Thirty-fifth Annual

CAROLINA POULTRY NUTRITION CONFERENCE

NOVEMBER 12, 2008

Sheraton Imperial Hotel
I-40 Exit 282 at Page Road
Research Triangle Park, North Carolina 27709
(919) 941-5050

Sponsored by
Carolina Feed Industry Association
North Carolina State University
NC Department of Agriculture & Consumer Services

“MAXIMIZING PROFITS DURING TIMES OF EXTREME FEED COSTS”

POULTRY NUTRITION CONFERENCE – Auditorium
WEDNESDAY, NOVEMBER 12

7:00 a.m. **Registration**
Auditorium Foyer

Morning Session
Dr. Peter Ferket – Session Chairman

8:00 a.m. **Welcome –** Tom King**,** CFIA President

8:30 a.m. **The Grain Supply Outlook for the Poultry Industry**
– Dr. Jerry Weigel, ExSeed Genetics LLC

9:15 a.m. **Future Considerations for Poultry Nutrition**
- Dr. Steve Leeson, University of Guelph

10:00 a.m. **Panel Discussion**

10:15 a.m. **Coffee Break and Poster Session**

11:00 a.m. **Enzyme Synergy in Poultry Nutrition**
- Dr. Marcelo Shang, UCA-INTA

11:45 a.m. **Morning Session Discussion**

12:00 **Lunch**

Afternoon Session
Dr. Edgar Oviedo-Rondón – Session Chairman

1:30 p.m. **Mechanisms of Action for Supplemental Enzymes**
– Dr. Craig Wyatt, AB Vista

2:15 p.m. **Enzyme Combinations to Optimize By-Product Use in Poultry Feed**
– Dr. Janet Remus, Danisco

3:00 p.m. **Ingredient Nutrient Uplift By Enzyme Supplementation**
- Dr. Nelson Ward, DSM

3:30 p.m. **Afternoon Discussion**

4:00 p.m. **Adjourn**

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The Grain Supply Outlook for the Poultry Industry

Jerry C. Weigel, Manager, Nutrition

BASF Plant Science
RTP, NC

By all accounts, the 2008 corn, wheat and soya crops are well on their way to being among the largest ever in the United States. According to the USDA, Oct. 10 Crop Production Report, farmers are expected to produce the second largest corn crop ever, the largest wheat crop in 10 years and the 4th largest soybean crop on record. The USDA has estimated the 2008 corn crop at 12.2 billion bushels with an average of 154 bpa. This is a huge difference and turnaround from the July, 2008 report of 11.7 billion bushels with an average yield of 148.4 bpa……**<u>but wait a news breaking report…..</u>**the USDA has taken an unprecedented action of revising the corn crop…….citing a discrepancy within the raw data reports between the Farm Service Agency (FSA) and the National Agricultural Statistics Service (NASS), thus last week they adjusted the grain balance sheet. What this means is reducing the corn acres by 1 million acres (78.177 mm acres as harvested, with Iowa losing 300,000 acres and lowered the trend line to 153.9 bpa or on a cents per bushel of $0.05/bu.) From a pricing standpoint the Feds are calling the corn range at the farm gate of $4.25-$5.25/bu. This has put doubts in the minds relative to many of the accuracy of our USDA reports. It also has put the corn market into the fundamental mode and bullish toward corn, but I feel this will adjust back to the bearish side as our combines continue to move through the fields of the Midwest. The US corn pricing is still somewhat sensitive to investor (Wall Street) confidence. Who knows what will happen here. It again tells us that we have to wait until our combines are in full harvest before suggesting what the crop will be!

We saw similar action on the soya side with the Feds reducing acres by 1.105 million acres to 74.4 million acres and a total production of 2.938 from 2.983 million bushels. In the soy pit one need to watch very closely to what the Chinese crushers will do…there is a lot of buying interest towards US beans, as we see the Chinese buyers buying for government stock piles. Watch any panic responses as we see strengthening in the US dollar.

We may also see some reduction in the availability of ethanol co-products like DDG/S as we see increases in exports and with the reduction of gasoline consumption slowing the blending to gas of ethanol will be reduced.

With DDG/S in mind and feeling that export opportunities will continue we need to look at the available inventory. In the 2007/08 marketing year we saw 22.8 million tons available or about 50% than the previous year. I have heard numbers for the 2008/09, which started 10-1-08 to be up another 50% or 31.3 million metric tons. The USDA is now taking into consideration the replacement for corn grain with corn co-products, mostly distillers grains.

With the above said about fuel ethanol and the heavy demand for corn, where are we now? We have seen corn fall from $7.65/bu., spot contract in June, 2008 to spot contracts for late

October, 2008 of $3.85. Many analysts will admit the ethanol demand will add as much a $1.00/bushel and yet others say it increases the price of corn by 20% or about $0.80/bu. The USDA has earmarked 4 billion bushels of corn for ethanol or about 30% up from 23% last year (3 billion out of 13.1 billion bushel crop). Well, just like other parts of the economy, the ethanol industry has suffered from the economic crisis. As a result of this volatility several plants have interrupted production and forced to restructure their balance sheets and a few even close their doors. We have seen several file for bankruptcy. Only time will sort this out, but I think we will see some consolidation in the ethanol industry. Just a word of interest and that is that US capacity to make ethanol has by 60% or 11.2 bgpy and if in fact if we bring the new and re-built plants the capacity will be increase to 13.8 bgpy. Where will the cellulose produced ethanol go and where will the tax and import tariff's end up?

Grain operations are also facing more scrutiny from the lendering community due to the credit crisis and these lenders are asking more and more specific questions about risk management before agreeing to loan any money.

A sharp drop in the futures market has freed up cash that grain elevators can use to get through the harvest season. Grain terminals and country elevators burn a lot of cash as farmers deliver crops they contracted months in advance. Also growers sell large volumes for harvest delivery which require operations of have cash ready. This could create margin call problems. With the futures prices going down, elevators are not having to contribute monies from margin calls but can keep it within their businesses. We are seeing many grain elevators becoming much more cautious in hedging strategies and forward contracting due to the exposure of margin calls.

What about the credit crisis at the farm gate? This is a very complicated situation this year as farm incomes are at all-time highs and the debt-to-assets ratio is the lowest. The issue is going to be how the farmer and the banker look at credit, whereas normally the farmer finances all his input costs and credit payments are due 60-90 days later and some cases post harvest. This type of flexibility has allowed farmers to manage sales of their old crop commodities to capture higher prices and use credit to cover early input costs. Many input companies have offered credit and farmers have used this delayed payment to hold for higher prices. We are seeing credit lock downs as credit becomes a bigger issue. It is my opinion the ability for farmers to obtain credit is going to much harder and certainly more scrutiny than in past years.

So with the above all mentioned what does farm cost look like even when sharply higher crop prices create windfall income gains. Well what the corn grower can expect this next crop year of……………..greater seed costs, fertilizer costs, fuel costs, crop insurance and chemical treatment costs. I am not even considering the potential higher cost for obtaining money. Iowa State estimates the cost of corn production in 2008 was 18% greater than 2007.

What about the future of yield potential for corn? Many have said we could easily double yields of corn by 2030 by the use of plant biotechnology. It has to be remembered that biotech crops will prevent yield loss. The biotech products like Round-Up or BT do this. There is no yield gene insertion into corn as of yet. These were the first generation biotech trait pipeline. This biotech pipeline will continue with yield potential, better nitrogen utilization, water efficiency, etc.

Okay, let us look at the 2009 corn planting crop and a total input cost of $850.00 acre with a yield of 180 bpa or $4.73/bushel transparent break even costs? Many growers will be watching the corn price to see what their floor price will be and try and hedge a floor price.

So how do we see the future? The key has to be to watch the financial markets. If world incomes drop, we will see less demand for exports and maybe a reduction in domestic demand. The grain markets in the US are distinctly tied to the financial markets. One problem not aligned to this is the cost of production will be greater than the marketed cost. If we see stabilization within the financial sector, my guess this will not occur. Those of you that watch corn pricing rationale need to spend time and understand grower mindset, such as when will the grower price his corn, how will they look at storage, will money be made in carrying corn(?) and the ability for the grower to capture credit.

In closing the nice thing……………….***American farmers like to plant corn!***

Future Developments in Poultry Nutrition

Dr Steve Leeson
Department of Animal and Poultry Science
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Developments in poultry nutrition have generally been driven by the need to sustain genetic potential within the confines of ever evolving systems of poultry production. Over the last 50 years we have developed quite sophisticated systems for quantitating the available nutrients in both ingredients and diets and this has allowed us to provide birds with quite precise levels of nutrients required for production. Only for energy are we perhaps one step removed from accurately describing the nutrient needs of birds for productive purposes.

As the genetic potential and the characteristics of poultry have evolved, so we have manipulated diet specifications to suit market needs. In general, such changes have been quite minimal, since maintenance needs for nutrients are fixed, and with few notable exceptions the composition of eggs and meat is difficult to change. Subtle changes in diet specifications are often a reflection of change in the metrics used to describe efficiency. Examples of the latter are the newer interest in egg solid yield as opposed simply to egg weight, and the nutrient requirements of birds for purposes of optimizing immune responsiveness rather than simply growth rate or classical feed efficiency.

At the feed mill our past goals have been aimed at assurance of consistency in nutrient content of feed, coupled with minimizing the content of anti-nutrients. While techniques such as Near Infra Red analysis allows for rapid identification of abnormal samples in terms of nutrients as complex as amino acids and even available energy, by and large our quality control programs have provide us with historical data that helps us to build more robust databases for consideration in future decision making.

External influences

All poultry companies have evolved to accommodate consumer and societal issues. The nutrient profile of poultry products now impacts poultry nutrition for production of specialty products, while the need to avoid natural and man-made antinutrients that impact both birds and humans, has had major impact on feed manufacture.

Perhaps the single largest factor to impact poultry production and poultry nutrition has been the mandatory or voluntary removal of antibiotics from feeds, spurred by reports from organizations such as the World Health Organization. Very much an emotional issue lacking sound scientific judgment, the current trend for less reliance on feed as a vector to dose meat birds with “antibiotics” is likely to be irreversible. It is difficult for long-term management of meat birds without recourse to use of antibiotics, and the current trend for dramatic increase in use of water-borne antibiotics is both worrying and presumably self-defeating if these products in fact pose a real health risk to humans. However it seems that consumers accept such dosing of birds as a positive welfare issue when bird health is an issue, a situation that highlights the fickle priorities of wealthy consumers. In this regard it seems that not allowing birds to free-range in many countries is now acceptable to consumers since the threat of personally contracting AI suddenly supercedes prior concerns about bird welfare.

Poultry nutrition has also been impacted in the move to produce specialty foods that are most often enriched with an array of nutrients. In general the fatty acid profile of eggs and meat mirror that present in the diet and so it is a relatively straightforward task to formulate diets that result in direct incorporation of diet fatty acids into poultry products. Such fatty acids are usually polyunsaturates and so there is the added challenge of limiting the rate of their oxidation in the feed, in the bird and in the resultant poultry products. Currently, flaxseed and fish oils have been the major ingredients incorporated into designer poultry feeds.

Government regulatory agencies are also impacting both feed formulation and feed manufacture. No one will argue that the process of feed manufacture today is becoming exponentially more complex in terms of accountability and traceability of feeds and their component ingredients. As yet, such regulations have had minimal impact on quality control procedures at the mill that are used to verify nutrient content of feeds. Once the novelty of reconciling pharmaceutical products diminishes, then regulatory agencies may well focus on accountability of nutrient supply. Currently we have little confidence in the composition of feeds leaving the mill, since our analytical procedures are more likely conducted with a view to developing an historical database. Regardless of analytical systems used, the most costly and important nutrient in feed, namely available energy still eludes us in terms of rapid, accurate and precise measurement.

The current cost of feed energy is another major issue facing animal production in general, and to a large extent is again a consequence of government intervention. For apparently political reasons, the US government has decided to provide sufficient subsidy to attract significant diversion of corn to ethanol production. There has been considerable discussion on the wisdom, morality and overall economics of this industrial process, but much like the aforementioned consumer issues, such decisions are not made on the basis of sound scientific, or in this case economic, reasoning. Unfortunately there are no ingredients available that can replace corn worldwide in the quantities now used by the poultry and swine industries.

Environmental concerns are obviously another current issue that have become a political agenda, fodder for the media, and consequently topical with consumers. Manure management has already received considerable attention in many countries leading to regulations that have impacted feed formulation. In addition to current concerns about phosphorous and nitrogen excretion, there may be comparable regulations attached to excretory rates of copper and zinc. Likewise the release of ammonia from poultry houses and stored manure is now being quantitated, presumably with the intent of mitigation partly by alteration to feed formulation.

Digestion, bird health and antibiotics

The perceived threat to human health from use of feed-borne antibiotics, growth promoters and certain anticoccidials has created significant interest in increasing our understanding of digestive physiology and the dynamics of the gut microflora. A basic tenant of gut microbiology suggests that bacteria, and especially pathogens have much greater difficulty in colonizing the gut of older birds that have a firmly established microflora, and this is the basis of the Nurmi concept of prevention. From a nutritional point of view there are perhaps steps that can be taken to influence early gut colonization. Unfortunately a clear understanding of how nutrition impacts gut microbiology is hampered by the fact that we know little about the nutrient requirements of pathogens vs. symbiotic organisms vs. those of the bird. Likewise, we are slow to identify the species of all bacteria that reside in the gut, a situation that obviously limits our attempts at microbial control or manipulation.

Presumably any nutrients indigestible to the bird will be potential nutrients for all bacteria including pathogens. Certainly the digestibility of diets by very young birds is up to 20% less than our expectations. To some extent this inadequate digestion of conventional broiler starter diets has led to the introduction of pre-starter diets that are composed of highly digestible ingredients. If nutrient supply to bacteria in the lower intestine impacts population size and/or proliferation of certain species, then perhaps we should start to consider the supply of indigestible, as well as digestible, nutrients in poultry diets, and especially for young birds. While an interesting theoretical concept, it has practical limitations during formulation.

There is renewed interest in fibre nutrition of all classes of poultry, in terms of both gut health and impact on microflora. With the advent of high nutrient-dense diets in the late 1970`s, the role of fibre was relegated in importance. The notable exception was the negative effect of non-starch polysaccharides (NSP`s) in small grains and means to overcome adverse effects of associated increase in digesta viscocity through use of exogenous enzymes. In both human and animal nutrition various fibre components are now being scrutinized for beneficial effects on gut health and potential to modify the gut micoflora. The difference in emphasis, from negative to positive attributes, relates to inclusion level. At low inclusion levels (perhaps less than 1%) there may be advantages to using NSP`s as a means of beneficially modifying the gut microfora, especially in situations where antibiotic growth promoters are not used. Fermentation of NSP`s to VFA`s such as butyrate may be one mode of action in controlling proliferation of pathogens and improving gut health**.** Butyrate resulting from the microbial fermentation of dietary components such as resistant starch, appears to be important for normal development of epithelial cells. Butyrate derived from fermentation of non-starch polysaccharides is credited with improved gastrointestinal health in humans and a reduced incidence of colon cancer. We have recently shown improved performance of broilers fed butyrate trigycerides in cocci-challenged birds. Our knowledge of the role of fibre in monogastric nutrition is somewhat hampered by rudimentary knowledge describing various fibre components, their solubility, and changes that occur with transit through the digestive tract.

Energy costs and bird response to energy

Energy costs are high because of demand for corn and the fact that there are no viable alternatives available worldwide. Coupled with the diversion of corn for ethanol production is the concomitant loss of availability of reasonably priced fats and oils that are being used as a feedstock for biodiesel.

A question often asked is what are the alternatives to corn and high-priced wheat in poultry diets? The answer is already established since we have a reasonable idea of the nutrient profile of all alternate ingredients. Limitation to their use is often inadequate supply. Distillers grains is a relatively new alternate ingredient in terms of quantity now being produced, although one wonders about the long-term viability of drying this product such that it can be used any great distance from the refinery. There is considerable variation in composition and nutrient availability in DDG`s, and so knowledge of the feeding value of locally produced products is essential.

With high-energy prices, there is often discussion about using lower energy diets. Traditionally this is not a valid assumption, since when feed prices are high it is often most economical to use diets as efficiently as possible, and this means high, rather than low, nutrient density. However this premise assumes an adequate supply, albeit at greater cost, of conventional ingredients. However, with constraints on ingredient supply it may be impractical to sustain normal levels of nutrient density, and so lower energy diets may be the only alternative.

Both layers and meat birds still eat quite precisely to their energy requirements. The key to successful use of lower energy diets lies in prediction of change in feed intake and corresponding adjustment to all other nutrients in the diet.

When only energy is reduced, then both broilers and layers consume less energy as diet energy level declines. Presumably this reduction in diet energy is in fact a consequence of reduced feed intake precipitated by excess or imbalance of other nutrients in the diet. When all nutrients are tied to energy then both broilers and layers exhibit a remarkable ability to maintain energy intake when confronted with a major decline in diet energy concentration. For both broilers and layers a reduction in diet nutrient density of 10-15% is practical in terms of the birds ability to adapt and perform adequately, assuming that this can be achieved economically.

The economics of using diets with lower nutrient density is invariably predicated on the unit energy price in corn or wheat vs. that in alternate lower energy ingredients. Alternate ingredients are very much dependent on local agronomics and or/supply of ingredients from various industrial processes. Corn Distillers Grains is an obvious potential ingredient in certain countries, and its nutritive value is now well documented . An ingredient that will be available in increasing quantity is glycerol, produced as a by-product of the biodiesel industry. The EU and USA together produce around 1m tones of glycerol, and this number will double for each 2% of diesel fuel replaced by biodiesel. Already supply exceeds current demand for conventional uses, and so it could be an attractive energy ingredient for the feed industry. As a carbohydrate with a GE of 4300kcal/kg it has potential to supply significant quantities of energy.

Conclusions

Current progress in genetic potential will likely continue unabated in the near future, and so the underlying decision has to be made as to whether or not to sustain this potential through feed formulation. The biological limit to increased genetic potential may well be calcium and bone metabolism. In broilers this relates to maintaining skeletal development in ever younger birds, since inadequate calcification will likely impact bird welfare and the efficiency of mechanical processing. For layers, the immediate past surge in egg numbers has miraculously been achieved without apparent compromise to shell quality. However, as we approach the situation of birds being in excess of 90% production after 52 weeks of lay, there are likely to be limits to the birds ability to sustain skeletal integrity sufficient to placate all segments of society. While current topical issues of diversion of corn, wheat and fat into industrial processes will to continue to impact feed ingredient prices and availability, economic development in Asia will likely be the major long-term factor influencing global feed prices and so our decisions in setting diet specifications.

Enzyme Synergy in Poultry Nutrition

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Introduction:

With other consequences, the recent worldwide economic crisis has demonstrated that the price of commodities (fuel and grains mainly) are not necessarily associated to strict rules of supply and demand. At the time the stock markets were falling and with little evidence of reductions in livestock production (beef, dairy, poultry and pig were not altered), the commercial value of both corn and soya dropped to almost half of their previous prices. A similar situation showed oil prices returning in a few days to a value of around $85/barrel.

These trends would clearly demonstrate that productivity in animal production can be easily modified by simple market speculations leaving to nutritionists few possibilities to control or predict grain prices. At the same time, this economic situation should serve to elevate the value of biological facts to a higher level particularly considering that irrespective of ingredient prices, feed cost represents between 50-70% of the total cost of production. In this regard, and more than ever, the ability of nutritionists to take advantage of technologies associated with the improvements of feed digestibility may certainly result in reinforcements of the competitiveness of companies dedicated to animal production.

Corn-soy diets and digestibility:

The increase in world soya production and processing has made soybean meals more available for poultry and pig production. Consequently corn-soy diets became widely fed at the time of serving as reference feed in terms of nutrient composition and digestibility. However, it is known that while digestibility of energy in corn is relatively high (In general over 90%), in soya (Meal of Full fat) energy digestibility range is 70-75%. This limitation in soya energy yield (And other plant protein sources) has been associated to the presence of antinutritional factors in the ingredient and to reduced enzyme activity at the animal level. Thus, the development of technologies orientated to improve nutrient (energy, protein, phosphorous) digestibility has been focused on by researchers and the economic importance has been recognized by private companies.

The contribution of exogenous enzymes:

Independently of the variation in absolute results, it should be recognized that the possibility of using exogenous enzymes in non-ruminant diets has provided nutritionists with a very important tool to improve feed digestibility, to moderate environmental contamination and to reduce feed cost.

Based on experimental results, several commercial approaches have been suggested for enzyme utilization in pig and poultry feeds. While some single enzymes are proposed to promote single effects on a particular chemical component of the diet, some others combine several enzymes (Naturally occurring or as a cocktail) in order to cope with more than one substrate. Independently of the approach, it is clear that enzymes are specific (maintain a given enzyme:substrate ratio) and are only effective when several conditions like temperature, humidity and ph are achieved. In other words, if similar enzymes are competing for a similar substrate the action of those enzymes would not be additive.

There have not been defined standard procedures for evaluating the effects of exogenous enzymes in animal feeds. However the importance of some aspects have been recognized at this time to set up experiments: to have “negative controls”, to formulate experimental diets below the nutrient recommendations for maximum performance, to clearly define the objective of the trials (Improve performance or maintain performance at a lower cost) to define the use of the enzyme “on top” of the diet or “up lifting” nutrient allowances.

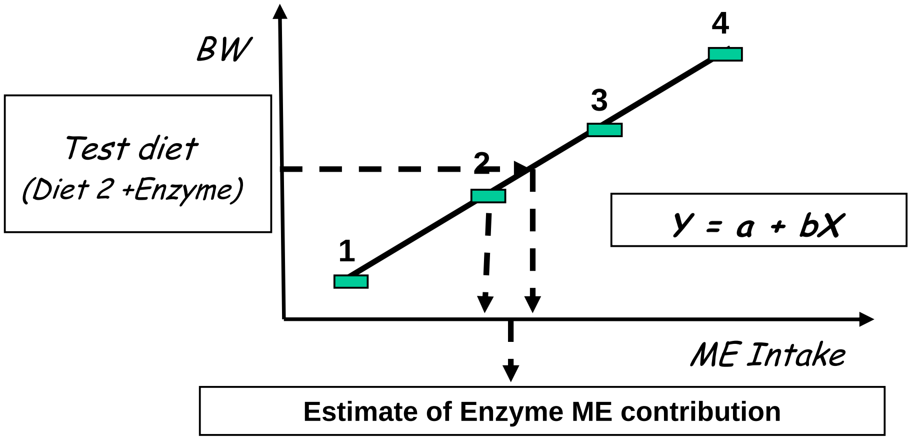
In general the results obtained from these trials together with field data demonstrates the benefits provided by the use of exogenous enzymes when feeding pig and poultry, particularly from the economic point of view.

Single action and synergy of exogenous enzymes;

Under certain economic conditions, nutritionists are tempted to incorporate in their diets more than one type of enzyme, assuming that independent enzyme effects may be additive. Unfortunately there is almost no scientific information in this field and the competition for similar substrate may reduce the benefits provided by single enzymes.

Based on these observations an experimental approach was defined to evaluate the improvements in energy yield due to enzyme (Single or combined) utilization in poultry diets. The trial consisted of feeding rations formulated to contain increasing levels of metabolizable energy to obtain a lineal response in terms of body weight gain (BWG). The enzyme to be evaluated was added “on-top” of one of the former diets (low in energy) in order to observe the effect on BWG. Any difference in performance between birds with this treatment (With enzyme) and those corresponding to the standard diet (No enzyme addition) would be due to the effect of enzyme addition. Regression analysis was performed and the procedure allows the estimation of the energy yield provided by the enzyme addition (Figure 1).

Figure 1: Procedure for exogenous enzyme evaluation



Some results:

The procedure indicated above was applied in a couple of experiments to evaluate the effect of adding more than one enzyme to poultry diets.

In the first growing trial (Universidad Central de Venezuela) 3 corn-soy diets were formulated to provide EM levels of 2.850, 2.950 and 3.050 cal/g (0 to 28 days) and 2.950, 3.050 and 3.150 cal/g (29 to 42 days) respectively. All diets considered a 7% up lifting in soybean meal ME, CP and AA due to the addition of a commercial enzyme. On top of a fourth diet (Similar to diet 1; 2.850 cal/g) a second enzyme was added, promoted to increase diet energy yield in 75 cal/g. The trial lasted 28 days and the result showed a lineal response in BW as intake of ME was increased. When plotting the data of treatment 4 in the corresponding equation, the estimate of ME yield due to the addition of the second enzyme was 56 cal/g of feed.

In a second 28 days trial (Instituto Nacional de Tecnologia Agropecuaria-Argentina) a similar experimental approach was followed except 4 corn-soy diets instead of 3 were formulated (2.750, 2.850, 2.950 and 3.050 cal/g). In this case the calculated increase in diet ME due to a second enzyme addition was 52 cal/g.

As a result of both experiments it was possible to conclude that the recommendation given for the second enzyme (75 cal/g) when used alone has to be reduced to around 60 cal/g when combined with the other enzyme.

Interpretation and some considerations:

The results showed that the use of enzymes that are competing for the same substrate can not be recommended by the simple addition of their single effects. In practical terms and due to lack of precision in identifying the action of the given enzymes, the effect of one of the enzymes should be fixed while the benefits of the other should be reduced. Unfortunately this assumption may impact on feed cost calculations requiring the knowledge and experience of each nutritionist to decide the correct commercial enzyme recommendations.

As mentioned, and irrespective of the economic variations, feed cost represents the largest proportion of total production costs. In addition, present trends in corn utilization for ethanol production in developed countries and soya demands from emerging countries may have modified the actual map of meat producing countries. In this regard it could be valid to speculate that competitiveness of grain producing countries would be higher than those of grain importing countries. In all cases, however, the possible reduction in corn and soya supplies would negatively impact on digestibility of animal diets. The proven technology associated with the use of enzymes (along or in combination) would certainly assist to economically increase feed digestibility in order to reach animal genetic potential.

Mechanisms of Action for Supplemental NSP and Phytase Enzymes in Poultry Diets

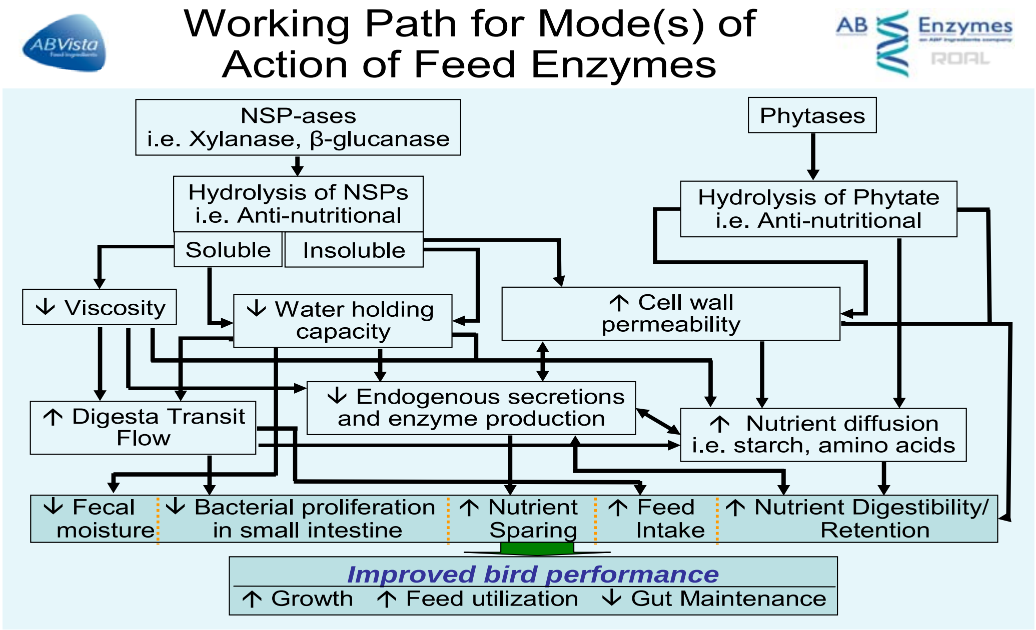
Craig L Wyatt, Terri Parr and Mike Bedford
AB Vista Feed Ingredients Ltd.

Introduction

Recently, there has been an availability shortage of many key raw materials or limitations resulting in significant price increases thus more than ever, a great deal of pressure has been placed on nutritionists and feed manufacturers to maximize their efficiency of nutrient utilization. In fact, ingredient costs have increased to such an extent that in many countries the final cost of feed has almost doubled. In most feeds the pressure has been on two nutrients in particular: energy and phosphorus. With limitations in the use of animal proteins in some markets and pressure from fertilizer use, phosphate prices have tripled in the past year. In addition, there has been a surge in the use of cereal grains and animal fats for biofuels significantly putting pressure on dietary energy costs. The net result is that the increase in shadow prices in least cost formulations (LCF) for energy and phosphorus has driven feed manufacturers to consider alternative options especially feed enzymes. The use of enzymes for releasing more energy from corn-based diets has increased especially in poultry in most markets compared to the past when the economic incentive was not great enough to overcome the skepticism. Some nutritionists are also formulating with considerably higher phytase dosages than utilized in the past. Not only does this latter activity increase phosphorus retrieval from phytate, but it will spare some energy as a result because of the removal of more phytate.

Most of the enzymes were derived from fungal sources, but today the degree of bacterial derived enzymes has increased especially with the new phytase products. In the past five years the market for poultry enzymes has changed significantly with phytases now leading followed by xylanases and then cellulases (glucanases) trailing in a distant third place. Other enzyme classes such amylases, proteases and mannanases are making up only a small proportion of the total feed enzyme market today. Although there are some enzymes that are positioned at targeting vegetable protein sources (such as alpha-galactosidases), their use to date is still small and the understanding of the mode of action remains limited. The main use of feed enzymes still targets cereal grains and in countries where wheat and barley predominate the diet enzyme use is almost universal. More effort has been put in developing a better understanding of how enzymes can enhance corn or sorghum and soybean meal diets for poultry and today this area is now increasing with the new better enzymes.

One fact that must be appreciated is that exogenous enzymes including phytases function through enhancing the availability and retention of nutrients present in the diet. If the nutrients “spared” such as phosphorus and energy do not happen to be the nutrients limiting animal performance, then there will be a small or no response upon addition of the enzyme. Since ingredients vary markedly in their nutritive value, it is quite conceivable that two separate diets of an identical formulation based on corn and soya but derived from different sources (locations) will respond differentially upon enzyme administration. Thus, one must consider feed enzymes including phytase as another tool to help reduce variation in performance. This paper will briefly discuss some of the possible mechanisms of action of feed enzymes shown in Figure 1 in diets fed to poultry and discuss some of the conditions which are known to influence the response observed.



Phytase

With the advent of commercial feed phytases which is an enzyme class not constrained by cereal type, its use has become more commonplace to the point where today it constitutes more than half of all feed enzyme sales [<$250 million]. Phytases have been fed to poultry and pigs to degrade plant phytate which would otherwise pass through the digestive tract relatively intact with minimal release of P resulting in high levels of bound P in manure. Moreover, since phytate is in the form of calcium and magnesium salts, and is able to chelate many cations, its hydrolysis has been associated with improved mineral utilization overall. Consequently, most people feel feeding a phytase source can be effective for increasing phosphorus and mineral digestibility and retention, and as a result the use of inorganic P sources can be reduced. In the past, phytase was simply used to provide phosphorus to the bird, and as such a small savings in feed costs was obtained by decreasing the use of inorganic phosphate sources in the diet. Alternatively, new data derived from feeding second generation phytase sources have shown improvements beyond phosphorus with increases in retention of calcium, energy and amino acids. The economics of such activities will differ markedly depending upon current prices of the nutrients spared but will result in a significant feed cost savings. In turn this leads to the challenge that the nutritionist must know “that all phytases work differently” leading to different performances in the animal. Review of the early phytase data, based significantly on the fungal derived phytases, suggest that there is a great deal of variability in the performance response when feeding these products (Rosen, 2002), including P digestibility (van der Klis et. al.,1997). This has lead to many nutritionists or feed formulators using a safety margin of lowering phosphorus and calcium levels in the nutrient matrix which significantly reduces the monetary value provided from feeding the phytase.

Mode of Action

Action in the acidic region of the gut: Many factors have been found to influence the response of the animal to these different phytases including the molecular properties themselves. Some commercial phytases have been found to have limited stability in the gastric environment and low specific activity compared to the new bacterial derived phytases. It has been clearly shown that phytate solubilizes in the acidic region of the chicken gut, thus this is the critical area of digestion where one needs a highly efficacious phytase to work (Tamim, et al., 2004). In addition to the phytase working in the acidic environment it needs to work quickly with a high affinity for the target substrate before being impacted by endogenous and exogenous proteases (i.e. pepsin). While the low pH environment in the gastric phase is ideal for making phytate susceptible to hydrolysis by phytase, the low pH also places a stress on all proteins, including enzymes. For some fungal phytases this appears to be a greater problem than for other phytases. The acidic phase (pH 2.5-4.5) of the intestinal tract is the only part where phytate is truly soluble and most likely susceptible to degradation. It is essential that a phytase maintains maximum activity across the pH range where phytate is most susceptible to hydrolysis (pH 4.5 and lower). Several new phytases have been developed and marketed (Quantum™ Phytase and Finase EC) that clearly are better at working in the optimal acidic pH range compared to other phytase sources giving a more efficacious and consistent response.

Phytate as an anti-nutrient: As more P is removed from phytate leading to more breakdown of intact IP-6, the less able it is to bind or chelate minerals, starch or proteins either directly or via ionic bridges (Selle & Ravindran, 2007). Decreasing the binding of these compounds through the use of phytase may directly improve the digestibility not only of phosphorus and divalent cations such as Ca, Zn and Mg, but also indirectly increase energy and nitrogen utilization. Recently, it has been suggested that phytate alone is more of a potent anti-nutrient than previously thought, and as such its presence results in a significant loss of endogenous nutrients and energy in the form of mucins, intestinal cells and perhaps pancreatic enzymes (Cowieson et. al., 2004). Exactly how it brings about such losses is probably multi-faceted, but nevertheless new data clearly indicates there are gains in bird performance from the hydrolysis of dietary phytate beyond that required to supply the P requirements of the bird from a typical phytase containing diet. More recent work has demonstrated the influence of phytate on the digestibility of energy and amino acids (Wu et. al., 2003; Selle et. al., 2003; Selle et. al., 2006), either via direct interaction with positively charged amino acids or through deprivation of activating cations from digestive enzymes or as mentioned above excessive losses of mucins. This would clearly suggest that phytate is an intestinal irritant. It is not clear, however, how this effect is brought about, although recent evidence suggests that in addition to the decrease in absorption there is a reduction in inflammatory response measures at the duodenal/jejunal junction on feeding phytase to laying hens (Koutsos et al., 2005). It is interesting that the effect of phytase on amino acid digestibility tends to be greatest on those amino acids which are prevalent in intestinal maintenance and turnover, namely cystine, threonine, proline and glycine when measured

(Selle et. al., 2006). This suggests that a greater proportion of the benefit of phytase is due to reduced endogenous losses rather than increased dietary amino acid digestibility.

Thus, destruction of phytate reduces the anti-nutritive effect in a directly proportional manner, and as a result energy and amino acids that would have been used in a maintenance activity can be directed towards productive energy (growth) instead. The savings in endogenous losses can be directed towards growth, so that a greater proportion of metabolized energy and amino acids are used to net energy and less for maintenance. It must be noted that this effect of phytase is mostly a post-adsorptive effect, and if this is consistently the case then it would explain why digestibility assays are not effective in determining the value in a phytase in sparing energy and amino acids for growth. Dietary phytate enhances and phytase reduces endogenous losses in what appears to be a dose dependent response, thus feeding phytase appears to favor shifting ME to NE of growth rather then NE of maintenance. Low P diets will depress feed intake and it has been shown that usually more phytase is required to equilibrate intake than the AvP with the positive control. This can result in digestibility trials lacking the precision such that the calculated P equivalency exceeds that achieved in commercial practice.

Commercial Application

Unlike the NSP'ase enzymes used for corn soy diets, the phytase levels fed in practice are well below that of the biological optimum. Although the magnitude will vary between phytase sources the benefit has been found to be linearly related to logarithmic increments in dose – thus improvement in P digestibility is doubled with a 10 fold increment in dose. Despite new data supporting additional nutrient matrices beyond P and Ca for most phytases into energy and amino acids, the economic incentive to increase the dosage of phytase used has not been sufficiently obvious until recently with increasing ingredient costs. A clear problem in the use of phytase in least cost formulation programs (LCF) is that whilst the benefit of the enzyme increases in a linear fashion with logarithmic increments in dose, the LCF program does not account for the logarithmic benefit. To demonstrate this issue, if a phytase is allowed to float as an ingredient with a fixed nutrient matrix for 500 units, and the LCF selects only 250 units of phytase, it will assume that the phytase has provided only 50% of the given matrix, whereas in reality the actual value is closer to 75% for such a dose. On the other hand, if the formulation program doubles the inclusion level to 1000 units using the 500 unit matrix then one will significantly over-value the nutrient matrix supported by that product (potential loss in performance). Use of any dose below the product maximum results in the LCF assuming that the enzyme delivers less value than it actually does in vivo. AB Vista has been looking at several different approaches to help formulators use the optimal amount of phytase. One solution is based on a dose model using shadow prices to best predict the optimal level of phytase for that situation. Another approach would be to place two phytase ingredients in the LCF program based on one phytase having the recommended 500 unit nutrient matrix and the second would be a new phytase (500 to 1000 unit) product which would have a matrix defined as the difference between the 500 and 1000 unit matrix products. Either approach is designed to help maximize the value that a feed manufacturer can extract from their phytase especially when high ingredient prices justify much greater inclusion levels of this enzyme.

CARBOHYDRASE(S) or NSP enzymes for corn based diets

Recently, with dramatic increases in the price of dietary energy many nutritionists have renewed interest in feeding NSP enzymes due to the potential cost savings they offer. It has been a hard market to penetrate through customer acceptance because most end-users think that the best diet to feed poultry is a corn-soy diet, whereas, diets based on barley or wheat are known to have negative issues like wet litter making the solution of feeding a specific enzyme more accepted with quick visible value (i.e. dry litter). The challenge has been the inconsistent and/or muted responses in certain diets to feeding these types of enzymes primarily based on xylanases, glucanases, cellulases and mannanases. A significant body of work has shown these products do work but their responses can be quite variable because such a response in corn-soya based diets requires the correct application adjustments. Based on the industry's experience with NSP enzymes, the response can be variable depending on what enzyme or cocktail of enzymes used, the quality of feed ingredients or substrate and if the enzymes are thermo-tolerant. The economics of such activities will differ markedly depending upon current market prices of the nutrients spared but will result in significant feed cost savings. In turn this leads to the challenge that the nutritionist must know "that all NSP enzymes work differently" leading to different performances in the animal.

Mode of Action

Enzymes targeting corn-soya diets were introduced many years ago to target several substrate components within the plant material including fiber, starch and some plant proteins. It is thought that NSP enzymes function through a composite of three separate activities, the contribution of each activity varying with ingredients and individual birds. The two main key functions for a corn-soya based diet would be plant (cereal) cell wall destruction, and stimulation of beneficial bacteria with changes in the fiber composition. The target for such a feed enzyme(s) product for corn-soya diets is not based on lowering intestinal viscosity derived from soluble cell wall polysaccharides since corn and soya contain low levels of soluble material. Instead of soluble fiber, it has been suggested that in corn based diets the target is the insoluble fiber component to help break open the cell wall material. Research has shown that in addition to the impact of soluble fiber, the insoluble fraction can have a direct and indirect effect on digestive function. Several authors have shown that insoluble fiber (i.e. cellulose) has an inhibitory effect in vitro on lipase and proteases from the pancreas, and this has also been found in vivo trials (Almirall et al., 1995; Schneeman et al., 1985). The feeding of varying levels of grass and other NSP levels has been shown to increase the mass of the intestines in chickens. These changes in the digestive tract will lower digestive efficiency and increase the level of nutrients used for gut maintenance, thus pulling nutrients away from growth. Whether the target is the soluble or insoluble component, the benefit brought about is due to enhanced digestibility and absorption, not only of the nutrients present in grain (like starch) but also from other ingredients, particularly added fats by improving the diffusion of nutrients within the intestinal lumen. The key functions described focus on how certain NSP enzymes function.

1. Cell Walls destruction: encapsulation of nutrients - The cell wall material in the starchy endosperm of corn and sorghum is constructed mainly of small amounts of cellulose encrusted with hemicellulose, the bulk of which is arabinoxylan with minor B-glucan components and lesser contents of mannans (Stone, 2004). Since poultry do not possess the

necessary enzymatic capacity to degrade plant cell walls, a lot of the content (especially starch and protein) within this material can effectively bypass digestion or not be broken down until the lower gut by bacteria. This factor of encapsulation is based on the fact that some endosperm cells in corn and other ingredients manage to avoid physical breakdown during the activities of grinding and pelleting in feed manufacturing, and gizzard activity. Effective degradation of this material requires the addition of sufficient amounts of the appropriate NSP enzyme activity such that "holes" are created in the cell wall. This allows water hydration and large enough amounts of pancreatic proteases and amylases enabling better digestion of the starch and protein more rapidly. Xylanases, and to a lesser extent cellulases (B 1-4 glucanases) have proven most effective in the field (Zanella et al., 2004; Leslie et al., 2007). Mannanases and pectinases have targeted the soy more so than the corn fraction of the diet, but with the same endpoint in mind (Jackson et al., 2004). Many studies have shown improvements in starch and to a lesser extent protein digestibility which is indicative of activity of the enzyme towards corn endosperm cell walls. It has been shown that corn starch digestibility in the upper part of the digestive tract can vary markedly across different corn sources probably related to differences in starch structure, protein matrix, and the handling of the corn post-harvest (drying and milling/processing techniques). These products focus on breaking down the cell wall material exposing more starch and protein for enzymatic digestion, reducing endogenous secretion (i.e. lowers mucin production) and altering the lower gut microbial populations. These effects of exposing more nutrients in the upper intestine, reducing the physical damage and lowering endogenous secretions by the gut villa does lower maintenance requirement spent on digestion and improve nutrient retention. However, in a corn-soya based diet one will need to use NSP enzymes (i.e. xylanase, glucanase) that are more effective at targeting and breaking down the insoluble fiver fraction. A direct benefit of feeding these enzyme products is through reducing the variability in birds and improvements in bird uniformity across the different feed batches.

2. Bacterial population stimulation - Exogenous NSP'ase(s) breakdown plant cell wall carbohydrates and reduce chain length producing smaller polymers and oligomers. At some point the fragments become small enough (ie short chain oligosaccharides) and numerous enough to act as a substrate (pre-biotic) for bacterial fermentation. Xylanases, mannanases and cellulases produce xylo-, manno- or gluco- oligosaccharides respectively. The benefit of such end products depends upon the type and quantity of the oligosaccharides produced, with different enzymes producing different oligosaccharides. These short chain oligosaccharides travel to the lower gut and become substrates for bacterial fermentation in the ileum and cecum which can be beneficial with VFA production and altering the bacterial population. Several papers have shown that use of enzymes significantly alters VFA production and the population profiles of bacteria in both the ileum and cecum (Apajalahti et. al., 1995; Choct et al., 1999; Apajalahti and Bedford 1999; Bedford & Apajalahti, 2001). Results have clearly shown the presence of medium to long chain oligomers will increase fermentation in the jejunum and ileum region however feeding a xylanase significantly decreases fermentation and shifts it to the cecum. If the diet is well digested in the upper part of the digestive tract by using NSP'ase, it appears that there is little starch present in the ileum, and more cell wall oligomers present in the ileum and cecum (Engberg et. al., 2004). However, one must be careful in selecting the feed enzyme because some products can be overdosed and reduce the size of the oligosaccharides down to far to mono-saccharides. If sufficient monosaccharides are produced it may result in osmotic diarrhoea and /or poor performance (Schutte, 1990).

These problems are most likely to occur with endo-xylanases products derived from a crude preparation containing substantial amounts of exo- rather endo-xylanase activity which are not too specific in their requirements for binding to substrate. As we learn more about these enzymes and their actions it is critical to obtain the right amount of short chain oligosaccharides (ie tri and di-saccharides) otherwise this alteration in the bacterial population in the lower gut may lead to increasing the gut maintenance requirements of the bird especially without growth promoters resulting in fewer nutrients for growth instead of a positive benefit.

Commercial Application

There are many different NSP enzymes on the market today, all of which differ markedly from one another. Even within the xylanase class, there are enormous differences in pH profiles, gastric stability, end products produced and their ability to attach to soluble and/or insoluble arabinoxylan structures. It is important that when making a choice the decision is based on the animal performance of the product in proper formulated diets and not simply based on in vitro assays or TME/AME digestibility studies which can truly mislead the true net energy response. The translation of such net energy response into a performance benefit has been very difficult to demonstrate because today's broilers do not respond to over-feeding energy. Nevertheless there have been studies demonstrating that not only ileal energy digestible levels of corn/soya diets can vary but also that inclusion of an appropriate enzyme can reduce this variation having a significant impact on body weight uniformity. The value for the reduction in performance variability will be different across end-users and will need to be evaluated individually. Recent work would suggest if the proper amounts of amino acids are present the bird will respond positively to the increase in energy release especially in the heavy strain birds. There also may be an interaction between rapid starch digestion and the need for more lysine. Several trials have shown that increasing the release of starch in the upper part of the digestive tract by feeding different starch sources or using enzymes to shift starch availability appears to respond better in higher lysine diets (Figure 2; Weurding et. al., 2002). These results indicate that nutritionists may need to adjust the diets to account for this release in nutrients to obtain optimal performance from products like enzymes.

Phytase and Corn/soya NSP enzyme combination application

The combined use of phytase and NSP enzymes has become a critical subject of interest with many questions focusing on application. While we have a significant understanding of the use of phytase in general fed alone in poultry diets, the database for the combined use of phytase with different NSP'ases is quite limited. Nevertheless, knowing that phytases (especially Quantum) and NSP'ases target different substrates releasing nutrients like phosphorus and starch, and are effective in different regions of the gastro-intestinal tract, their indirect benefits of reducing gut maintenance (i.e. lower mucin production) may overlap, thus, one might deduce that the combined effect may be complimentary but not directly additive. Independently published research has shown that the interaction of feeding these products together based on mineral retention improves the responses giving better

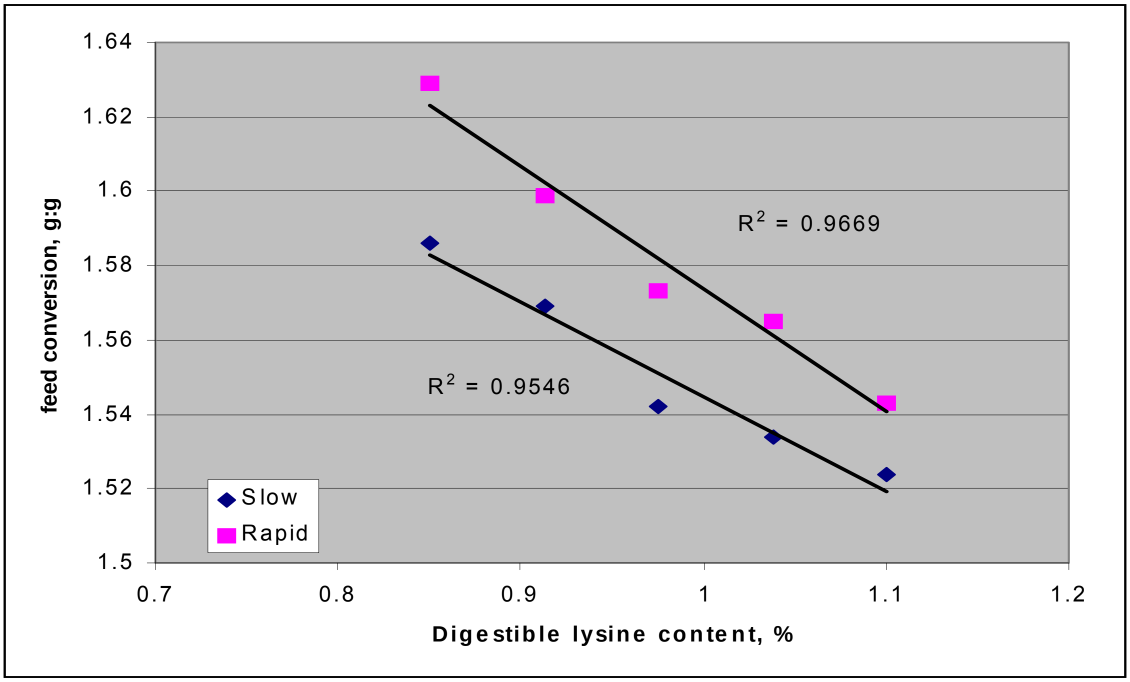


Figure 2. Effect of starch source and digestible lysine levels on feed conversion in 30 d broilers.

results than feeding either product alone. However, the data clearly showed there was no additive benefit on overall animal performance. In fact, the ability of the NSP'ase to physically break open the fiber components of the diet may improve the access of phytase to phytate increasing its hydrolysis and the action of both enzymes could be complementary, however there was no additional increase in net energy.

Thus, not knowing for certain the level of energy and amino acids resulting from the feeding of NSP'ase in corn-soya based diets; many people use a safety margin with these products. Current recommendation would be when feeding the proper NSP'ase product and phytase (i.e. Quantum) together, the net energy response between the two should be complementary in the bird. Based on feeding trials with phytase and NSP'ase it is suggested that the combined use of these enzyme products may only result in partial additivity for energy thus approximately 80% of the total. For instance 1 kcal ME of phytase and 1 kcal ME of an NSP enzyme when fed together may only result in 1.6 kcals ME as opposed to an additive effect of 2 kcals.

Conclusions

There are many feed enzyme products available for use in poultry diets containing corn, wheat, soybean meal and other ingredients. The enzyme efficacy can vary substantially depending upon the type (e.g. phytase or xylanase) and source of the products. Also, the response can vary depending if the product is thermo-tolerant and able to survive the normal pelleting process (Parr and Wyatt, 2006). The intrinsically thermo-tolerant enzymes are clearly the best solution, with compromises arising from the use of either coating or post pelleting application which vary with each product. In addition to surviving feed processing the enzyme needs to be able to work in the right areas of the digestive tract in a highly efficacious way to result in an improvement in nutrient digestion and retention. Thus, it is important for the end-user to remember that not all enzymes within a class (e.g. phytase and xylanase) work the same resulting in different animal responses and different total values.

Typically the response to phytase is more predictable across all diets compared to a xylanase product used specifically for corn-soy containing diets. This is a result of the fact that the target substrate is better defined for a phytase, and consequently resulting in a more consistent desired effect. Corn, soybean meal and other plant–based ingredients lack clear “targets” until now. We have a better understanding that one needs to breakdown the insoluble cell wall material which will result in a consistent nutrient increase compared to enzymes targeting the soluble fraction. Digestibility experiments do not provide a clear picture of the potential value of an enzyme product under commercial conditions because the response is coming from net energy (i.e. gut maintenance). There are dietary, feeding processing and/or environmental factors which can limit or extend the value of these products. The end user is encouraged to work closely with the specific products to obtain the optimal outcome desired based on the mechanism of action.

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Enzyme Combinations to Optimize Byproducts Use in Corn-Based Poultry Feed

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Introduction

Although there are a variety of potential substrates for enzymes in poultry diets (see Table 1), the primary ones generally targeted in practical corn-based diets are phytate, arabinoxylans and starch. Mannans and oligosaccharides may also be potential substrates but dietary content of these may be more variable across ingredients than the aforementioned three. Beta-glucans are normally considered when feeding viscous cereals such as barley or oats as the primary cereal or at levels at or above 10% of the diet for either cereal. Due to the level of β-glucans and the insolubility of these nonstarch polysaccharides (NSPs) in typical US ingredients used in corn-based diets, β-glucans are not normally a NSP of concern for most diets. Cellulose is not a practical target in poultry as no enzyme system currently exists that would efficiently and cost-effectively fully release glucose from this NSP within the constraints of the bird's gastrointestinal tract.

Enzyme use is well documented across different types of poultry diets. Example papers on amylase (Ritz et al., 1995; Jiang et al., 2008), protease (Ghazi et al., 2002; Ghazi et al., 2003; Wang et al., 2008), xylanase (Mathlouthi et al., 2002; Cowieson et al., 2005), beta-glucanase (Mathlouthi et al., 2002), mixes of two or more of the aforementioned activities (Pettersson and Åman, 1992; Vranješ et al., 1994; Zanella et al., 1999; Hong et al., 2002; Mathlouthi et al., 2002; Meng et al., 2005; Cowieson and Ravindran, 2008a, b) as well as phytase (Onyango et al., 2005; Liu et al., 2008a, b) are among the many that can be found in the scientific literature. However, trials often examine one type of enzyme in isolation. For example, many of the published phytase papers do not examine relationships between phytase and various carbohydrases or proteases in practical diets. Given that many cereals, cereal byproducts or vegetable protein byproducts can vary in phytate (Table 2) as well as NSPs (Tables 3, 4, 5), it would seem logical that combinations of phytase, nonstarch polysaccharidases (NSPases) or other activities may provide a benefit in practical diets. Some recent papers published in the poultry research press have examined phytase with and without carbohydrase inclusion in corn-based diets (Cowieson and Adeola, 2005; Olukosi et al., 2007) or wheat-based diets (Ravindran, 2001; Wu et al., 2004). From a practical standpoint, many commercial companies in the poultry business are using phytase currently so demonstration of carbohydrase or protease efficacy in the presence of a phytase has become more important. Although many enzyme trials have focused on simple cereal/soy

Table 1. Potential enzyme substrates

Antinutrient	**Where found**	**Problem**	**Content**	**Enzyme**
Phytate	**All plant-based ingredients**	**Binds P and other cations, increases endogenous loss**	**Varies but is generally higher in vegetable meals and cereal byproducts**	**Phytase**
Arabinoxylans	**Cell walls of plant based ingredients**	**Relatively resistant to digestion and may reduce nutrient digestibility; soluble causes viscosity**	**Moderate to low – depending on ingredient**	**Xylanase, Arabinofuranosidase**
β-glucans	**Cell wall of cereals such as barley or oats**	**Soluble form causes extremely high viscosity**	**Moderate to low and not found in corn, milo**	**β-glucanase**
Mannans	**Soybean meal, yeast cell walls**	**Resistant to digestion**	**Variable**	**β-mannanase**
Oligosaccharides	**Soybean meal, etc.**	**Resistant to digestion**	**Variable**	**α-galactosidase**
Cellulose	**Plant ingredients**	**Insoluble and resistant to digestion**	**High**	**Cellulase, Cellobiohydrolase**
Starch	**Cereals, cereal byproducts primarily**	**Structural resistance, retrogradation or protein binding**	**High**	**Amylase, debranching activities (protease)**
Protein	**Corn, milo, vegetable meals**	**Resistant proteins to digestion: storage proteins, protein ANFs, etc.**	**Variable**	**Targeted proteases**

diets (for example: Wu et al., 2004; Cowieson et al., 2005; Olukosi et al., 2007; Cowieson et al., 2008a; Jiang et al., 2008), the responses noted with enzyme addition have ramifications for diets containing byproducts as these diets may have higher levels problem substrate(s) present. There are published papers on corn-based diets containing several different byproducts or vegetable meals, these provide evidence of enzyme efficacy (Dipeolu et al., 2005; Yuan et al., 2008). Unfortunately, there are a limited number of trials that have looked at inclusion of only one byproduct in the presence of feed enzymes.

Table 2. Percent Phytate Phosphorus in Feed Ingredients.

Ingredient	NRC, 1994	Eeckhout & De Paepe, 1994	Kornegay, 2001[1]	Liao *et al.,* 2002	Slominski *et al.,* 2004
Corn	0.20	0.19	0.24	0.24	NA
Wheat	0.24	0.22	0.27	0.26	0.26
Wheat bran	0.95	0.97	0.92	0.81	NA
Wheat millrun[2]	NA	NA	NA	NA	0.57
Wheat midds	0.55	0.53	NA	NA	NA
Wheat screenings	NA	NA	NA	NA	0.33
Rice bran	1.28	1.10	NA	1.07	NA
Sorghum	NA	0.19	0.24	0.22	NA
Barley	0.19	0.22	0.27	0.23	NA
Oats	0.22	0.21	0.29	0.26	NA
Millet	0.20	NA	NA	0.17	NA
Triticale	0.20	0.25	NA	0.27	NA
Rye	0.22	0.22	NA	0.22	NA
Rye bran	NA	0.79	NA	0.60	NA
Soybean meal	0.40	0.32	0.39	0.37	NA
Soybeans (heated)	NA	0.26	NA	NA	NA
Canola meal	0.87	0.40	0.70	0.63	NA
Sunflower meal	0.84	0.44	0.89	0.69	NA
Peanut meal	0.50	0.32	0.48	0.46	NA
Cottonseed meal	0.88	NA	0.84	0.82	NA
Peas	NA	0.17	NA	0.23	NA
Flax meal	NA	0.42	NA	0.69	NA
Corn gluten meal	0.36	NA	NA	0.36	NA
Corn gluten feed	NA	0.47	NA	NA	NA
Wheat gluten feed	NA	0.56	NA	NA	NA
Sesame meal	1.03	NA	NA	1.03	NA
Alfalfa dehy. meal	NA	0	NA	0.01	NA
Coconut meal	NA	0.18	NA	0.27	NA
Bakery meal	NA	NA	NA	NA	0.24
Corn DDGS	0.10	0.19	NA	NA	NA

NA = not available

[1] Kornegay's values are adapted from data of Ravindran, V. (1996); Ravindran *et al.,* (1994); Ravindran *et al.,* (1995).

[2] Midds and millrun are very similar although millrun may have a higher portion of bran.

Vegetable byproducts and antinutritional issues

Typical byproducts used in feed formulation can vary widely by region. But in general, typical problems involve NSPs, phytate, starch and amino acids where plant-based byproducts are concerned. In these ingredients, digestibility and antinutritional factors

Table 3. Select NSP levels in cereals reported as % dry matter unless otherwise noted

Ingredient	sAX (%)	iAX (%)	tAX (%)	sAX (AF)	iAX (AF)	tAX (AF)	sβ-gl (%)	tβ-gl (%)	iCW (%)	tNSP [1] (%)	Reference
Wheat (Rialto)	0.8	-	-	-	-	-	0.24	-	12.3	-	Mathlouthi et al., 2002
Wheat	-	-	-	-	-	-	-	-	-	10.0	Slominski et al., 2004
Wheat	-	-	4.46	-	-	-	-	-	-	8.8	Meng et al., 2005
Triticale	0.48	-	-	-	-	-	0.18	-	10.4	-	Mathlouthi et al., 2002
Rye	1.44	-	-	-	-	-	0.75	-	14.6	-	Mathlouthi et al., 2002
Rye	2.3 to 2.9	5.3 to 6.9	7.6 to 9.8	-	-	-	-	2.3	-	13	Voragen et al., 1992
Rye	-	-	-	2.6	4.1	6.7	-	-	-	9.0	Cowieson and Adeola, 2005 [2]
Oats	0.13	-	-	-	-	-	4.35	-	31.1	-	Mathlouthi et al., 2002
Barley	0.3	-	-	-	-	-	2.43	-	14.1	-	Mathlouthi et al., 2002
Barley (hulless)	0.48 to 0.56	2.52 to 3.44	3 to 4	-	-	-	4 to 5	4 to 5	-	13	Voragen et al., 1992
Corn	0.03	-	-	-	-	-	0.05	-	9.6	-	Mathlouthi et al., 2002
Corn (US)	-	-	-	0.1	3.8	3.9	-	-	--	6.1	Cowieson and Adeola, 2005 [2]
Corn	-	-	5.35	-	-	-	-	-	-	9.3	Malathi and Devegowda, 2001 [3]
Sorghum	0.28	2.52	2.8	-	-	-	-	0.3	-	5	Voragen et al., 1992
Sorghum	-	-	2.77	-	-	-	-	-	-	9.7	Malathi and Devegowda, 2001 [3]

[1] Total NSP includes both water soluble and insoluble NSPs.

[2] Cowieson and Adeola, 2005 presented data on an as fed basis

[3] Malathi and Devegowda, 2001 reported total pentosans of which arabinoxylans would be classified under.

sAX = soluble arabinoxylan; iAX = insoluble arabinoxylan; tAX = total arabinoxylan; β-gl = soluble beta glucan; tβ-gl = total beta-glucan; iCW = insoluble cell wall components; tNSP = total NSP; AF = % as fed

Table 4. Select NSP profile of various byproduct ingredients reported as % dry matter unless otherwise indicated

Ingredient	sAX (%)	iAX (%)	tAX (%)	sβ-gl (%)	tβ-gl (%)	iCW (%)	tNSP [1] (%)	Fiber (%)	Reference
DDGS	-	-	11.4	-	-	-	22.7	-	Ward et al., 2008
Bakery byproduct	-	-	-	-	-	-	9.5	14.6	Slominski et al 2004
Wheat screenings	0.35	-	-	0.05	-	19.5	-	-	Mathlouthi et al., 2002
Wheat screenings	-	-	-	-	-	-	11.1	21.6	Slominski et al 2004
Wheat mill run	-	-	-	-	-	-	24.6	42.4	Slominski et al 2004
Wheat bran	0.50	-	-	0.07	-	40.6	-	-	Mathlouthi et al., 2002
Wheat bran	3	27	30	-	-	-	45	-	Voragen et al., 1992
Wheat midds	-	15.04	-	-	-	-	26.0	-	Danisco database
Rice bran	0.06	-	-	0.08	-	19.4	-	-	Mathlouti et al., 2002
Rice bran	-	-	19.2	-	-	-	60	-	Voragen et al., 1992
Rice bran, deoiled	-	-	10.65	-	-	-	59.97	-	Malathi and Devegowda, 2001 [2]
Oat bran	0.39	4.05	4.44	-	7.72	-	15.1	16.9	Pettersson and Åman, 1992
Oat bran, extracted	0.48	4.78	5.26	-	8.40	-	17.6	19.3	Pettersson and Åman, 1992

[1] Total NSP includes both water soluble and insoluble NSPs; [2] Malathi and Devegowda, 2001 reported total pentosans of which arabinoxylans would be classified under. sAX = soluble arabinoxylan; iAX = insoluble arabinoxylan; tAX = total arabinoxylan; β-gl = soluble beta glucan; tβ-gl = total beta-glucan; iCW = insoluble cell wall components; tNSP = total NSP;

Table 5. Select NSP profile of various byproduct ingredients reported as % dry matter unless otherwise indicated.

Ingredient	**sAX (%)**	**tAX (%)**	**sAX (AF)**	**iAX (AF)**	**tAX (AF)**	**sβ-gl (%)**	**tβ-gl (%)**	**iCW (%)**	**tNSP [1] (%)**	**Reference**
Soybean meal	**0.11**	**-**	**-**	**-**	**-**	**0.06**	**-**	**21.1**	**-**	**Mathlouthi et al., 2002**
Soybean meal	**-**	**3.22**	**-**	**-**	**-**	**-**	**-**	**-**	**14.8**	**Meng et al., 2005**
Soybean meal	**-**	**-**	**0.3**	**3.0**	**3.3**	**-**	**-**	**-**	**12.6**	**Cowieson and Adeola 2005 [2]**
Soybean meal	**-**	**4.21**	**-**	**-**	**-**	**-**	**-**	**-**	**29.02**	**Malathi and Devegowda, 2001 [3]**
Rapeseed meal	**0.27**	**-**	**-**	**-**	**-**	**0.05**	**-**	**40.9**	**-**	**Mathlouthi et al., 2002**
Rapeseed meal	**-**	**8.85**	**-**	**-**	**-**	**-**	**-**	**-**	**39.79**	**Malathi and Devegowda, 2001 [3]**
Canola meal	**-**	**4.69**	**-**	**-**	**-**	**-**	**-**	**-**	**17.1**	**Meng et al., 2005**
Sunflower meal	**0.13**	**-**	**-**	**-**	**-**	**0.18**	**-**	**52.0**	**-**	**Mathlouthi et al., 2002**
Sunflower meal	**-**	**11.01**	**-**	**-**	**-**	**-**	**-**	**-**	**41.34**	**Malathi and Devegowda, 2001 [3]**
Peanut meal	**-**	**6.11**	**-**	**-**	**-**	**-**	**-**	**-**	**29.50**	**Malathi and Devegowda, 2001 [3]**
Peas	**0.07**	**-**	**-**	**-**	**-**	**0.11**	**14.8**	**-**	**-**	**Mathlouthi et al., 2002**
Peas	**-**	**2.54**	**-**	**-**	**-**	**-**	**-**	**12.7**	**-**	**Meng et al., 2005**

[1] Total NSP includes both water soluble and insoluble NSPs.

[2] Cowieson and Adeola, 2005 presented data on an as fed basis

[3] Malathi and Devegowda, 2001 reported total pentosans of which arabinoxylans would be classified under.

sAX = soluble arabinoxylan; tAX = total arabinoxylan; β-gl = soluble beta glucan; tβ-gl = total beta-glucan; iCW = insoluble cell wall components; AF = % as fed are almost always involved in the decision process of when and how much to use to help reduce cost while not jeopardizing performance. Toxic antinutritional factors such as gossypol, mycotoxins, etc. are obvious limiting factors. But issues on NSP-related effects on nutrient uptake, GI passage rate and subsequent bird performance to problems in starch, amino acid or phosphorus (mineral) availability are also key in the decision process. It should be noted in any discussion on usage of byproduct, particularly at higher levels, that good ingredient quality monitoring programs should be in place.

Nonstarch polysaccharides

Nonstarch polysaccharide issues are best addressed by the appropriate NSPase (table 1). However it is fair to note that the degree of and type of benefit of NSP enzymes may depend on the nature of the NSP present, i.e. soluble (Mathlouthi et al., 2002) or insoluble (Jaroni et al., 1999), or even a factors like particle size (Mavromichalis et al., 2000; Aulrich and Flachowsky, 2001). In addition, heat processing can affect physical characteristics of NSPs (Cowieson et al., 2005; González-Alvarado et al., 2008) Viscous fiber prolongs gastric emptying and slows transit time (Malkki, 2001), which in growing animals alters nutrient digestibility and generates performance losses. For corn-based diets, insoluble NSPs predominate in many of the ingredients typically used (see tables 3, 4, 5). Insoluble fiber/NSPs can affect gut transit time, gut motility and may also hinder the ability of endogenous enzymes to gain access to their respective substrates (Choct, 2001). Insoluble NSPs do not cause viscosity but these cell-wall components can encapsulate nutrients inside intact cell walls. Correspondingly, the finer the grind or particle size, the more of these encapsulated nutrients may already be released. Another way of addressing intact cell walls is to add an NSPase such as xylanase to the feed, with the intention of this enzyme acting to open intact cell walls via its action on the NSP arabinoxylan, thus allowing endogenous enzymes access to previously ‘hidden’ nutrients.

The mechanism of action may be related to the ability of insoluble fiber to ‘bind’ or ‘hold’ water, thus influencing gut bulk (physical fill) and potentially motility. Some consider wheat bran or rice bran as possibly beneficial in laxation for humans (Dikeman et al., 2006), although this is typically not the reason for use in poultry diets. *In vitro* tests on water binding and water holding capacity of various feed ingredients has noted differences across major feedstuffs. In general, corn was found to have lower water holding capacity than does soybean meal or other protein meals whereas potato flakes were found to have the highest water holding capacity (Partridge, 2001). All ingredients, but grass meal and sunflower meal, responded with reductions in water holding capacity greater than 10% when a carbohydrase/protease product was added to the *in vitro* test system (Partridge, 2001). Aulrich and Flachowsky (2001) examined the relationship between particle size and water binding as well as water holding capacity of wheat bran. These authors found that as particle size when from 1 mm to 0.25 mm, there was a reduction in both water binding and water holding capacity of wheat bran. In addition, if an NSPase mix of xylanase and β-glucanase were applied to the wheat bran, a linear reduction in water binding and water holding capacity was noted with increasing enzyme inclusion (Aulrich and Flachowsky, 2001). Interestingly enough, these authors also noted in the porcine *in vitro* stomach and small intestine simulations that NSPase supplementation improved release of ‘bound’ protein in wheat bran.

Since nutrient flow to the villi on the gut wall depends on gut motility and corresponding convective movement of water and nutrients, insoluble fiber may affect feed intake, nutrient absorption and ultimately bird performance. So in this scenario, NSP enzymes act to alleviate the negative water binding/holding effects of insoluble fiber/NSPs in the GI tract, which in turn helps to reduce more normal transit time and nutrient flow. A fairly extreme example of how insoluble NSPs can affect growth of the bird in the absence of NSPases can be seen in the work of Pettersson and Åman, 1992. These authors used a diet containing 68% oat bran or extracted oat bran with or without NSPase addition and noted dramatic improvement in chick performance with enzyme addition even considering that they pair fed one of the treatment pairs (see table 6).

Table 6. Can chicks raised to 20 days grow on diets containing 68% oat bran or extracted oat bran if NSPases* are added?[1]

Treatment	**Gain (g)**	**FI (g)**	**F:G**	**Frequency of sticky droppings (day 7)**
Oat bran	**195 a**	**413 bc**	**2.63 b**	**33 b**
Oat bran + NSPases[2]	**282 b**	**416 b**	**1.71 a**	**4 a**
Extracted oat bran	**177 a**	**380 b**	**2.73 b**	**27 b**
Extracted oat bran + NSPases	**452 c**	**688 a**	**1.66 a**	**6 a**

Dietary total arabinoxylans = 3.34 (88.6% insoluble) for oat bran diet; 3.66% (88.8% insoluble) for extracted oat bran

Primary ingredients consisted of oat bran, corn starch, fishmeal, soybean meal. Enzyme supplemented birds were pair-fed with corresponding control.

*NSPases = xylanase and β-glucanase added in combination at 0.2% of the diet

a,b, c: means within a column without a common letter differ at $P<0.05$.

[1] Data is from Pettersson and Åman, 1992.

[2] The enzyme supplemented oat bran diet was pair fed to match its control. The extracted oat bran + enzyme was not pair fed.

Phytate

Phytic acid has long been known as an antinutrient due to the presence of bound phosphorus on its structure but also its ability to bind positively-charged substances (e.g. Kornegay, 2001 or similar articles for review) and alter secretion of endoengenous enzymes (Dilworth et al., 2004; Liu et al., 2008 b). High phytate levels depressed chicks weights and G:F (Liu et al., 2008 b) as well as nutrient digestibility (Ravindran et al., 2006). Plus high phytate reduced activity of disaccharidases and Na+K+ATPase in the duodenum (Liu et al., 2008 b). The mode of action of phytate in increasing endogenous nutrient loss has become clearer over the last 5 years, which helps explain why phytase may affect nutrients other than those that could be directly bound to phytate. In addition, new research suggests that phytase addition to nutritional marginal diets may help improve lymphocyte numbers as well as antibodies in the sera and mucosa of broilers (Liu et al., 2008a). In all, it should be realized that the higher the dietary phytate level, the greater the potential for phytate to generate digestive issues and potentially increase maintenance cost of digestion. Use of phytase helps alleviate issues with this substrate and is known to generate feed cost savings via lower inclusion of inorganic phosphorus.

However, it should be considered that higher phytate diets have greater potential for negative effects of this substrate, so it may be beneficial to consider higher phytase inclusion per ton feed to help alleviate these effects.

Starch

Starch is the easiest of the carbohydrates to digest but is not fully utilized by poultry at the terminal ileum. Noy and Sklan (1994) estimated that 11 to 18% of the starch may be undigested at the terminal ileum in chicks of 4 to 21 days of age. However, crystalline structure, component ratio of amylase to amylopectin, protein binding, cell wall encapsulation or even gelatinization can all affect how efficiently starch is digested by the bird. Cowieson (2005) reviewed these factors for corn and Weurding and coworkers (2001a, b) noted clear differences in the rate and extent of starch digestion for various feed ingredients.

In cereal byproducts, starch content is often highly variable and may vary widely in quality. In bakery byproducts, care must be taken by the manufacturer to ensure that already cooked items containing starch are not retrograded during the second cooking process. In addition, there is potential for the formation of Maillard reaction products between glucose and amino acids such as lysine, although this depends on the type of bakery goods included in the mix and the content of reactive materials. Maillard reaction products are also a great concern in DDGS. Partially reacted products will assay as lysine but are not wholly bioavailable as lysine. The amino acid lysine is a concern here due to it having two amino groups that can be reactive whereas other amino acids, except proline, have one amino group. Regrettably, there is no enzyme that will counteract the effect of the Maillard reaction on lysine after the Schiff base step.

Protein antinutritional factors

For poultry feed, proteases may target issues in vegetable protein meals, protein antinutritional factors or storage proteins found in plant-origin materials. It is known that a targeted protease(s) can be used to help degrade lectins, trypsin inhibitors (Hessing et al., 1995) and other protein ANFs (Ghazi et al., 2002). Proteases also may be used to target storage proteins. These proteins ‘store’ or bind starch and are one possible cause of starch that is resistant to digestion (Brown, 1996).

Vegetable byproducts: where are we now?

Table 7 shows general recommendations for select byproducts that may be encountered in the field. Some of these are discussed further in this paper but the reader is strongly encouraged, when using higher levels of byproducts, to have a strong quality control program in place to help ensure successful usage. In addition, it may be helpful to evaluate what antinutritional factors that byproduct(s) may be bringing into the diet particularly as level increases. This can help make the decision of the type of enzyme(s) that may be needed to remedy digestive and performance issues clearer. Relatively few trials have examined enzyme use in high byproduct type diets. Logically this may be due historic need to ‘prove’ response in what was the status quo type diet – simple corn/soy. Some byproducts merit a few extra words as these typically exhibit high variance.

Table 7. Recommended maximum levels of select byproducts and highest levels tested successfully with enzymes (corn-based diets)

Ingredient	Species	Maximum (no enzyme)	Reference	Highest level with enzymes	Successful Parameter	Activities	Reference
Bakery meal	Meat birds	10% to 4 wks, 15% to market	Leeson & Summers, 1997				
	Layers	10%	Leeson & Summers, 1997				
Corn DDGS (modern)	Broiler	6% starter 12 to 15% in other phases; 15% all phases	Lumpkins et al., 2004; Wang et al., 2008	10% in all phases	Gain, F:G, bone breaking strength	X, A, P, Phy	Moran and Lehman, 2008
				40% in starter	Tibia ash	Phy	Martinez-Amezcua et al., 2006
	Turkey	10 to 20% in market birds	Noll, 2004				
	Layer	10 to 15% depending on diet density; Up to 15% but an age issue may be present at 52 wks; 20% light colored DDGS	Lumpkins et al., 2005; Roberson et al., 2005 Cheon et al., 2008	20% (entire cycle)	Egg weight, lay rate in 44-68 wk phase where controls struggled	X, BGL	Świątkiwicz & Koreleski, 2006
Dehydrated alfalfa	Broiler, Turkey	5% to 4 wks, 10% to market	Leeson & Summers, 1997	3%	Performance	X, A, P, Phy	Moran and Lehman, 2008
	Layer	5%	Leeson & Summers, 1997				
Wheat bran	Meat birds	8% to 4 wks, 20% later	Leeson & Summers, 1997	7.9% starter, 14% later (ducks)	Performance, digestibility	X, A, P,	Hong et al., 2002
	Layer	10%	Leeson & Summers, 1997	16% wheat midds	Performance, egg weight, villi height	X, P	Jaroni et al., 1999a, b
Wheat shorts	Meat birds	10% to 4 wks, 20% later	Leeson & Summers, 1997		See above		
	Layers	20%	Leeson & Summers, 1997		See above		

X = xylanase; A = amylase; P = protease; Phy = phytase; BGL = β-glucanase

Bakery Byproducts

As a whole, bakery byproducts can vary considerably in composition depending on the relative sources of raw material around the production plant. Typical bakery byproduct raw materials may consist any of the following: raw doughs, partially cooked doughs, doughnuts, breads, muffins, cookies, crackers, candy, snack foods, chips, cakes, inedible flour, unsalable nuts, etc. Since the component ingredients vary in composition, the final bakery byproduct will vary from plant to plant. In their examination of bakery product originating from different feed manufacturers, Slominski and coworkers (2004) noted that high variability existed in starch fat, NSP and phytate P. These authors noted a difference in NSP content ranging from 3.3 to 17.0% across the 12 samples analyzed (mean = 8.7% with a standard deviation of 5.53 and coefficient of variation of 63.6%). Toal fiber showed the highest variance, although the authors only analyzed 8 of the 12 samples. The range in total fiber was 6.9 to 32.7% with a SD of 9.57 and CV of 71.4%. Phytate P content ranged from 0.03 to 0.47% (mean = 0.22%, SD = 0.15 and CV of 69.5%). For starch, the range ran from 24.7 to 49.3% with a mean of 37.8 (SD of 8.29 and CV of 21.9%). Fat content ranged from 4.2 to 10.2% with a mean of 8.0% (SD of 18.2 and CV of 22.7%). Protein was less variable with a mean of 11.9% (SD of 1.35, CV of 11.3%). Dry matter was very consistent at 91.6 (SD of 9.8, CV of 1.06%). Not unsurprisingly, there was an 85% correlation between NSP and starch wherein increases in NSP meant reduced starch levels in bakery meal (Slominski et al., 2004). But no relevant correlation was present for starch vs. fat in this byproduct.

Since it is typical that xylanases and/or amylases are added in the mixing of doughs to enhance the production process, it is an open question as to how little or much these processing enzymes have improved nutrient value of components in bakery meal. Much depends on type of bakery product as raw or partially cooked doughs from bread or pizza crust would logically have different potential for NSPs and starch availability than would cakes, cookies and candies.

The potential for bakery as an energy source in poultry diets is clear. However, users should monitor proximate composition, salt and similar nutrients. But also it may be helpful to periodically measure NSP or at least NDF and ADF content of incoming bakery byproduct from each of their suppliers' plants.

Wheat byproducts

Traditionally wheat byproducts are been restricted to diets for layers, breeders, ducks or older turkeys due to concern about fiber and starch contents. Although Leeson and Summers (1997) suggest that wheat byproducts may be used at up to 10% of diet in young birds. Wheat mill run (similar to midds but with more bran), for example, contains about 22.4% total NSPs (Slominski et al., 2004; CVof 13.2% on 6 samples). The bulk of which are insoluble NSP rather than the viscosity-causing soluble NSP. Phytate P levels also run high in wheat mill run at 0.52% (Slominski et al., 2004). Like wheat midds, starch and fat contents are the most variable components. Starch averaged 26.4% (SD of 5.24, CV of 19.8%) whereas fat had a mean of 4.3% (SD of 1.02, CV of 23.7%; Slominski et al., 2004). The challenge with any of the wheat byproducts is that the starch, NSP level and perhaps even phytate can vary considerably from flour mill to flour mill. So again, monitoring programs are essential with this byproduct. In table 7, corn-based diets containing midds and feed enzymes have been successfully used in duck and layer to aid bird performance.

DDGS:

For the US, DDGS is mainly made from corn. In Canada, the cereal used in fuel ethanol production can be wheat, corn or blends of the two depending on location. In their NSP screen of US-origin DDGS from modern ethanol plants, Ward and coworkers (2008) noted that arabinoxylans and cellulose were the predominant NSPs. The reported value of 11.4% arabinoxylans (dry matter basis) from Ward and coworkers (2008) agrees with that of the Dansico database which averages 11.7% total arabinoxylans. Insoluble NSPs predominated in US DDGS for all NSPs measured but fucose and ribose (Ward et al., 2008). Lysine is of special concern in this ingredient as it can irreversibly react with starch via the Maillard reaction, which renders the reacted product unavailable to the bird. A screen of 20 Minnesota-origin DDGS samples revealed that while mean lysine digestibility averaged 72%, the range was between 59 and 84% (Parsons, 2006). In a related project examining if P availability could be increased in DDGS, amino acid availability was also examined. Not unsurprisingly, autoclaving or oven drying DDGS reduced lysine availability although it did improve P availability (Parsons, 2006). Thus lysine deserves special consideration when formulating diets with DDGS as enzyme supplementation will not restore availability of Maillard-reacted lysine. As with other byproducts, use of digestible amino acids in formulation is strongly recommended.

Use of DDGS in poultry feeds has varied by age and species of the bird in question (Table 7 or please see the website www.ddgs.umn.edu). But in pelleted feeds for broilers, DDGS has been associated with reduced pellet quality at levels greater than 15% (Wang et al., 2008). Particle size of the DDGS, starch content as well as its oil content affect pellet quality, so all should be considered when using higher levels of DDGS as poorer pellet quality can impact performance. However, research is relatively limited on how NSPases and/or phytase may impact the nutritional value of diets containing this byproduct. Martinez-Amezcua and coworkers (2006) noted that 1000 or 10000 units phytase/kg feed improved tibia ash and threonine digestibility of broiler chicks fed a corn based diet with 40% DDGS but not AME. Work by Moran and Lehman (2008) using xylanase, amylase, protease and phytase supplementation to a corn/soy/10% DDGS diet for broilers raised to 56 days of age noted improvements in weight gain, weight-corrected F:G as well as bone breaking strength in these birds (Table 8). For laying hens, some trials have reported reduced egg production with feeding 15 to 20% DDGS in older layers (Roberson et al., 2006, Świątkiwicz & Koreleski, 2006). Addition of NSPases in the 44 to 68 wk feed phase helped offset the drop in lay rate and daily egg mass noted by Świątkiwicz and Koreleski (2006) in diets with 20% DDGS vs. in the nonsupplemented diet with 20% DDGS.

The type of fermentation process and possible use of fractionation present in the fuel ethanol industry means the user needs to be certain of the type of corn DDGS they are receiving as the new fractionation products differ in nutrient profile (Kim et al., 2008).

Table 8. Examination of a carbohydrase, protease, phytase product in broiler diets containing 10% DDGS[1]

Period	**Treatment**	**Gain (g)**	**wcF:G***	**Tibia breaking strength (kg)**
0 to 8 wks	**Positive control (PC)**	**3895 b**	**1.98 ab**	**33.8 b**
	PC + Enzymes[1]	**4078 a**	**1.87 b**	**36.8 a**
	Negative control (NC)**	**3736 c**	**1.99 a**	**30.6 c**
	NC + Enzymes [1]	**4195 a**	**1.94 ab**	**38.7 a**

a, b, c: separate letters within a column indicate differences at P<0.05 or less.

* Weight corrected feed: gain was calculated using 100 g difference in weight = 3 pts.

**Negative control was reduced in aP, Ca, AME and key amino acids.

[1] Data are adapted from Moran and Lehman, 2008

[2] Enzymes = 0.05% Avizyme 1502, 500 units Phyzyme XP/kg feed

Monitoring other key factors such as protein, fat, starch, fiber, ash, dry matter, total and bioavailable amino acids (particularly lysine), sodium, phosphorus, phytate and sulfur is strongly recommended. Mycotoxin monitoring is also recommended as those from the corn itself are not destroyed in the fermentation process. Plus, mold growth due to improper storage or drying can occur. Periodic analysis of NSP profile may also be helpful particularly as the productions methods (and fractionation) evolve over the next few years.

Closing thoughts

As higher levels of byproducts are used in diets, problem substrates correspondingly will increase. As enzymes typically have demonstrated efficacy in simple cereal diets, these benefits should continue as target substrates increase for key enzyme activities. However, the user needs to be aware of just what those substrates are and how much is coming into their diets to determine the appropriate enzyme or enzymes use. Regrettably, several key common byproducts are highly variable in composition which means good ingredient monitoring systems need to be in place and that targeted enzymes may have a role to help maintain nutritional uniformity.

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Ingredient Nutrient Uplift by Enzyme Supplementation

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Excessive feed costs have prompted many nutritionists to consider enzymes as a tool to lower inputs, with energy and P (phosphorus) being the two focal points. This coincides with tremendous advances in enzyme technology, protein engineering and fermentation that generate products with heightened efficacy. The large number of publications in recent years is a testament to the interest in enzymes for both development and application (Engster, 2008) because enzyme use can serve an economical function through improved utilization of substrates in feeds.

The application of matrix values is warranted for enzymes in a manner no different than for corn, SBM and other ingredients. Phytases are widely accepted to improve the utilization of P from phytate, thus these products often carry a matrix to reflect the efficacy and inclusion rate in the feed. The NSP (non-starch polysaccharide) portion in plant ingredients is the target for the "NSP enzymes", and while the relatively high energy availability for corn (~85%) has restricted the use of NSP enzymes in corn-based diets, this is quickly changing.

Alternatively, enzymes could be used to improve feed conversion, body weight or other performance variables, but feed represents 2/3s of production costs. Meat supply is plentiful at a time when feed costs are high; hence, prudence favors a reduction in feed costs for economy, and therefore, a nutrient uplift or matrix is the preferred route in the application of enzymes.

These matrix values are generated from animal studies designed to determine the amount of P, ME (metabolizable energy) and other components that are positively affected by an enzyme. While feed prices drive the focus toward the use of matrix values, due diligence is required since an enzyme's ability does not magically increase to reflect the crisis mode of today's ingredient prices.

Phytase

Typical corn/SBM broiler diets with 3-5% meat and bone meal contain 0.22 to 0.27% phytate P. Thus, phytase provides considerable opportunity to increase P availability, being that phytase supplementation has traditionally focused on the replacement of about 0.1% available P. However, the high cost of phosphate has pressured phytase levels upwards to replace 0.12 to 0.15% P. Considerations to supplant 0.2% P have met considerable resistance, partly because of insufficient supporting data, and partly because the cost of the level of phytase needed may exceed the P being replaced.

The P replacement value for fungal and bacterial phytases is basically linear up to about 0.1-0.12% P, followed by a plateau response. Thus, if 1X phytase = 0.1% P, then 2X phytase = approximately 0.12-0.15%. Shirley and Edwards (2003) tested up to the equivalent of about 20X phytase in a study in which broilers were fed corn/SBM diets. With nonlinear regression analysis on log-transformed phytase levels, N retention, F/G, and AME responded linearly, while all other variables (including tibia ash) responded quadratically. Tibia ash is considered the most sensitive variable on which to base the P replacement value for phytase, but weight gain, F/G and other attributes can be used.

A number of studies have been reported an improved trace mineral utilization when feeding phytase, but the variation can be considerable. Until recently, cost of trace mineral supplementation has been low, such that savings from a trace mineral matrix value would have been minimal from a least-cost standpoint.

ME matrix values for phytase products have a considerable range, largely due to interpretation or the philosophy of the supplier. For fungal phytases, ME recommendations are from about 5 to 25 kcal/lb (pound) final feed. For bacterial phytases, the disparity ranges from one commercial bacterial phytase with 25 kcal ME/lb final feed, while another's recommendation has no increase in ME.

An improved ME from phytase should be a direct correlation with phytate degradation by phytase. Thus, if two phytases at a given amount result in 0.1% P, than that given amount of phytase should impact ME equally for both phytases. Phytate is the antagonist, and once removed to the equivalent of 0.1% P, regardless of the form of phytase, the resulting improvement in ME logically would be the same.

Our experience with across different fungal and bacterial phytases indicates a fairly low ME value can be gained, regardless of the phytase. An addition level of 1X or 2X (with 1X = 0.1% P) has given no significant difference in ME value across fungal and bacterial phytases. Owing to the wide disparity in philosophies to assign commercial values across companies, matrix values for ME and amino acids have virtually no value when evaluating commercial phytases.

In addition, the evaluation of phytase should focus on the cost to replace a given amount of P "per ton feed", since considerable modifications in phytase analyses make the use of units (specifically FTU) for comparison purposes especially confusing (Ward and Campbell, 2007).

Crop Activity. Most of the phytase activity and phytate breakdown occurs in the crop, proventriculus and gizzard, with minimal activity in the small intestine. Liebert et al. (1993) noted about 50% or more of the activity of fungal *A. niger* occurred in the crop.

Considerable phytate hydrolysis occurs in the crop for *P. lycii* fungal phytase, as opposed to two different bacterial *E. coli* phytases (Glitso et al., 2007; Sorenson et al., 2005). Both phytases were added separately to a corn/SBM feed to replace ~0.1% P. The crop contents were taken to determine phytate disappearance for the three groups: Control (no phytase), fungal *P. lycii* and bacterial *E. coli*.

With *E. coli* phytase, 37% of the phytate was degraded, whereas the *P. lycii* group experienced a decrease of 78% (Figure 3). In other words, the *P. lycii* appears to be doing most phytate hydrolysis in the crop.

Based on the ability of *P. lycii* to release P in the crop, the University of DE and University of MD tested the effect of lighting on phytase efficacy (Saylor et al, 2008), since lighting programs are known to stimulate meal feeding (crop fill). One program was designed to encourage crop fill and the other was the 21L:3D traditionally used with most battery studies.

P. lycii and an *E. coli* phytase were supplemented to the feed to replace ~0.1% P as separate treatments, and feed levels were confirmed by analysis.

Overall, as compared to the constant lighting, the chicks on the intermittent lighting performed with increased ($P<0.05$) efficiency, no difference in body weight, a 16% lower feed consumption and a lower ($P<0.05$) tibia ash.

With the intermittent program, a significant ($P<0.05$) increase occurred in 21-day tibia ash of *P. lycii* birds over those fed *E. coli* (Table 1). Body weights and F/G did not differ across phytase sources. The lighting experiment agrees with the study which found *P. lycii* to accomplish more phytate degradation in the crop than did the bacterial phytase. Commercial conditions utilize some degree of intermittent lighting, all of which favor meal feeding (crop fill). Crop weight as a percent body weight was greater ($P<0.002$) for birds with intermittent lighting as opposed to 23 hrs light recently (Dalal et al., 2008). Comparative trials with all-light programs may not allow for these differences to be expressed, yet under commercial conditions, such difference may become more evident since intermittent lighting are common commercially.

Table 1. The Effect of Lighting Program and Phytase Source on 21-day Broiler Performance

Dietary Program	N	aP, %	Weight Gain, g/bird	F/G	Tibia ash, %
Constant Lighting					
PC	8	0.45	596^{a}	1.44^{cd}	51.99^{ab}
NC	8	0.28	533^{cd}	1.55^{ab}	46.52^{f}
Fungal Phytase	8	0.28	562^{abc}	1.52^{ab}	50.78^{bc}
Bacterial Phytase	8	0.28	549^{cd}	1.52^{ab}	50.24^{cd}
Intermittent Lighting					
PC	8	0.45	585^{ab}	1.40^{d}	51.29^{ab}
NC	8	0.28	527^{d}	1.49^{bc}	45.59^{g}
Fungal Phytase	8	0.28	568^{bcd}	1.45^{cd}	49.48^{d}
Bacterial Phytase	8	0.28	558^{bcd}	1.45^{cd}	48.50^{e}
SEM			12	0.02	0.26

^{abcdefg}Different superscripts within a column $P<0.05$
Saylor et al., 2008

NSP Enzymes

More wheat than normal has been used in the U.S. during this calendar year, for which xylanase was used in the diets to avoid wet litter conditions, lowered feed intake and other performance-related problems related to soluble NSP. Amylase enzymes can also elicit improvements with such ingredients. Depending on the level of wheat and xylanase fed, and the type of wheat, an ME matrix value of up to 30-40 kcal/lb final feed is not uncommon. Amino acid responses have also been attributed to xyalanses in wheat-based diets, thus sometimes have been included in a matrix.

While they lack the viscous nature of NSPs found in wheat, barley and rye, NSP in non-viscous cereal grains pose a physical barrier between the intestinal enzymes and cell components (Hesselman and Aman, 1986). In doing so, starch, protein, oil and other nutrients are encapsulated within the plant cell. Energy gained from the complete NSP digestion of the cell walls is insignificant. Instead, the greatest nutritional value is expected from the released components inside the cell. Some evidence suggests that NSP can stimulate mucin secretion through an increase in goblet cells (Satchithanandam et al., 1990), and possibly disrupt and hinder normal digestive processes. Choct (2001) outlines other detrimental aspects of insoluble NSP.

Corn. For the most part, however, the insoluble NSP portion of ingredients presents the greatest challenge in most U.S. diets. For corn grain, this would be mainly the arabinoxylans and cellulose. We recently analyzed two groups of U.S. corn grain (n = 23). The arabinoxylan content was close to 4%, while the cellulose fell within a range of 2-4%. These two components, along with about 1% pectins, comprise 90% of the NSP in corn, or 9-10% of the dry matter (Malathi and Devegowda, 2001).

SBM. The oligosaccharides and polysaccharides are of concern in SBM due to their indigestible nature and level (18-21%; Bach Knudsen, 2001). The oligosaccharides are mainly α-galactosides (raffinose and stachyose) and are not digested by endogenous enzymes. These make up 6% of the SBM dry matter, and are associated with wet litter due to bacterial degradation in the lower intestinal tract. The ratio of ME to gross energy in SBM is 0.51 for poultry (NRC, 1982), indicating about 51% of the gross energy in SBM is used for metabolic functions. The removal of oligosaccharides with ethanol resulted in 10-15% or more improvement in TME (Coon et al., 1990).

Corn DDGS. In a group of 30 samples from throughout the U.S., the total NSP content of corn DDGS was 23.1% of the dry matter, and the insoluble NSP comprised 88% of this (Ward et al., 2008). The arabinoxylan and cellulose accounted for 85% of the NSP in corn DDGS.

Thus, due to the complexity of NSP in typical corn/SBM diets, a multiple-enzyme addition would likely be the most beneficial for an energy matrix value or uplift. For corn grain and corn DDGS, xylanases and cellulases utilize the arabinoxylans and cellulose as substrates, while pectinases, mannanase and galactosidases target the pectins in SBM. Malathi and Devegowda (2001) reported that groups of enzymes high in pectinases released more sugars in SBM than other combinations tested.

Amino Acid Effect

Through its degradation of phytate, phytase can permit a greater utilization of proteins (Ward 2006). A reduction in phytate may also reduce excessive mucin secretion, thus could make the animal more “nutrient efficient”. A number of researchers have reported beneficial effects on amino acid digestibility (Zanella et al., 1999; Saleh et al., 2005; Meng and Slominski, 2005). In one study, the apparent ileal digestibility of amino acids was significantly higher in the corn/SBM diets supplemented with a combination of endo-xylanase, α-amylase and ß-glucanase (Rutherfurd et al., 2007). Overall, amino acid digestibility was improved by more than 5%. In the absence of a supplemental protease, this increase is attributed to the degradation of the fiber matrix surrounding the protein, thus allowing intestinal proteases greater access. Enzyme supplementation did not influence ileal endogenous amino acid loss, evidence that the improved amino acid digestibility occurred by the actual breakdown of protein by proteases already present.

Across trials, carbohydrases in corn/SBM diets generally show a 2-3% improvement for amino acids. Some products may include “uplift” for amino acids, but ME historically has been the focus for carbohydrases. These products recommend in the vicinity of 30 kcal/lb final feed, depending on inclusion rates and other aspects.

Synergism, Additivity and Antagonism

For the most part, there is little evidence that phytase + carbohydrases work synergistically (the final outcome is greater than the sum of the parts; 1 + 1 >2) across all diets. Theoretically, and considering the binding of phytate within the fibrous matrix of some ingredients, one could expect carbohydrases to make phytate more available to phytase, thus work synergistically with the carbohydrases. This is more apt when NSP content is higher than in corn/SBM diets, or when viscosity is an issue for wheat, rye or barley diets.

An additive effect is more likely between two unrelated enzymes (the final outcome is equal to the sum of the parts; 1 + 1 = 2). Questions exist as to whether we truly see this; some studies are confounded without suitable negative controls and treatments. The simple addition of the ME value for phytase and the ME for carbohydrase products can be a "stretch", especially if one or the other is already excessive. Between phytase and carbohydrases, the latter would have the greater potential for improving ME in most diets, regardless of the ingredients.

One would not necessarily expect antagonism (final outcome is less than the sum of the parts; 1 + 1 < 2), but this aspect has not been completely studied. We have recently concluded work which finds protease + phytase to work effectively with no obvious antagonism of the protease on phytase function. Other proteases likely need similar confirmation.

Currently, a prudent position seems in order when adding all enzymes to the same feed: phytases for P, Ca; carbohydrases for ME; proteases for amino acids. By no means, however, is the issue resolved as to how various enzymes work together, and how the "uplift" or matrix will be ultimately be modeled.

Parting Comments

The use of enzymes in poultry diets is widespread, owed largely to the cost of ingredients. Yet, without advances in the technology of enzymology, protein engineering and fermentation, the extensive use of enzymes would not be feasible. Practical use of enzymes by nutritionists usually includes matrix values focused on those nutrients or components that act as substrates. The carbohydrases offer a grand opportunity for enhancing economic value of ingredients, with ME being the primary focus for corn/SBM type diets. Amino acid nutrition may be improved by carbohydrases, although not always, but this is generally viewed as a secondary effect through the breakdown of the various complex carbohydrates. Strong support for synergism across enzymes is lacking, and additive effects are not always obvious. Until resolved, matrix values and uplift should be focused on the specific substrates for specific enzymes.

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