ADVANCES IN THE USE OF ENZYMES AND DIRECT-FED MICROBIALS IN POULTRY NUTRITION

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Introduction

Dealing with variability is a key factor determining the success of companies in animal agriculture, including poultry meat and eggs production. Factors like ingredient prices and currency exchange rates have high levels of volatility and a significant impact on profitability. Internal factors such as the variability in feed efficiency and outputs of animal production operations, as well as the quality of the feed ingredients, also play an important role to determine the economic results of poultry companies. To stay viable, modern companies are forced to adopt processes to manage those sources of risk. Reducing variability in animal production not only allows prediction of economic results, but also gives improvements in efficiency by reducing the gap between potential performance of current breeds, and what they can actually achieve in commercial production. Feed additives play a role in improving efficiency and reducing variability. Enzymes in particular, have the potential to complement the endogenous enzyme machinery of the animal to allow digestion and absorption of nutrients that otherwise would not be absorbed. Those effects, however, are not always as consistent or predictable as nutritionists would expect, partially because the avian gut systems involve interactions with the gut microbiota and the gut mucosa, which are dynamic and depend on physiological and environmental factors. Greater digestion is not always translated into greater absorption, growth or feed efficiency. This presentation reviews recent research, which suggests complementarity between Direct Fed Microbials (DFMs) and exogenous enzymes for the absorption of nutrients and performance of birds with different health status, and discusses some potential modes of action.

The roles of enzymes and DFMs

Exogenous enzymes have been attributed a number of gut health related benefits, not always with enough evidence supporting those claims. Xylanases have been shown to have pre-biotic effects in different species including poultry, which are exemplified by reductions of *Campylobacter jejuni* in chickens fed diets containing xylanase (Fernandez, 2000). Indeed, there have been a number of studies on probiotic effects of xylo-oligosaccharides and arabinoxylo-oligosaccharides in humans, rats, and in-vitro digestive tract simulations, which demonstrate a variety of prebiotic mechanisms with possible beneficial effects in the host (Broekaert et al., 2011). Those mechanisms include selective stimulation of beneficial bacteria and production of short-chain fatty acids (SCFA). The link between the solubilisation of xylo-oligomers and the effects of such oligomers in the gut appears obvious, but differences in the activity of xylanase enzymes might create differences in their ability to produce those end-products.

Improvements in the digestibility of nutrients due to exogenous enzymes may have direct influence on enteric disease. Increased undigested protein appears to be a predisposing factor for dysbacteriosis, in particular related to necrotic enteritis (Dahiya et al., 2007). It follows that enzymes which increase the digestion and absorption of protein in the higher portion of the digestive tract can potentially reduce the growth of pathogenic bacteria in jejunum, ileum and large intestine. Nonetheless, it is also possible that sub-clinical gut health challenges, which are normally present in commercial production, may reduce the ability of exogenous enzymes to translate the benefits of increased digestion of substrates into increased absorption of nutrients. Recent work from Teeter et al. (2011) aimed to quantify the energy losses due to mal-absorption and increased maintenance requirements in mild coccidia infections in broilers. From their work, it was clear that mal-absorption accounts for a significant proportion of those losses. It appears then plausible that an important factor explaining variable responses of some groups of exogenous enzymes, carbohydrases and proteases in particular, in commercial poultry production is the presence of sub-clinical enteric disease.

Exogenous enzymes have some practical advantages as additives for poultry nutrition, such as the ability to target substrate and hydrolyze it quickly, the fact that their activity can be measured and standardised, and their functionality can be engineered to work in the animal intestine after pelleting. DFMs, although lack the specificity that purified exogenous enzymes have, do have the advantage of being live organisms and as such, have the ability to reproduce and adapt to the environment and the substrates present for growth. Additionally, they can produce effects on immuno-modulation and modulation of bacterial communities (Lee et al., 2010), which can promote a healthy intestine. Combining those advantages may create an adequate environment in the bird's intestine to maximize absorption and efficiency of nutrient utilization, and reduce variation in individual bird performance.

Study 1. Nutrient Digestibility (Romero et al., 2013)

Two studies with 11-d-old and 21-d-old Ross-308 male broilers evaluated the digestibility of nutrients in response to dietary combinations of xylanase, amylase, protease and a *B. subtilis* DFM. Day old chicks were administered a live coccidiosis vaccine and assigned to one of six dietary treatments with eight replicate cages and eight birds per cage. Four chickens per cage were selected at 11 d and 21 d for collection of ileal digesta samples. A 3x2 factorial arrangement was used. Three enzyme levels were: 1) no enzyme, 2) xylanase from *T. reesei* and amylase from *B. licheniformis* (XA), or 3) XA plus protease from *B. subtilis* (XAP). Two levels of DFM were: 1) no DFM, or 2) a combination of spores from three defined strains of *B. subtilis* (DFM, 7.5 x 10^4 CFU/g of feed). Diets contained corn, soybean meal, corn DDGS, and wheat middlings. Apparent ileal digestibility of energy and protein were measured at both 11 and 21 d using TiO_2 (0.3%) as marker. Ileal digestibility of fat and starch, and AME_n (total collection) were evaluated only at 21 d.

At 11 d, a main effect of enzyme was present for ileal digestible energy (IDE), where XA and XAP increased IDE by 118 and 128 kcal/kg DM compared to treatments without enzymes. No effects on protein digestibility and no interactions were detected at 11 d. At 21 d, main effects of enzymes and DFM were detected on IDE and AME_n with increments on digestibility for both enzymes and DFM versus unsupplemented diets, with no interactions present. Ileal protein and fat digestibility was affected only by enzymes at 21 d ($P<0.05$), whereas starch digestibility was affected by DFMs, enzymes, and exhibited an interaction ($P<0.05$). Interestingly, the DFM + XAP treatment increased IDE by 152 kcal/kg DM, but only 110 kcal/kg DM were explained by increments in the digestibility of starch, fat and protein, suggesting an effect on fibre disappearance, which was subsequently confirmed by evaluation of NSP digestibility.

This is one of the first studies that demonstrate complementary effects of enzymes and DFM on nutrient digestibility in broilers. Evidently age, and potentially the levels of maturity of the gastro intestinal tract and microbial communities, and the ingredients in the diet may be factors that influence such complementarity. Interestingly, this study suggested that these DFMs and enzymes may act together to increase the solubilisation of fibre, particularly in older birds. However, even in younger birds, DFMs have the potential to increase starch digestibility. Overall, it appears that DFMs may help to increase the robustness of the enzyme response on energy digestibility, part of which may be explained by increase solubilisation of dietary fibre.

Study 2. Growth Performance (Walsh et al., 2013)

A 42 d performance trial using 792 Ross 308 chicks was conducted to assess the effects of exogenous xylanase and amylase carbohydrases and protease in combination with a *B. subtilis* DFM on the growth and feed efficiency of broiler chickens. Day old chicks were administered a live coccidiosis vaccine via drinking water and assigned to one of six treatments with six replicate pens in a randomized block design. A 3x2 factorial arrangement of treatments was used. Three enzyme levels were: no enzyme, xylanase from *Trichoderma reesei* and amylase from *Bacillus licheniformis* (XA), or XA plus a protease from *B. subtilis* (XAP).Two levels of DFM were: no DFM, or a combination of spores from three strains of *B. subtilis* applied at 7.5 x 10^4 CFU/g of feed. Diets were based on corn, soybean meal, corn DDGS (7%), and wheat middlings (7-10%). Body weight (BW) gain, feed conversion ratio (FCR) and feed intake (FI) were measured weekly until 42 d.

An interaction between enzymes and DFM was evident for BW gain to 42 d (P<0.05). All the treatments containing DFMs had greater BW gain compared to the negative control (NC) at 42 d. XAP alone increased BW gain compared to the NC diet (P<0.05), but XA did not. The DFM exhibited an increase in BW gain of chickens from wk 1 onwards versus treatments without DFM (144 vs. 131 g), whereas XAP increased BW gain compared to the NC only after 28 d. DFM increased feed intake until 28 d, while neither XA nor XAP exhibited main effects on feed intake. Nonetheless, both XA and XAP reduced FCR from 28 d (P<0.05), and only XAP reduced FCR from 0 to 42 d as a main effect (P<0.05). DFM did not affect FCR and no interactions on FCR were present.

DFMs increased feed intake and growth of young chickens, presumably due to effects on the gut health status, whereas enzymes increased feed efficiency, particularly in older broilers. Similar to what the previous study suggested, the role of DFMs and enzymes appeared to be influenced by age. It can be hypothesised that DFMs may have a greater effect during the early life of the chickens when their immune system is still naïve and bacterial communities are not fully established, whereas effects of enzymes on nutrient digestion and absorption can be fully captured in older birds with a more mature gastro intestinal tract and greater levels of feed intake.

Study 3. Performance under enteric challenge (Mathis et al., 2013)

This study assessed the feeding of a *Bacillus* DFM and / or different feed enzymes on performance, Necrotic Enteritis (NE) lesion scores, and mortality of broilers subjected to a *Clostridium perfringens* (CP) challenge. Fifty Cobb X Cobb 500 male coccidia vaccinated chicks were initially placed in pens. Bird weights and feed consumption were measured on d 12, 23, 35, and 42. A commercial grade diet with a three phase program was formulated and used as the basal, which was incorporated into the following treatments: no enzyme, Amylase from *Bacillus licheniformis* (A), Protease from *Bacillus subtilis* (P), and a combination of xylanase from *Trichoderma reesei*, A, and P (XAP; Danisco Animal Nutrition). The treatments were repeated with the addition of a combination of spores from three defined strains of *B. subtilis* (DFM) and all treatments were CP challenged except for one treatment with no enzymes or DFM. The DFM was applied at 7.5x10^4 CFU/g feed. On d 20, 21, and 22 all CP challenged birds were dosed with *C. perfringens* at 1.0 X $10^{8\text{-}9}$. On d 23, three pre-selected birds were examined for the degree of NE lesions.

A moderate NE infection developed with 11 % NE mortality in the no enzyme, no DFM, CP challenged group. All DFM treatments reduced the clinical effects of the *C. perfringens* with significantly lowering NE mortality and improving performance on d 23, 35, and 42 compared to all non-DFM, CP challenged treatment fed birds. The XAP without DFM fed birds had significantly reduced % NE mortality compared to the no enzyme, no DFM, CP challenged treatment. On d 42, the FCR and BWG of birds fed the XAP diets with and without DFM were better than the CP challenged birds fed no enzymes. The birds fed protease non-DFM diets also had better performance than CP infected birds with no enzyme. The combination of XAP and DFM in a CP challenge had the best performance, which was comparable to that of non-challenged birds. The reduction in NE with DFMs was aided by the feed enzymes singularly or more effectively with a combination of enzymes.

In this enteric challenge model, the role of the DFMs on gut health was highlighted. Even though different enzymes can have a significant improvement on performance under this type of challenge, it appears that their potential is limited. In these conditions, the role of the DFMs, and potentially other additives improving gut health, may be linked to reducing the negative the negative effects of the disease on appetite and absorption, which are necessary conditions to capture any potential improvement of substrate hydrolysis due to exogenous enzymes.

Mechanisms of action

Research on mechanisms of action explaining potential complementarity between DFMs and enzymes has been initiated recently. Murugesan (2013), performed a series of small experiments to study mechanisms of action of DFMs and enzymes in broiler chickens and laying hens. Studying intestine samples in Ussing chambers, it was observed that trans-epithelial electrical resistance (TER) was increased and apparent permeability coefficients were reduced due to the supplementation of chickens with a *Bacillus* DFM in the presence of an enzyme combination (XAP), suggesting increased intestinal integrity. The ileal nutrient flow of glucose and lysine was also increased, without a significant change in L-glutamine flow, which suggested positive changes in the ability of the intestine to transport nutrients. Additionally, combinations of Bacillus DFMs and XAP enzymes have shown to increase the concentrations of butyrate in ceacal digesta of broiler chickens (Murugesan, 2013; unpublished data, Danisco Animal Nutrition, 2012), which may be linked to changes in fibre digestion. Immune related effects of these types of combinations still need additional study, although the effects of particular DFMs have been studied in depth (Lee, 2010).

A complicating factor to understand the mode of action of DFM and enzyme combinations is the great variation on enzyme and DFM functionality, which makes difficult to reach broad generalizations. Even small variations in the sequence of enzyme proteins can produce large variations in functionality, and strain differences in DFMs can also be major. It appears that factors like dose, both for enzymes and DFMs, specificity for substrate, metabolic capabilities and life cycle of DFMs, diet composition, age, and health status can affect the relative value of different combinations. It cannot be assumed that any combination of these additives can maximize value or be robust under different commercial situations. Therefore, optimization of combinations for robustness under commercial conditions is needed, as well as additional research on the specific synergies between DFMs and enzyme, which may be driven by nutrient utilization and gut health.

Summary

DFM and enzyme technologies have shown complementary effects on nutrient digestibility and growth performance in broiler chickens, which seem to be more evident in conditions of enteric disease challenge. Those effects may be explained by the direct effects of DFMs on intestinal health, which can create the correct environment for enzymes to increase the absorption of nutrients. Additionally, there is evidence of complementarity between DFMs and carbohydrase and protease enzymes on the digestion of fibre, which could imply both an increase of the digestion and absorption of energy yielding nutrients from the diet and possibly release pre-biotic oligosaccharides.

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