INTRODUCTION

Enzymes are proteins with a complex 3-dimensional structure that accelerate chemical processes. They exert their catalytic effect under specific environmental conditions such as pH, temperature, and humidity and have substrate specificities. Enzymes make possible controlled sequences of chemical reactions in biological systems but are not spent during the reaction; they catalyze and thus return to their original state when the reaction is complete, allowing for a very small concentration of enzymes needed compared with the substrate concentration. For an enzymatic reaction to occur in the intestinal tract, the right conditions have to be present. That is, the substrate must be in a chemical and physical form that the enzyme can work.

An often overlooked basic concept is that enzymes will only work if a suitable substrate is available at the correct chemical and environmental conditions. Very few publications on the use of enzymes in poultry feeds include substrate amounts and even less directly document the impact of enzyme use on substrate degradation. For example, 37 papers were published in Poultry Science between January 2010 and December 2013 that had the word phytase in their title, abstract, or both. Of these, one was an erratum, one was an in vitro assay, one was a review, one did not add a phytase, and one was a meta-analysis. Of the 32 remaining papers published, 14 (44%) did not analyze the enzyme in the diets as fed, 20 (63%) did not analyze the substrate in the diet, and 26 (81%) did not analyze the remaining substrate in either intestinal content or excreta. Only papers by Svihus et al. (2010), Woyengo et al. (2010), Casteel et al. (2011), Delezie et al. (2012), Cowieson et al. (2013), and Olukosi et al. (2013) analyzed for the substrate remaining in the intestinal content or excreta. Published work on phytase use in poultry diets increased dramatically starting in the mid 1980s, and thousands of papers have been published in numerous journals on this subject since then (Narcy and Létourneau-Montminy, 2012). It is astounding, given the number of research studies done yearly on phytase and given that the analytical methods exists and are readily available for both the enzyme phytase and the substrate phytate, that we still have such a high proportion of papers that do not even analyze for the enzyme or substrate in the diet.

Possible Substrates for Exogenous Enzymes

Why is it important to understand substrates if we are to optimize exogenous enzyme efficacy?1

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ABSTRACT The use of exogenous enzymes in feeds for poultry has increased dramatically between 1990 and 2013. Today, the use of enzymes is broad, going beyond phytases and β-glucanases and xylanases to include other carbohydrases and proteases as well as lipases. The number of scientific articles and publications related to enzymes in feed clearly shows that this has been an area of intense and broad interest for scientists and nutritionists. However, knowledge of the different substrates available in the feed and how these substrates change depending on feed ingredient selection has not received the same level of attention. Understanding substrates is key to better developing and implementing exogenous enzymes. Of importance today is to potentiate endogenous digestive capabilities and use exogenous enzymes to optimize nutrient digestion and use. Our aim with this symposium was to call attention to the importance of having a more in-depth knowledge about substrates and to fill the large gaps in our current understanding of the digestive processes in poultry.

Key words: xylanase, protease, amylase, phytate phosphorus, amino acid

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An often overlooked basic concept is that enzymes will only work if a suitable substrate is available at the correct chemical and environmental conditions. Very few publications on the use of enzymes in poultry feeds include substrate amounts and even less directly document the impact of enzyme use on substrate degradation. For example, 37 papers were published in Poultry Science between January 2010 and December 2013 that had the word phytase in their title, abstract, or both. Of these, one was an erratum, one was an in vitro assay, one was a review, one did not add a phytase, and one was a meta-analysis. Of the 32 remaining papers published, 14 (44%) did not analyze the enzyme in the diets as fed, 20 (63%) did not analyze the substrate in the diet, and 26 (81%) did not analyze the remaining substrate in either intestinal content or excreta. Only papers by Svihus et al. (2010), Woyengo et al. (2010), Casteel et al. (2011), Delezie et al. (2012), Cowieson et al. (2013), and Olukosi et al. (2013) analyzed for the substrate remaining in the intestinal content or excreta. Published work on phytase use in poultry diets increased dramatically starting in the mid 1980s, and thousands of papers have been published in numerous journals on this subject since then (Narcy and Létourneau-Montminy, 2012). It is astounding, given the number of research studies done yearly on phytase and given that the analytical methods exists and are readily available for both the enzyme phytase and the substrate phytate, that we still have such a high proportion of papers that do not even analyze for the enzyme or substrate in the diet.

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Extensive work has been published using enzymes, but overall there is very poor knowledge of substrates, how substrates may be different between ingredients, and how substrates may change as they move through the gastrointestinal tract (GIT). The results of a survey sent out to attendees of the 2013 Poultry Science Association Annual Meeting support this. Out of 201 people that replied that they had attended the symposium, 40% replied that they had limited knowledge on the different substrates and consider that the literature on substrates was scarce (Figure 1).

Phytate Phosphorus, Phytic Acid, and Phytic Phosphorus

The enzyme phytase has a specific substrate, phytic acid and its chelated forms phytin and phytate. Phytic acid interacts with soluble proteins at low pH, forming inositol 6 phosphate-protein aggregates that decrease the digestibility of the protein bound to phytic acid as well as decrease the ability of phytase to remove phosphate groups and make them usable by the animal (Yu et al., 2012). Soluble proteins bind with inositol 6 phosphate through electrostatic charges at low pH or through a salt bridge at high pH (Graf, 1986). The binding of inositol 6 phosphate with protein can reduce the availability of protein and changes the ionization of the complex, thus potentiating the ability of phytin to complex with minerals (Graf, 1986). This can change the efficacy of different phytases to hydrolyze phytic acid and release phosphate groups.

In its acidic form, phytic acid has the capacity to chelate multivalent mineral cations (Taylor, 1965; Nelson and Kirby, 1987; Maenz et al., 1999). The solubility of the phytic acid metal complexes is pH dependent. Most phytate-mineral complexes are soluble at low pH (less than 3.5) and solubility decreasing above a pH of 3.5, and maximal insolubility occurs at a pH of 7 (Champagne, 1988). The approximate pH of the intestine, where absorption of metal ions takes place, coincides with the pH at which these complexes precipitate and thus these precipitated phytate-mineral complexes are poorly accessible for hydrolysis or absorption in the intestine (Champagne, 1988; Angel et al., 2002; Tamim and Angel, 2003; Tamim et al., 2004). In the GIT of broilers the pH changes as the digesta moves distally from the crop (Angel et al., 2013). In the proventriculus and gizzard, the pH are usually below 3.5, but in the proximal duodenum the pH increases to 5 and by the end of the duodenum the pH in a 22 d of broiler is 6 (Angel et al., 2013). As the pH increases above 4, after the gizzard in the intestinal tract of broilers, the phytic acid chelates with cations, resulting in a precipitated substrate that decreases the ability of the phytase to release phosphates both in vitro and in vivo (Tamim and Angel, 2003; Tamim et al., 2004). Location of phytin within the seed and its chemical associations with other nutrients influence its availability as well as readiness to interact with other components of the digesta as it moves through the changing chemical environments present in the GIT (O’Dell and De Boland, 1976; Cheryan, 1980; Reddy et al., 1982).

Different ingredients have varying amounts of phytate phosphorus (Nelson et al., 1968; Tahir et al., 2012) and different location of phytate phosphorus within the plant (O’Dell and De Boland, 1976; Erdman, 1979; Reddy et al., 1982; Ravindran et al., 1995). The actual number of analysis for phytate phosphorus and total phosphorus in different ingredients is surprisingly small (G. Pesti and R. Alhotan, University of Georgia, Athens, unpublished data) even for corn and soybean meal. We still lack an understanding of how the chemical and physical characteristics of phytate in different ingredients affect their breakdown in the digesta by phytases.

Fiber and Nonstarch Polysaccharides

Fiber structure varies among different plants and within the same plant species, different tissues, or even the same tissue at different developmental stages (Santiago et al., 2013). This can partially explain why knowledge about NSP appears to be the lowest among the substrates covered in this symposium with only 31% of attendees responding that they have good knowledge of NSP.

Even within dietary fiber (DF) there are at least 2 definitions: physiologic and chemical. The physiologic definition of DF is the dietary components resistant to degradation by vertebrate digestive enzymes and the chemical definition is the sum of NSP and lignin (The-ander et al., 1994). Based on the different definitions we can conclude that analytical methods and results will...

![Figure 1](image.png)

Figure 1. Survey responses on how much attendees know about the different substrates. The survey had 201 responses.
also diverge. Bach Knudsen (2001) also stated that “although modern analytical techniques enable quantification and characterization of the physical and chemical properties of DF in the plant materials, understanding of the nutritional significance of these measurements is far from complete.”

**Starch**

Starch can constitute up to 50% (DM basis) of a poultry diet, making it the most abundant single component. Starch as a substrate for exogenous enzymes has been poorly explored in part due to the relatively high starch digestibility in most poultry diets. However, Svilhus (2014) suggested that more information is needed, especially in older birds that have higher feed consumption. Work by Svilhus (2011a) showed a negative correlation between feed intake and starch digestibility. This impact seen in older birds suggests that exogenous amylases added to the feed may improve starch digestibility in the finisher and withdrawal phases of broiler production. Sorbara et al. (2009) also emphasized the importance of testing different enzymes for different phases because different ingredients and the proportion of them in the diet change with concomitant changes in substrate concentrations. As energy requirements increase with bird age, the proportion of grain increases compared with starter diets. Therefore, there is a potential for exogenous amylases in the latter feed phases where feed consumption as well as starch content of the diet increase. Starch is the diet component that by far provides the most energy to the bird, and any increase in its digestibility will improve performance and decrease feed cost.

**Protein and Amino Acids**

Protein and amino acid nutrition in poultry has been one of the most prominent areas in poultry nutrition in the last decade. The most effective methods to determine protein and amino acid digestibility or disappearance are debatable. Whether the methods to determine protein and amino acid digestibility are also the best methods to determine the effectiveness of exogenous proteases is still not clear. Proteases are expected to be most beneficial with poor quality proteins or large peptides. Today, proteases are evaluated by apparent, standardized, or true ileal amino acid digestibility. Nevertheless, the development of an assay more suitable to evaluate a protease should be considered in the future.

**Importance of the Gastrointestinal Environment and Passage Rate to Digestion**

Enzyme and substrate information alone does not provide the complete digestion picture. It is important to understand how the chemical environment changes along the length of the GIT, how nutrients in ingredi-
a conservation of enzymes, a fact that minimizes nutritional and energetic costs to the bird and highlights the importance of retrograde movement in the digestive capacity of poultry.

To optimize nutrient digestibility, we must understand the effect of diet characteristics (Svihus et al., 2004, 2010; Amerah et al., 2007; Svihus, 2011a,b) as well as light programs (Classen et al., 2010; Svihus et al., 2013) on feed passage through the different sections of the GIT. Zyła et al. (2004) hypothesized that the limitations to complete dephosphorylation of phytate in poultry were intestinal pH and passage rate. Changing passage rate to allow more time for endogenous and exogenous enzymes to act upon their substrates in an environment that optimizes their activity would likely improve digestibility. Svihus et al. (2013) used intermittent feeding programs to increase crop fill and residence time and reported no interaction between feeding method (intermittent vs. continuous) and phytase use in the diet. Previously, Svihus et al. (2010) had reported increased efficacy of phytase as residence time in the crop increased through intermittent feeding programs. Quansah et al. (2010) reported no phytase efficacy change in an experiment where intermittent lighting and fumaric acid were used.

Modifying intestinal pH in the different segments of the GIT through the use of fiber (Jimenez-Moreno et al., 2009), particle size (Gabriel et al., 2003) or acids (Rynsburger, 2009; Quansah et al., 2010) can help potentially to improve nutrient digestibility by providing a chemical environment that is closer to that which optimizes the activity of exogenous enzymes. The use of fiber or large particle size diet components (Svihus et al., 2004; Svihus, 2011b) to change gizzard pH clearly can have an impact on intestinal health and on protein digestion and possibly improve phytase efficacy. Intestinal pH changes with age (Hurwitz et al., 1972; Rynsburger, 2009; Angel et al., 2013) and variability between animal is high (Angel et al., 2013). In the broiler at young ages when pH in the proventriculus and gizzard is higher (Rynsburger, 2009; Angel et al., 2010; Angel et al., 2013) than after 15 to 20 d of age the potential is there to decrease pH through diet or water use of acids (Rynsburger, 2009; Quansah et al., 2010).

Optimizing the conditions (residence time, digesta physical, and chemical conditions) in the digestive tract to optimize substrate breakdown by endogenous or exogenous enzymes will be a fundamental part of optimizing diet digestibility.

CONCLUSIONS

Having as much information and understanding of the substrate and its interactions with other components of the diet as well as with the intestinal tract and microbiota is key if we are to optimize diet digestibility and use of enzymes and new technologies. Clearly there are limits in what we know about certain substrates that are poorly utilized in poultry diets and about what limits the digestion and absorption of others such as proteins and their constituent amino acids. There is also limited understanding by poultry professionals, as shown from the survey responses, about not only the substrates themselves but also how to best improve their utilization by poultry.

REFERENCES


