enzyme action, the use of colored substrates, immunological methods (ELISA), and assays based on the ability of an enzyme to reduce the viscosity of WSNSP (Cowan and Rasmassen, 1993; Headon and Walsh, 1993). Cowan and Rasmassen (1993) reported that among the different methods, the release of dye from the substrate was one of the easiest and most sensitive methods but that it was not of sufficient sensitivity to readily detect enzymes in a feed. The ELISA procedure was capable of detecting enzymes in feed but antisera to all of the different enzymes are not available and some of the ELISA showed a weak reaction to inactivated enzyme. Measurement of the liberated reducing sugars by the enzymes was not sufficiently accurate due to the high background level of reduced sugars in feed relative to that produced by the enzyme itself. Currently a suitable viscosity

assay for feed enzymes has not been reported. These authors concluded that the only practical method to assay for enzyme in a feedstuff was to extract the enzyme from the feed followed by the use of the colored dye method. This assay, however, must be modified to include an extended incubation time to allow for the low levels of enzyme activity that is usually present in the feed.

Although, none of the above methods have been shown to be useful for the assay of enzymes in feed, all can be used to assay enzymes in commercial products prior to addition to diets. It is not possible, however, to evaluate different products on the basis of stated activity values as each of the above assays will yield different activities for the same product. Also within a given assay, activity values from laboratory to laboratory may be vastly different, depending on purity of substrate, assay conditions and technical differences. There is clearly a need to develop a standardized enzyme assay that is simple, accurate and reproducible from laboratory to laboratory. It is important that there also be an association between activity values obtain for a feedstuff and its effect *in vivo*.

Government regulatory bodies, Enzyme Manufactures, the Feed Industry and Professional Societies should therefore make a concerted effort to develop standardized assays for feed enzymes. This is important as it would enable the following:

(i) Permit the manufacture and purchasers of enzymes to establish the comparative value of enzymes based on their activity values.

(ii) Enable the livestock producer to determine the amount of active enzyme product that was added to the diet or the amount that survived the rigors of the environment including heat treatment during processing.

(iii) It would also provide a means of assessing the survivability of the enzyme in different sections of the gastrointestinal (GI) tract, especially in the section where it is most efficacious. The benefits of this are discussed in the next section.

Site of action of enzymes

There is a lack of fundamental information on the site at which enzymes produce most of their beneficial effects within the GI tract. It is not known, for example, if the main site of action of enzymes in chickens is in the crop, proventriculus, gizzard, duodenum, ileum, or rectum, or in all or part of the GI tract. This information would be most beneficial as it would assist in the selection and use of enzymes that are appropriate for the target substrate under the conditions that occur at the site where they are most efficacious. The type of enzymes selected for use in poultry, for example, could be very different if their major site of action is the crop compared to the lower sections of the GI tract especially with regards to their ability to resist proteolysis and a low pH, and the optimal pH at which they act. Also the optimal properties for specific feed enzymes may be different in different species of animals. Enzymes that are effective in the chicken may not be effective in the pig due to differences in their susceptibility to inactivation at a low pH and by proteolytic enzymes.

Production of new forms of enzymes

The current generation of enzymes have been found to be highly beneficial but their usefulness will undoubtedly be increased when new forms of the same enzymes are available. Enzymes from alternate sources other than those currently used such as those produced by microorganisms in the rumen of cattle or from thermophilic organisms will have the following properties: (i) high activities (substrate turnover rates per unit of protein) under the conditions where they act; (ii) high level of resistance to inactivation by heat treatment, low pH and proteolytic enzymes; (iii) inexpensive to produce; (iv) long shelf-life under ambient storage conditions.

These enzymes will probably be made available by the production of recombinant enzymes. This involves the cloning and isolation of specific complimentary DNA (cDNA) encoding for the specific enzyme of interest and the transfer of the cDNA to microbial strains that can be produced in large amounts at a low cost in large scale fermentors (Ward and Conneely, 1993). The use of recombinant DNA technology coupled with site-directed mutagenesis will enable the production of enzymes

tailored to the specific requirements of the animal in which it is to be used. This powerful technology was developed by Dr. M. Smith and coworkers, the 1993 Canadian Nobel Prize Laureate, for their seminal work on mutagenesis at a specific position in the DNA sequence (Hutchison *et al.,* 1978).

A new area is the generation of a second type of tailor-made enzymes. This involves the production of catalytic antibodies from immunoexpression libraries using a microorganism such as *Escherichia coli* as the expression system. This technology will provide the basis for the production of specific bioengineered antibodies in fermentors that have enzyme-like properties designed to meet the specific requirements for each feedstuff and each class of livestock and poultry (Lerner *et al.,* 1992; Mayforth, 1993). Other modifications of these procedures will undoubtedly be developed.

Alternate sources of enzymes

As indicated above, enzymes will not only be produced directly from fungi that have been improved using traditional methods but will be expressed in microorganisms such as bacteria and in plants such as canola seed. The availability of an abundant supply of enzymes in the seed of canola would dramatically reduce their cost to the livestock producer.

An alternate strategy for the hydrolyses of antinutritive compounds in animal feeds is the construction of transgenic monogastric animals able to digest cellulose, -glucans, xylans or phytic acid. Forsberg *et al.* (1993) has reported that it should be feasible to use bacterial DNA constructs to express and secrete glycanases in rat pancreatic acinar cells lines. The major challenge will be to obtain a sufficiently high level of expression of the glucanase gene(s) and other genes in pancreatic cells to effect adequate hydrolysis of the glucans, xylans, etc. in the intestine. Of particular interest would be the introduction of a phytase gene into domestic animals.

Role of synergistic interaction among enzymes

Nearly all of the research on the response of poultry to enzymes, with the exception of phytase, has been with crude fungal extracts high in desired activities such as -glucanase or xylanase but also having a considerable amount of other enzymes including proteases. Research should be carried out with different combinations of pure enzymes (i.e., devoid of synergistic enzyme activities) to determine if the principal enzymes have a synergistic, antagonistic or additive effect. For example, the ability of enzymes to reduce the viscosity of the water-soluble arabinoxylans in wheat or rye may not only depend upon the amount of endo-xylanase in the preparation but also that of arabinofuranosidase and possibly -glucanase, acetylxylan esterase and feruloyl esterase (Forsberg *et al.,* 1993). It is also conceivable that preparations high in protease activity would have a negative effect as they would enhance protein digestion including that of the added enzymes. The availability, in the future, of cloned enzymes that are devoid of other enzyme activities will enable researchers to determine the benefits of using various cocktails of enzymes for the improvement of the nutritive value of cereals. Such studies will become more important with a shift from the use of crude fungal enzymes to that of enzymes produced by recombinant DNA technology.

Nature of the interaction of enzymes with dietary constituents

Recent studies indicate that the presence of a saturated fat (tallow) compared to an unsaturated fat (soybean oil) in diets that contain a high level of WSNSP can dramatically depress chick performance. Studies by Dänicke *et al.* (1995), for example, demonstrated that the enhancement of growth and fat digestibility due to enzyme supplementation of a soybean diet, although large, was completely over-shadowed by the enormous response obtained with a tallow-based diet. Similar results were also reported by Schutte *et al.* (1995). These observation indicate that the response obtained when enzymes are added to the diet are not only affected by the viscosity of the WSNSP but also the presence of other dietary ingredients such as tallow. Further research should be directed to establishing the nature of these interactions as they will provide fundamental new information on the mode of action of enzyme and on the optimization of diets under different dietary conditions which in turn will be of considerable economic importance.

Modeling studies with enzymes

Recent studies by Zhang *et al.* (1996) have demonstrated that it is possible to predict the response of poultry to a particular feed enzyme using a simple linear model. The model predicts that the response to enzyme supplement is a function of its concentration when converted to a logarithmic value and that a doubling or halving of the response to enzyme treatment can only be achieved by varying the amount of enzyme by a factor of 10-fold, not two fold as may be expected. It was also shown that it was possible to simultaneously predict response to enzyme supplement for diets that contain any given amount of enzyme and any proportion of two cereals. This model can be readily adapted to least cost-analysis, provided accurate input data are available, and therefore would provide a basis of estimating economic return per unit of enzyme added to the diet. Further research, however, is required to verify the model, to further simply its use and to obtain base-line values. This model should provide a sound basis for prediction of responses obtained with any given amount of enzyme for any given feedstuff.

Studies with other animals

A very limited number of species of animals have been evaluated with regards to their response to enzymes. Although studies with chickens have clearly established the benefits of enzyme as feed additives, only a limited number of studies have been carried out with other species of poultry such as turkeys, ducks, geese, ostriches, etc. Also very few studies have been carried out with species of animals such as fish, eels, alligators, turtles, other exotic animals, pets such as dogs and cats, fur bearing animals, etc. that have a simple stomach. Enzymes may be particularly beneficial in species of animals that tend to be carnivorous as they tend to have stomachs with smaller large-intestines and therefore they do not house a large population of anaerobic microorganism capable of hydrolyzing complex carbohydrates. Further studies also need to be carried out with pigs of different ages particularly when fed different fat sources.

Finally, the use of recombinant rumen microorganism that contain over expressed and bioengineered cellulases and hemicellulases as inoculum for ruminants may increase efficiency of forage utilization in ruminants. Clearly there appears to be many possibilities for use of enzymes in other species of animals. Also, there are probably many enzymes other than those that have been used to date that can be effectively used in the livestock industry.

Target substrates in cereals

It has been hypothesized by numerous researchers that the principal mode of action of most enzymes is the destruction of gel-form polysaccharides leached from cereal cell walls in amounts which depresses performance (Annison and Choct, 1991; Bedford, 1993; Chesson, 1993). An alternate explanation has been proposed by Hesselman and Aman (1986). They proposed that the -glucans and arabinoxylans which form the endosperm wall of cereals, act to restrict access of enzymes to nutrients. They postulated that the disruption of intact walls and the release of entrapped nutrients is the major factor responsible for the improvement of nutritive value ascribed to exogenous enzymes. Chesson (1993) has postulated that a single enzyme should be effective if the beneficial effects of enzymes are attributable solely to viscosity reduction. The reason is that since viscosity is partially a function of chain length as it is only necessary to break the chain in a few sites to substantially reduce or destroy its gel-forming capacity. However, if disruption of intact cell walls and release of entrapped nutrients make a greater contribution to the beneficial effects of enzyme supplementation than viscosity reduction, then many more enzymes may be required. Some of the required enzymes have been discussed above and by Marquardt (1996). Research should establish which of the two effects are most important as these factors have important implications in the economics of enzyme usage. Experiments involving the use of different combinations of purified enzymes should provide information on the significance of the viscosity of the diet compared to the entrapment of nutrients within the cell walls as factors responsible for the antinutritive effects of nonstarchy polysaccharides.

Physiological and endocrine induced changes

Essentially, no studies have been carried out in the effect of the viscous WSNSPS on the output of pancreatic and other intestinal secretions including enzymes and bile salts, on the immune response, on hormone levels including growth hormone, thyroid hormone and insulin and on glucose tolerance, and on the ability of exogenous enzymes to counteract these effects. The inclusion of WSNSP in the diet in the presence and absence of enzymes will provide a sound basis for further interpreting the effects of the viscous nondigestible polysaccharides on physiological responses in animals. This will not only have practical benefits but will result in the extrapolation of results to humans where there is great interest in the ability of fibrous foods to alter nutrient uptake. Dr. Han (1996) has initiated a comprehensive study in this area and has demonstrated an effect by WSNSP on many of these parameters. Extensive studies will be required to completely characterize these effects.

Conclusions

The use of enzymes in the past several years as supplements to feeds has expanded dramatically. The research that has lead to this development has been mostly carried out at Universities and in Research Institutions and has been funded in a large part by the industry in cooperation with government agencies. Although dramatic progress has been made in the past decade on the use of enzymes in poultry and livestock many areas require additional research so as to exploit the full potential of this very powerful and beneficial technology. Areas of research and development that should be carried out in order to provide a basis for the better use of enzymes include the development of more sensitive and accurate assays, a more precise identification of the most desirable catalytic properties of enzymes that are required for different classes of livestock and poultry and the effects that enzymes have on the physiological and endocrine responses of animals fed cereal-based diets. Research in the future should clarify some of these problems. Also a better understanding of the mode by which enzymes produce their beneficial effects, the nature of the interaction of enzymes with different dietary components and the development of simple models to predict response to enzyme treatment would greatly facilitate their use. In the future many new enzyme products will become available, many of which will be produced by recombinant DNA technology. They will have superior stability and catalytic properties and will be available at a relatively low cost. They will also find wide application with many different classes of poultry, livestock, and other types of animals. Enzymes in addition to improving animal performance will also be shown to have many other beneficial effects including reduced pollution of the environment. Enzymes as feed additives not only have had a very great impact on the livestock industry but will continue to provide an ever increasing range of benefits in the future. There are many challenges in this rapidly expanding field.

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