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APPLICATION OF FEED ADDITIVES IN POULTRY PRODUCTION AND ITS POSSIBLE EFFECTS

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ABSTRACT: Intestinal health is very critical for wellbeing, welfare and performance of poultry. Generally, due to absence of available technologies, our knowledge about the variety of microbial species inside the chicken gastrointestinal system had been simplified. New technologies suitable for microbial species analysis have developed our idea. Food-borne conditions require reduction in bacterial infections by ingredients of animal origin. After the restriction of antibiotics use in poultry feed, there is a challenge for scientists to look for alternatives to reduce the accumulation of pathogenic microorganism. The use of alternative products to antibiotics have been increased due to consumer awareness and bacterial resistant to increase chicken well-being. Alternative to antibiotics are prebiotics, probiotics, essential oils, enzymes, volatile fatty acids, etc. However, new technologies should be developed for advancing the selection of these products for practical application in the chicken industry and more research about these products is necessary to promote chicken production in the absence of antibiotics.

Keywords: In-feed antibiotics, feed additives, gut health, microbiota, poultry performance.

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INTRODUCTION

The principal aim of poultry production is to provide enough safe foods for the utilization of human. Within the past, to promote the growth of chicken antibiotics have been added in the feed as growth promoters. Due to bacterial resistance, antibiotics are restricted to use in feed. Due to this reason, scientists are now focusing on alternative products to antibiotics. Alternative products are the best way to promote chicken production and performance instead of antibiotics. The products are like probiotics, prebiotics, synbiotics, herbal essential oils, and enzymes. Feed additives are extensively used in chicken feed to promote the health and performance of birds and to stimulate the growth and feed efficiency (Abouelfetouh, 2012). Specific raw materials have positive effects on birds performance and wellbeing, primarily by maintaining the host intestinal microbes health (Nawab *et al*., 2018).

POULTRY INTESTINAL MICROBIOTA

A healthy intestinal microbe constitutes a barrier, restricts the development of pathogenic bacteria by producing fatty acids and vitamins, and stimulates the immune functions. A variety of microbes live in the gut of humans and other mammals mostly they are bacteria, but other microorganisms also present which have a collaborative relationship with its host and help the host for utilization of nutrients, pathogens resistance, immune system development and metabolism of host (Billy 2015; Brestoff and Artis, 2013; Kiarie *et al*., 2013). The habitats for microbes to live in are -: lumen of intestine; mucosal epithelium layers that cover it; crypts deep mucus layer; and intestinal epithelial cells surface (Pluske *et al.*, 2012). This relationship is very complicated; it depends upon intestinal microbes. It may have positive or negative effects on birds. Intestinal microbes produce metabolites (e.g., derivatives of bile acid, vitamins, and organic acids) that affect the normal host functions. Bacteria of the genus *Lactobacillus* and *Bifidobacterium* provide safety against intestinal infections. Gut problems are reduced, and chicken health is promoted by increasing the active components of the gut microbiotas (O'Hara and Shanahan, 2007; Liu *et al.*, 2018). Given this scenario, for keeping the balance of microbes in the gut, certain nutrients play a significant role and determine whether a pathogenic bacterium proliferates. Due to the lack of abilities to identify the bacterial species and their mode of action by which they affect the host health, causes a barrier in studying the gut microbiome. Research about host-microbe interaction will be beneficial because it can help in the identification of many more gut bacteria which are linked with excellent performance and health in birds (Torok *et al.*, 2011). The gut microbiota can positively influence the integrity of the intestinal barrier with its metabolic, trophic and defensive functions. The permeability of intestine increased as a result of intestinal barrier dysfunction inducing a change from "physiological" to "pathological" inflammation (Frank *et al.,* 2007; Kim *et al.,*2016). Damage to the intestinal barrier is due to Physiological and psychological stressors resulted in increased permeability of the intestine, have an effect on intestinal microbes composition and damage to intestinal pathogens (Lennon *et al.,* 2013). When there is a stress factor than the number of beneficial bacteria, like *lactobacilli* and *bifidobacteria*, have been decreased (Min *et al.,* 2004; Liu *et al.,* 2018).

MANAGEMENT AND TREATMENT USING ALTERNATIVE PRODUCTS

Bacteria develop resistance due to continuous and over use of antibiotics in poultry feed industry (Cao *et al.,* 2010). After hatching conditions, transportation stress and other stress production conditions may weaken the immune system of birds, leading the development of pathogenic bacteria, which have an adverse effect on chicken health. *Salmonella* spp. has the ability to cause contamination through the food chain among other pathogen species (Humphrey 2006; Liu *et al.,* 2017). For balance and healthy gut microbiota, the use of alternative products is the best way to minimize pathogen growth. These alternative products are essential oils, enzymes, probiotics, prebiotics, synbiotics, etc.

Essential oils (EO): Essential oils are sweet smelling and oily substances, which are derived from plant species and associated with herbs. Oregano, thymol, thyme, garlic, and cinnamaldehyde are used as essential oils (Krishan and Narang, 2014). Essential oils (EOs) have properties as follow: antibacterial ability, antioxidant, anti-inflammatory, anti-carcinogenic and stimulation of digestion (Viuda-Martos *et al.,* 2011; Zhai *et al.,*2018). Fermentation, extraction or expression are the techniques by which we obtained essential oils; however, on an industrial level, steam distillation is most commonly used.

Properties and activities of essential oils

Antibacterial activity: The antibacterial function of the diverse chemical compounds in EOs is a combined effect on different parts of the cell; it is not resulted by a single mechanism. EOs work better against Gram-positive as compared to Gram-negative bacteria (Gracia-Valenzuela *et al.,* 2012) because gram-negative bacteria have an outer membrane which prevents the entry of hydrophobic compounds.

Antiparasitic activity: Individual plants and their Eos have antiparasitic properties, e.g., garlic (*Allium sativum*), onion (*Allium cepa*) and mint (*Mentha spp.*), their EOs and seeds have positive effects against GIT parasite. (Karadas *et al.,* 2014) found that concentration of carotenoids in liver and coenzyme Q10 is improved when we fed a combination of EOs including carvacrol, cinnamaldehyde and capsicum oleoresin to broiler chickens.

Anti-inflammatory activity: Anti-inflammatory properties of EOs are due to phenolic compounds. Terpenoids and flavonoids have anti-inflammatory abilities. The metabolism of inflammatory prostaglandins suppressed by these substances (Cao *et al.,* 2010).

Immunomodulatory activity: Rahimi *et al.* (2011) reported that supplementation of garlic in broiler diets at the rate of 0.1% increased the weight of spleen and bursa of Fabricius, improved antibody titer of Newcastle disease (ND) virus and improved hypersensitivity response.

Use of EOs in poultry nutrition: The EOs as single or mixture may be used as a growth promoter in chicken production. A study by (Rezaei-Moghadam *et al.,* 2012) has shown that antioxidant levels of serum and immunity of the chicken improved by the supplementation of turmeric. Abdel-Wareth *et al.* (2019) showed that 3% garlic as a supplement in feed increases the development and performance of broiler. Garlic powder enhances the production of egg and weight of egg in layer chicken (Jawad *et al.,* 2013). The weight of egg and immune status is improved in heat stress condition by the use of a mixture of essential oil and organic acid administration in layer birds feed (Nawab *et al.*, 2018). In the study of Cao *et al.* (2010), the bird mortality is reduced by over 6% unit from week 1 to week 3 by using EOs mixture of thymol and cinnamaldehyde (TC). However, over week 4 to 6, there was no mortality noticed. Performance and carcass yield was improved with rosemary oil in broiler feed (Yeşilbag *et al.*, 2011; Zhai *et al.*,2018).

Enzymes: In the late 19th century, enzymes were discovered and after discovery used in industry since the early 1900s. Most of the enzyme are fermentation products of basophilic microorganisms. A study shows that feed enzyme benefits the global feed market the estimated US \$3–5 billion per year (Adeola and Cowieson, 2011). Enzymes are protein in nature, which boost up chemical reactions. The digestibility of fiber, phytate, protein, and another component of feed is improved by adding enzymes (Yang *et al.,* 2009). Phytases, carbohydrases, proteases, and lipases are enzymes which are mostly used in animal production. Carbohydrase enzyme has 2 dominant enzymes: xylanase and glucanase (Kiarie *et al.,* 2013). Other commercially available carbohydrases include a-amylase, b-mannanase, a-galactosidase, and pectinase. The share of different enzymes in the market is as follow: phytase taking 60 % share, carbohydrase 30 % and the rest (proteases, lipases, etc.) 10 % (Adeola and Cowieson, 2011). The enzymes work in 2 phases. Enzymes speed up the digestion process and minimize the substrate available for bacteria thus reducing the number of bacteria; this is the first phase, which occurs in the ileum. The second phase occurs in the caecum, where beneficial bacteria feed on soluble, poorly absorbed sugars produced by enzymes. Volatile fatty acids produced by these bacteria may be helpful to minimize *Salmonella* numbers, and possibly *Campylobacter* spp (Yang *et al.*, 2009; Salem *et al.*,2017). Increase the thickness of the digesta, reduces nutrient digestibility, change in beneficial bacteria and change in normal intestine physiology occurs by adding cereals rich in NSP. By the addition of enzymes in feed, the thickness of the content is minimized and improved the uptake of nutrient and animal performance. The relationship between feed enzymes and gut microbes can better be understood in fig1. Third most expensive nutrient in diets for non-ruminants is the Phosphorous. 65 % phosphorous of plant origin feedstuffs is in bound form and not available to the chicken without enzymatic degradation. Phytase enzyme is required to release the phosphorous from the bounded form in poultry and other mammals (Abdel-Wareth *et al.*, 2019). Liu and Kim, (2017) stated the effects of diet type (maize vs wheat), multi-carbohydrase enzyme supplementation (without or with) and *C. perfringens* challenge (none and challenged) in broiler chickens. They check the growth performance, gut colonization of *C. perfringens* and gut lesions. Good FCR observed in those birds which are supplemented with maize-based formulated diets than those which are supplemented on wheat-based formulated diets. Enzyme administration minimizes the growth suppression linked with the pathogen challenge, with the most dominant effect observed in birds supplemented with wheat-based formulated diet. High jejunal digesta viscosity observed with wheat-based diets which were minimized by the addition of enzymes (Jia *et al.*, 2009; Liu and Kim, 2017).

Probiotics: Probiotics are called as direct-fed microbes. Probiotic are 'live organisms,' when administered in particular amounts, have a beneficial effect on the health of the host (Wang *et al.*, 2017). *Bacillus, Bifidobacterium, Enterococcus, Escherichia, Lactobacillus, Lactococcus, Streptococcus*, and variety of yeast species are commonly used as probiotics. The probiotics which are used in poultry feed have been shown in table-1. The mechanism by which probiotics perform their action is: "competitive exclusion" and immune modulation. Competitive exclusion involves the synthesis of antimicrobial products that inhibit pathogenic microbes and compete for substrates and attachment sites. By considering this mechanism, positive results are observed when probiotics have been tested for controlling Salmonella colonization in broilers. In the USA, Canada, and Europe probiotics progress in law has been made. For the safety criteria of probiotics; however, no standard dose is available. Expected qualities and security criteria of probiotics have been shown in table-2. The different strains of probiotics have been used to modulate the poultry gut microbes and from the prevention of infection. Croom *et al.* (2015) reported that compared with control birds, the birds fed with direct microbes have longer villus length and perimeter in the jejunum in starter feed. Direct-fed microbials resulted in increases thickness of intestinal muscle by 33% vs. controls. Gracia *et al.* (2016) showed *in vivo* by using a culture-dependant technique probiotic effectively reduced caecal population of the pathogenic microbes *Campylobacter jejuni*. When *Lactobacilli* were given orally to one-day-old chicks, it results in decrease mortality 60% to 30% due to necrotic enteritis. Eggs production also tested for probiotic use; Atela *et al.* (2018) showed that a mixed species of *Lactobacillus acidophilus, L. casei, Bifidobacterium thermophilus, and Enterococcus faecium*, increased the size of an egg and reduced the cost of feed in layer birds. The use of lactobacilli probiotic administration, result in increased body weight gain (BWG) of female birds by 12% but increased FCR by 4% and mortality rate by 29% under heat stress condition. Egg production and quality of eggs increased by the use of probiotics (Jawad *et al.*, 2013). Probiotics also have effects on either innate or acquired immunity or both by directly interacting with the gut mucosal immune system. Gut defense function may strengthen by the use of *Lactobacillus*-based probiotic enhancing local cell-mediated immunity against pathogenic bacteria. Generally, probiotics can improve infection against Newcastle disease (ND) virus; infectious bursal disease (IBD) virus and red blood cells (RBCs) of sheep (Wang *et al.*, 2017).

Prebiotics: Prebiotics are feed particles that have a positive effect on the host by choosing and increasing the activity of bacteria in the later part of the intestine (Kırkpınar *et al.*, 2018). A prebiotic should have following characteristics: not be degraded or absorbed in the stomach, or small intestine should choose for beneficial commensal bacteria in the later part of the intestine, and should produce positive effects within the host after fermentation of the product (Gaggìa *et al.*, 2010). By using prebiotics only the growth of beneficial bacteria is stimulated which are normally present in the intestine that's why prebiotics have an advantage over probiotics. Fructooligosaccharides (FOS), oligofructose, mannan oligosaccharide (MOS) and inulin are used as prebiotics. Others prebiotics include gluco-oligosaccharides, stachyose, and oligochitosan (Ricke, 2018). Evolution of the prebiotic concept has been shown in table 3. The prebiotics is not very commonly used in poultry. However, now the trend has been changed to check their benefits on intestine health, bird's performance and reduction of pathological bacteria. Intestinal functions performed by prebiotics have been shown in table 4. (Chen, 2005) showed increased body weight, feed conversion ratio, the weight of carcass and decrease yolk cholesterol by administration chicory fructans to laying hens; also reduced the *Campylobacter* and *Salmonella* species. Cao *et al.* (2010) stated that in broilers the number of *C.perfringens* and the level of bacterial endotoxin reduced by feeding fructan (0.5%) as prebiotics. The standard dose for prebiotics as growth-promoter is not known; however feeding a higher level of prebiotics decreased growth rate, digestibility and metabolizable energy of chicken (Biggs *et al.,* 2007; Teng and Kim, 2018). There is also an interaction between bird sex and prebiotics. According to a study by Chen, (2005), in female birds, body weight and FCR increased by 10% and 9%, respectively by the administration of oligofructose but no positive results were seen in males. Janardhana *et al.* (2009) demonstrated immunomodulatory effects on intestine associated lymphoid tissue by supplementation with MOS and FOS, same as the antibiotic-treated group, without any effect on performance. MOS may increase the production and compete for the binding site to inhibit the entry of Gram-negative bacteria in intestine. In turkey the mitigating stress results by an *E.coli* and transport is reduced by a combination of MOS and β-glucans, the feed intake, egg weight, and disease resistance improved in laying chicken, FCR, antibodies titers against Newcastle disease and immune functions improved in broiler chickens by feeding β-glucans (Bhutto and Moshaveri, 2017).

Synbiotics: Synbiotics are a mixture of probiotics and prebiotics that give benefit to the host by favoring the survival and growth of the probiotic organism in the GIT. Synbiotics which are used are Fructo-oligosaccharides and bifidobacteria, lactitol and lactobacilli (Tavaniello *et al.,* 2019). A recent study focuses on the applications of synbiotic products in the poultry field. The average daily gain and FCR were improved by adding FOS and *B. subtilis* to the feed. Rubio, (2013) evaluated the results of synbiotic on broiler chickens. Body weight, carcass percentage, and FCR were significantly increased compared to the control group, longer villus length in duodenum and ileum were also observed. Generally, all scientist agreed that than the individual preparations, the products in combination (synbiotic product) have better positive effect (Awad *et al.,* 2009; Tavaniello *et al.,* 2019). Thus, future research is required to develop a new combination of products which have positive effects on the host.

Volatile fatty acids: Butyric acid has antibacterial and development functions in the intestinal epithelium. Butyric acid enters the Gram-negative bacterial cell membrane and results in its antimicrobial activity (Cortyl, 2014). Butyric acid is used as an alternative product to antibiotic as growth promoters in chicken production. Administration of butyric acid resulted in lower shedding of Gram-negative bacteria. The intestinal microbial infections also treated, including salmonellosis by the use of butyric acid (Fernández-Rubio *et al.,* 2009); increase performance; improve resistance to necrotic enteritis and villus length morphology change in chickens (Timbermont *et al.,* 2010).

USE OF ALTERNATIVE GROWTH PROMOTERS IN CHICKEN PRODUCTION

To maintain the effectiveness of the antibiotics the treatment using alternative products is essential. For sustaining the future of production, the feed additives are important to maintain gut welfare. However, the efficacy of feed additives has been contradictory. The positive effects of feed additives are improved FCR, innate immunity development, stimulated an immune response, and reduced mortality (Sugiharto, 2016). Alternative products remove food hazards and improved animal performance. The addition of feed additives in poultry feed reduced the production costs and more economical for producer and consumer as compared to the use of antibiotics in feed. Finally, by the use of alternative products, we can prevent many diseases (Nawab *et al.,* 2018).

Table-1: The probiotics used in poultry diet.
Adapted from (Gaggìa *et al.* 2010)

Genus	Species
Lactococcus	*L. lactis subsp. cremoris (Streptococcus cremoris)*
	L. lactis subsp. Lactis
Lactobacillus	*L. acidophilus*
	L. brevis
	L. crispatus
	L. fermentum
	L. murinus
	L. plantarum
	L. salivarius
Leuconostoc	*L. citreum*
	L. mesenteroides
Enterococcus	*E. faecalis*
	E. faecium
Bacillus	*B. cereus*
	B. licheniformis
	B. subtilis
Streptococcus	*S. infantarius*
	S. salivarius subsp. salivarius
	S. thermophilus
Kluyveromyces	*K. fragilis*
	K. marxianus
Saccharomyces	*S. cerevisiae*
	S. pastorianus
Aspergillus	*A. orizae*
	A. niger

Table-2: Expected qualities and safety criteria of probiotics.
Adapted from (Gaggìa *et al.* 2010)

Non-toxic and non-pathogenic
Accurate taxonomic identification
A normal inhabitant of the targeted species
Production of Antibacterial substances
The antagonist of pathogenic bacteria
Stimulate immune responses
Health-promoting properties
Survival, pooling and active at the destination point, which involve:
Bile and gastric juice protection
In the gastrointestinal tract remains intact
Adherence to the mucosa of epithelium
Compete with the pathogenic microbes
Genetically stability
Amenability of the strain and stability of the desired characteristics during processing, storage, and delivery
Viability at high populations
Desirable organoleptic and technological properties when included in industrial processes

Table-3: Advancement in the concept of prebiotics.
Adapted from (Bindels, Delzenne, Cani, & Walter, 2015)

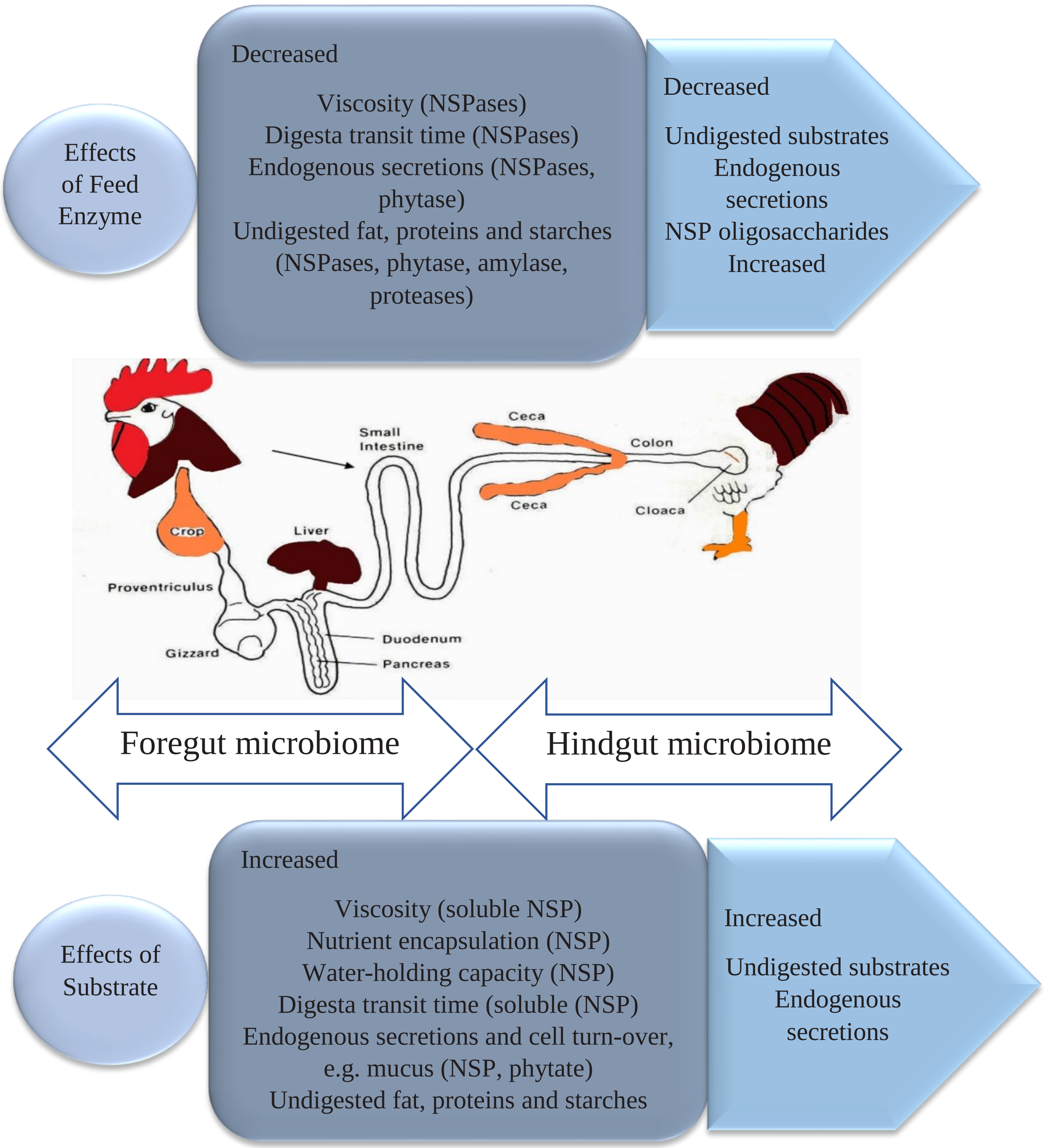
Reference	Definition	Year	The material used as prebiotics	Positive effects
(Gibson *et al.*, 2010)	Dietary prebiotic: the metabolized particles that help in particular changes in the composition of the GIT microbiota, thus giving beneficial effects upon host health	2010	Inulin FOS tGOS Lactulose Candidate	Effects on the gastrointestinal tract Focus on health with no mention of "wellbeing." Continues to adhere to "selective fermentation" in disagreement to the FAO definition
(Pineiro et al., 2008)	Nonviable food particles that give benefit to the host with modulation of gut microbiota	2008	Inulin FOS, GOS, SOS, XOS, IMO, lactulose, pyrodextrins, dietary fibers, resistant starches, other nondigestible oligosaccharides	Eliminate the selectivity qualification and the restriction to the GIT Changes reason by association Does not require the to be fermented by gut microbiota, and therefore it does not distinguish among substances that change the gut microbiota composition by inhibitory action.
(Roberfroid, 2007)	Selectively metabolized particles that provide particular changes, both in the composition and activity in the gastrointestinal microflora that	2007	Inulin tGOS	Made no changes to the definition, but specifically stated that only two dietary oligosaccharides fulfill the

	confers benefits upon host wellbeing and health			requirement for prebiotic classification
(Reid, 2003)	Selectively fermented ingredients that allow specific changes, both in the composition and activity in the gastrointestinal microflora that confer benefits upon host wellbeing and health	2004	Inulin FOS tGOS Lactulose	Extension of the original definition to include the entire GIT The first time that changes in "composition." Were included, and the term "wellbeing."
(Reid, 2003)	Non-digestible substances that provide a beneficial physiological effect on the host by selectively stimulating the favorable growth or activity of a limited number of indigenous bacteria	2003	FOS tGOS Lactulose	Extension of the original definition to include other body sites and not just the colon Changed "improves host health" with "beneficial physiological effects."
(Gibson, G. R. and Roberfroid, 1995)	A non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and activity of one or a limited number of bacteria in the colon, and thus improves host health	1995	FOS	NA

Table-4: Intestinal functions provided by prebiotics.
Adapted from (Gaggìa *et al.* 2010).

	Dietary fibers and gastrointestinal functions
Effects on upper GI tract	Decrease absorption of glucose and low glycaemic index Increased oro-caecal retention time Delayed gastric emptying Limitation to digestion Stimulation of intestinal hormonal peptides secretion Small intestinal epithelium hyperplasia
Effects on the lower GI tract	Production of fermentation end products (mainly SCFAs) Stimulation of saccharolytic fermentation Act as food for colonic microbiota Act as substrates for colonic fermentation Stimulation of colonic hormonal peptides secretion Dominate effect on stool production Acidification of the colonic content Colonic epithelium hyperplasia Regularization of stool production (frequency and consistence) Stimulation of ceco-anal transit

Figure-1: The relationship between feed enzymes and the GIT microbiome.
Adapted from (Kiarie *et al.* 2013)



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Conclusions: Feed additives improve the health and performance of birds by favoring the growth of beneficial bacteria or by minimizing the growth of pathogens. Feed additives also have effects on intestine microflora, which affect the digestive functions and improve the growth of birds by different mechanisms. However, additional new research is required in the development of new technologies for a better selection of alternative products to ensure better chicken production.

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