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Dietary NSP nutrition and intestinal immune system for broiler chickens

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Currently broiler chickens are genetically selected for improved feed conversion ratio and rapid growth rate. This rapid growth rate is generally based on a high rate of feed intake and not due to an increased nutrient digestibility. Increased body weight gain, in turn, is found to be negatively correlated with antibody response (total antibody response and specific anti-sheep red blood cell (SRBC)) in broilers. The interactions between commensal bacteria and GALT immune systems are considered to be the basic mechanism of the host against invading pathogens; achieved by forming bio films and by binding to the intestinal epithelium and therefore effectively blocking the sites from pathogens. Apart from the fact that NSP may shift microbial populations toward beneficial micro-organisms to stimulate the immune system, recent evidence has assumed that exogenous xylanase majorly degraded NSP to oligosaccharides and released more phenolics to act as anti-oxidants. Furthermore, it has been suggested that the dietary fibre anti-oxidants existing in insoluble NSP may actually quench the soluble radicals that are continuously formed in intestinal tracts. During the first week post-hatch, the intestinal immune system may be developed by colonization with beneficial bacteria and enhanced competition with invading pathogens. NSP and associate substrates might not only provide substrates for beneficial bacteria but play an important part in removing free radicals.

Keywords: broiler; intestinal immune system; NSP; oxidative stress

Introduction

Modern broiler chickens have been genetically selected for improved feed conversion ratio and rapid growth rate. Today's fast growing broiler chickens can reach 2 kg body weight within 35 days, using only 3 kg feed (Choct, 2009). This rapid growth rate is generally based on a high rate of feed intake but not due to the increased nutrient digestibility (Klasing, 2007). Thus, increased feed intake, in particular during the early period of growth, is perhaps the single most important factor determining feed efficiency for broiler chickens. On the other hand, this high feed intake rate is a heavy burden on the

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gut and cardiovascular systems in broiler chickens due to the increased oxidation rate of macro-nutrients. In fact, a negative correlation was found between increased body weight and antibody response (total antibody response and specific anti-sheep red blood cell (SRBC)) in broilers (Koenen *et al.*, 2002).

The total ban on in-feed antibiotics and the relatively poor sanitation conditions in floor pens in many countries have provided more opportunities for broilers to be exposed to pathogens. Consequently, these fast growing broiler chickens have actually faced a disadvantage in terms of intestinal health. Considering that the criteria used to formulate broiler feeds do not usually take into account the importance of nutrition in maintaining bird health, the modification of nutrition for broiler chickens to improve broiler intestinal immune function will play an important part in the modern poultry industry.

In general, the nutritional impact on the poultry immune system has been extensively reviewed recently (Klasing, 2007; Kogut, 2009; Kogut and Klasing, 2009). It is clear that the intestinal immune system benefits greatly from the commensal micro flora by competitively excluding pathogens. Thus, it makes sense that the dietary composition may be the foundation for the establishment of the commensal micro flora and the development of intestinal immune system in young birds (Bar-Shira *et al.*, 2005). However, the effects of non-starch-polysaccharides (NSP) and oligosaccharides on the immune system in broiler chickens have not been fully elucidated. Choct (1997) indicated that the depolymerisation of soluble NSP significantly reduces fermentative micro flora in the small intestine. Considering the fact that nutritionists are forced to include more and more plant-based by-products in their diet formulation for poultry, an understanding of the mechanisms through which NSP and its associate substrates influences the intestinal immune system and hence the overall health status of poultry, will be of benefit to poultry researchers and producers.

Overview of broiler chicken immune system and development

The immune system consists of specific cells and tissues produced or transformed from lymphoid organs that recognize and respond to invading micro-organisms, parasites, and other antigenic materials (Korver, 2006). Broiler chickens, like other animals, have non-specific (innate) and specific (acquired) immune systems. It is widely believed that the innate system usually recognises infectious microbes and the acquired system are subsequently activated to purge invading pathogens (Kogut and Klasing, 2009).

The structure and relevant functions of the innate and acquired immune systems in chickens have been summarized in several reviews (Korver, 2006; Kogut, 2009; Kogut and Klasing, 2009). Briefly, the innate response, by using germ line coded receptors to recognise invading pathogens, is rapid and critical for disease resistance and susceptibility. The acquired immune system consists of cell mediated and humoral mediated reactions through T lymphocytes (T-cell) and B lymphocytes (B-cell), respectively. For broiler chickens, the primary lymphoid organs include the thymus and bursa of Fabricius (bursa). Encounter of lymphocyte cells with their respective antigens (Ag) and T-cell receptors then migrate to the secondary lymphoid tissues, including the spleen and gut-associated lymphoid tissues (GALT) (Janeway *et al.*, 2005; Sklan, 2005). The GALT, a component of mucus-associated lymphoid tissue, consists of organized tissues and freely dispersed lamina propria lymphocytes (Liebler-Tenorio and Pabst, 2006). The organized tissues are usually made up of Peyer's patch (pp), caecal tonsils, bursa of Fabricius, Meckel's diverticulum, Harderian gland and various lymphoid aggregates located throughout the digestive tract.

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The immune system of the bird is partly developed at hatch. The primary immune organs, such as the thymus and bursa, are both present and are already populated by lymphoid tissue. However, the secondary immune organs, such as the spleen, caecal tonsils, Meckel's diverticulum, Harderian gland, and the diffuse lymphoid tissue of the gut are incomplete at hatch and will take one to two weeks to fully develop, and then start by circulating IgA and IgG antibodies or begin to mature T-cells. Therefore, IgM is the dominant immunoglobulin at hatching which is derived from maternal IgM deposited in the yolk sac (Dibner *et al.*, 1998). Obviously, feeding on the day of hatch may provide an early antigen stimulus and thus facilitate rapid differentiation of the humoral response or mucosal tolerance. However, this period of low immune response against antigens present in diets may be important for the development of tolerance to antigens that birds should not respond to (Maiorka *et al.*, 2006).

It is well known that intestinal colonization by various bacteria commences immediately after hatch (Brisbin *et al.*, 2008), although a 'mature' flora is not established until 14 days of age (Torok *et al.,* 2009). Thus, interactions between commensal bacteria and GALT immune systems are considered to be the basic mechanism of the host against invading pathogens (Forchielli and Walker, 2005), achieved by forming biofilms and by binding to the intestinal epithelium and effectively blocking the sites from pathogens (Brisbin *et al.*, 2008). Invading pathogens are then trapped in the mucosa where they are inactivated by IgA or other secreted products including T-cells. The inactivated pathogens are passed out of the digestive tract later (Korver, 2006). Consequently, the complete development of acquired immune response in GALT will rely on commensal bacterial stimulation during 1-2 weeks post-hatch. Considering that this barrier function of the gut epithelium depends on the presence of IgA, until it is present, the hatchling is more susceptible to invading pathogens (Dibner *et al.*, 1996). Thus a successful GALT immune system will be assumed to permit beneficial bacteria to colonise as soon as possible and protect against pathogens by occupying available attachment sites. Obviously, before this barrier function is completely developed, mainly the maternal IgM and innate immune responses protect the host from invading pathogens (Bar-Shira and Friedman, 2005). In many cases, maternal IgM and innate immune responses are sufficient to resolve the challenge. Furthermore, the genetic selection of rapid growth for broiler chickens has resulted in little or no differences in macrophage and natural killer cell function, *i.e.* the innate immune responses (Rauw *et al.*, 1998). Interestingly, Bar-Shira *et al.* (2005) demonstrated that functional maturation of chicken hindgut preceded that of the small intestine and chicken hindgut in particular caeca might play more important role in maintaining health than we assumed previously.

The gut NSP nutrition and the enteric immune system

The healthy chicken gut harbours more than 640 different species of bacteria (Apajalahti *et al.,* 2004), and dietary NSP are the main substrates for those organisms. Considering that the efficient GALT immune system heavily relies on beneficial bacteria competing with pathogens, NSP and exogenous NSP degrading enzymes have significant impacts on the broiler chicken immune system and hence gut health. It is becoming increasingly apparent that NSP are more complex than just being anti-nutrients or nutrient diluents (Choct, 1997). In general, soluble NSP impart anti-nutritive activities due to their viscous nature and exogenous enzymes have been widely used to counter this (Choct, 2001; Choct, 2006; Józefiak *et al.*, 2007). The current exogenous NSP degrading enzymes are able to partially depolymerise soluble NSP into smaller polymers and reduce digesta

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viscosity, instead of degrading them into monomers. This suggests that a considerable amount of oligosaccharides and other low-molecular weight carbohydrates is produced *in situ* when birds are fed diets based on viscous grains like wheat, barley, rye and triticale. Indeed, Choct (2006) speculated the effects of these carbohydrates might have on gut health and production in poultry.

However, it is not clearly understood how the degradation products of NSP in response to enzyme supplementation affects the dynamics of the gut microflora. Bedford and Apajalahti (2004) demonstrated that in birds fed a reasonable quality wheat-based diet, xylanase supplementation resulted in a 60% reduction in microbial numbers, which was almost comparable to that achieved by an antibiotic. Half a decade has passed since the publication of this existing piece of research and we are still not clear about the mode of action whereby enzymes act as an alternative to in-feed antibiotics. A couple of possible explanations can be put forward. Firstly, it is possible that a more complete digestion of nutrients due to the action of enzymes means that little substrate remains at the end of the intestine to sustain a large population of organisms, effectively 'starving' some of the microbes out of existence. Secondly, somewhat contrary to the first point but perhaps more plausible is that an increased amount of low-molecular weight carbohydrates produced from NSP by enzymes leads to the production of short chain fatty acids (SCFA) (Sakata, 1987). SCFA, among other activities, can inhibit the proliferation of certain organisms, an effect that may explain results of enzymes reported by Bedford and Apajalahti (2004). Furthermore, low-molecular weight carbohydrates produced from NSP by enzymes indirectly stimulate the immune system by encouraging the growth of enteric lactic acid bacteria, which, through their cell wall components, evoke an immune response, especially on the intestinal mucosal surface (Santos *et al.,* 2008). It appears to indicate that not all fermented NSP but only those fitting the criteria of pre-biotics (oligosaccharides) produce an acid pH which benefits intestinal host defence. The pre-biotics can also directly stimulate an up-regulation of regulatory (IL-10) and protective (INF-g) cytokines (Forchielli and Walker, 2005).

Recently Kogut (2009) indicated that the genetic selection of today's broiler chickens for rapid growth had resulted in a diminished inflammatory response. It is reasonable to assume that the current diets for broiler chickens may not provide sufficient or suitable substrates for beneficial bacteria (endogenous probiotics) to colonise and compete with pathogens. The fact that eggs and human milk are full of oligosaccharides may confirm that newly-hatched broilers need sufficient and suitable oligosaccharides for beneficial bacteria substrates. Traditionally, the total load of intestinal micro flora has been effectively controlled by the use of in-feed antibiotics, alleviating microbial composition for nutrition with the host. Without the use of in-feed antibiotics, however, alternatives such as enzymes, prebiotics and probiotics are required to modulate gut microbial balance and maintain gut health.

Klasing (2007) and Kogut (2009) have claimed that all nutrients in the diet play a fundamental role in sustaining an optimal immune response and the addition to the diet of many non-essential nutrients causes larger changes in immunity than manipulation of many essential nutrients. Therefore, insoluble NSP are not inert nutrients as previously assumed. In fact, there has been a tremendous amount of research undertaken in recent years, elucidating the effects of structural materials in feed *i.e*., plant materials that consist mainly of insoluble NSP, such as course oat hulls (Hetland *et al.,* 2003; Choct, 2006). More recently, Gonzaleź-Alvarado *et al.* (2007) indicated that broiler chickens needed minimal NSP to improve their gut health due to the fact that NSP could increase gizzard weight and decrease pH value in the gizzard. It is believed that a more acidic gizzard pH may not only kill ingested pathogens but could allow beneficial

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bacteria in the crop to out-colonise pathogens in the lower digestive tract (Engberg *et al.*, 2004).

Effect of stress on the intestinal immune system

When the immune system mounts defence functions against invading pathogens, phagocytes, as well as T-cells and B-cells release a multi-component enzyme complex which is responsible for the production of reactive oxygen species. While these radicals are killing invading pathogens, they also have the potential to damage host tissue (Costantini and Møler, 2009). This increased oxidative stress is a side-effect of immune responses. It is known that immune cells rely heavily on cell to cell communication, particularly via membrane-bound receptors, to work effectively. Cell membranes are rich in polyunsaturated fatty acids which, if peroxidised, can lead to a loss of membrane integrity, altered membrane fluidity and result in alterations in intracellular signalling and cell function (Hughes, 1999). Thus, sufficient antioxidants may protect the host from unnecessary cell damage due to immune responses. The causes of stress in animals are varied including heat, negative energy balance, transportation, pregnancy, and mixing with unfamiliar animals. For broiler chickens, the high rate of growth may also be considered a type of stress. Various immune cells are affected when an animal is stressed. In particular, animals subjected to restraint stress fail to mount a protective immune response (Anglen *et al.*, 2003). Therefore, antioxidants are important for the correct reaction and functionality of avian intestinal immune systems.

In addition to the commonly used dietary antioxidants, like vitamin E and organic selenium, there is increasing evidence that numerous cell wall components, such as NSP, phenolics and even phytate, have appreciable antioxidant properties. For example, cereal fructans have the ability to stabilise membranes under stress conditions, with size and species dependent differences between fructans. In addition, mixtures of fructans, as they occur in living cells, may have protective properties that differ significantly from those of the purified fractions (Hincha *et al.*, 2007). Phytate, which is generally considered as an anti-nutritional factor, has recently been suggested as having antioxidative activity due to its high binding affinity for Fe^{2+} (Stodolak *et al.*, 2007). This is because the control of free Fe is important for intestinal health maintenance due to the fact that freely available Fe diminishes or destroys normal resistance to bacterial infection (Adams, 2006). In addition, phenolic acid in cereal bran has shown strong antioxidant activity (Kim *et al.*, 2006) because it exists as ferulic acid, substitute of the arabinose side chains are substituted at the 5' position (Hegde *et al.*, 2006). In an *in vitro* study, using exogenous multiple NSP degrading enzymes to hydrolyze the palm kernel meal and rice bran, resulted in increased total phenolics and anti-oxidative activity in palm kernel meal and rice bran (Bao, 2009). It is further suggested that such an antioxidant may quench soluble free radicals that are continuously formed in intestinal tracts (Vitaglione *et al.*, 2008).

Conclusions

This brief review has thrown together a number of disparate ideas to highlight areas of research surrounding NSP, hoping to prompt future research exploring novel aspects of feed components and gut health.

It appears to be obvious that NSP and associated substances are important for today's fast growing broiler chickens in the development of a competent intestinal immune

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system and a robust gut health. This is important for poultry production devoid of in-feed antibiotics. There are a number of underlying reasons for this:

- Genetic selection for rapid growth may have placed a greater demand on the immune response of the gut in broilers.
- As grow-out period gets shorter, early nutrition becomes more crucial as it defines the type of microflora that birds will have, which, in turn determines robustness of the internal immune system.
- Nutriceuticals that change or modify diet components, such as NSP and associate substances, produce effects beyond apparent production parameters, which often modulate immunity and other factors affecting gut health.

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