Abstract
Feed is one of the largest items of expenditure and accounts up to 70% of the total poultry production. In order to minimize the cost of feeding, several feed additives were used to enhance poultry production. Probiotic is one such feed additive which eliminates the use of low-dose antibiotics and lead to better and safe poultry production. It avoids the health hazards of antimicrobials to human as well as poultry. Probiotics are live microbial food/feed ingredients that have a beneficial effect on health and stimulate the growth of beneficial microorganisms and reduces the amount of pathogens, thus improving the intestinal microbial balance of the host and lowering the risk of gastro-intestinal diseases. They are “mono or defined mixed culture of live microorganisms which when applied, beneficially affect the host by improving the properties of the indigenous micro biota”. Probiotics improve feed intake, growth performance, meat quality, egg production, and egg quality. Probiotic have cholesterol lowering potential, antimutagenic, antihypertensive, anti-osteoporosis, and immunomodulatory effects. However, contra indicatory effect of probiotics on various parameters has also been observed by a number of researchers. These probiotic are prepared from various species i.e. Lactobacillus bulgaricus, L. acidophilus, L. casei, L. helveticus, L. plantarum, L. casei, Streptococcus thermophilus, Enterococcus faecium, Enterobacteria faecalis, Bifidobacteria species, Saccharomyces cerevisiae, Toulopsis sphaerica etc. and Lactobacillus, Bifidobacterium, Leuconostoc, Enterococcus, Lactococcus, Bacillus, Saccharomyces, Aspergillus and Pediococcus species are some commonly used species in poultry production. However, an accurate dosage of administration has yet to be established despite the wide-use of probiotics. In this review, the history, function, characteristics, species, mode of action and the effect of probiotics on different parameters in poultry is discussed.

Keywords: Broiler, probiotic, feed additives

1. Introduction
The use of antibiotics in poultry feed as growth promoter and as therapeutic agent has shown major advances from the year 1950. However, its use as growth promoter in farm animals has been restricted in many countries around the world because of growing concern about the development of resistance against bacterial populations and the disturbance to indigenous gut flora.[19, 2, 15]. Consequently, there is a growing interest in finding viable alternatives for growth enhancement and disease prevention in the poultry.[1, 11]. Thus, the researchers have been compelled to look for some alternative sources which could fulfill the desired goals of feed additives in animal production.[61]. In animal nutrition, Probiotics are defined as viable microorganisms used as feed additives, which lead to beneficial effects for the host by improving its microbial balance.[19]. Probiotics stimulates the growth of beneficial microorganisms and reduces the amount of pathogens thus improving the intestinal microbial balance of the host. Probiotics are reported to have also antimutagenic, anticarcinogenic, hypcholesterolemic, antihypertensive, anti-osteoporosis, and immune modulatory effects.[7]. Probiotics alleviates the problem of lactose intolerance, the enhancement of nutrients bioavailability, and prevention or reduction of allergies in susceptible individuals.[31, 7]. Probiotic bacteria have also been shown to produce molecules with antimicrobial activities, such as bacteriocins, that target specific pathogens, or even inhibit the adhesion of pathogens or the production of pathogenic toxins.[56, 53]
2. What are probiotics?
Probiotic are defined as: “a mono or mixed culture of live micro-organisms which when applied to animal or humans, benefitably affect the host by improving the properties of the indigenous gastrointestinal microbiota, but restricted to products that (a) contain live micro-organisms (e.g.: freeze dried cells or fresh or fermented product), (b) improve the health and well-being of animals or man (including growth promotion of animals) and (c) can have their effect on all host mucosal surfaces, including the mouth and gastrointestinal tract (e.g: applied in food, pill, or capsular form), the upper respiratory tract (e.g: applied as an aerosol) [27]. The definition is very broad and provides a basis for the use of numerous bacteria and yeast for enhancement of health and well-being in host animals.

3. History of Probiotics
The thought that intestinal bacteria played a role in maintenance of health [42], while studying on “lactic acid bacteria” in fermented milk products and their use to increase longevity and maintenance of youthful vigour in humans. It was reported that total exclusion of S. typhimurium from maggots of blow flies, although not related to either human or food animals, demonstrated that one species of bacterium more vigorously competed for receptor sites in the intestinal tract than did another species. It was observed that S. typhimurium would only survive if there was a reduction or elimination of normal intestinal microflora. Since, that time, several terms have been developed to describe the concept of competitive exclusion through the use of defined probiotics or undefined mixtures from adult chickens [24]. In some European countries, faecal and caecal contents have been used to induce competitive exclusion in growing poultry [76]. In recent years, defined cultures have become increasingly important for use as probiotics. Before development of these products for the poultry industry, there were numerous Probiotic products with either single or multiple organism composition. The in-ovo and ex-ovo use of Lactobacillus reuteri in poultry showed that it was product that has the unique distinction of being the only Probiotic that can be applied directly to the chicken [14].

Table 1: Desirable characteristics and function of Probiotics applied to poultry.

<table>
<thead>
<tr>
<th>Desirable Probiotic Characteristics</th>
<th>Desirable Probiotic Function</th>
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<tbody>
<tr>
<td>Host</td>
<td>Exclude (prevent colonization) or kill pathogenic bacteria</td>
</tr>
<tr>
<td>Non-pathogenic</td>
<td>Stimulate the immune system</td>
</tr>
<tr>
<td>Tolerate processing and storage</td>
<td>Reduce inflammatory reactions</td>
</tr>
<tr>
<td>Resist gastric acids and bile salts</td>
<td>Enhance animal performance</td>
</tr>
<tr>
<td>Readily bind to epithelium and mucus</td>
<td>Decrease carcass contamination</td>
</tr>
<tr>
<td>Persistent viability in Gastrointestinal tract</td>
<td>Increase production of volatile fatty acids</td>
</tr>
<tr>
<td>Produce inhibitory substances against other bacteria</td>
<td>Increase Vitamin B synthesis</td>
</tr>
<tr>
<td>Alter microbial activity</td>
<td>Improve nutrient absorption</td>
</tr>
<tr>
<td>Modulate immune response</td>
<td>Decrease diarrhoea</td>
</tr>
</tbody>
</table>

4. Species commonly used as Probiotics
Important species used as Probiotics are:
- **Aspergillus oryzae**
- **Bacillus subtilis**
- **Bifidobacterium spp.**
- **E. coli**
- **Enterococcus faecalis**
- **Enterococcus faecium**
- **L. acidophilus**
- **L. bulgaricus**
- **L. casei**
- **L. helveticus**
- **L. plantarum**
- **L. salivarius**
- **Saccharomyces acidophilum**
- **Saccharomyces cerevisiae**
- **Streptococcus thermophilus** [43, 77, 9, 26],

Just as not all strains of bacteria are the same, not all Probiotics are the same. Probiotic bacteria are either anaerobic or facultative [8]. The crop, pro-ventricular and gizzard have very few anaerobic bacteria due to the presence of oxygen and hydrochloric acid [60]. The small intestine contains a large number of facultative anaerobes such as Lactobacillus, Streptococci and anaerobes like Bacteroides and Bifidobacterium spp. Probiotics colonise three different regions within the gastrointestinal tract; enterocytes, caecal and colonic epithelium [22] and the most heavily colonised region of the gastrointestinal tract is the colon and caecum with $10^{10}$ to $10^{11}$CFU/ml [28].

5. Characteristics of Probiotics
A good Probiotic should have the following characteristics:

a. The culture should be acid and bile resistant and should contain a minimum of $30,109$CFU [9].

b. It should be strain specific. The culture should possess high survival ability and multiply fast in the conditions within the poultry gut [9].

c. The culture should not have any side effects. It should be neither pathogenic nor toxic to the host [57].

d. Be durable enough to withstand the stress of commercial manufacturing process and distribution [57].

e. The culture should have the ability to reduce pathogenic micro-organisms [57].

f. It should be able to modulate immune response [57].

6. Mode of action
The possible modes of action of Probiotics were extensively reviewed by many researchers [35, 68, 15] and they found that the two basic mechanisms by which probiotics act to maintain a beneficial microbial population include “competitive exclusion” and immune modulation.

(I) Competitive exclusion involves competition for substrates, production of antimicrobial metabolites that inhibit pathogens and competition for attachment sites.

Various mechanisms have been proposed which include:

a) The nutrients are more efficiently absorbed and less is utilized by the gut.

b) More nutrients are available to the host because of a reduced intestinal microflora.

c) There is reduction in harmful gut bacteria.

d) Production of growth suppressing toxins or metabolites is reduced.

e) Microbial de-conjugation of bile acids is decreased.

The beneficial effects of probiotics are mediated by their mechanism of action through which they inhibit the growth and proliferation of pathogenic bacteria. The most common manner of inhibition is by lowering the pH of the gut. It was
found that the production of primary metabolites, such as organic acids and hydrogen peroxide are involved in the suppression of bacterial cultures [19], and that volatile fatty acids (VFAs) are equally effective in the suppression of pathogenic gut flora [8]. Similarly it was reported that probiotic produce VFAs and organic acids as part of their natural breakdown and metabolism of nutrients in the gut digesta. These organic acids lower the pH below that is essential for the survival of pathogenic bacteria such as E.coli and Salmonella spp. Another mechanism is through the competition for adhesion sites on the intestinal epithelium, thus preventing formation of the colonies of pathogenic bacteria [25, 51, 9]. This competitive exclusion of harmful bacteria is achieved through colonisation of favourable sites of adhesion such as intestinal villus and Colonic crypts or excretion of mucins (MUC2 and MUC3) from goblet cells which inhibits the adherence of entero-pathogenic bacteria [8].

(II) One most important mechanism involved in producing beneficial impacts on the hosts body is the stimulation of the immune system with elevated humoral and cellular immune responses which is achieved through increased production of T-lymphocytes, CD-cells and antibody secreting cells, expression of pro and anti-inflammatory cytokines, interleukins, IFN-gamma, natural killer cells antibody production [55-52].

Another mode of action of probiotics is lowering the activities of the intestinal and faecal β-glucosidase and β-glucuronidase bacterial enzymes which are involved in the formation of toxins in the body [55].

7. Effect of probiotics
a) Effect on the performance

Recurrent work has been done on effect of probiotic on performance of poultry and it was revealed that inclusion of L. sporogens (100mg/kg feed) resulted in an improvement in body weight and feed conversion (FCR) [55] while probiotics (Lactobacillus and S. faecium) also improved the growth rate [43, 9] in broiler chicken. Body weight and FCR were also improved in response to feeding of Lactobacillus [17], Lactobacillus salivarius [82] based probiotics in broiler chicken. It was observed that mixture of Lactobacillus acidophilus, Lactobacillus bifidus and Streptococcus faecalis [51] and also Lactobacillus, Bifidobacterium coliforms, and Clostridium species [50, 69] significantly improved body weight gain in broiler chicken. In layers FCR was improved linearly with increasing levels (0.1% and 0.2%) of probiotics (Lactobacillus spp+ Enteroccus faecium+Bifadobacterium bifidum+ Aspergillus oryzae) [77]. Probiotic (Thexap and Saccharomyces cerevisiae) [50, 80] increased FI and showed positive effects on performance of Japanese quailsand.

Probiotics may be used single or as multisstrains, some workers acquire higher body weight in broiler flocks that received multistrain compared to control group [66]. There was rise in feed and water consumption in laying hens fed with Liquid Probiotics Mixed Culture (LPMC) containing two type microorganisms, Lactobacillus and Bacillus species [59]. It was also reported that FI (feed intake) increase in chicken fed with multisstrain probiotics compared with that in control group fed basal diet [80].

Others reported that FI, body weight (BW) and feed conversion ratio (FCR) values of different treated groups were approximately similar and lacked significance with those fed with Saccharomyces cerevisiae [64] or other probiotics [3, 78] in poultry. Probiotic supplementation doesn’t improve chickens’ feed intake [41] these inconsistent results, maybe because of type of diet ingredients which affect probiotic’s growth or their metabolites [74]. Table 2 shows that Probiotics did not consistently improve growth performance and/or mortality rate of birds.

<table>
<thead>
<tr>
<th>Researcher</th>
<th>Items</th>
<th>Control</th>
<th>Probiotics</th>
<th>Improvement (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[40]</td>
<td>BWG (g/bird)</td>
<td>1892</td>
<td>1920</td>
<td>+1</td>
</tr>
<tr>
<td></td>
<td>FCR (g/g)</td>
<td>1.75</td>
<td>1.74</td>
<td>0</td>
</tr>
<tr>
<td>[46]</td>
<td>BWG (g/bird)</td>
<td>2216</td>
<td>2237</td>
<td>+1</td>
</tr>
<tr>
<td></td>
<td>FCR (g/g)</td>
<td>1.81</td>
<td>1.78</td>
<td>+2</td>
</tr>
<tr>
<td>[47]</td>
<td>BWG (g/bird)</td>
<td>2784</td>
<td>2720</td>
<td>–2</td>
</tr>
<tr>
<td></td>
<td>FCR (g/g)</td>
<td>1.62</td>
<td>1.63</td>
<td>0</td>
</tr>
<tr>
<td>[74]</td>
<td>ADG (g/bird)</td>
<td>49.99</td>
<td>49.65</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>FCR (g/g)</td>
<td>1.83</td>
<td>187</td>
<td>+3</td>
</tr>
<tr>
<td>[37]</td>
<td>BWG (g/bird)</td>
<td>2151</td>
<td>2251</td>
<td>+5</td>
</tr>
<tr>
<td></td>
<td>FCR (g/g)</td>
<td>1.96</td>
<td>1.78</td>
<td>+9</td>
</tr>
</tbody>
</table>

Body weights gain (BWG); Feed conversion ratio (FCR); Average daily gain (ADG).

Probiotics can be used in inactivated or live form and it is observed feeding of inactivated probiotics could have similar beneficial effects compared to live probiotics, when used at a certain concentration in broiler chicks. Bacillus coagulans improved growth performance, FCR and meat quality of broiler chicken [81] while supplementation of live yeast culture of Saccharomyces cerevisiae (0.4% and 0.8%) improved FCR in layer birds [56] and dietary supplementation of live yeast culture of L. Sporogenes (100mg/kg diet) also enhanced feed efficiency in white leghorn birds [54].

b) Stimulation of immunity

Probiotic micro-organisms in the gut stimulate the immune system of birds in one of two ways [23], they can migrate through the gut wall as viable cells, where they multiply to a limited extent or the released antigens from the dead organisms are absorbed and stimulate the immune response directly. Probiotics have a positive effect on the host immune response either through the increased activity of macrophages with enhanced ability to phagocytise organisms and increased production of systemic antibody e.g. IgM and interferon or by the effect of probiotics on the host immune system which can be estimated by the level of macrophage enzyme. Many researchers found that a significantly higher antibody titres and coetaneous basophilic hypersensitivity was observed in layer birds fed diets supplemented with probiotics (100mg/kg feed) [55] and the supplementation of probiotics in layers increased cellularity of Payer’s patches in the ileum, an
indication of stimulation of the mucosal immune system which secretes immunoglobulin (IgA) in response to antigenic stimuli [48]. The effect of supplementation of inactivated L. acidophilus and L. casei enhanced the IgA titres in the serum of broiler chicks, Newcastle disease antibody titres, T-lymphocyte percentage and immune organ relative weights increased significantly. In addition it was reported that Lactobacillus based probiotic cultures improved the number of macrophages in the caecum as well as increasing the phagocytic activities against Salmonella enteritidis suggesting that probiotics have the ability to modulate the immunity of broilers [29]. It was reported that broilers fed Protexin supplemented diets had higher antibody titers against influenza disease, infectious bursal disease and Newcastle disease virus, respectively compared with the controls [16, 49 and 79]. Moreover [63], it was also reported that there was higher blood IgM against SRBC when probiotics were included in a broiler diet. However, some workers failed to show improvements in the overall broiler humoral immune status at systemic level in response to probiotic supplementation [85].

In turkeys basal diet supplemented with probiotics mixture containing Lactobacillus acidophilus, Lactobacillus casei, Enterococcus faecium and Bifidobacterium thermophilus elevated the concentration of IgG and IgM levels and the enhancement of the immunoglobulins level contributed to more positive growth performance, production and resistance of the animals towards diseases [6].

c) Effect on Parasitic status
Probiotics effectively enhanced the resistance of birds against growth depression due to coccidiosis, it was found that feeding of diets supplemented with probiotic reduced the level of Eimeria tenella and E. acervulina infection [38].

(d) Relation with Enteric infection:
In poultry Salmonella spp. contamination of poultry products primarily originates from the gastrointestinal tract specifically the caeca where there is high microbial activity. Salmonella is one of the most important foods borne zoonotic disease around the world [56]. Poultry meat and eggs are recognized as a vehicle for human Salmonella, the application of probiotics as a tool for preventing this disease was actively explored [10]. Probiotics have been extensively used to control pathogenic Salmonella in chickens to reduce mortality [5]. Live yeast culture of Saccharomyces cerevisiae (0.4% and 0.8% level) decreased the intestinal load of Escherichia coli, Klebsiella spp, Staphylococcus spp, Micrococcus spp, Campylobacter spp and Clostridium perfringens in layers [20]. Further the immunological properties of probiotics have been extensively studied demonstrating that certain Lactobacilli spp augment systemic and mucosal immunity against enteropathogens leading to the production of secretory IgA [60]. Based on this mechanism, probiotics have been tested for their efficacy at controlling Salmonella colonization in broilers and the results are positive and constant Table 3.

<table>
<thead>
<tr>
<th>Researcher</th>
<th>Number of chicks</th>
<th>Reduction (%) in the colonization</th>
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</thead>
<tbody>
<tr>
<td>[29]</td>
<td>840</td>
<td>60</td>
</tr>
<tr>
<td>[71]</td>
<td>210</td>
<td>44</td>
</tr>
<tr>
<td>[18]</td>
<td>344</td>
<td>50</td>
</tr>
<tr>
<td>[4]</td>
<td>Six commercial blocks</td>
<td>31</td>
</tr>
<tr>
<td>[10]</td>
<td>720</td>
<td>39</td>
</tr>
</tbody>
</table>

Probiotics are also used for the prevention of Campylobacter infections in poultry. C. jejuni is considered to be one of the major causes of food borne bacteria and selection of bacteria from chicken is able to produce Anti- C. jejuni metabolites [12]. It was observed that on feeding 250mg of purified bacteriocins (per kg feed) to broiler chicks the bacteriocins obtained from Lactobacillus salivarius and Paenibacillus polymyxa substantially reduced C. jejuni colonisation in live birds [72]. In addition Bifadebacterium longum possesses high probiotic properties and marked anti-campylobacter activities both in vivo and in vitro and is an excellent feed additive for poultry for the reduction of food bone campylobactera infection in humans [63]. Numerous studies showed that probiotics can exert antimicrobial effect against pathogenic bacteria via production of metabolites such as short chain fatty acids (SCFAs) and bacteriocins. Increased concentration of butyric acid has been demonstrated to reduce Salmonella infection in poultry animals whereas elevated concentration of SCFAs as a result of probiotic Bacillus subtilis effectively reduced coliform counts while increased population of Lactobacillus in broiler chickens [75]. C. perfringens infections can be reduced or abolished by using natural feed additives, such as probiotics (yeasts or bacteria), plants/herbs [17], molecules of plant origin : for example, essential oils [73] or Annatto extracts [20], organic acids [23, 73], enzymes [32, 18], lysozyme [39], or molecules of microbial origin, such as yeast extract and antimicrobial peptides. These beneficial micro-organisms possess certain favourable characteristics that allow for the expression of several mechanisms that prevent pathogen from colonizing the intestinal tract and these mechanisms are listed as follows:

1. Creation of micro-ecology that is hostile to other bacterial species.
2. Elimination of available receptor sites.
3. Production and secretion of antimicrobial metabolites and
4. Competition for essential nutrients.

8. Stress factors affecting probiotic performance
Use of probiotics for poultry production is not without risks and limitations. There are many stress factors in the environment of newly hatched poultry species that could reduce the effectiveness of the maternal antibody defence mechanism and normal colonization of the gut by beneficial micro-organisms effectively allowing the colonization of pathogens during the early post-hatch stage. This seems to be somewhat ironic because there is evidence that probiotic can limit the consequences of exposure to stressors of many types. Some stress factors and causes of the stress are listed in Table 5. The factors listed show that there are high probabilities that newly hatched chicken will face a situation in commercial as well as in experimental settings that will alter the development of natural gut associated beneficial micro-organisms.
17. Engberg RM, Grevesen K, Ivarsen E, Fretté X, Christensen LP, Højberg O. The effect of Artemisia annua on broiler performance, on intestinal microbiota and on the course of a Clostridium perfingens infection applying a necrotic enteritis disease model. Avian
Pathology. 2012; 41:369-376.


