

Review

Feed formulations to reduce N excretion and ammonia emission from poultry manure

K.H. Nahm *

Feed and Nutrition Laboratory, College of Life and Environmental Science, Taegu University, Gyong San 712-714, South Korea

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Abstract

This summary focuses on reducing nitrogen (N) and ammonia emissions from poultry manure through the use of improved amino acid digestibilities and enzyme supplementation. Proper feed processing techniques, phase feeding, and the minimization of feed and water waste can contribute to additional minor reductions in these emissions. Reductions in environmental pollution can be achieved through improved diet formulation based on available nutrients in the ingredients, reducing crude protein (CP) levels and adding synthetic amino acids. Use of amino acid and CP digestibilities can reduce N excretion up to 40% and a 25% increase in N digestibility can be achieved with enzyme supplementation in broiler diets. Digestibilities can be measured by two methods: the excreta and ileal amino acid digestibilities. Both methods allow amino acid levels to be reduced by 10% or more. Enzyme supplementation decreases intestinal viscosity, improves metabolizable energy levels, and increases amino acid digestibilities. Many feed manufacturers still use total amino acid content to formulate feeds. To meet amino acid requirements, crystalline amino acids are needed. The use of feather, meat and bone meal must not be overestimated or underestimated and the limiting amino acids such as cystine, tryptophan, and threonine must be carefully analyzed.

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1. Introduction

Under ideal conditions, dietary levels of nutrients poultry require for growth, maintenance, or productive functions could be balanced, resulting in minimal fecal waste and, therefore, minimal concerns about environmental pollution. It is unfortunate that there are no perfectly balanced diets nor are there feed groups that are highly digestible by animals. Atmospheric ammonia is a major aerial pollutant from poultry buildings (Kristensen and Wathes, 2000).

In order to improve nutrient utilization and decrease environmental pollution from poultry wastes, potential imbalances within the poultry feeding operation must be recognized and corrected. Afterwards poultry litter and manure

management must also be maximized. Sutton (2002) reported that some producers may need the advantages of a feed-management program if they want to reduce manure nutrient excretions and odors such as maximizing nutrient retention and reducing manure output, formulating diets based upon available nutrients in the feed ingredients, reducing the crude-protein levels and adding synthetic amino acids, as well as using the proper amount of specific carbohydrates during microbial fermentation (Sutton et al., 1999).

Nitrogen (N) pollution is one of the greatest concerns for the public, for excesses excreted by animals can pollute not only water, but also air. Cromwell and Coffey (1995) and Creswell and Swick (2001) demonstrated that use of dietary synthetic amino acids can potentially reduce N excretion by approximately 40%. The concept of “ideal protein”, and use of synthetic amino acids, can result in N excretions from livestock being minimized (Mahan and Howes, 1995).

* Tel.: +82 815 478 5069.

E-mail address: KHNAhm1@cs.com

According to [Lenis and Schutte \(1990\)](#), the crude protein (CP) level of a typical swine ration can be reduced by 3 percentage points (e.g., from 16% to 13% CP) by replacing soybean meal with synthetic amino acids and corn, without negative effects on animal performance. Similar results were observed in poultry ([Firman and Boling, 1998](#)). Optimal amino acid recommendations for most poultry diets were higher than those published by [NRC \(1994\)](#) ([Sklan and Noy, 2005](#)). [Kerr \(1995\)](#) reported amino acid supplementation of low-protein diets for both poultry and swine on average reduced N excretion by 8.5% per one % unit reduction in CP, regardless of body weight. [Schutte et al. \(1993\)](#) determined that for each percentage point that N is reduced in feed, N excretion is reduced by 10%. Environmental conditions affect the availability of organic substrates (e.g., acetate) and their reducing power (e.g., H₂), and ecological forces can also influence carbon isotope discrimination by sulfate-reducing bacteria ([Londry and Des Marais, 2003](#)). In turkeys, [Parks et al. \(1996\)](#) demonstrated that amino acid supplemented diets with CP levels at 90% of [NRC \(1994\)](#) reduced N excretion by 16.4% without adversely affecting growth performance, but breast meat yield decreased from 26.5% to 25.5%.

Amino acids are the building blocks of proteins. There are more than 20 amino acids that comprise proteins. A proportion of dietary amino acids is excreted as undigested fecal matter, and individual feed ingredients differ widely in this respect. Diets may be formulated more closely to the requirements of the bird by knowing the digestibility coefficient (DC) for individual amino acids and the requirement of digestible amino acids for a defined production target. Alternative protein sources with low DC may be used in diets based on digestible amino acids, resulting in N outputs decreasing, while at the same time reducing the costs of the rations used by operators ([Parsons, 1999](#)).

When added to a diet with different proportions of barley and oats, a multi-enzyme preparation, that includes the activities of cellulase, beta-glucanase and proteinase, can result in improved egg production and feed conversion of laying hens in post-peak lay ([Nasi, 1988](#)). Barley and oats have higher fiber levels than wheat and therefore, a lower energy value. In barley and to a lesser extent oats, the endosperm cell walls contain a variable amount of viscous gel-forming beta-glucan, an anti-nutritive factor that causes sticky droppings, that reduces nutrient utilization and decreases growth rate ([Nasi, 1988](#)). Pentosans have been identified from several cereals including wheat, sorghum, barley, rye, oats and triticale ([Reddy, 1992](#)). Enzymes are used to alter the anti-nutritional factors from feed ingredients used in poultry diets. The main anti-nutritional factors in grains are phytate, beta-glucans and pentosans (arabinoxylans), xylose, mannose and galactose ([Coelho, 1996](#)).

Barley beta-glucans increase digesta viscosity and consequently decrease the absorption of nitrogen (N) and carbohydrates ([Burnett, 1966](#)). [Potter et al. \(1965\)](#) observed significant responses to an uncharacterized fungal enzyme

supplement and attributed the increased barley metabolizable energy (ME) to increased digestibilities of protein, fat and N-free extract. [Friesen et al. \(1992\)](#) reported that the nutritive value (bioavailable energy and protein) of cereal grains such as wheat, barley, oats and rye can be improved by enzyme supplementation to the diet of young chicks.

A large volume of published data on the amino acid digestibility of feed ingredients and enzyme supplementation for poultry is now available. The aim of this paper is to introduce the optimal recommendations for feed formulation and their utilization to reduce N output and associated ammonia emissions.

2. Critical nutrients related to environmental pollutants

2.1. Nitrogen

Reducing amino acid requirement yields a reduction feed costs as well as a measurable reduction in the N composition of poultry manure. Of the N consumed, approximately 70–75% is lost or excreted ([Parsons, 1995](#)). Inefficiencies in digestion or absorption are associated with substantial losses in N.

A major source of N loss occurs due to the constant protein turnover associated with muscle metabolism. On a daily basis, approximately 5% of body protein is turned over through degradation and resynthesis. Another large source of N loss is amino acid catabolism. Catabolism of amino acids takes place inevitably or obligatorily due to metabolic insufficiency or overconsumption of amino acids in excess of requirements ([Parsons, 1995](#)).

2.2. Phosphorus

A major concern for surface water quality is eutrophication of lakes and streams. Eutrophication occurs when mineral and organic nutrients reduce the dissolved oxygen to levels that favor plants over animal life. The limiting nutrient for algae and other aquatic plant growth is phosphorus (P) ([Sharpley et al., 1994](#)). Overgrowth of certain blue green algae causes concern since they produce toxins that are a potential health hazard for humans and animals ([Kotak et al., 1993](#)).

2.3. Zinc and copper

Repeated application of poultry and swine manures to land may eventually result in accumulation of excessive levels of copper (Cu) and zinc (Zn), and these minerals may be toxic to agronomic crops and certain species of foraging animals. Unlike excessive land application of N and P, Zn and Cu remain bound to soil and do not migrate into water supplies except during soil erosion occurrences. Accumulation of Zn and Cu will occur and eventually result in an unsustainable situation for certain crops unless these minerals are removed from the land via plant products ([Sims and Wolf, 1994](#)).

There is some concern that the long-term application of high rates of Cu could be toxic to crops grown on coarse-textured soils or crops grown on fine-textured soils subject to anaerobic conditions (Meeks et al., 1975). Application of poultry wastes at levels that increase the soil available P levels could result in P-induced Zn deficiency in soils with low levels of Zn or where Zn-sensitive plants are grown (Sims and Wolf, 1994). Meeks et al. (1975) reported that citrus trees fertilized with turkey manure developed Zn deficiency symptoms while adjacent trees not treated with manure remained healthy. The Zn deficiency could also be induced by addition of P fertilizer.

3. Dietary manipulation to enhance nutrients

3.1. Minimizing feed and water waste

In general, there is a 1.5% increase in manure N and P content for each 1% increase in feed wastage (Ferket et al., 2002). Significant animal feed waste can result from poor feeder design and position or feed form, and this waste ultimately ends up in manure or litter. If feeders are overfilled, adjusted too low, or poorly designed, poultry will waste a significant amount of feed (Beyer et al., 2001). Feeder height should be adjusted so the top of the feed pan is level with the base of the birds neck, and the feeder pan should only be filled 25% (Ferket et al., 2002).

For all living plants and animals, water is the primary nutrient. The amount of minerals excreted by animals is not affected by the amount of liquids consumed and excreted, but manure or litter processing and disposal costs are affected by water levels in the excreta. Excessive water in broilers and turkeys excreta requires much more work and bedding materials to maintain proper litter quality. Excessive water levels in poultry excreta can also increase the incidence of pests (bacteria, litter beetles, flies, etc.) that can cause flock health problems. Excess water in the house results in increased bacterial growth, ammonia emission, and air quality problems, while it also adversely affects the volume and nutrient quality of the litter to be disposed. Leaks in watering systems require immediate repair and efficient water nipples should be used and adjusted to minimize spillage. Drinker rinse water should not be poured into the litter during cleaning.

3.2. Feed processing

Grinding and pelleting are effective methods for improving feed utilization and for decreasing dry matter and nutrient excretion in poultry manure. When feed particle size is reduced, surface area of the feed ingredient is increased, which allows for greater interaction with digestive enzymes. There was a significant interaction between diet type and soy particle size when the corn–soybean meal (SBM) diets were fed because the coarse SBM increased plasma P levels, while there was little effect when semi-purified diets were fed (Kilburn and Edwards, 2004). They suggested that large

particle SBM may be more efficiently utilized than fine particle SBM. In comparing maize particle size (Kilburn and Edwards, 2001), fine maize diets had higher ME values and there was a significant interaction between particle size and food form as pelleting improved the ME value of coarse but not fine maize diets.

There is special interest in the role of the gastrointestinal tract (GIT) when high fiber diets are utilized. An experiment was conducted to study the effects of inclusion of oat hulls in diets on whole or ground wheat for broilers and layers (Hetland et al., 2003). According to these scientists, fiber concentration was considerably higher in the gizzard contents than in the feed. No effect of hulls or whole wheat inclusion was found, indicating that all particles are ground to a certain critical size before leaving the gizzard. Mujahid et al. (2004) reported that treating (roasting or pelleting) rice bran with up to 250 ppm of antioxidants had non-significant effects on broiler performance.

Pigs have been shown to prefer pelleted feed over mash feed, which improved feed efficiency by 8.5% due to reductions in feed wastage (Vanschoubroeck et al., 1971). Protein digestibility was also improved by 3.7% following pelleting. Similar responses were observed in poultry (Beyer et al., 2001) where increased amino acid bioavailability may occur when corn or SBM is expanded under different cone pressures. Increases in cone pressure increased the true ME (TME), amino acid availability, protein solubility and starch gelatinization. Since these products were processed separately, further study is needed to determine if there is an interaction of nutrients from different sources in the gelatinization phase. There is likely to be an interaction of protein, starch and fat particles when ingredients are placed under pressure or high temperatures.

3.3. Feed formulation with enzymes

Poorly digested cereals are more greatly influenced by enzyme addition than well digested cereals (Lyons, 1995; Lobo, 2000). For the feed compounder, enzyme addition has presents two consequences: (1) there is a reduction in variation between the best and worst samples of a given grain, (2) the feed formulation nutrient matrix values may be elevated by enzyme addition (Bedford, 2000).

Based on their ME levels, grains can be ranked in the following order for digestibility: corn > wheat > triticale > barley > oats > rye. Barley, oats and rye have not been used extensively in the past in poultry diets due to poor and variable bird performance and due to their propensity to cause unmanageable wet litter conditions (Friesen et al., 1992; Bedford, 1995; Grimes and Crouch, 1997). The negative characteristics of these cereals have generally been attributed to their fiber content and various non-starch polysaccharides.

Three focal points of pollution are odor (ammonia and other compounds), manure N and P and broilers lose approximately 30% of consumed dry matter, 25% of gross energy, 50% of N and 55% of P intake as extracted waste

(Ivey, 1992). Enzymes destroy anti-nutritional factors or increase the digestibility of indigestible nutrients so utilization of an enzyme system saves energy and improves amino acid digestibility (Lyons, 1995; Silversides and Bedford, 1999). In addition, reductions in digesta viscosity that enzymes provide is especially significant for improving fat digestion and for increasing the availability of fat-soluble vitamins as well as improving the efficiency of absorption of natural dietary pigments – chlorophylls and xanthophylls – that are essential for good yolk color in eggs and broiler beaks as well as feet (Anonymous, 1996).

Cowan et al. (1996) reported that increased N retention resulted in improved energy availability and ME, and that they are also improved by the addition of enzymes to the diet (Bio-Feed plus CT, Novo Nordisk A/S, Denmark), but this did not show a clear dosage response with the apparent metabolizable energy, nitrogen corrected apparent metabolizable energy (AMEn) of the selected ingredients (which contained 30% of wheat middlings and 25% of plant protein sources). These scientists indicated that improved N retention did not directly correlate with protein digestibility, but that improved protein retention may contribute to the reduction in dietary protein. Gdala et al. (1997) found out that supplementation of piglet diets based on barley and wheat with soybean meal (CS) or soybean meal and rapeseed meal (CPSR) with a xylanase or an enzyme mixture (alpha-galactosidase + xylanase + protease) increased xylose digestibility of the CS diet and xylose, arabinose and mannose digestibilities and dry matter (DM) content of the CPSR diet. Pectin was more rapidly and extensively digested than cellulose, arabinoxylans and xylan polysaccharides but the fermentation yielded similar proportions of acetate, propionate and butyrate in the large intestine.

There are environmental benefits to having reduced nutrient levels in manure output. N output from broiler farms can also be impacted by the addition of dietary enzymes that provide improved protein digestibility. The addition of a complex enzyme to broiler diets (Allzyme Vegro, Alltech, Inc., UK) increased true digestibility of all amino acids except valine (Charlton and Pugh, 1995), where substantial increases in the bioavailability of many amino acids for broilers including most limiting amino acids (lysine, methionine, cystine and threonine) were reported.

Enzyme supplementation is most commonly done by direct addition of an enzyme or enzyme combination to the feed. The most effective enzyme addition to feed has been when the enzyme action was directed at a specific feed component (Tables 1 and 2). Data from these tables indicates that different species require different levels of dietary enzyme supplementation and different basal diets also require different levels of enzyme supplementation.

3.4. Phase feeding (PF)

Nutrients that animals do not digest and assimilate for body maintenance and tissue growth are excreted. In order

to optimize dietary nutrient balance it is essential to accurately estimate nutritional requirements. Unfortunately, it is difficult to obtain these estimates because nutritional requirements are constantly changing due to many environmental or age related factors plus yearly changes in the genetic characteristics of our different poultry types.

In order to determine the optimum proportion of time to feed broiler starter (23% protein), grower (20% protein), and finisher (18% protein) diets that would optimize production and processing variables, an experiment was designed with various feeding times for the three component diets based on a 48 day production cycle (Roush et al., 2004). In this study, broilers were fed a starter and finisher diet for 30 and 18 days, respectively, in order to optimize production and processing variable. Trace plots showed that the production and processing variables were not very sensitive to the grower diet. Pope and Emmert (2002) studied the effects of phase feeding (PF) on heat stressed broilers (high temperatures were 15 h at 35°C and 9 h at 23.9°C, while normal was 24 h at 23.9°C) during the grower and finisher periods. Diets were based on NRC (1994) requirements for lysine, sulfur amino acids (SAA) or threonine. The results indicated that birds exposed to high environmental temperatures could have dietary lysine, SAA and threonine levels reduced every other day under a PF program without adversely affecting growth performance or carcass yield. In another study by Pope and Emmert (2002), reducing lysine, SAA and threonine levels every other day in a PF program supported maximum growth performance during an extended finisher period. Pope and Emmert (2002) also indicated that CP could be reduced by PF10 (PF10 is the diet that recommends reducing by 10% the NRC (1994) recommendations relative to broilers fed NRC (1994) and PF diets. A series of 11 diets (PF10) were formulated to contain 10% less lysine, SAA and threonine than those contained in PF diets, with dietary amino acid concentrations lowered every other day of the experiment. No differences in percentage carcass composition were observed when broilers were fed PF or PF10 diets. PF and PF10 diets reduced CP intake and nitrogen excretion from 43 to 63 days.

Evaluation of the dietary amino acid requirements of animals is a continuous process, but N utilization will require greater attention in the future since it is complicated by numerous factors including genetic characteristics, environmental management, physiological stress and the digestibility of feedstuffs involved.

3.5. Estimating amino acid requirements

The preferred method to estimate amino acid availability is by employing one of two types of digestibility assays: excreta and ileal, where fresh excreta from intact or caecotomized birds can simply be collected for digestibility of the nutrients contained in the feed, or digesta from the distal part of the ileum can be collected and compared with the levels of feed nutrients for measuring ileal digestibility.

Table 1
Enzyme supplement and the effects on broiler performance

Author and year	Type of enzyme	Amount of enzyme	Increased performance	Based diets
Wang et al. (2005)	Xylanase and β -glucanase	0, 200, 400, 600, 800 or 1000mg/kg enzyme	<ol style="list-style-type: none"> 1. Improved performance, daily gain, feed conversion linearly with increasing levels of enzyme supplementation 2. Decreased the size of the digestive organs and GIT 	Barley-based diets
Daskiran et al. (2004)	Endo- β -D-mannanase	0.05% of diet	<ol style="list-style-type: none"> 1. Did not affect final body weight 2. Improved the 14-day feed:gain ratio 3. Reduced total dry fecal output 	Corn–soy-based starter diets
Jackson et al. (2004)	β -Mannanase	0, 50, 80, 110MU/ton feed (1 MU = 10^6 enzyme activity units)	<ol style="list-style-type: none"> 1. 50 MU/ton is not sufficient 2. 80 MU/ton improved gain and feed conversion 3. 110 MU/ton showed no significant response 	Corn–soybean based diets
Lazaro et al. (2004)	Xylanase and β -glucanase	Two enzyme doses (0 vs. 500ppm)	<ol style="list-style-type: none"> 1. Impaired broiler performance 2. Increased digesta viscosity and incidence of leg disorders 	Rye-based diets
Gracia et al. (2003)	α -Amylase	40ppm	<ol style="list-style-type: none"> 1. Improved digestibilities of nutrients and performance of broilers 2. Reduced relative pancreas weight, intestinal viscosity and relative weight of all organs 	Corn–soybean based diets
Gracia et al. (2003)	Enzyme complex 1. Xylanase 2. Protease 3. α -amylase	Two levels of enzyme supplementation (0 or 500ppm)	<ol style="list-style-type: none"> 1. Broiler performance was improved 2. Heat processing and enzyme supplementation increased apparent retention of nutrients, AME of the diet, villus height 	Barley-based diets
Lee et al. (2003)	β -Mannanase	Three levels (0, 1 \times and 4 \times) 1 \times = 1.09×10^5 units/kg feed	<ol style="list-style-type: none"> 1. Reduced intestinal viscosity 2. Alleviates the deleterious effects 	Industry-type broiler diets
Von Wettstein et al. (2003)	(1,3-1,4)- β -glucanase	545 or 469g/kg nontransgenic barley instead of maize	<ol style="list-style-type: none"> 1. Allow the reduction of the transgenic grain ingredient to 0.2g/kg diet 2. Make the ingredient comparable to that of trace minerals added to standard diets 	Barley-based diets
Kocher et al. (2002)	Commercially available product	<ol style="list-style-type: none"> 1. Recommended dosage 2. At five times the recommended dosage (High dosage) 	<ol style="list-style-type: none"> 1. Enzyme A: High dosage improved AME, reduced excreta moisture, and improved ileal protein digestibility 2. Enzyme B: High dosage reduced ileal protein digestibility and increased amount of free sugar 	A nutritive value of soybean meal
Silva and Smithard (2002)	Xylanase	15,000 and 45,000 units/kg feed	<ol style="list-style-type: none"> 1. Improved growth performance 2. 25% increase in the N digestibility 3. A doubling of the fat digestibility 	Rye-based diets
Marron et al. (2001)	Xylanase (Avizyme)	None Avizyme (Finnfeed) 1g/kg feed Avizyme (Liquid) 0.5g/kg feed	Both enzymes forms gave similar improvements in performance in absence of copper sulfate	Wheat-based diets
Jin et al. (2000)	<ol style="list-style-type: none"> 1. <i>L. acidophilus</i> 2. A mixture of 12 <i>L.</i> strains 	<ol style="list-style-type: none"> 1. A basal diet + 0.1% dried culture of <i>L. acidophilus</i> 2. Basal diet + 0.1% dried culture of a mixture of 12 <i>L.</i> strains 	Reduced the intestinal and fecal β -glucuronidase and fecal β -glucosidase	Industrial-type broiler diets

Because amino acids are absorbed from the large intestine, the amino acid profile of digesta at the terminus of the ileum is not necessarily the same as in the feces (Dalibard and Paillard, 1995). Rodehutsord et al. (2004) reported that a linear regression approach be adopted to study amino acid digestibility of individual feedstuffs in broiler chickens. Digestibility determined this way does not need any correction for basal endogenous loss. Farrell et al. (1999) showed no significant difference in growth rate that was related to the total or digestible amino acid levels present in any of the diets. It is clear, however, that diets formulated to 100% of digestible amino acid specifications are over-specified by at least 10%.

Williams (1995) reported that hind-gut microbial fermentation influences amino acid digestibility. The lower the digestibility of the protein supplement, the more microbial fermentation affects digestibility. Excreta or total gastrointestinal tract digestibility is therefore not a good estimate of the amount of amino acids actually absorbed in the small intestine. In a comparison of intact with cecectomized cockerels there was little difference between fecal and ileal digestibility of amino acid for cereals, slight differences for oilseed meals, but significant differences for some animal meals (Williams, 1995).

Digestibility determination based on excreta collection has been shown to overestimate the uptake for some amino

Table 2
Enzyme Supplementation and the effects on each type of poultry performance

Author and year	Type of poultry	Type of enzyme	Amount of enzyme	Increased performance	Based diets
Mandal et al. (2005)	Pullets, Guinea fowl, Quail	Commercial enzyme preparations	Two levels added (0 or 0.57 g/kg feed)	The apparten metabolizable energy (AMEN) value of rapeseed meal did not improve, while sunflower seed meal increased	Solvent-extracted rapeseed and sunflower seed meal
Wu et al. (2005)	Layers	β -Mannanase	Recommended levels	1. No significant differences in overall average egg production and egg mass among treatments 2. Increased average egg production and egg mass during 5–8 weeks 3. No significant differences in feed intake, production performance and body weight	Corn–soybean meal
Lazaro et al. (2003)	Layers	Fungal β -glucanase/xylanase	Four enzyme concentrations (0, 250, 1250, 2500 mg/kg feed)	No effect on egg production of feed efficiency	Wheat, barley and rye-based diets
Mathlouthi et al. (2003a)	Turkeys	Xylanase β -glucanase	560 and 2800 IU/kg feed of xylanase and β -glucanase, respectively	Improved body weight gain and feed efficiency	Wheat or wheat and barley-based diets
Mathlouthi et al. (2003b)	Layers	Xylanase and β -glucanase, used as two enzyme preparations	1. Wheat/barley based diet with 20 mg 2. Corn/soybean meal with 20 mg	1. Decreased viscosity of wheat, barley, corn and soybean meal 2. Other beneficial effects were not significant	1. Wheat/barley based diets 2. Corn/soy-bean meal
Odetallah et al. (2002a)	Turkeys	Enzyme mix supplementing β -glucanase units (BGU) and endo-xylanase units (EXU)	BGU: 300 g/ton feed, EXU: 300 g/ton feed, EXU: 100 g/ton feed, BGU 150 g/ton feed	Marginal improvement in growth performance depending upon enzyme formulation	Wheat-based diets
Odetallah et al. (2002b)	Turkeys	Mannan-endo-1,4- β -mannosidase	With or without 100 million units (MU) Hemicell/ton (1 MU = 10 ⁶ enzyme activity U)	Some of the adverse effects of antinutritional factors of SBM on turkey growth performance can be alleviated by enzyme supplementation	SBM-44 or SBM-48 was found in the basal diet
Zhang et al. (2001)	Pullets	Five preparations using: <i>T. viride</i> , <i>T. reesei</i> , <i>A. niger</i> (Finizym), <i>A. niger</i> (SP 249), <i>Bacillus subtilis</i>	1. 0.003125% 2. 0.00625% 3. 0.0125% were added respectively	1. Can accurately evaluate profitability 2. Selects the most profitable cereal	Barley-based diets
Zhang et al. (2000)	1-d-old single combed white leghorn cockerels	Xylanase	Six amounts of two enzymes: 1. 0, 0.25, 0.75, 2.25, 6.75, and 20.25 g/kg feed 2. 0, 0.1, 0.3, 0.9, 0.27, 8.1 g/kg feed	1. Evaluate the efficacy of enzyme preparations 2. Predict chick performance	Rye-based diets

acids in some feeds (Kadim et al., 2002). The degree of over-estimation varied from 8.9% (apparent digestibility of threonine in soybean meal) to 66% (apparent digestibility of aspartic acid in wheat). Digestibility values measured at the terminal end of the ileum have been proven to be more reliable measures of amino acid availability than those measured in the excreta. β -glucanase improved apparent ileal digestibility of amino acids in dried barley but not in air-tight stored or ensiled barley (Perttila et al., 2001). They reported that amino acid digestibilities were lower in broilers than cockerels and that the effect of barley preservation on feeding value of barley was different for broilers and cockerels. Farrell et al. (1999) demonstrated differently and stated that diets formulated on a total or digestible (excreta) amino acid basis gave no differences in egg production or broiler production parameters with no effect for level of inclusion. However, reducing amino acid specification should clearly

provide a reduction in feed costs (as was the case) and consequently the cost per kg of gain was also reduced.

The digestibility coefficients of amino acids in eight feed ingredients have been shown to increase with advancing age of broiler chicks (Huang et al., 2005). Broiler diets (Kluth et al., 2005) containing 300 g/kg of peas had significantly lower energy digestibilities than did the pea-free basal diet. The pea cultivar with the lowest amino acid digestibility caused the lowest energy digestibility at both levels of pea inclusion. Chen et al. (2005) determined the ileal digestibility of arginine and lysine in acutely heat-stressed broilers using diets varying in their arginine:lysine ratio, NaCl concentration, and methionine source. Their results indicated that dietary NaCl (3 g/kg of additional NaCl) could affect the apparent ileal digestibility of arginine and lysine at certain arginine:lysine ratios and the response may be influenced by the methionine source.

Precision amino acid nutrition and diet acidification can significantly reduce the amount of ammonia emission from animal waste (Ferket et al., 2002). A large volume of data has been published on the amino acid digestibility of common ingredients used for poultry (Lemme et al., 2004), but diet formulation based on total amino acid content is still widely used (Bryden et al., 2000). As an example, Table 3 presents the application of the digestibility coefficients to recommendations for broilers. However, Table 4 shows how environmental temperature can effect the amino acid requirements of laying hens while Table 5 compares selected amino acid recommendations in the growing chick. Amino acid supplementation in each poultry group

Table 3
Example of formulation specifications for broilers (Dalibard and Paillard, 1995)

	Starter	Grower	Finisher
Metabolizable energy (kcal kg ⁻¹)	3100	3150	3200
<i>Total amino acid, %</i>			
Lysine	1.18	1.03	0.94
Methionine	0.55	0.45	0.39
Methionine + cystine	0.91	0.81	0.76
Threonine	0.76	0.69	0.65
Tryptophan	0.22	0.20	0.19
Arginine	1.30	1.05	0.96
Isoleucine	0.89	0.75	0.69
Leucine	1.65	1.39	1.26
Valine	0.98	0.86	0.80
<i>Digestible amino acid, %</i>			
Lysine	1.00	0.88	0.80
Methionine	0.51	0.42	0.36
Methionine + cystine	0.79	0.71	0.66
Threonine	0.65	0.59	0.56
Tryptophan	0.19	0.17	0.16
Arginine	1.17	0.95	0.86
Isoleucine	0.79	0.65	0.61
Leucine	1.50	1.24	1.14
Valine	0.85	0.75	0.69

Table 4
Example of formulation specification for laying hens (Dalibard and Paillard, 1995)

Temperature	18 °C		30 °C	
	105	110	90	95
Feed intake (g day ⁻¹)				
Metabolizable energy (kcal kg ⁻¹)	2850	2750	2900	2750
Protein, %	15.0	14.5	17.8	16.8
<i>Total amino acid, %</i>				
Lysine	0.74	0.71	0.87	0.82
Methionine	0.36	0.35	0.42	0.42
Methionine + cystine	0.64	0.61	0.74	0.71
Threonine	0.50	0.47	0.58	0.55
Tryptophan	0.16	0.15	0.19	0.18
Digestible protein, %	13.2	12.8	15.7	14.8
<i>Digestible amino acid, %</i>				
Lysine	0.63	0.60	0.74	0.69
Methionine	0.33	0.32	0.39	0.37
Methionine + cystine	0.56	0.53	0.64	0.62
Threonine	0.43	0.40	0.50	0.47
Tryptophan	0.14	0.13	0.16	0.16

resulted in differences in the ammonia production and performance of each species studied (Tables 6–9).

4. Improving feed digestibility and nutrient bioavailability

An enzyme is an organic catalyst, or compound composed of some organic components that accelerate chemical reactions. Enzymes are proteinaceous and they are susceptible to the types and causes of degradation that proteins experience such as destruction from heat, extreme pH ranges, and high urea concentration.

Feed enzymes used are derived from either bacteria or fungi (Table 10). The development of feed enzymes has greatly increased the use of feed ingredients such as barley, wheat, rye, soybeans, etc., in commercial animal production. These feedstuffs are rich in nutrients but also in anti-nutritional factors (ANF), and in a way producers have been forced to use these ingredients along with enzymes in their formulation of high-density diets needed for intensive production of mono-gastric animals such as chickens and pigs (Campbell and Bedford, 1992). However, it must be recognized that the response obtained with the use of such enzymes may vary considerably, as there are many factors that may influence their actions. Ingredient, cultivar sources, types of soils, dietary ingredients included, type of feed processing, age of the animal, etc., may significantly affect the effectiveness of feed enzymes (Puchal and Mascarell, 1999). Only a certain percentage of any nutrient in a feedstuff is absorbed or digested, so bioavailability is closely related to the true and apparent digestibilities.

4.1. ANF and digesta viscosity

ANFs are commonly referred to as non-starch polysaccharides (NSP) which can be classified as arabinoxylans (pentosan) and beta-glucans. The arabinoxylans, which consist of a linear backbone of xylose substituted with arabinose, are responsible for the bulk of the problems encountered in birds fed wheat or rye based diets, whilst the beta-glucan, a linear polymer of glucose with kinks in its structure, are responsible in oats and barley (Bedford, 1995). The negative effects of beta-glucans and arabinoxylans on digesta are due to the creation of large entanglements that result in an elevation in viscosity of the contents of the small intestine (Bedford, 1995).

Any feed which contains ANF has an increased viscosity which disturbs starch digestion and utilization. Primary factors affecting starch digestion are soluble cell-wall polysaccharides, which impede digestion of all nutrients including starch. Factors which affect starch utilization include presence of ANF in grains, nature of grain starch and digestive capacity of the animal (Classen, 1996). Intestinal viscosity is a major factor limiting bird performance (Bedford and Morgan, 1996).

The long NSP polymers entangle and have a high water-holding capacity, resulting in an increase in intestinal viscosity. When sufficient levels of barley, wheat, triticale or

Table 5
Selected values for the amino acid requirements of the growing chick

	Dean and Scott (1965)	Zimmerman and Scott (1965)	NRC (1966)	ARC (1963)	Hewitt and Lewis (1972b)	Selected values
Threonine	0.65	0.65	0.70	0.55	0.53	0.60
Glycine	1.60	1.20	1.00	1.00	0.61	0.60
Valine	0.82	0.82	0.85	0.80	0.79	0.80
Methionine (Met)	0.45	0.35	0.40	0.28	0.39	0.40
Met + cystine	0.80	0.70	0.75	0.70	0.79	0.75
Isoleucine	0.80	0.80	0.75	0.50	0.61	0.70
Leucine	1.20	1.20	1.40	1.50	1.34	1.20
Phenylalanine (Phe)	0.68	0.50	0.70	0.60	0.72	0.70
Phe + tryrosine	1.31	0.95	1.30	1.20	1.27	1.30
Lysine	1.10	0.95	1.10	1.00	0.85	0.90
Histadine	0.30	0.30	0.40	0.35	0.40	0.40
Arginine	1.10	1.00	1.20		0.85	0.85
Tryptophan	0.23	0.15	0.20	0.15	0.17	0.17

Values are expressed as percentages (Hewitt and Lewis, 1972a).

rye are fed to poultry, substandard performance, sticky feces and pasty beaks are attributed to the NSP components of these diets. The antinutritive character of NSP is demonstrated by a negative relationship between NSP, energy level, and nutrient digestibility, while its poor digestibility is shown by fecal recoveries of 86% (Ward, 1996). When various cereals have been fed with SBM, the resultant foregut viscosities are reported in Table 11. A gel-like viscosity in the intestinal tract is formed by the water-soluble portion of the NSPs. The definition of viscosity is “the internal fluid resistance of a substance and it can be envisioned as a gelatinous, thick, syrupy or sticky substance”. Increased solution viscosity is known to reduce the rate of nutrient diffusion (Fengler and Marquardt, 1988), and depress feed passage rate (Salih et al., 1991). Zeamation (a 22-kDa protein isolated from *Zea mays* that shows antifungal activity against human and plant pathogens) did not inhibit human α -amylase activity and only inhibited mammalian trypsin activity in high molar concentrations (Schimoler-O'Rourke et al., 2001).

The cell-wall carbohydrate fraction is mainly formed from heteropolymers like beta-glucan and arabinoxylan (pentosan) which are present in most grains, but their total amount and proportions vary considerably. Beta-glucans predominate in barley and oats but arabinoxylans are at higher levels in wheat, rye and triticale. Phenolic acids (mainly ferrulic acid) are primarily esterified to the arabinoxylans (Classen and Bedford, 1991). Some unicellular organisms encyst to protect themselves from a harmful environment, and this cyst wall usually contains chitin, but in some cases, as in *Acanthamoeba*, it consists of cellulose instead (Linder et al., 2002). When determining the utilization of nutrients such as starch by poultry, the soluble fractions of beta-glucan and arabinoxylan found in cereal grains are considered of major importance. There is an increase in digesta viscosity when beta-glucan and arabinoxylans solubilize after ingestion (Teitge et al., 1991). This increased digesta viscosity is a major factor influencing the nutritional value of rye, barley and oats, and recently simi-

lar evidence has become available for wheat (Bedford and Classen, 1992). The second effect is that the cell walls prevent or slow access of endogenous enzymes to nutrients (Hesselman and Aman, 1986).

4.2. Enzyme utilization with NSP

Improvements ($P \leq 0.05$) in gain, feed/gain (F/G), intestinal viscosity, digesta dry matter and digestibility of amino acid, ME, protein, fat and dry matter are associated with multienzyme addition (Gohl et al., 1978). A further increase in productive value with enzyme supplementation can be achieved by: (1) solubilization of cell-wall polysaccharides rendering them available for hindgut fermentation, (2) elimination of nutrient encapsulating effect of the cell wall, (3) hydrolysis of certain types of carbohydrate-protein linkages, and (4) release of readily available sugars by oligosaccharide hydrolysis (Slominski, 1995). Enzyme treatment decreases moisture content of excreta, which, together with improved dry matter digestibility, reduces the total amount of excreta produced and, therefore, reduces management and environmental problems (Marquardt et al., 1996).

The use of beta-glucanase enzymes is important in broiler diets containing high levels of barley, or in some cases, rye and wheat. Barley typically contains between 2% and 6% beta-glucan and yields highly viscous solutions (Dunne, 1995). Wheat and rye contain between 4% and 8% of pentosan (arabinoxylan) gums which are not readily metabolizable by the young chicken in trials reported by Marquardt et al. (1996). Research (Gohl et al., 1978; Belyavin, 1994; Pack et al., 1998; Lobo, 1999) has demonstrated the benefits of enzyme supplementation when directed at a specific feedstuff.

In poultry nutrition today, most enzymes can be classified as carbohydrases (beta-glucanase, xylanases, hemicellulase, pentinase, alpha-galactosidases, inulase) which are enzymes that act on different carbohydrates, particularly the types of carbohydrates (hemicelluloses, oligosaccha-

Table 6
Examples of amino acid levels influencing broiler performance or ammonia emission from manure

Authors and year	The amount of amino acid supplemented	Influencing ammonia or performance
Chamruspollert et al. (2004)	<ol style="list-style-type: none"> 1. Tem affects responses to dietary Arg and Met and suggests that the higher tem slowed the Arg metabolism of chicks through the creatinine synthesis pathway 2. In Exp. 1: Arg (15.2 and 25.2g/kg diet) Met (3.5 and 5.5g/kg diet) at 22°C and 32°C. In Exp. 2: Arg (15.2, 25.2, 35.2g/kg diet) Met (3.5, 5.5 and 7.5g/kg diet) at 25°C or 32°C 	Performance
Chavez et al. (2004a)	Sodium methionine aqueous solution, dry Met hydroxy analogue, liquid Met hydroxy analogue, DL-Met. Starter and grower diets were formulated to contain 0.5% and 0.38% Met activity	Significant for odor production (Odorous volatile concentration)
Chavez et al. (2004b)	52%, 45.9%, 88% and 98% Met activity. All diets were formulated to contain either 0.8% (Exp. 1) total Met activity or 0.5% Met activity in Starter and 0.38% Met activity in growers (Exp. 2), but others met NRC (1994) requirements	Odor-related compounds, excreta, total dietary Met
Chavez et al. (2004c)	Same as above. Dry Met Hydroxy analogue, Sodium Methionate, Liquid Met hydroxy analogue, D, L-Met	Odorous volatile concentrations, excreta
Ciftel and Ceylan (2004)	<ol style="list-style-type: none"> 1. Estimated Thr requirements for feed conversion efficiency increased as dietary CP increased according to an exponential performance model. This model increased higher Thr requirements than those of broken-line models for growth performances 2. CP: 191.3 or 179.7g/kg feed for 0–3 weeks, 176.7 or 165.4g/kg feed for 3–6 weeks. Thr: 6g/kg feed for 0–3 weeks, 5.4g/kg feed for 3–6 weeks 	Performance
Fatufe et al. (2004)	<ol style="list-style-type: none"> 1. Depending on genotype 2. They differed in lysine concentration from 3.8 to 16.8g/kg 	Performance
Kidd et al. (2004)	The 21–42 day threonine need across was estimated as 0.74% total or 0.65% digestible	Feed intake is higher, but not in BW gain or breast meat yield
Vieira et al. (2004)	<ol style="list-style-type: none"> 1. Reducing diets with SAA/Lys of 77% impairs performance 2. Increasing diets with SAA/Lys of 77% was optimum 	Performance
Corzo et al. (2003)	1.00% as required by NRC (1994) is in agreement	Live production and meat yield are good, but carcass and skin are not
Kalinowski et al. (2003)	<p>0–3 weeks of age</p> <ol style="list-style-type: none"> 1. Met required is 0.50% 2. Cys required is 0.39% (slow-feathering males) and 0.44% (fast-feathering males) 	Performance
Sterling et al. (2003)	The amino acid requirements of broilers are a constant proportion of CP levels	Comment: Two levels of lysine per CP level (35 and 48g lysine/kg CP)
Chamruspollert et al. (2002)	<ol style="list-style-type: none"> 1. Met requirement of male chicks needed to be higher than that of females, but not significant 2. In Exp. 1: 0.01%, 0.15%, 0.3% supplementation. In Exp 2.: 0.1%, 0.13%, 0.15%, 0.2%, 0.25% supplementation 	Performance
Lemme et al. (2002)	Liquid MHA-FA was 72% (weight gain), 51% (feed conversion), 48% (carcass yield) and 60% (breast yield) as efficacious as DLM on a weight-for-weight basis	Performance
Acar et al. (2001)	<ol style="list-style-type: none"> 1. Excessive individual amino acids in diets suppress the appetite and early rapid growth to alleviate or minimize metabolic disorders 2. The high level of His decreased the percentage of pectoralis minor muscle yield, whereas the high level of Lys and Met increased the percentage of liver compared to those fed the basal diet 	Performance
Labadan et al. (2001)	<ol style="list-style-type: none"> 1. The requirements for Lys and Arg were similar except for the earliest age group for which the Lys requirement appeared for be slightly higher than that of Arg 2. Calculated digestible Lys and Arg requirements: 1.24% and 1.19%: up to 2 wks of age. 1.11 and not determined: 2–4 wk of age, 0.92 and 0.91%: 3–6 wk of age, 0.75 and 0.78%: 5–8 wk of age 	Performance
Rosa et al. (2001a)	There was no apparent difference in the Trp requirement of young broilers due to genetic stock ($P>0.05$)	Performance
Rosa et al. (2001b)	<ol style="list-style-type: none"> 1. The Trp requirements of high yield and classic broilers (males and females) were similar and greater than for leghorn strains studied 2. The Thr requirement was $0.71 \pm 0.01\%$ for BWG and $0.71 \pm 0.01\%$ for FCR for the males and $0.72 \pm 0.01\%$ for BWG and $0.71 \pm 0.01\%$ for FCR for females 	Performance
Si et al. (2001)	<ol style="list-style-type: none"> 1. The BW was significantly increased at 21–42 days by addition of 10.1% lysine above NRC but not at 56 days 2. Increasing the level of EAA resulted in significant improvements in feed conversion at 21, 42 and 56 days 	Performance
Abdallah et al. (2000)	Basal diet+0.31% (starter) and 0.26% (grower) DL-methionine, Basal diet+0.48% (starter) and 0.40% (grower) liquid MHA-FA (ratio 65:100 on weight/weight basis)	Performance

Table 6 (continued)

Authors and year	The amount of amino acid supplemented	Influencing ammonia or performance
Alleman et al. (2000)	Genetically lean chickens require more diets more concentrated in NEAA than fat chickens and that there seems to be an effect of NEAA on breast muscle development	Performance
Schutte and Pack (1995)	0.75% apparent sulfur AA or 0.78% true digestible AA	Performance

rides, etc.) that have been identified as components of the group known as NSP. Other types of enzymes have been successfully studied including those affecting digestibilities of fat (lipase), phosphorus (phytase), proteins (proteases), etc. However, ingredient type, cultivars, soil type, dietary ingredients, processing, age of the animal, etc., may significantly affect the effectiveness of feed enzymes (Puchal and Mascarell, 1999). The level of improvement is related to enzyme type and to dosage, and correlates well with the substrate specificity of the various enzymes present (Cowan et al., 1996).

4.3. Oligosaccharides and enzyme supplementation

Beta-glucan and arabinosylan (pentosan) are neutral detergent soluble fiber (NDSF) which are found in the cell wall, while oligosaccharides are considered neutral detergent soluble carbohydrate (NDSC) which are found in the cell contents (Hall, 2001). The term oligosaccharide refers

to glycosides composed of 3–10 monosaccharide units. Many oligosaccharides are indigestible in the small intestine, but they are fermentable in the large intestine (mainly in the colon) by various bacterial species (Gibson and Roberfroid, 1995; Orban et al., 1997). These are lactulose, galactooligosaccharides, fructooligosaccharides (FOS), isomaltooligosaccharides, soybean oligosaccharides, lactosucrose, mannonoligosaccharide (MOS), senitooligosaccharides, and xylooligosaccharides. Among these nine, FOS and lactosucrose have so far been focused on in human, dog and pig research for reducing N excretion and ammonia emissions (Sutton et al., 1996, 1998).

There is evidence that FOS alters volatile fatty acid (VFA) patterns in the lower gastrointestinal tract (GIT) of the bird (reducing the proportion of acetate and increasing the proportion of propionate), decreases total number of aerobes (predominantly coliform), increases *Bifidobacteria* (Howard et al., 1995), and reduces amount of odorous compounds from swine manure (Hidaka et al., 1986). A similar

Table 7

Examples of amino acid levels influencing layer performance or ammonia emissions from manure

Authors and year	The amount of amino acid supplemented	Influencing ammonia or performance
Liu et al. (2004)	The average bioavailability of MHA-FA relative to DLM was 88% on a molar basis	Performance
Pesti et al. (2003)	When PMN and Thr were mixed: A. Feed consumption and egg production were identical, B. BW and Egg Wt. improved, C. Egg sizes were in different, D. PMN-fed hens had better interior quality, E. Haugh units remained better in PMN. F. Egg specific gravity was slightly lower when PNM was fed, G. Purified Thr was used	Performance
Faria et al. (2002)	1. The Thr requirements to produce 1 g of egg mass were 8.76 and 9.44 mg in Exps. 1 and 2, respectively 2. Eight experimental diets: 0.53%, 0.50%, 0.48%, 0.45%, 0.42%, 0.40%, 0.37%, 0.35% of Thr. 3. Seven experimental diets: 0.58%, 0.53%, 0.50%, 0.48%, 0.45%, 0.43%, 0.40% of Thr	Performance
Elwinger and Wahlstrom (2001)	Met supplementation (the basal diet + 0.12% DL-Met) improved feed conversion rate and decreased the occurrence of peak injuries in the cloaca region	Performance
Coon and Zhang (1999)	1. 14% CP + Met + Lys + Iso + Val fed to layers was equal to layers fed 18% CP control diet 2. N loss in excreta was 15% less for layers fed 14% CP diets supplemented with amino acid compared to layers fed 18% CP 3. The MN layer Arg and Met ratios and Lys are higher whereas the TSAA, Iso, Try, Val are slightly lower than the NRC ration 4. The digestible requirement of Lys, Arg, Val and Thr for maximum egg mass production was 705 mg dig. Lys, 1070 mg dig. Arg, 731 mg dig. Val, and 560 mg dig. Thr	Performance
Farrell et al. (1998)	1. The concept of low protein diets in livestock production is attractive because they maximize the use of dietary protein and reduce nitrogen excretion 2. 119–170 g/kg feed	N excretion
Keshavarz and Jackson (1992)	1. Overall egg production and egg wt of birds fed the sequence of 14%, 13%, and 12% CP supplemented with Met, Lys and extra levels of Try and Iso or of the birds fed the sequence of 15%, 14%, 13% CP supplemented with Met and Lys were not different from those fed the positive control (20%, 16%, and 14% CP at 0–6, 6–12, 12–18 weeks of age) 2. Egg mass and body wt. were inferior	Performance

Table 8
Examples of amino acid levels influencing pullet performance or ammonia emissions from manure

Authors and year	The amount of amino acid supplemented	Influencing ammonia or performance
Song et al. (2003)	The availability of amino acids in CHOC is equal or superior to that in CC and that the available energy for poultry is higher in CHOC than in CC	Performance
Yaghobfar and Zahedifar (2003)	<ol style="list-style-type: none"> 1. The calculated EAAL from regression analysis caused an increase in the TAAA value in maize 2. The amounts of metabolic and EAAL excretion varied in voided excreta from unfed birds 3. These differences would be related to genetics, age, sex and environmental temperatures 	Performance
Halle (2001)	<ol style="list-style-type: none"> 1. A reduction in body weight was observed by feeding pullets a low-Met (0.3/0.27) and low-Lys (0.85–1.0/0.55–0.7%) diet in either the starter or grower phase 2. The highest reduction rate of the immature body weight (18 wk of age) affected egg production rate through 46 wk of age 	Performance
Shirley and Parsons (2000)	<ol style="list-style-type: none"> 1. Pressure processing of MBM decreases the digestibility of AA for poultry 2. Pressure processing of MBM to reduce potential BSE infectivity will decrease the nutritional value of MBM 	Performance
Keshavarz and Jackson (1992)	Satisfactory growth with considerable savings of protein and lysine intake can be obtained by using low-protein, amino acid-supplemented diets (lysine and methionine) during the growing period	Performance

Table 9
Examples of amino acid levels influencing turkey performance or ammonia emissions from manure

Authors and year	The amount of amino acid supplemented	Influencing ammonia or performance
Wylie et al. (2003)	<ol style="list-style-type: none"> 1. Dietary CP (from 300 to 180g/kg feed) was preferentially partitioned to feather rather than muscle growth in the male line in contrast to a traditional line of turkeys in which the growth of feathers and muscle were affected equally 2. When CP level was 180–260g/kg of feed, feather growth was maintained as much as possible at the expense of body growth and the amino acid concentrations of the ration was less than that required to maximized gain in large male line turkeys 	Performance
Waibel et al. (2000a)	<ol style="list-style-type: none"> 1. For turkeys fed 60% of NRC (1994) CP, BW gain was severely depressed 2. The combination of Thr, Ile, Val, Tyr and Arg at NRC (1994) levels resulted in nearly complete BW recovery at each age 	Performance
Waibel et al. (2000b)	<ol style="list-style-type: none"> 1. In low-CP diets compared to NRC (1994) containing Met and Lys at NRC (1994) requirements, supplemental Thr resulted in improved BW, 2. Whereas Thr, Ile, Val Trp and Arg at NRC (1994) levels returned BW, but not BMV to normal CP control levels 	Performance
Potter and Shelton (1988)	<ol style="list-style-type: none"> 1. The TSAA requirements of medium white turkeys during 8–12 and 12–16 week periods were about 0.93% and 0.75%, respectively 2. The protein requirement was about 30.3% at one day of age and decreased 0.61% and 0.78% units per week for males and females, respectively 	Performance
D'Mello and Emmans (1975)	<ol style="list-style-type: none"> 1. The Arg and Lys requirements for maximum growth of the 3-week-old turkeys (British United) were 1.175% Arg and 1.55% Lys 2. The Arg required to support a growth rate of about 20g/day and Arg was utilized for growth 	Performance
Warnick and Anderson (1973)	<ol style="list-style-type: none"> 1. Growth rate with the diets containing all EAA at the minimum requirement levels was only about 87% of that noted with the diet with a total of 14% EAA 2. When any one of the EAA was reduced from the higher to the minimum requirement levels, poult performance was reduced slightly 	Performance

trend was noted when chickens were supplemented with a carbohydrate complex, lactosucrose (0.15% of the diet) for 62 days (Terada et al., 1994). This study found that not only were cecal numbers of *Bifidobacteria* (per gram of wet feces) increased, but that lactosucrose supplementation reduced the numbers (per gram of wet feces) of lecithinase-positive clostridia including *C. perfringens*, *Bacteroidaceae* and *Staphylococci* spp.

Feeding oligosaccharides provides positive changes on the microbial ecology of the colon including increasing the number of desirable bacteria (e.g., *Bifidobacteria* and *Lactobacilli*) and decreasing the number of pathogenic or less desirable bacteria (Morris and Boeker, 1983; Tabor and Tabor, 1985). Chicken dietary trials (Terada et al., 1994) showed that lactosucrose supplementation (0.15% of diet) decreased cecal concentration ($\mu\text{g/g}$ wet cecal contents) of

Table 10
Main feed-enzyme producing microorganisms (Puchal and Mascarell, 1999)

Name of microorganism	Enzyme
<i>Aspergillus niger</i>	α -Amylase
<i>Aspergillus ficuum</i>	β -Glucanase
<i>Aspergillus candidus</i>	Cellulose
<i>Aspergillus sydowl</i>	Phytase
<i>Aspergillus oryzae</i>	α -Amylase, neut. protease
<i>Bacillus ficheniformis</i>	α -Amylase
<i>Bacillus subtilis</i>	Phytase
<i>Trichoderma viridae</i>	Xylanase, β -glucanase, neut. protease
<i>Saccharomyces cerevisiae</i>	α -Galactosidase
<i>Humicola insolens</i>	β -Glucanase

Table 11
Foregut viscosities (centipose units) determined in 21-day old broilers fed various cereals in combination with SBM (Bedford, 1995)

Cereal	Minimum	Maximum
Corn	1.5	4.5
Wheat	3	45
Triticale	4	50
Barley	6	225
Rye	70	>1000

ammonia (from 150 to 75), phenol (from 33 to 20) and *p*-cresol (from 91 to 60). These results also showed that lactosucrose decreases numbers of *C. perfringens* in chicken feces and due to these decreased numbers the lactosucrose is thought to be effective in decreasing amine production in the colon.

The S85 type strain of *Fibrobacter succinogenes*, a major ruminal fibrolytic species, was isolated 50 years ago from a bovine rumen and has been used since as a model for extensive studies. As expected, fibrolytic genes of strains of the genus *Fibrobacter* as well as the cellulase and xylanase activities are better conserved in closely related phylogenetic isolates (Bera-Maillet et al., 2004). Aro et al. (2003) reported the effect of deletion of the *Trichoderma reesei ace1* gene encoding the novel cellulose regulator ACEI (a type of *Trichoderma reesei*) that was isolated based on its ability to bind to and activate in vivo in *Saccharomyces cerevisiae* the promoter of the main cellulose gene, *cbh1*. Deletion of *ace1* resulted in an increase in the expression of all the main cellulose genes and two xylanase genes in sophorose- and cellulose-induced cultures, indicating that ACEI acts as a repressor of cellulose and xylase expression.

Unfortunately, published research examining the potential role of oligosaccharides, and their relationship with enzymes on improving the microbial ecology of the chicken GIT are limited. However, it is possible that oligosaccharides could improve fermentation in the chicken GIT with enzyme supplementation. In order to determine this, however, future research will be required. Some areas of research are clearly needed including: dose–response functions; discontinuous dosage effects; specific disease influences; other pronutrients (e.g., exogenous enzymes,

microbials, etc.) and/or limiting nutrients; and comparisons with other oligosaccharides (Rosen, 2005).

4.4. Reducing N excretion and ammonia emission in poultry manure and litter

4.4.1. Reducing N excretion

Significant reductions in N emission can be achieved by reducing dietary crude protein levels and balancing digestible amino acid profile requirements with synthetic amino acids (Ferket et al., 2002). Dietary manipulation greatly influences excretion of N in feces and urine. Amino acid digestibility of feedstuffs for poultry varies greatly (Parsons, 1992) and the amount of N excreted into excreta may be reduced by feeding ingredients that have highly digestible amino acids. Endogenous N and amino acid losses are increased by increasing dietary protein and fiber levels. Reducing dietary CP levels and supplementing with synthetic amino acids have reduced manure N excretion in pigs from 25% to 30% (Lenis, 1993; Hartung and Phillips, 1994).

Layer diets with reduced dietary protein contents significantly and linearly decreased fecal N content to about 50% of the intake. Considering the N fecal/intake ratio, the layers fed 150 g/kg CP showed improved N utilization at each sampling time (Meluzgi et al., 2001). Lippens et al. (2002) concluded that a retardation of the early growth of fast growing broiler chickens can, in certain circumstances (after the period of restrictions all birds were fed *ad libitum*), reduce mortality and increase performance and N retention. One study (Sklan and Noy, 2005) examined optimal lysine and sulfur amino acid supplementation in the first week posthatch in broilers and the relationships between essential amino acids and dietary crude protein on performance at 7 days. Increasing essential amino acids in a constant ratio to crude protein enhanced performance during the 7 days posthatch.

Although it is possible to reduce dietary CP levels by 3–4% for broilers and layers, there are biological limits to the amount of dietary protein that can be replaced with synthetic amino acids (Patterson, 2002). Work summarized by NRC (1994) suggests that calculated digestible amino acid requirements are 8–10% lower than requirements for total amino acids.

In the global N cycle, bacterial denitrification is recognized as the only quantitatively important process that converts fixed N to atmospheric N gas, N₂, thereby influencing many aspects of ecosystem function and global biogeochemistry (Thamdrup and Dalsgaard, 2002). By shunting N directly from ammonium to N₂, anaerobic ammonium oxidation promotes the removal of fixed N in the oceans. This process can explain ammonium deficiencies in anoxic waters and sediments, and it may contribute significantly to oceanic N budgets.

Dong et al. (2002) suggested that in addition to anaerobic bacteria, which possess that complete denitrification pathway for N₂ formation in the estuarine sediments, there may be two other groups of bacteria: nitrite denitrifiers,

which reduce nitrite to N_2 via N_2O , and obligate nitrite-denitrifying bacteria, which reduce nitrite to N_2O as the end product. Intracellular nitrate cannot be directly utilized by sediment denitrification, and the probable fate of the intracellular nitrate is hypothesized to be from a dissimilatory reduction to ammonium (Sayama, 2001). Presence of nitrate-accumulating sulfur bacteria, therefore, may lower nature's self-purification capacity (denitrification) and exacerbate eutrophication in shallow coastal marine environments.

The taxis response of *Rhodobacter sphaeroides* 2.4.1 and 2.4.3, *Rhodopseudomonas palustris*, and *Agrobacterium tumefaciens* to nitrate and nitrite was evaluated by observing the macroscopic behavior of cells suspended in soft agar and incubated under various conditions (Lee et al., 2002). A taxis response to nitrogen oxides was observed in *R. palustris* and *A. tumefaciens*. *R. palustris* exhibited a taxis response to nitrite but not to nitrate, while *A. tumefaciens* exhibited a response to both compounds. Cebren et al. (2003) reported that degradation of organic matter by heterotrophic bacteria and subsequent oxygen depletion occurs immediately down stream of the effluent outlet, whereas nitrifying bacteria apparently need to build up a significant biomass before ammonium oxidation significantly depletes the oxygen. The Acheres wastewater treatment plant, located just down stream of Paris, discharges its effluents into the lower Seine River. These effluents were found to contain large numbers of heterotrophic bacteria, organic matter, and ammonium and are a source of nitrifying bacteria.

The effects of $ZnSO_4$ and ZnO supplementation of broiler diets on growth performance and loss of uric acid N and total N from manure was studied by Kim and Patterson (2004). In their study, Zn treatment (1500 ppm Zn as $ZnSO_4$) significantly reduced N loss in poultry manure without affecting weight gain, feed consumption and feed efficiency of the chicken, ZnO (1500 ppm Zn as ZnO) was found to be a better Zn source for preventing nitrogen loss to the atmosphere without causing detrimental effects on growth performance.

4.4.2. Reducing ammonia emission

By reducing N excretion in urine as urea, which is the primary precursor for ammonia volatilization, and shifting the N excretion into feces, which is primarily in the form of bacterial protein, ammonia volatilization is reduced (Sutton et al., 1998). Using precise nutrition techniques and feeding low-protein diets with supplemental amino acids can reduce ammonia emissions. Reducing protein levels by 1 percentage point resulted in a 10% reduction in ammonia emissions (Van der Peet-Schwering et al., 1997). Den Brok et al. (1997) demonstrated that benzoic acid in feed decreased ammonia emissions by 40% while increasing the feed/gain in market pigs from 2.93 to 2.83.

Poly (r-D-glutamic acid) (PGA)-producing strains of *Bacillus* species were investigated to determine their ability to contribute to reducing the amount of ammonium nitrogen in liquid manures and their ability to convert some of

the ammonium into this poly-amino acid as transient depot for nitrogen (Potter et al., 2001). These problems are mainly due to the high content of ammonia nitrogen. *Bacillus licheniformis* ATCC9945 and *Bacillus subtilis* were able to grow in liquid manure and to produce PGA in the presence of sodium glucose. However, growth and nitrite production from ammonium did not occur at pH values below 7. Growth on urea occurred at pH values in the range 4–7.5, but ceased when urea hydrolysis was not complete, even though ammonia, released during urea hydrolysis, remained in the medium (Burton and Prosser, 2001). Direct competitive interaction takes place between algae and ammonia-oxidizing bacteria (AOB), and benthic algae have been shown to be superior competitors because they have higher N uptake rates and grow faster than AOB (Risgaard-Petersen et al., 2004). Avrahami and Conrad (2003) concluded that ammonia oxidizer populations are influenced by temperature. In addition, they confirmed that N fertilizer also influences the community structure of ammonia oxidizers. *Nitrosospira* cluster 1 was absent in one soil treated with less fertilizer, while *Nitrosospira* cluster 9 was only found in the sample given less fertilizer.

5. Feed formulation with digestible amino acids and enzyme complexes

Feed formulation based on digestible amino acids and enzyme supplementation improves daily gain and feed conversion of growing birds more than total amino acid usage in feed formulation of diets. Use of ileal digestibility values in diet formulation improved the accuracy of formulation and prediction of animal performance (Williams, 1995). He indicated that ileal digestibility measurements represent a good compromise between the requirement for a rapid, economical determination of digestibility of amino acids in feed and measurement of availability of amino acids for tissue synthesis.

Formulation of poultry diets on a digestible amino acid basis and enzyme supplementation should be superior to formulation on a total amino acid basis due to differences in amino acid digestibilities among ingredients, especially when ingredients have NPS. The poultry industry, however, has been slow in changing from total amino acid formulation to digestible amino acid formulation. This has been due to the lack of a good data base of amino acid digestibilities and little or no data on digestible amino acid requirements (Parsons, 1999).

Research results have varied on the economic benefits of using digestible amino acids to formulate diets. Rostango et al. (1995) showed economic benefits of formulating diets with digestible rather than total amino acids, but no benefit was seen with diets containing highly digestible amino acids. There is a marked impact on excreta volume and composition by reducing the DM content of the intestinal tract with supplemental feed enzymes. When broilers were offered a wheat or wheat/barley based diet with or without enzymes, fresh excreta weights were reduced 17–28%, and

the DM out was reduced 12–15% by the use of enzymes (Wyatt and Harker, 1995). No consistent advantage was shown in using digestible amino acids in diets containing high and low quality meat and bone meals fed to broilers (Wang and Parsons, 1998). They concluded that the method used to measure true amino acid digestibility may overestimate amino acid bioavailability.

Ferguson et al. (1998) demonstrated with broilers that litter N could be reduced more than 16% when dietary CP was reduced by 2%, while maintaining similar levels of dietary amino acids. Turkeys had similar limitations, suggesting that the amino acid requirements of these birds are not fully understood and meeting CP requirements are therefore critical and necessary to realize full performance (Patterson, 2002).

Fewer amino acids are excreted in the feces when the dietary amino acid composition closely matches bird requirements for maintenance, growth and production of meat and eggs. Variability in amino acid digestibility among different animal proteins and samples of the some meals can be very large. Parsons (1999) found that the three most deficient amino acids were the same for both animal meals, namely cystine, tryptophan and threonine. Cystine and tryptophan were much more deficient than the others, and cystine was more limiting than tryptophan in poultry byproduct meal but tryptophan was first limiting in meat and bone meal. He also reported that the type of processing system and processing temperature significantly affected amino acid digestibility of feather meal, meat and bone meal and poultry byproduct meal. The most striking effects were observed for meat and bone meal. In SBM-based diets, the concentration of lysine which maximized body weight gain (BWG) of broiler chickens was less than or equal to the lysine concentration reached by the proportions of corn and SBM needed to meet dietary CP constraints (Sterling et al., 2005).

Another approach suggested by Baker and Han (1994) is to deliver an “ideal protein” whereby the protein portion of the diet meets bird requirements for each amino acid with no excess or deficiencies. Wijtten et al. (2004) concluded that the weight gain and feed conversion efficiency of male broilers respond to higher dietary ideal protein levels than would be expected from single lysine requirement studies in the literature. Table 12 illustrates the lysine and other amino acid requirements estimated for turkeys, chicks and pigs. Experiments conducted to investigate effects of dietary manipulations to improve growth performance and the whole-body composition of broiler chicks fed low-protein diets supplemented with crystalline amino acids (Bregendahl et al., 2002) failed to support growth performance equal to that of high-protein control diets. In order to benefit from increased digestibility values due to the use of enzymes, feed formulation must be based on digestible, not apparent amino acid values (Puchal and Mascarell, 1999). Total protein levels should also be reduced accordingly or changed to use digestible protein in order to maximize the potential of enzyme supplementation. Enzymes are added

Table 12
Estimated ideal protein ratios for starting hen turkeys (chick and pig ratios for comparison)^a (Firman and Boling, 1997)

Amino acid ^b	Chick	Pig
Lysine	100	100
SAA	72	60
Threonine	67	65
Valine	77	68
Arginine	105	–
Histidine	31	32
Isoleucine	67	60
Leucine	100	111
Phe + Tyr	105	65
Tryptophan	16	18

^a Expressed as a percentage of the lysine requirement.

^b Estimates based on previous research with low protein turkey diets and the estimates for other species.

to rations of poultry and swine to enhance digestion and absorption of nutrients (Inborr, 1990; Swick, 1991) and to reduce pollution (Swick, 1991).

No single recommendation for feed formulation can be expected to be applicable to broilers, layers, pullets and turkeys under all circumstances. A major difficulty confronting feed formulators is the conversion of optimum intakes and amount of amino acids with enzyme supplementation into dietary concentrations. This difficulty would be resolved if feed intake by poultry could be predicted accurately. Predicting the primary-, secondary-, tertiary- and other limiting amino acids under varying environmental and nutritional circumstances would be a prerequisite in calculating optimum dietary concentrations of nutrients in feeds for poultry.

6. Conclusions

Supplementation with synthetic amino acids has been shown to reduce N excretion by up to 40% (Cromwell and Coffey, 1995; Creswell and Swick, 2001) and up to 50% in layer diets (Meluzzi et al., 2001). Enzymes destroy anti-nutritional factors or increase the digestibility of indigestible nutrient factors. According to Kerr (1995), supplementing synthetic amino acids to poultry and swine diets could result in an 8.5% reduction in N excretion for every 1% reduction in dietary CP. Reductions in N excretion result in decreased ammonia emissions of growing-finishing swine (Sutton et al., 1998). Digestibility may be measured in excreta, which allows nutrients to pass through the large intestine, or from the distal part of the ileum. Both methods allow for reductions in amino acid and protein levels in feed formulations. Lowering N levels in feed formulations will result in decreased N excretion and ammonia emissions from poultry manure.

Poultry diets containing barley, rye, wheat, triticale and oats are commonly used, but inclusion of these ingredients in their raw state increase intestinal viscosity. Enzyme supplementation may be utilized with these feed ingredients to improve the digestibility of the NSP portions of these

feedstuffs and thereby poultry production. The most common enzymes utilized are carbohydrases (beta-glucanase, xylanase, hemicellulase, pentinases, alpha-galactosidase, and inulinase) and phytase, but proteases and lipases are also used to increase digestibility of feeds.

Recent research in poultry production has focused on the use of oligosaccharides. Oligosaccharides are made up of 3–10 monosaccharide units and are known to play a role in fermentation in the large intestine. Further research is needed into the use of enzyme supplementation to improve the utilization of oligosaccharides in poultry diets.

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