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REVIEW ARTICLE

TRENDS OF BIOTECHNOLOGICAL APPLICATIONS IN POULTRY NUTRITION AND FEED IMPROVEMENTS

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ABSTRACT

The increasing cost of feed ingredients necessitate improving poultry feed utilization to enhance production. To fulfill increased world food demand and to overcome the consequences related to natural and industrial changes, scientists have been trying to select and improve both feedstuff and livestock with the application of biotechnology. The trends of biotechnology application in livestock involve traditional breeding, biochemical and physical techniques in nutrition to increase the abundance (availability) of feed and to improve the utilization of nutrients in those feeds to maximize animal production. Additionally, animal nutrition studies are conducted to determine the safety of human food and modeling of some human diseases. Manipulating animal ability to absorb and utilize more nutrients starts from the plant breeders and continues until where those nutrients are utilized in the body. Microorganisms that have symbiotic life with livestock organism are also transgenically manipulated to improve nutrition and feed utilization. The different biotechnological applications used in poultry industry to improve poultry nutrition and feed includes; engineering of bird's genes, micro flora of birds and their enzymes, use of antibiotics, Probiotics, prebiotics and others. Hence, biotechnology application is paramount in poultry nutrition and feed improvement for efficient utilization and sustainable development of the poultry industry in the future.

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INTRODUCTION

The world population is dramatically increasing since 19th century. This escalated the feeding competition between human and animals. It is due to the major components used in animal feeds, especially in poultry diets are also being used in human nutrition. This lead to decreased availability of quality feed resources and increasing price of poultry feeds. Particularly, the rapid increment in the global consumption of poultry products leading to intense expansion of poultry and feed companies and this trend is expected to continue. Such growth in the poultry industry is having a profound effect on the demand for feed materials (Avindran and Blair, 1991). Due to limited natural resources, alternative biotechnological application should be sought in poultry nutrition and feed improvement to combat the present and future challenges in the sector. The addition of new genes to feedstuff also gives nutritionists more application tools for improving nutrition and animal health through feeds. Therefore, this review discusses the past and present biotechnological applications in poultry industry to improve poultry nutrition and feed, while not all inclusive, provides a brief discussion of some of the potential applications of biotechnology that have been demonstrated to date with future implications.

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Biotechnological applications in poultry

The animal biotechnology in use today is built on traditional breeding techniques that date back to 5000 B.C.E. Such techniques include crossing diverse strains of animals (known as hybridizing) to produce greater genetic variety. Today, the application of biotechnology is on the stage of engineering micro flora of birds and their enzymes to improve poultry nutrition and manipulate the genes of feed which include antibiotics, Probiotics, prebiotics and enzymes.

Antibiotics

Feed additives have been widely used to increase the performance of animals, and used in poultry nutrition feeding practices extensively (Collington *et al*., 1990; Khan *et al*., 2007) not only to stimulate the growth and feed efficiency but to improve the health and performance of birds (Fadlalla *et al*., 2010). In past, several antibiotic growth promoters had been used in poultry feed aiming to prevent disease, to improve growth performance, and to increase some useful microorganism in intestinal microflora. The growth promoter effect of antibiotics was discovered in the 1940s, when it was observed that animals fed dried mycelia of *Streptomyces aureofaciens* containing chlortetracycline residues improved their growth. The mechanism of action of antibiotics as growth promoters is related to interactions with intestinal microbial population (Dibner and Richards, 2005;

Niewold, 2007). The use of antibiotics as growth promoters became public health issue by the World Health Organization (1997) and the Economic and Social Committee of the European Union (1998) after used for 50yrs and led to withdraw in 2006 even though controversially used in other countries even today. However, because of the emergence of antibiotic resistance microorganisms, researchers have been investigating other alternatives for antibiotics; to interact with the intestinal microflora, including, probiotics, prebiotics (Patterson and Burkholder, 2003; Kocher, 2006), and enzymes (Bedford, 2000; Hruby and Cowieson, 2006) or acidification of diets (Ricke, 2003; Diebold and Eidelsburger, 2006). Recently, spices, plant extracts and herbs known as Essential oils (EOs) are received increasing attentions.

Probiotics and prebiotics in poultry production: Probiotics

Probiotics are mono or mixed cultures of live microorganisms which, when administered in adequate amounts, confer a health benefit on the host (FAO/WHO, 2002). The use of Probiotics in birds was set by Nurmi & Rantala (1973), who observed that when intestinal content of the healthy adult birds were orally administrated to birds at one day of age, it changed their sensitivity to *Salmonella* sp. The use of Probiotics in poultry production is to improve performance in broiler chickens and to increase egg production in laying hens in addition to reduce intestinal colonization by pathogens such as *Salmonella* sp. The main genus of bacteria identified in gastrointestinal tract of birds and used as probiotics are: *Bacillus*, *Bifidobacterium*, *Clostridium*, *Enterobacter*, *Lactobacillus*, *Fusobacterium*, *Escherichia*, *Enterococcus* and *Streptococcus*. In poultry production, manners of Probiotics administration to birds more commonly observed are: by the feeds, by drinking water, by pulverization on the birds, inoculation via cloaca or in embryonated eggs (*in ovo*), among others, and the manner of administration has effect on intestinal colonization capacity. Except if submitted to stress situation, bacteria which colonize gastrointestinal tract of the birds since their birth, tend to remain there for the rest of their lives. Therefore, *in ovo* inoculation is an aspect of using probiotics for birds. *In ovo* inoculation of Probiotics *Lactobacillus casei*, *Lactobacillus plantarum* and *Enterococcus faecium* at 10^6 UFC egg^{-1}, at 16 days of incubation and the performance of challenge of chicks at one day of age via stomach with 13.6 x 10^6 UFC mL^{-1} of *Salmonella enteritidis*, improved performance of animals fed Probiotics when compared to control treatment (Leandro *et al*., 2004). Moreover, in the same way, it was observed that from 7 to 21 days of age, *Salmonella* sp. was identified only in challenged animals which were not fed probiotic. It is suggested that probiotic avoided bacterium colonization in the gastrointestinal tract of the birds. In chicks emerging from incubators, pH concentration and the presence of volatile fatty acids, which are one of the main protection barriers of the animal organism, are not sufficient chemically to avoid that pathogens enter in their organism. Moreover, the small variety of the birds' intestinal microbiota in this phase is considered as a limiting factor for the digestion and for the possibility of intestinal colonization by enteric pathogens. Thus, probiotic supplementation seems to be a beneficial action for the animal performance and health of birds from commercial incubators. An efficient immune response is related to the presence of immunomodulaters in the diet, which will act by reducing immune stress and then reducing nutrient mobilization to activities which are not related with production (meat or eggs), permitting in addition to a greater survival in stress situations, the non-harmful effect on animal performance. The use of yeast *Saccharomyces boulardii* in the diet for broiler chickens reduced the level of *Salmonella* sp. from 53.3% to 40.0% at stress conditions in transportation to slaughter (Line *et al*., 1998). The use of yeast *Saccharomyces cerevisiae* var. *chromium* reduced negative effects of caloric stress on broiler chickens (Guo and Liu, 1997). The results of studies with probiotics in poultry production have been showed to be rather contradictory regarding to its efficiency. Not always are positive results observed by using probiotics. Those vary with age of the animal, type of probiotic used, viability of the microorganisms, storage conditions, level and manner of administration, in addition to the low challenge in relation to the experimental condition concerned to sanity, management and other stressful conditions. The addition of *Bacillus subtilis* into the diet increased weight gain and feed conversion (Fritts *et al*., 2000). The addition of *Lactobacillus* increased weight gain and improved feed conversion of supplemented animals (Kalavathy *et al*., 2003). The use of yeast *Saccharomyces boulardii* in *Salmonella enteritidis* infected broiler chickens improved feed efficiency by 10% when compared to control treatment, and by 12% in animals supplemented with *Bacillus cereus* var. toyoii (Gil de los Santos, 2004). Concerning to carcass quality of broiler chickens, the beneficial effect of probiotic use was also observed.

Mode of actions and characteristics of ideal Probiotics

Probiotics show numerous essential ways of action, an antagonistic action towards pathogen bacteria by alteration of gut pH, through antimicrobial effect by secretion of products which inhibit their development, such as bacteriocins, organic acids and hydrogen peroxide, production of short chain fatty acids (SCFA) in the intestine, regulation of the immune system of the host, regulation of gut microbiota, and diverse metabolic effects (Vamanu and Vamanu, 2010; Ferreira *et al*., 2011). Additional mode of action is competitive exclusion which represents colonization ability and adhering competition in the intestinal mucous membranes to avert adhesion and invasion of pathogens and, which is a key performance parameter, inhibition of their colonization and replacement of already adhered ones; competing for available nutrient and growth factors (Patterson and Burkholder, 2003). List of ideal probiotics characteristics and their valuable effects are presented in Table 1.

Table 1. Characteristics of ideal probiotics and their desirable properties (Alloui *et al*., 2013)

Probiotics	
Properties	Positive influences
Belong to host origin	-Change intestinal microbiota
Non pathogenic	-Induce immune system
Resist processing and storage	-Decrease inflammatory reactions
Endure gastric acid and bile	-Prevent pathogen proliferation
Epithelium or mucus attaching capability	-Enhance animal performance
Persist in the intestinal tract	-Reduce carcass contamination
Produce repressive compounds	-Decrease ammonia and urea excretion
Immune response regulation	
Modify microbial activities	

Most used probiotic genera

A variety of microbial species have been used as probiotics with nearly 20 known species, which beneficially affect the host by improving its intestinal microbial balance (Bhupinder and Saloni, 2010). Various types of probiotic bacteria include species of *Bacillus*, *Bifidobacterium*, *Lactobacillus*, *Lactococcus*, *Streptococcus*, other bacteria and a kind of yeast species, and indefinable mixed cultures. *Lactobacillus* and *Bifidobacterium* species have been used above all in humans, whereas species of *Bacillus*, *Enterococcus*, and *Saccharomyces* yeast have been the most predominant organisms used in livestock (Ferreira *et al*., 2011). However, recently, enhanced performance was noticed when feeding *Lactobacillus* to livestock (Vicente *et al*., 2007; Vicente *et al*., 2008).

Prebiotics

Prebiotics were defined as non-digestible food (feed) ingredients that beneficially affect the host by selectively stimulating the growth and/or activities of one or a limited number of bacteria in the gut, thereby improving host health (Gibson and Roberfroid, 1995). However, more recent definitions stated that a prebiotic is a selectively fermented ingredient that allows specific changes, in both the composition and activity in the gastrointestinal microbiota which confers benefits to the host (FAO/WHO, 2002). The common point of these definitions is that prebiotics are characterized by a selective

Table 2. Type of commercial feed enzymes and with target substrates used in poultry feeds (Ravindran V., 2013)

Enzyme	Target substrate	Target feedstuff
Phytases	Phytic acid	All plant-derived ingredients
β-Glucanases	β-Glucan	Barley, oats, and rye
Xylanases	Arabinoxylans	Wheat, rye, triticale, barley, fibrous plant material
α-Galactosidases	Oligosaccharides	Soybean meal, grain legumes
Proteases	Proteins	All plant protein sources
Amylase	Starch	Cereal grains, grain legumes
Lipases	Lipids	Lipids in feed ingredients
Mannanases, cellulases, hemicellulasespectinases	Cell wall matrix (fiber components)	Plant-derived ingredients, fibrous plant materials

effect on the microbiota, and thus can improve the host health. Prebiotics include mainly oligosaccharides, sugar molecules of three to six chains, and soluble fiber. These carbohydrates are found naturally in fruit and vegetables (Charalampopolus and Rastall, 2009). Numerous types of oligosaccharides, including inulin, fructooligosaccharides (FOS), galactooligosaccharides (GOS), soya-oligosaccharides (SOS), xylo-oligosaccharides (XOS), pyrodextrins, isomalto-oligosaccharides (IMO) and lactulose, are commonly considered as prebiotics. However, the majority of studies up to date focus to inulin, FOS and GOS (Macfarlane *et al*., 2008). A collection of prebiotics exists and the most reported in a large number of studies and with the most reliable evidence accumulated for the effects of prebiotics have been non digestible oligosaccharides (NDOs) from different origin and chemical properties (Chen and Chen, 2004; Macfarlane *et al*., 2008; Zduńczyk *et al*., 2011).

Characteristics of prebiotics and their mode of actions

Prebiotics beneficially interact with animal's physiology by selectively stimulating favorable microbiota in the intestinal system. This may have valuable effects in reducing the incidence of enteric pathogens. However, from the available published evidence, the exact mechanism involved in prebiotics to reduce pathogenic infections is still unclear. Competitive exclusion of pathogens by increasing numbers of microbiota that are associated with a healthy host can produce a variety of bacteriocins that have a detrimental effect on the pathogen by promotion of macrophages, stimulation of antibody production, and antitumour effects (Vamanu and Vamanu, 2010). Contrary to these microbiota dependent theories, it may be that prebiotics are able to directly affect the pathogen or host in a microbiota independent manner. It has been suggested that the principal mechanisms of prebiotics is immunomodulation, that includes selective growth of lactic acid-producing bacteria, resulting in an increased concentration of short chain fatty acid (SCFA) like acetate, propionate, and especially butyrate which is the preferred energy source of colonocytes and stimulates gut integrity. High fermentation activity and high concentration of the short chain fatty acid (SCFA) is correlated with a lower pH, which is associated with a suppression of pathogens and increased solubility of certain nutrients (Józefiak *et al*., 2004). This increase of SCFA with immune cells, direct contact in the digestive tract and the change in mucin production contribute to lower incidence of bacteria moving across the gut barrier (Lee and Salminen, 2009). This phenomenon may inhibit some pathogenic bacteria and reduce colonization of some species like *Salmonella* and *Campylobacter* (Charalampopolus and Rastall, 2009).

Action of prebiotics in animal organism

The principal effect of prebiotics is to stimulate the resident microbiota of host to proliferate, to stop harmful bacteria, and to share health benefits to the host. The supplementation of poultry and pig diets with oligosaccharides is generally associated with stimulation of microbiota proliferation. Specifically, in pigs, GOS has been associated with increasing the numbers of bifidobacteria in both *in vivo* and *in vitro* evaluations (Charalampopolus and Rastall, 2009). Prebiotic oligosaccharides supplementation of the diets, such as FOS, may have an effect on improvements in the gut microbial population, including a reduction in *Salmonella* colonization. This suggests that supplemental dietary FOS may be a viable option in both *Salmonella* control and antibiotic free programs.

Enzymes

Feeding enzymes to poultry is one of the major nutritional advances in the last fifty years. Enzyme use in poultry diets has a long history, with the first report of an enzyme product, known as Protozyme, being used in the 1920s. What is not widely appreciated is that most of the early feed enzyme research originated from the United States, with pioneering research demonstrating the value of the addition of enzyme preparations to barley-based diets reported during the 1950s and 1960s (Jensen *et al* 1957, Willingham *et al* 1961, Jensen *et al* 1968) and early studies on the use of phytases in improving phosphorus availability from plant feed ingredients conducted during the early 1970s [Rojas and Scott. 1969]. The enzyme products evaluated in these studies were crude enzymes and, importantly, available only in small quantities. It was another 10 to 20 year before the non-starch polysaccharide (NSP) enzymes and phytases became available in commercial quantities. It is the culmination of something that nutritionists realized for a long time but until 1980's it remained beyond their reach. Indeed, the theory of feed enzymes is simple. Plants contain some compounds that either the animal cannot digest or which hinder its digestive system, often because the animal cannot produce the necessary enzyme to degrade them. Nutritionists can help the animal by identifying these indigestible compounds and feeding a suitable enzyme. These enzymes come from microorganisms that are carefully selected for the task and grown under controlled conditions (Wallis, 1996).

Types of Enzyme Available for Poultry

Some of the enzymes that have been used over the past several years or have potential for use in the feed industry include cellulose (ß-glucanases), xylanases and associated enzymes, phytases, proteases, lipases, and galactosidases (Table 1). Enzymes in the feed industry have mostly been used for poultry to neutralize the effects of the viscous, non-starch polysaccharides in cereals such as barley, wheat, rye, and triticale. These anti-nutritive carbohydrates are undesirable, as they reduce digestion and absorption of all nutrients in the diet, especially fat and protein. Recently, considerable interest has been shown in the use of phytase as a feed additive, as it not only increases the availability of phosphate in plants but also reduces environmental pollution. Several other enzyme products are currently being evaluated in the feed industry, including protease to enhance protein digestion, lipases to enhance lipid digestion, ß-galactosidases to neutralize certain anti-nutritive factors in non-cereal feedstuffs, and amylase to assist in the digestion of starch in early-weaned animals.

Benefits of Enzymes: Benefits of using feed enzymes to poultry diets include; reduction in digesta viscosity, enhanced digestion and absorption of nutrients especially fat and protein, improved Apparent Metabolizable Energy (AME) value of the diet, increased feed intake, weight gain, and feed gain ratio, reduced beak impaction and vent plugging, decreased size of gastrointestinal tract, altered population of microorganisms in gastrointestinal tract, reduced water intake, reduced water content of excreta, reduced production of ammonia from excreta, reduced output of excreta, including reduced N and P (Ouhida *et al*, 2000; Gill, 2001; Odetallah, 2002; Gracia, *et al.,* 2003; Saleh, *et al.,* 2003; Odetallah, *et. al.,* 2005 and Wang *et al*., 2005).

Impact on Environment: Enzymes have been approved for use in poultry feed because they are natural products of fermentation and therefore pose no threat to the animal or the consumer. Enzymes not only will enable livestock and poultry producers to economically use new feedstuffs, but will also prove to be environmentally friendly, as they reduce the pollution associated with animal production. As well as contributing to improved poultry production, feed enzymes can have a positive impact on the environment. In areas with intensive poultry production, the phosphorus output is often very high, resulting in environmental problems such as eutrophication. This happens because most of the phosphorus contained in typical feedstuffs exists as the plant storage form phytate, which is indigestible for poultry. The phytase enzyme frees the phosphorus in feedstuffs and also achieves the proteins and amino acids bound to phytate. Thus, by releasing bound phosphorus in feed ingredients, phytase reduces the quantity of inorganic phosphorus needed in diets, makes more phosphorus available for the bird, and decreases the amount excreted into the environment.

Essential Oils (EOs)

The EOs is mixture of fragrant and volatile compounds, which are usually originated from plant, and are named with the aromatic characteristics considering the origin of plant (Oyen and Dung, 1999). The EOs as single or mixture may be used as a growth promoter in broiler production. Many studies have shown positive effects of dietary EO on body weight gain. Supplementing the dietary EOs would stimulate the growth performance of broilers (Cross *et al*., 2002; Bampidis *et al*., 2005). Broilers supplemented with a mixture of laurel, oregano, sage, citrus and anis EOs, or a mixture of EOs significantly improved feed conversion (Cabuk *et al*., 2006a). Also, in a broiler trial that examined mixtures of oregano, cinnamon, cayenne pepper, thyme, and combination of organic acids and plant extracts in comparison to nutritive antibiotic avilamycin in broiler chickens, the birds supplemented with the plant extracts showed higher body weight gain and increased feed consumption as compared to the other groups. Rezaei–Moghadam *et al*. (2012) has also reported that supplementation of turmeric increases serum antioxidant levels and immune status of the birds (Madpouly *et al*., 2011). Elagib *et al*. (2013) reported that addition of 3% garlic as feed additive could significantly enhance growth and performance of broiler chicks. Addition of a blend of EO from basil, caraway, laurel, lemon, oregano, sage, tea and thyme in a diet meeting the nutrient requirements of broilers would improve the body weight gain with positive effects on feed to gain ratio (Khattak *et al*., 2014). Essential oils are found to have antibacterial ability, and also exhibit antioxidant, anti-inflammatory, anti-carcinogenic, digestion stimulating, and hypolipidemic activities (Viuda-Martos *et al*., 2010). Thus, EOs can also be used as growth promoters in poultry production for broilers and layers (Cross *et al*., 2007; Kirsti *et al*., 2010).

Implications of Biotechnology in poultry nutrition

A successful poultry production system, irrespective of scale and sophistication, requires a continuous enterprise cycle, which can be better achieved by embracing the important elements of sustainability both physical and financial. Systems of the future, in the context of poultry nutrition, will need to apply greater focus on “resource sufficiency” not scarcity, which will necessitate the constant consideration and evaluation of alternative protein and other nutrient input sources, for example a reduced reliance on the traditional proteins – fishmeal and soybean– in broiler and layer diets. There will remain the need to achieve and exploit efficiencies throughout the system, particularly opportunities for further feed conversion economies through improved general flock management. A more holistic and integrated approach to the development of feeding programmes will assist the poultry industries and individual producers of chicken products to pursue their goals of enhanced production within a sustainability context. Endemic and local occurrence of infectious diseases will remain an omnipresent threat, and on occasion will stifle the progress achieved in bird performance through the adoption of improved biotechnology in nutrition and feed strategies.

Conclusion

The trends in application of biotechnology in animal nutrition and feed have been continually improving. It improved challenges of low productivity, low performance, food conversion ratio, and disease preventions. Additives to poultry nutrition, such as enzymes, probiotics, and antibiotics in feed, are already widely used in intensive production systems worldwide to improve the nutrient availability of feeds and the productivity of poultry products. Alternative to antibiotics, essential oils are emerging as potential plant products with multifunctional benefits. The use of biotechnology also important in releasing the available nutrient for efficient utilization of feedstuff and reduce the load of phosphorous and nitrogen on the environment related with poultry production. In conclusion, successful and sustainable poultry production is achieved with the wise application of up-to-date biotechnologies with further innovation for efficient utilization of available resource, with reduced risk of food safety, animal welfare and environmental pollution.

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