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Technical aspects of enzyme utilization: Dry *vs* liquid enzymes

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SUMMARY - It is difficult to draw conclusions on the general technical literature on enzymes, as new developments are rapidly taking place. The general assumption, however, is that liquid enzymes are generally less stable than solids although they may have greater activity than their solid counterparts. In general practice it is assumed that the loss in activity of liquid enzymes due to thermal and mechanical treatments can be substantially minimized once incorporated into the feed, as their hydric content is decreased. The feed acts therefore as a stabilizing agent. With present manufacturing processes of enzymes, they can easily be sprayed or added on to special carriers, thus making them more resistant to thermal treatments, although this carries and additional cost in their production. It is therefore generally concluded that the choice of a liquid enzyme, either directly in its liquid form or sprayed or absorbed onto a solid carrier, is in many cases a simple commercial decision. It seems clear that, apart from the intrinsic characteristics of the enzyme utilized, the same enzyme, when applied onto a solid carrier, be it either the feed itself or an adequate carrier, increases its resistance to temperature. On the other hand, when the thermal process of the feed exceeds 90°C, liquid enzymes may present some advantages in terms of activity losses.

Key words: Enzyme supplementation, liquid enzymes, dry enzymes, enzyme stability.

RESUME *- "Aspects techniques de l'utilisation d'enzymes : Enzymes secs et liquides". Il est difficile de tirer des conclusions en ce qui concerne la littérature technique générale sur les enzymes, car de nouveaux progrès ont lieu très rapidement. Il est cependant généralement admis que les enzymes liquides sont normalement moins stables que les enzymes solides bien qu'elles puissent avoir une activité plus grande que les enzymes solides. Dans la pratique générale, on accepte que la perte d'activité des enzymes liquides due aux traitements thermiques et mécaniques peut être substantiellement minimisée après les avoir incorporées à l'aliment pour bétail, car leur teneur en eau diminue. L'aliment agit donc comme un agent de stabilisation. Etant donné les processus actuels de fabrication des enzymes, elles peuvent facilement être nébulisées ou ajoutées dans des substrats porteurs spéciaux, ce qui les rend plus résistantes aux traitements thermiques, quoique ceci entraîne un coût supplémentaire dans leur production. La conclusion générale est donc que le choix d'une enzyme liquide, que ce soit directement sous sa forme liquide ou nébulisée ou absorbée par substrat solide, est bien souvent une simple décision commerciale. Il semble clair que, outre les caractéristiques intrinsèques de l'enzyme utilisée, la même enzyme lorsqu'elle est appliquée moyennant un substrat solide, que ce soit l'aliment lui-même ou bien un substrat adéquat, augmente sa résistance à la température. D'autre part, lorsque le processus thermique d'élaboration de l'aliment dépasse 90°C, les enzymes liquides peuvent présenter quelques avantages en termes de perte d'activité.*

Mots-clés : *Supplémentation enzymatique, enzymes liquides, enzymes secs, stabilité des enzymes.*

Enzymes are protein molecules that play a definite role in catalyzing specific chemical reactions in living beings. Among the practical considerations to consider, when trying to qualify an enzyme, we should point out their activity, stability and substrate specificity. There are presently a large number of enzyme preparations marketed within the scope of the agricultural and food markets. Most of them come from the world of molds, bacteria or yeasts and some of them can even be classified as of animal or vegetal origin (Kworr, 1987; Staton, 1988) Among the microorganisms most commonly used to produce enzymes we could list: *Aspergillus niger*, *Trichoderma viridae* and *Bacillus subtilis* (Table 1).

Table 1. Principals microorganisms producers of enzymes

Aspergillus niger	α-amylase, β-glucanase, cellulase
Aspergillus ficuum	fitase
Aspergillus candidus	
Aspergillus sydowi	
Aspergillus oryzae	α-amylase, neutral protease
Bacillus licheniformis	α-amylase
Bacillus subtilis	α-amylase, neutral protease, β-glucanase
Trichoderma viridae	xilanase, β-glucanase, cellulase

These enzyme preparations, developed initially for the food industry, have seen their marketing strategies expanded to include the animal feed business, both in the monogastric as well as the ruminant species, as has been reviewed by several authors (Chesson, 1987) and more recently by Vanbell *et al.* (1990); Campbel and Bedford (1992) and Wenk (1993). Experiments conducted with animals bring forward data to show the limited digestibility of some nutrients from vegetal origin in the small intestine of pigs and poultry (Pettersson and Aman, 1989; Low and Longland, 1990). This loss of nutrients can be due to an insufficient enzyme production, as is the case in the very young animal, to an inadequate provision of natural enzymes or even to an interference, at intestinal level, with indigestible substances from cellular walls (Hesselman and Aman, 1989).

Improvements to be expected from enzyme supplementation are derived from the foreseeable increment in the nutritive value of the diet, mediated through an improvement in the digestibility and absorption of most nutrients but very particularly from polysaccharides, proteins and phosphorus present in the different raw materials used in the feeding of monogastric animals. Through their use one should expect not only quantitative improvements in their productivity (Rosi *et al.*, 1987; Rotter *et al.*, 1988; Inborr, 1989) but a significant reduction in environmental pollution (Headon, 1993). In as far as improvements of digestibility of cell wall polysaccharides, which has received maximum interest, most of the work conducted to date has been in the field of poultry nutrition and specifically on those enzymes capable of acting against ß-glucans of barley and oats (Hesselman and Aman, 1989; Van der Klis, 1993) as well as on soluble pentosans of rye and wheat (Thacker *et al.*, 1992a; Morgant *et al.*, 1993), both groups of polysaccharides considered to have antinutritive properties.

The utilization of specific enzymes in diets formulated with grains and cereals make it possible to reduce their negative effects on digestion and absorption of nutrients (Brufau and Francesch, 1991), thus improving significantly most productive parameters (Francesch, 1991; Thacker *et al.*, 1992a and 1992b). Through their use, therefore, the nutritive value of certain raw materials can be increased, making it possible to increase their level of utilization, at the expense of more expensive ingredients, without impairing the productivity of animals

Technological aspects of enzyme utilization

Enzyme stability

The enzyme molecular structure can be easily altered by external factors. We must therefore exercise a rigorous control over any factor which, through the feed productive chain, can modify the activity of the enzyme and thus interfere with the expected positive response. We know that whatever process that results in heat production, specially above 70° C. or even long storage periods, can cause denaturalization of the enzyme and thus render the molecule either partially or totally inactive for the use intended. Vitamins, minerals, trace elements as well as some prooxidant agents, which can be normally found in feed premixes, can also modify enzyme stability and thus decrease enzyme activity (Inborr, 1990).

Thermostability

Thermostability can be defined as the capacity of the enzyme to resist, during a given period of time, the temperature of the process to which it is subjected.

Thermal stability is an important factor to consider in view of the extended usage of heat producing processes in the production of feeds for swine and poultry (pellleting, extrusion, etc.). However, there is both scientific as well as practical evidence, that when temperatures range between 70 and 90ºC, enzyme activity continues to be unaltered and significant improvements can still be obtained (Classen *et al.*, 1991; Francesch *et al.*, 1991; Mascarell, 1994).

Enzyme producers are generally confronted with the need to guarantee the stability of their enzyme preparations, as well the amount of heat that their enzymes can withstand without deterioration. Although criteria used to measure stability vary with products, it can be concluded that within temperatures between 50-80ºC any commercial enzyme should present good thermal stability. At this point it may be stated that it has been repeatedly shown that enzymes in solid form are much more stable to heat than their corresponding liquid presentations (Inborr, 1990; Kung, 1993), withstanding temperatures as high as 90ºC during 30 minutes without any significant losses.

Enzyme stability at different gastro-intestinal pH

Enzyme stability can be substantially modified during transit through the different gastro-intestinal compartments, due to sudden and significant pH changes as well as to the action of proteolytic enzymes, both from endogenous as well as microbial origin. These possible effects are extremely important as it is in these digestive compartments where their action is going to take place.

Enzymes to be used in animal feeding are therefore selected on the basis of the pH and temperature which are going to be found in the intestinal areas where the enzyme will develop its maximum activity. While it is easy to guarantee, inside the animal, a constant temperature (38 -40ºC), the same does not apply to pH values, as these will change from acid to basic depending on intestinal sections, being the stomach, with its clear acid pH (2-5), as well as the duodenum, considerably more basic (6-8), the areas where the enzymes will be most likely put to work (Mascarell, 1994) (Fig. 1).

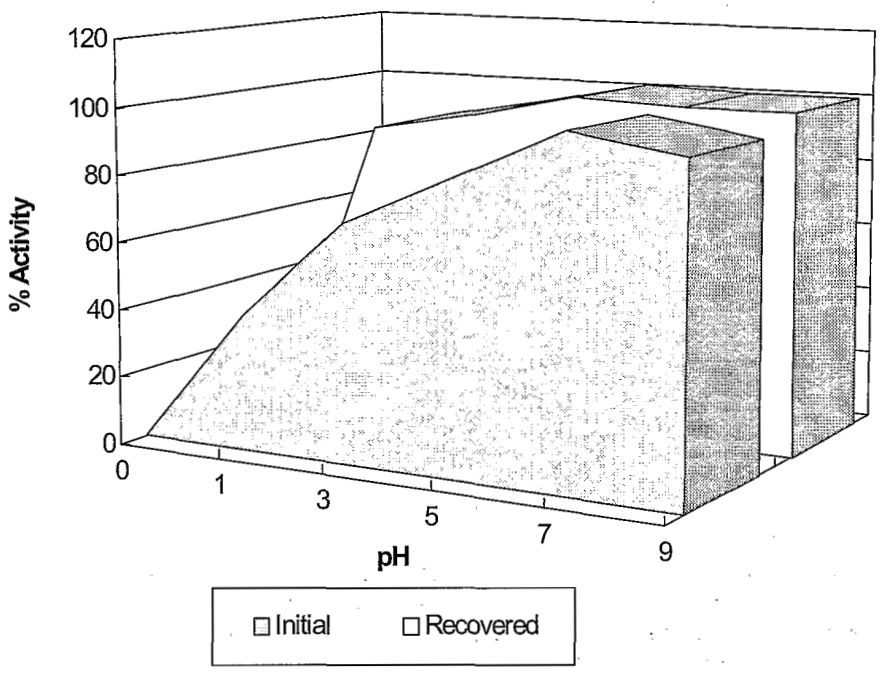

Fig. 1. Initial and recovered activities of bacterial ß-glucanase at different pH va lues.

Origin and activity

Enzymes used in animal feeding are almost exclusively from microbial origin, being from either fungal or bacterial origin and normally come as a complex mixture of other molecules with collateral activities. It is at this point important to remember that enzyme origin determines their molecular structure and that, due to the undissociable biochemical profile of that structure, this will determine their pattern of activity.

Thus we see that fungal enzymes normally require a pH below 5 for optimal activity, while the pH required by bacterial enzymes is generally closer to neutrality (Fig. 2). At the same time, and from the standpoint of thermal stability, we know that bacterial enzymes are more resistant to heat than those derived from fungi, even though some clear exceptions are known.

It is therefore quite possible that from a strictly marketing standpoint, different enzyme preparations may be presented with the same enzyme activity denomination and yet have different composition, origins and activity patterns, which may significantly condition their real and practical activity. These considerations have motivated the need for titration curves for each commercial product, which may make it possible to correctly interpret recommendations on their use as well as analytical results when comparative studies are made.

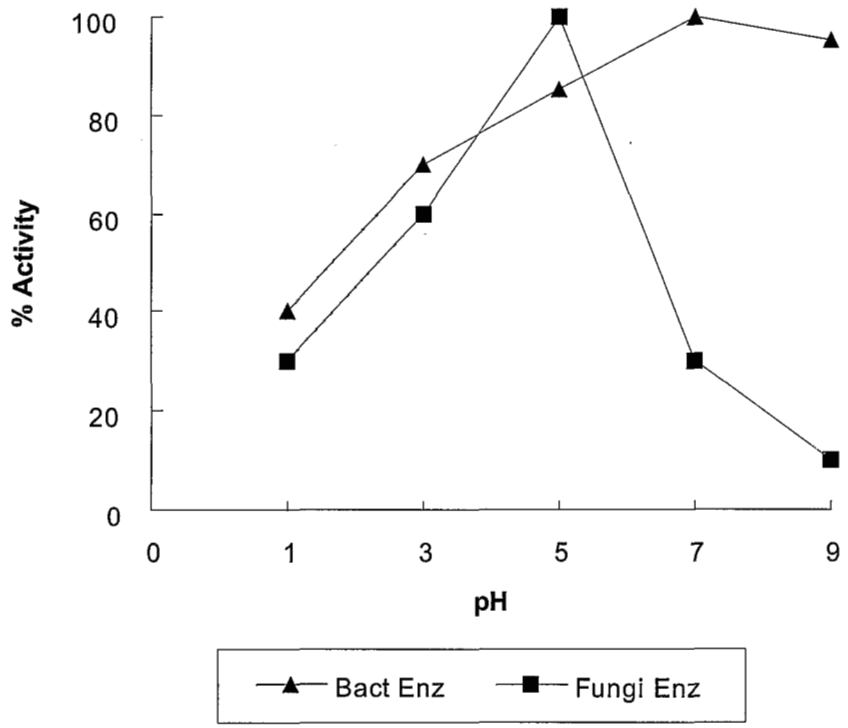

Fig. 2. Activity standards curve of bacterial and fungal ß-glucanase at different pH values.

Substrate specificity

Besides all considerations on origin, stability, etc. the specificity of substrate should also be considered if optimal results are to be obtained. Of all enzymes in use today, those with greatest importance and interest are undoubtedly the group known as hemicelulases, name normally applied to the different types of enzymes, which are integrated into the greater depolimerase denomination (amilolytic, pectinolytic and celulolytic enzymes) (Voragen *et al.*, 1982).

This type of denomination, however, becomes confused, especially when trying to establish relationships between enzyme and substrate. Contrary to the case of cellulases or amylases we

should emphasize that the designation of the vegetal cell wall polysacharases should be made with the chemical name of their substrate (glucans, xylans, etc.) rather than by their solubility (hemiceluloses, pectins, mucilags, etc.). Due to the fact that hemicelulases do not have hemicelulose as such, as specific substrate, we define as hemiceluloses different polysaccharides located in the cell wall (arabans, galactans, glucomanans, etc.) and since these specific compounds are not normally available in a purified form, we should refer to their corresponding hydrolytic enzymes as glucanases, xylanases, etc. (Brillouet and Hoebler, 1986).

Enzyme activity

Analysis of enzyme activity, as a quality control determination and evaluation of enzyme activity and stability is an important point to consider, not only from the standpoint of enzyme definition but even from a commercial viewpoint since the potential user should be able to refer his selection of enzyme to objective qualities and practical aspects of the different available products. This explains the numerous attempts to standardize these analysis, through assays requiring easily available substrates and methods reflecting physiological reality, with the ultimate objective of obtaining simple and reliable data.

Unfortunately the number of assays and analysis methods available is too large and variable, practically as many as there are enzymes in the market. This means that from a practical standpoint it becomes extremely difficult to carry out comparative analysis and draw direct conclusions from analytical results. If we add to this complexity the fact that the enzymes are used at very small dose levels, plus the known difficulty in recovering them from the feed once they are added, we will easily understand the variations and discrepancies normally encountered in this type of analysis. It is expected that advances in analytical facilities as well as new methods, such as liquid chromatography or gel filtration will contribute to increase reliability of analysis.

Enzyme activity is normally analyzed through the direct or indirect determination of the rate of disappearance of the specific substrate, or of the enzyme itself, at different time intervals or through the analytical quantification of the resulting compound, after a given period of time. This last method is presently used by most enzyme producers.

Most analytical methods in use today can be grouped in four different type analysis, when enzymes for animal feeding are considered. The dinitro salicilate method, which detects reducing sugars appearing as a direct effect of enzyme activity on carbohydrates, under known temperatures and pH (Bailey, 1988). A second method bases its effect on the use of coloured substrates, normally a polysaccharide and a dye, such a Congo Red. These techniques make it possible to follow the rate of the reaction, since these substrates remain insoluble in the absence of enzyme activity and begin to release coloured products, as soon as enzyme activity begins (Wood, 1981).

A third method refers to the use of radial diffusion techniques, on agar gels. Chemically coloured polysaccharides are added to the agar gel and the enzyme preparation to be analyzed is deposited on little wells practized on the agar. Enzyme activity can be followed visually, as while the enzyme acts concentrical decoloration rings are formed around the wells and these can be measured and directly correlated to the concentration and activity of the enzyme. This method is currently used to evaluate enzyme activity in mixed feeds (Edney *et al.*, 1986)

We should finally describe viscosimetry (Somogy, 1960) methods as the last group of enzyme determination techniques. In these, enzyme activity is determined through the reduction in viscosity of a solution as a function of time. Viscosity determinations can be used to estimate the effects of enzyme supplementation of feeds containing barley or oats on practical diets (Pérez Vendrell *et al.*, 1991)

While viscosity assays are used rather frequently in the feed industry, due to their effect being taken as a clear reflection of what takes place in the living animal, they are too time consuming and therefore their practicality is reduced. New methods are however appearing in the literature, with similar accurities on sensitivity and enzyme specificity as viscosity determinations, that will soon permit not only enzyme tritation in commercial products but also in supplemented complete feeds (McCleary, 1991)

Residual activity

Residual activity is defined as the activity found in a commercial product or mixed feed, once the enzyme is added and after having the product subjected to a thermal or mechanical process, in relation to the initial amount added.

The proportion of enzyme normally recovered after a process of pelleting or even after simple mixing is about 40 to 50% of the initial activity added (Fig. 3). This is assumed to be due to the great affinity of specific sites in the enzyme molecule for certain components of regular diets (Golovchenko *et al.*, 1992). It has been reported that some enzymes, such as ß-glucanases show a great affinity for cellulose, binding each other intimately, although in such a way that their enzyme activity is not affected. Therefore the activity of the enzyme is not affected even if the enzyme cannot be completely recovered by analytical methods. That is why biological tests are then normally required in order to obtain a correct reading of their activity.

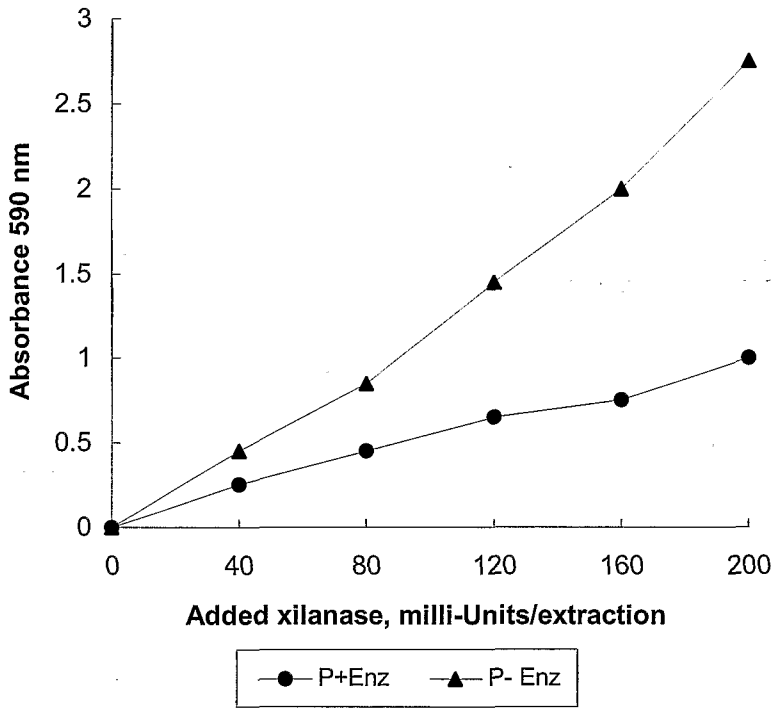

Fig. 3. Curves of recovery of xilanase (*T. longibrachiatum*) with or without pelleted feed.

Feed processing

We have seen that enzymes are proteins with catalytic properties that can disappear as a result of changes in their three-dimensional structure. They are susceptible to degradation by environmental factors, such as pH, high temperatures and microbial contamination.

Because of evident positive results, enzymes are quite extensively used in poultry and pig diets. However, since the processes of compounded feed manufacturing involve exposure to high temperatures and pressure (pelleting, extrusion, expansion, etc.) these processes have to be considered as very aggressive to the enzyme, from the standpoint of enzyme stability.

Enzymes are regularly added to the feed mix in either one of two accepted methods. Some companies prefer to add the enzyme in a solid form, as a component of the premix formulation. In this case it will pass through the pelleting process, assuming no significant losses will be produced during pelleting. Other companies however, prefer to add the enzyme once the pellets have been produced

and cooled. In this case, the enzyme is supplied as a liquid solution, which is sprayed onto the pellets and no risk is taken during the pelleting process.

Other processes, such as milling and crumbling, even though they form part of feed processing, are not considered from the standpoint of enzyme stability, as milling is very rarely applied to already mixed feed and even in that case, as it is also the case with crumbling, the heat generated in those steps is not high enough to presumably affect enzyme stability.

Pelleting

Pelleting can be defined as a mechanical process in which the effects of friction, pressure and extrusion coincide along with a given temperature rise. Pelleting can be considered as the most important development achieved in the history feed production since its very beginning.

In spite of the obvious benefits obtained from pelleting, one should not forget that the better the pelleting process, if we base it on the improvement in digestibility and reduction in its microbial contamination, the greater is going to be the loss of micronutrients and additives such as enzymes.

While referring to enzyme supplementation, we know now that the steam applied for pelleting is a key factor in the maintenance of their activity. Several studies have shown that the effect of pelleting on enzymes has to be considered in three well defined steps of the process. The first one would be the preconditioning chamber, where steam applied will generate an increment in heat and humidity; the second occurs at the level of the pellet die, where heat is produced while the feed is forced through the die perforations and the third and last is cooling of the pellets, where temperature must fall rapidly until room temperature is achieved.

Of all these, we know that the steam applied for conditioning is the main factor to consider in enzyme stability (Eeckhout *et al*., 1995). When the level of humidity increases, enzymes may become hydrated in such a way that their thermal stability decreases. This will occur mainly in the preconditioning chamber, as the amount of steam applied to prepare the feed for pelleting will increase its moisture content and therefore render the enzyme more sensitive to oncoming mechanical stress.

We can therefore conclude that, in as far as the enzyme is concerned, the more significant losses in activity will occur in the preconditioning chamber and that a positive correlation has been found between the temperature in the preconditioning chamber and enzyme inactivation (Table 2).

Table 2. Feed residual ß-glucanase activity after preconditioning (% A Ac.) and pelleting (% Apl.), referred to initial, prepelleting activity

Temp. Ac. (ºC)	Moisture	% A Ac.	Temp. Pl. (ºC)	% A Pl.
50	10.0	76	72	60
60	11.2	56	74	46
70	11.2	38	80	25
80	12.0	25	81	19
90	12.4	16	91	12

It must be considered that to adequately pellet mixed feeds included in class I (such as poultry and swine feeds), which normally have a high cereal and starch level, temperatures in the preconditioning chamber are supposed to reach 80ºC, if the nutritional improvements associated with pelleting are to be achieved. It is therefore obvious that with that type of feeds, the enzymes to be used must be capable of resisting high temperatures.

As previously stated, the pellet die is another area where the enzyme is bound to suffer from a thermal stress. In general the temperature of the meal going into the pellet die chamber may show a

rather wide range of variation, generally between 50 and 90ºC, depending mostly on the steam pressure applied in the conditioning chamber and the moisture content of the feed going to be pelleted. If we consider losses at this point, which is the one normally taken in experimental studies on thermal stability of enzymes, we can state that 20 to 60% of the initial activity is lost during this part of the process although when comparative studies between losses in the conditioning chamber and pellet die are done the real loss in this point only represents 10% of total losses observed during the whole process of pelleting.

Expansion and extrusion

Extrusion as well as expansion is a hydrothermal process that can be included in the high temperature-short time processes where pressure is modified. Most expanders work under 25-40 bars of pressure and temperatures between 90-130ºC, with treatment times between 5 and 20 seconds (Angulo and Puchal, 1995), expansion having become a regular process for meal before pelleting. In wet extrusion, feeds are subjected to high levels of moisture (20-35%), with pressure and heat treatment generated by the extruding screw and steam that can be maintain during several minutes. As a result of these effects, both raw materials and feeds see their nutritional values increased, either through inactivation of toxic components or increased fiber and starch digestibility, as well as control of enterobacteria and Salmonella contamination, although these positive effects may be correlated with a significant destruction of vitamins, aminoacids and unavoidably, enzymes (Pickford, 1992) (Table 3).

Table 3. Influence of expander and pelleting temperature on protected and unprotected en zymes

Temperature (ºC)		Recovery (%)	
Expander	Pelleting	Protected	Unprotected
85	68	100	95
95	75	80	61
105	83	31	0

Wet/soupy feeds

Liquid feeding of pigs is becoming increasingly popular, mainly in those areas were industrial by-products, in a liquid form can be obtained at competitive prices to reduce cost from traditional feeding systems. Since enzymes need water to be active, liquid feeding not only provides excellent opportunities for application of feed enzymes, but also from a biochemical and physiological point of view.

The possibility of enzyme activity outside the gastrointestinal tract, when enzymes are used in a liquid form, might skip the inactivation associated with temperature and both stomach pH and intestinal proteolytic enzymes. In this regard it should be considered that liquid feeding is more adequate for enzyme activity than dry feeding conditions since keeping the mixed liquid feed in the tank for a brief period of time, before it is pumped, can be considered as an enzyme pretreatment. With liquid feeding, optimal temperatures (35-40ºC) as well as adequate agitation, necessary for quick action of enzymes in a brief incubation period (30-35 min.), are easy to maintain prior to feed distribution. plus the fact that optimal conditions for the enzyme to be used are also easy to adjust.

Unfortunately this type of feeding requires expensive installations but otherwise allows the utilization of perishable or hard to handle by-products and permits reducing feeding expenses. From the scientific point of view, not very many experiments with liquid feeding of pigs have been reported, and only a few include enzyme utilization. However, results from experiments with growing/finishing pigs have shown quite consistent improvements in feed efficiency (Inborr, 1991) (Table 4).

Table 4. Liquid feed trial on commercial farms

	Control	Enzyme
Daily weight gain, (g)	886	868
Feed conversion	2.65	2.58

Strategies

The most effective method to modify enzyme stability, although the most costly and difficult, is through action on the enzyme producing microorganism, with direct selection on the initial steps of enzyme synthesis, thus modifying the intrinsic characteristics of the enzyme obtained (Classen *et al.*, 1991; Ward, 1993). Enzyme activity can be modified through biotechnological methods, till the necessary profile of activity is obtained. Although enzyme activity is defined in a general way as applied to feeds, we must realize that both the origin and type of strain utilized may substantially modify the type of activity that the enzyme will carry out in the intestinal tract.

The need to adequate the enzyme to the physico-chemical characteristics of the substrate and environment on which this substrate is to be found, has stimulated biotechnologists to develop techniques in order to do so. The use of recombinant strains (Ward and Conneely, 1993) to obtain the so-called hybrid enzymes, with significant modifications regarding their thermostability and pH resistance has repeatedly appeared in the technical literature as techniques easily accomplished. This approach, even though very promising, is rigidly controlled by EEC regulations, which controls the utilization of enzymes obtained through the use of genetically modified microorganisms, for fear of their pollution potential if released into the environment.

Pre-pelleting application

Enzymes can be inmovilized and protected until their target is reached, by means of substances, normally known as "carriers", which will either stabilize and/or absorb the enzyme. Uncompleted enzyme retrieval from these carriers can be another of the causes of the loss of enzyme activity, although this type of problem seems to have been adequately solved through the use of specific carriers (Kung, 1993; Gadient and Tritsch, 1995).

When considering the concept of enzyme additives, one should bear in mind that there are two factors, which can modify, and in fact they normally do, the expected results. On the one side there is the enzyme with its own biochemical properties and on the other the carrier or substance utilized to give the enzyme a solid form and adequate volume. One should therefore realize the importance of the nature of the carrier, along with its interaction with the enzyme it will "carry", not only from a commercial but from a functional standpoint.

Enzymes can therefore exist as simply "carried on", absorbed, micronized, etc., each of them supposed to be an improved form from the previous one., from the standpoint of stability and reliability. In many cases this specification has been utilized in a general form, without much definition, as it is generally accepted that a purified enzyme, taking as such that coming from the original culture medium, can be transformed into solid forms, on organic or inorganic carriers. In these cases we should refer to "absorbed enzymes", and these, from a practical standpoint, do not normally show good thermal stability, and they generally have losses of up to 50% of their activity when subjected to feed processing, losses that from an economical point of view make these products practically unacceptable. The reason for this different behavior can probably be explained in the sense that the enzyme that is absorbed on a solid product normally remains on the external surface of the carrier and is therefore more easily subjected to inactivation.

The enzymes known as "second generation", "thermo-resistant" or "coated" enzymes are normally enzymes subjected to micronization or coating processes. These enzymes are physically treated so as to obtain a micronized enzyme, in a solid form, which later on can be incorporated onto a carrier or

subjected to a coating process with products such as fats, starches, etc. That is why the carrier utilized becomes a fundamental part of the product because it does not only contribute to the solid structure of the product but helps stabilize its enzyme activity, making it resistant to extreme temperatures and pH. This also explains why enzymes in solid form have presently a very good thermal stability, being capable of resisting temperatures close to 90°C, even for periods over 30 minutes (Cowan, 1993) (Fig. 4).

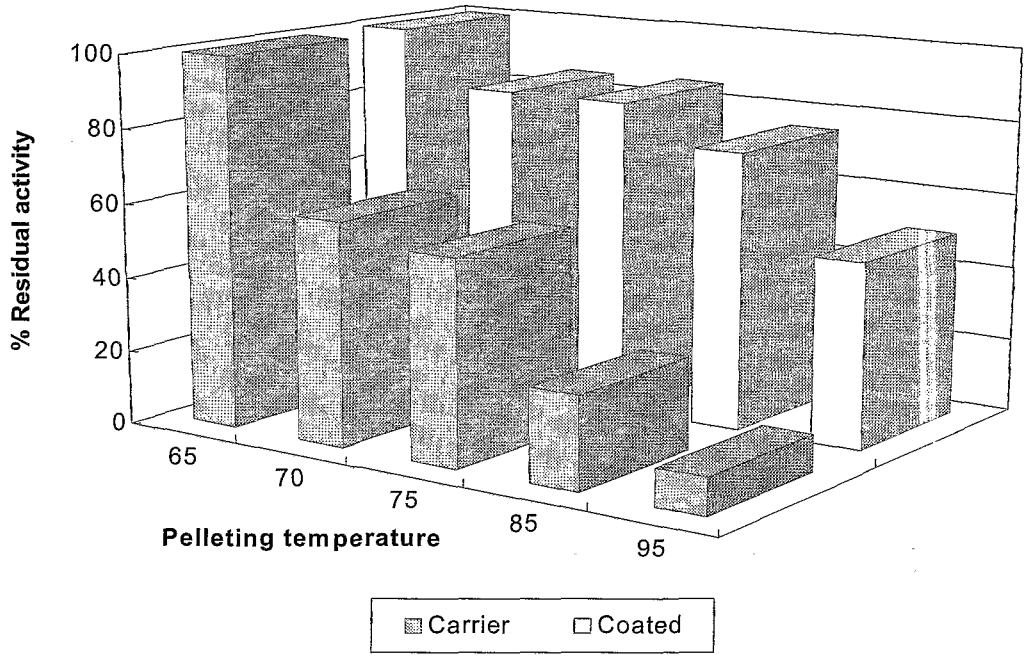

Fig. 4. Stability of carrier enzyme products *vs* second generation coated enzymes at pelleting temperatures.

It is therefore understandable that, in view of the aforementioned effects of carriers and enzyme processing, we may find great differences in thermal stability of the different commercial products. If we contemplate only those enzymes generally used in animal feeding and their supposed thermal stability, we can conclude that xylanases and ß-glucanases are normally quite resistant, while α-amylases and α-galactosidases are more sensitive to thermal stress (Gadient and Tritsch, 1995).

Post-pelleting application

The obviously apparent loss of activity due to pelleting or extrusion has motivated the possibility of incorporating enzymes after the thermal processes are done, directly in liquid form, a technical strategy that seems quite adequate when very aggressive processes are considered. However, before this strategy is applicable, several factors should be considered. The first one is the system used to incorporate the product, that is, a system that permits a liquid dosification which guarantees a homogenous distribution of the enzyme and therefore an intimate contact of the enzyme with the substrate. The requirements for dosification are very strict and therefore define the spraying system to be used. In this review we will only deal with general considerations, overseeing the technical aspects of the systems presently in use (Fig. 5), without getting into the description of their advantages and disadvantages (Table 5).

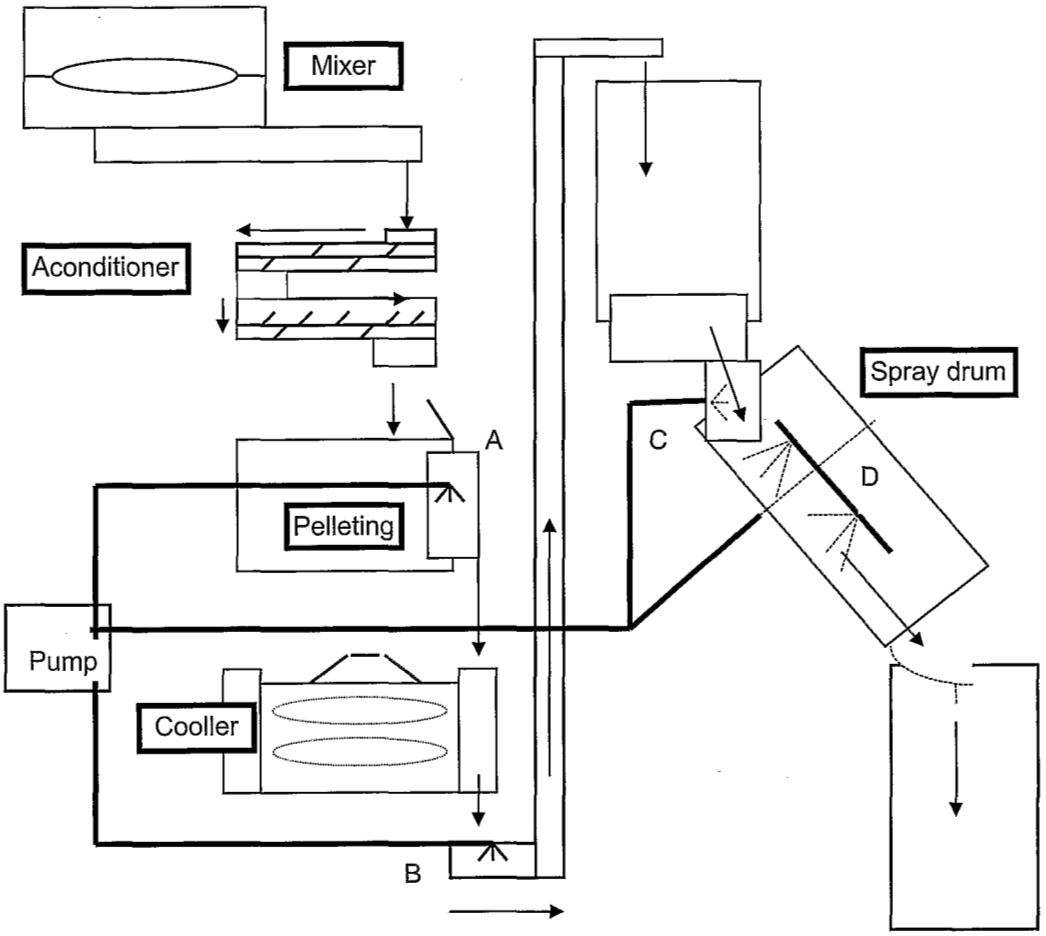

Fig. 5. Post-pelleting application (Liquid enzymes)
A: Die
B: "On line" After cooling
C: "On line" before refat
D: Spray drum (fat or enzyme)

Table 5. Percentage of recovering after a pelleting process with pre-pelleting or post-pelleting application of a liquid enzyme

	65ºC	80ºC
Pre-pelleting		
Control (MIXER)	80	65
Post-pelleting		
MATRIX	95	110
COOLER	120	120

The spraying process is normally defined by the dose level required as well as the number, size and distribution of the spraying nozzles and the working procedures of the system, which can be either of the batch or continuos type. Trying to obtain a liquid spraying system that will reach the maximum number of feed particles is, of course, the main objective. In the continuos type system, the liquid is applied in the form of a liquid curtain, with turbulence movements, modifying if necessary the time that the feed is exposed to the enzyme. In the batch system it is only necessary to control both spraying equipment and time of application.

On the other side we should consider the type of feed to be treated, as the type of pellet produced and the percentage of fines will definitely affect the homogenous distribution of the additive. In as far as the feed particle or pellet is concerned, the larger the size of the pellet the greater the homogeneity of distribution, because it becomes easier to spray on each one of them. In the case of small size particles, it is more difficult to obtain a completely homogenous distribution of the enzyme over all particles. It is therefore important to contemplate both the type of feed to be produced and the animal to which it is going to be fed, because if reduced rations are to be fed homogeneity of the feed becomes particularly important (e.g. piglets and chickens). The percentage of fines is another aspect of feed quality that we should bear in mind, because due to their great surface area it is possible that up to 20% of the enzyme may end up absorbed on that surface and therefore lost to the animal, if fines are not recovered.

Handling of liquid and solid enzymes

The first liquid enzyme preparations that appeared in the market, produced, in spite of the great expectations, a great level of confusion due to their poor stability in storage, with significant reductions in their shelf life. Improvements in design and manufacturing process of liquid enzymes have substantially improved their activity and stability beyond six months, thus making it possible to keep a minimum stock for regular feed production, specially if fermentative processes, known to be the cause of enzyme deterioration, are minimized. It is therefore essential that an adequate hygiene is observed during production and warehousing.

The developments in biotechnology have recently changed enzymology in the sense that it has ceased to be the handling of chemical entities hardly defined and uncontrollable in their activity. Modern technology has provided the tools to handle these products with full knowledge of their chemical structures and activities thus providing the opportunity to derive maximum profit from their use. While advances in the design and stability of enzymes are the basis for their improved efficacy when used in animal feeding, we should not forget that feed processing continues to be an important factor in their effectiveness.

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