



E-ISSN: 2320-7078

P-ISSN: 2349-6800

JEZS 2018; 6(3): 1293-1300

© 2018 JEZS

Received: 27-03-2018

Accepted: 28-04-2018

Madeeha Untoo

Division of Livestock Production and Management, Faculty of Veterinary Sciences and Animal Husbandry Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shuhama, Alusteng, Srinagar, Jammu & Kashmir, India

MT Bandy

Division of Livestock Production and Management, Faculty of Veterinary Sciences and Animal Husbandry Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shuhama, Alusteng, Srinagar, Jammu & Kashmir, India

Insha Afzal

Division of Livestock Production and Management, Faculty of Veterinary Sciences and Animal Husbandry Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shuhama, Alusteng, Srinagar, Jammu & Kashmir, India

S Adil

Division of Livestock Production and Management, Faculty of Veterinary Sciences and Animal Husbandry Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shuhama, Alusteng, Srinagar, Jammu & Kashmir, India

IA Baba

Division of Livestock Production and Management, Faculty of Veterinary Sciences and Animal Husbandry Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shuhama, Alusteng, Srinagar, Jammu & Kashmir, India

Asra Khurshid

Division of Livestock Production and Management, Faculty of Veterinary Sciences and Animal Husbandry Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shuhama, Alusteng, Srinagar, Jammu & Kashmir, India

Correspondence**Madeeha Untoo**

Division of Livestock Production and Management, Faculty of Veterinary Sciences and Animal Husbandry Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shuhama, Alusteng, Srinagar, Jammu & Kashmir, India

Review: Potential of probiotics in poultry production

Madeeha Untoo, MT Bandy, Insha Afzal, S Adil, IA Baba and Asra Khurshid

Abstract

Feed is one of the largest items of expenditure and accounts up to 70% of the total poultry production, there is also constant increase in the cost of poultry feed ingredients. 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, anticarcinogenic, hypocholesterolemic, 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. salivarius*, *L. plantarum*, *L. faecalis*, *Streptococcus thermophilus*, *Enterococcus faecium*, *Enterobacteris faecalis*, *Bifidobacteria species*, *Saccharomyces cerevisiae*, *Touloopsis 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, hypocholesterolemic, 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 [36, 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, beneficially 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 completed 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.

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

[67, 70, 33, 44, 58]

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. lactis*
- *L. plantarum*
- *L. salivarius*
- *Saccharomyces acidophilum*
- *Saccharomyces cerevisiae*
- *Streptococcus thermophiles* [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-ventriculus 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:

- The culture should be acid and bile resistant and should contain a minimum of 30,109CFU [9].
- It should be strain specific. The culture should possess high survival ability and multiply fast in the conditions within the poultry gut [9].
- The culture should not have any side effects. It should be neither pathogenic nor toxic to the host [57].
- Be durable enough to withstand the stress of commercial manufacturing process and distribution [57].
- The culture should have the ability to reduce pathogenic micro-organisms. [57]
- 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:

- The nutrients are more efficiently absorbed and less is utilized by the gut.
- More nutrients are available to the host because of a reduced intestinal microflora.
- There is reduction in harmful gut bacteria.
- Production of growth suppressing toxins or metabolites is reduced.
- 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 [34].

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 (*L.acidophilus* 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+ Enterococcus faecium+Bifadobacterium bifidum+ Aspergillus oryzae*) [77]. Probiotic (*Thepax* and *Saccharomyces cerevisiae*) [50, 80] increased FI and showed positive effects on performance of Japanese quailsand.

Probiotics may be used single or as multistrains, 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 multistrain 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 2: Growth performance and/or mortality rate of birds to probiotic supplementation

Researcher	Items	Control	Probiotics	Improvement (%)
[40]	BWG (g/bird)	1892	1920	+1
	FCR (g/g)	1.75	1.74	0
[46]	BWG (g/bird)	2216	2237	+1
	FCR (g/g)	1.81	1.78	+2
[47]	BWG (g/bird)	2784	2720	-2
	FCR (g/g)	1.62	1.63	0
	Mortality (%)	7.02	4.76	+32
[74]	ADG (g/bird)	49.99	49.65	0
	FCR (g/g)	1.93	1.87	+3
	Mortality (%)	8.84	7.27	+18
[37]	BWG (g/bird)	2151	2251	+5
	FCR(g/g)	1.96	1.78	+9

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 [26] 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 [21], 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 [45]. 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 food 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 [26]. 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 [62]. 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 3: Effectiveness of probiotics in the prevention of *Salmonella* colonization in broilers in research station and/or commercial.

Researcher	Number of chicks	Reduction (%) in the colonization
[29]	840	60
[71]	210	44
[56]	344	50
[4]	Six commercial blocks	31
[30]	720	39

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 *Bifidobacterium 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 borne *campylobacteria* infection in humans [65]. 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

pathogens 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.

Table 5: Factors that limit effectiveness of probiotics in poultry

Stress factors affecting probiotic performance	Causes of stress
Nutritional	Improper formulation of diets. Poor quality proteins and other nutrients. Poor water quality. Nutrient degradation. Molds and mycotoxins. Other toxic substances.
Environmental	Excess cold. Excessive heat. High levels of chlorine or fluorine in drinking water. Excessive humidity. Ammonia. Poor ventilation. Wet litter. Excessive dry litter. Lack of maintenance of water supply. Pathogenic microbes in overwhelming number.
Physical and Immunological	Poor chick quality. Immunological diseases.
Managerial	Setting of dirty eggs. Hatching too early. Late removal from hatcher. Poor beak trimming. Toe trimming. Overcrowding. Vaccination. Poor disinfection. Poor litter management. Cannibalism. Lack of removal of dead birds, interrupted feed and water supply.
Use of Antibiotics	Uncontrolled antibiotic use. Antibiotic destruction of normal intestinal microbes. Non-specific enteritis of viral origin.
Lack of association with mother hens	Hatchery supplies chicks that have never been on the ground with the mother hen require longer times for development of normal intestinal microbial population.

[15]

9. Conclusion

The use of antibiotics in poultry feed as growth promoter has been reduced due to the development of resistance against bacterial population. The probiotics are considered as alternative feed additives to antibiotics and can be defined as microbial feed supplements.

The beneficial effects of probiotics are as a result of improved immune function, better feed utilization, absorption of nutrients, resistance to infectious diseases and beneficial changes that occur in the intestinal.

Although, the beneficial effects of probiotics have been documented but the exact mechanism through which they produce these effects are still not exactly clear. There by warranting further research in this region.

10. References

1. Al-Shami MA, Salih ME, Abbas TE. Effects of dietary inclusion of alfalfa (*Medicago sativa* L.) leaf meal and Xylam enzyme on laying hens' performance and egg quality. *Research Opinions in Animal & Veterinary Sciences*. 2011; B1:754-759.
2. Ayed MH, Ghaoui F. Efficiency of supplementing *Saccharomyces cerevisiae* var. *ellipsoideus* for improved

growth performance and carcass yield in broilers. *Research Opinions in Animal & Veterinary Sciences*. 2011; 1:371-374.

3. Babazadeh D, Vahdatpour T, Nikpiran H, Jafargholipour MA, Vahdatpour S. Effects of probiotic, prebiotic and synbiotic intake on blood enzymes and performance of Japanese quails (*Coturnix japonica*). *Indian Journal of Animal Sciences*. 2011; 81(8):870-874.
4. Blankenship LC, Bailey JS, Cox NA, Stern NJ, Brewer R, Williams O. Two-step mucosal competitive exclusion flora treatment to diminish salmonellae in commercial broiler chickens. *Poultry Science*. 1993; 72:1667-1672.
5. Callaway TR, Edrington TS, Anderson RC, Harvey RB, Genovese KJ, Kennedy CN *et al*. Probiotics, prebiotics and competitive exclusion for prophylaxis against bacterial disease. *Animal Health Research Reviews*. 2008; 9:217-225.
6. Cetin N, Güçlü BK, Cetin E. The effects of probiotic and mannanoligosaccharide on some haematological and immunological parameters in turkeys. *Journal of Veterinary Medicine Series A*. 2005; 52:263-267.
7. Chiang SS, Pan TM. Beneficial effects of *Lactobacillus paracasei* subsp. *paracasei* NTU 101 and its fermented products. *Applied Microbiology and Biotechnology*. 2012; 93(3):903-16.
8. Chichlowski MJ, Croom BW, McBride L, Daniel G, Davis, Koci MD. Direct-fed microbial Prima Lac and salinomycin modulate whole-body and intestinal oxygen consumption and intestinal mucosal cytokine production in the broiler chick. *Poultry Science*. 2007; 86:1100-1106.
9. Choudhari A, Shinde S, Ramteke BN. Prebiotics and probiotics as health promoter. *Veterinary World*. 2008; 1:59-61.
10. Cox A, Pavic JM. Advances in enteropathogen control in poultry production. *Journal of Applied Microbiology*. 2009; 108:745-755.
11. Dibaji SM, Seidavi A, Asadpour L. Effect of dietary inclusion of the synbiotic Biomin IMBO on broilers' some blood metabolites. *Research Opinions in Animal and Veterinary Sciences*. 2012; 2:10-13.
12. Doyle MP, Schoeni JL. Isolation of *Campylobacter jejuni* from retail mushrooms. *Applied and Environmental Microbiology*. 1986; 51:449-450.
13. Eckert NH, Lee JT, Hyatt D, Stevens S, Anderson MS, Anderson PN *et al*. Influence of probiotic administration by feed or water on growth parameters of broilers reared on medicated and nonmedicated diets. *Journal of Applied Poultry Research*. 2010; 19:59-67.
14. Edens FW, Parkhurst CR, Casas IA, Dobrogosz WJ. Principles of ex ovo competitive exclusion and in ovo administration of *Lactobacillus reuteri*. *Poultry Science*. 1997; 76(1):179-196.
15. Edens FW. An alternative for antibiotic use in poultry: probiotic. *Revista Brasileira de Ciência Avícola*. 2003; 5(2):75-97.
16. El-Baky AAA. Clinicopathological and immunological effects of multistrain probiotic on broiler chicken vaccinated against avian influenza virus. *Global Vet*. 2013; 10:534-541.
17. Engberg RM, Grevsen 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 perfringens* infection applying a necrotic enteritis disease model. *Avian*

- Pathology. 2012; 41:369-376.
18. Engberg RM, Hedemann MS, Steinfeldt S, Jensen BB. Influence of whole wheat and xylanase on broiler performance and microbial composition and activity in the digestive tract. *Poultry Science*. 2004; 83:925-938.
 19. Fuller R. Probiotics in man and animals. A review. *Journal of Applied Bacteriology*. 1989; 66:365-378.
 20. Galindo-Cuspinera V, Westhoff DC, Rankin SA. Antimicrobial properties of commercial annatto extract against selected pathogenic, lactic acid, and spoilage microorganisms. *Journal of Food Protection*. 2003; 66:1074-1078.
 21. Ganguly S. Supplementation of prebiotics, probiotics and acids on immunity in poultry feed: a brief review. *World Poultry Science Journal*. 2013; 69:639-648.
 22. Gaskin H. The commensal microbiota and developments of enterocytes defense in the mammalian intestine. 9th International Symposium Digestive Physiology of Pigs. 2003; 1:57-71.
 23. Geier MS, Mikkelsen LL, Torok VA, Allison GE, Olnood CG, Boulianne M *et al.* Comparison of alternatives to in-feed antimicrobials for the prevention of clinical necrotic enteritis. *Journal of Applied Microbiology*. 2010; 109:1329-1338.
 24. Greenberg B. Salmonella suppression by known populations of bacteria in flies. *Journal of Bacteriology*. 1969; 99(3):629-635.
 25. Guillot JF. Probiotic feed additives. *Journal of Veterinary Pharmacology*. 2003; 26: 52-55.
 26. Hassanein SM, Soliman NK. Effect of Probiotic (*Saccharomyces cerevisiae*) Adding to Diets on Intestinal Microflora and Performance of Hy-Line Layers Hens. *Journal of American Science*. 2010; 6:159-169.
 27. Havenaar R, Huis In't Veld, MJH. Pro-biotics: A general view. In: Lactic acid bacteria in health and disease (Ed.: Wood, J.B.J.). Elsevier Applied Science Publishers, Amsterdam. 1992, 1.
 28. Heczko U, Abe A, Finlay BB. Segmented filamentous bacteria prevent colonization of enteropathogenic *Escherichia coli* O103 in rabbits. *Journal of Infectious Diseases*. 2000; 181:1027-1033.
 29. Higgins JP, Higgins SE, Vicente JL, Wolfenden AD, Tellez G, Hargis BM. Temporal Effects of Lactic Acid Bacteria Probiotic Culture on Salmonella in Neonatal Broilers. *Poultry Science*. 2007; 86:1662-1666.
 30. Hinton M, Mead GC. Salmonella control in poultry: the need for the satisfactory evaluation of probiotics for this purpose. *Letters in Applied Microbiology*, 1991; 13(2):49-50.
 31. Isolauri E, Sütas Y, Kankaanpää P, Arvilommi H, Salminen S. Probiotics: Effects on immunity. In *American Journal of Clinical Nutrition*. 2004; 73: 444-450.
 32. Jackson ME, Anderson DM, Hsiao HY, Mathis GF, Fodge DW. Beneficial effect of beta-mannanase feed enzyme on performance of chicks challenged with *Eimeria* sp. and *Clostridium perfringens*. *Avian Diseases*. 2003; 47:759-763.
 33. Jenkins DJA, Kendall CWC, Vuksan V. Inulin, oligofructose, and intestinal function. *Journal of Nutrition*. 1999; 129(7):1431S-1433S.
 34. Jin LZ, Ho YW, Abdulla N, Jalaludin S. Digestive and bacterial enzyme activities in broilers fed diets supplemented with *Lactobacillus* cultures. *Poultry Science*, 2000; 79:886-891.
 35. Jin LZ, Ho YW, Abdullah N, Jalaludin S. Probiotics in poultry: Mode of action. *Worlds Poultry Science Journal*. 1997; 53:351-368.
 36. Joerger RD. Alternatives to antibiotics: Bacteriocins, antimicrobial peptides and bacteriophages. *Poultry Science*. 2003; 82, 640-647.
 37. Kalavathy R, Abdullah N, Jalaludin S, Ho YW. Effects of *Lactobacillus* cultures on growth performance, abdominal fat deposition, serum lipids and weight of organs of broiler chickens. *British Poultry Science*. 2003; 44:139-144.
 38. Lee SH, Lillehoj HS, Dalloul RA, Park DW, Hong YH, Lin JJ. Influence of pediococcus-based probiotic on coccidiosis in broiler chickens. *Poultry Science*. 2007; 86:63-66.
 39. Liu D, Guo Y, Wang Z, Yuan, J. Exogenous lysozyme influences *Clostridium perfringens* colonization and intestinal barrier function in broiler chickens. *Avian Pathology*. 2010; 39:17-24.
 40. Liu JR, Lai SF, Yu B. Evaluation of an intestinal *Lactobacillus reuteri* strain expressing rumen fungal xylanase as a probiotic for broiler chickens fed on a wheat-based diet, *British Poultry Science*. 2007; 48(4):507-514.
 41. Mansoub NH. Effect of Probiotic Bacteria Utilization on Serum Cholesterol and Triglycerides Contents and Performance of Broiler Chickens. *Global Veterinaria*. 2010; 5(3):184-186.
 42. Metchnikoff E. *The Prolongation of Life*. Heinemann, London, UK, 1907.
 43. Mohan B, Kadirvel R, Natarajan A, Bhaskaran M. Effect of probiotic supplementation on growth, Nitrogen utilization and serum cholesterol in broilers. *British Poultry Science*. 1996; 37:395-401.
 44. Monsan P, Paul F. Oligosaccharide feed additives. In: Wallace RJ, Chesson A, editors. *Biotechnology in Animal Feeds and Animal Feeding*. VCH, New York, 1995, 233-245.
 45. Mountzouris KC, Tsitsirikos P, Palamidi I, Arvaniti A, Mohnl M, Schatzmayr G *et al.* Effects of probiotic inclusion levels in broiler nutrition on growth performance, nutrient digestibility, plasma immunoglobulins, and cecal microflora composition. *Poultry Science*. 2010; 89:58-67.
 46. Mountzouris KC, Tsitsirikos P, Kalamara E, Nitsch S, Schatzmayr G, Fegeros K. Evaluation of the Efficacy of a Probiotic Containing *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, and *Pediococcus* Strains in Promoting Broiler Performance and Modulating Cecal Microflora Composition and Metabolic Activities. *Poultry Science*. 2007; 86-2:309-317.
 47. Murry AC, Hinton AJ, Buhr RJ. Effect of botanical probiotic containing *Lactobacilli* on growth performance and populations of bacteria in the ceca, cloaca, and carcass rinse of broiler chickens. *International Journal of Poultry Science*. 2006; 5(4):344-350.
 48. Nahashon SN, Nakau SS, Mirosh LW. Production variables and nutrient retention in single comb white leghorn laying pullets fed diets supplemented with direct-fed microbials. *Poultry Science*. 1994; 73:1699-1711.
 49. Naseem S, Rahman SU, Shafee M, Sheikh AA, Khan A. Immunomodulatory and growth-promoting effect of a probiotic supplemented in the feed of broiler chicks vaccinated against infectious bursal disease. *Brazilian Journal of Poultry Science*. 2012; 14:109-113.

50. Nikpiran H, Vahdatpour T, Babazadeh D, Vahdatpour S. Effects of *Saccharomyces Cerevisiae*, Thepax and Their Combination on Blood Enzymes and Performance of Japanese Quails (*Coturnix Japonica*). Journal of animal and plant sciences. 2013; 23(2):369-375.
51. O'dea EE, Fassenko GM, Allison GE, Korver DR, Tannock GW, Guan LL. Investigating the Effects of Commercial Probiotics on Broiler Chick Quality and Production Efficiency. Poultry Science. 2006; 85:1855-1863.
52. Oyetayo VO, Oyetayo FL. Potential of probiotics as biotherapeutic agents targeting the innate immune system. African Journal of Biotechnology, 2005; 4(2):123-127.
53. Pan D, Yu Z. Intestinal microbiome of poultry and its interaction with host and diet. Gut. Microbes. 2014; 5:108-119.
54. Panda AK, Rao SSR, Raju MVLN, Sharma SS. Effect of probiotic (*Lactobacillus sporogenes*) feeding on egg production and quality, yolk cholesterol and humoral immune response of White Leghorn layer breeders. Journal of the Science of Food and Agriculture. 2008; 88:43-47.
55. Panda AK, Reddy MR, Rama SV, Praharaj NK. Production performance, serum/ yolk cholesterol and immune competence of White Leghorn layers as influenced by dietary supplementation with probiotic. Tropical Animal Health and Production. 2003; 35:85-94.
56. Pascual M, Hugas M, Badiola JI, Monfort JM, Garriga M. *Lactobacillus salivarius* CTC2197 prevents *Salmonella enteritidis* colonization in chickens. Applied Environmental Microbiology. 1999; 65:4981-4986.
57. Patterson J Burkholder K. Application of prebiotics and probiotics in poultry production. Poultry Science. 2003; 82:627-631.
58. Piva A. Non-conventional feed additives. Journal of Animal and Feed Science. 1998; 7(1):143-154.
59. Raka Pambuka S, Sjojfan O, Ekaradiati L. Effect of Liquid Probiotics Mixed Culture Supplements through Drinking Water on Laying Hens Performance and Yolk Cholesterol. Journal of World's Poultry Research. 2014; 4(1):05-09.
60. Rastall R. Bacteria in the gut: Friends and foes and how to alter the balance. Journal of Nutrition. 2004; 134:2022-2026.
61. Rehman S, Durrani FR, Chand N, Khan RU, Fawad Ur Rehman. Comparative efficacy of different schedules of administration of medicinal plants infusion on hematology and serum biochemistry of broiler chicks. Research Opinions in Animal & Veterinary Sciences. 2011; 1:8-14.
62. Revollo L, Ferreira AJP, Mead GC. Prospects in *Salmonella* Control: Competitive Exclusion, Probiotics, and Enhancement of Avian Intestinal Immunity. Journal of Applied Poultry Research. 2006; 15:341-351.
63. Rhee KJ, Sethupathi P, Driks A, Lanning DK, Knight KL. Role of commensal bacteria in development of gut-associated lymphoid tissues and preimmune antibody repertoire. Journal of Immunology. 2004; 172:1118-1124.
64. Saadia MH, Nagla KS. Effect of Probiotic (*Saccharomyces cerevisiae*) Adding to Diets on Intestinal Microflora and Performance of Hy-Line Layers Hens. Journal of American Science. 2010; 6(11):159-169.
65. Santini C, Baffoni L, Gaggia F, Granata M, Gasbarri R, Di Gioia D *et al.* Characterisation of probiotic strains: An application as feed additives in poultry against *Campylobacter jejuni*. International Journal of Food Microbiology. 2010; 141: S98-S108.
66. Sherief MA, Sherief MSA. The Effect of Single or Combined Dietary Supplementation of Mannan Oligosaccharide and Probiotics on Performance and Slaughter Characteristics of Broilers. International Journal of Poultry Science. 2011; 10(11):854-862.
67. Simmering R, Blaut M. Pro- and pre-biotics- the tasty guardian angels? Applied Microbiology and Biotechnology. 2001; 55(1):19-28.
68. Simon O, Jadamus A, Vahjen W. Probiotic feed additives, effectiveness and expected modes of action. Journal of Animal and Feed Sciences. 2001; 10:51-67.
69. Song J. Effect of a probiotic mixture on intestinal microflora, morphology and barrier integrity of broilers subjected to heat stress. American Historical Review. 2014; 119(2):581-588.
70. Stavric S, Kornegay ET. Microbial probiotics for pigs and poultry. In: Wallace RJ, Chesson A, editors. Biotechnology in Animal Feeds and Animal Feeding. VCH, New York, 1995, 205-231.
71. Stern N, Cox N, Bailey J, Berrang M, Musgrove M. Comparison of mucosal competitive exclusion and competitive exclusion to reduce *Salmonella* and *Campylobacter* spp. colonization in broiler chickens. Poultry Science 2001; 80:156-160.
72. Stern NJ, Eruslanov BV, Pokhilenko VD, Kovalev YN, Volodina LL, Perelygin VV *et al.* Bacteriocins reduce *Campylobacter jejuni* colonisation while bacteria producing bacteriocins are ineffective. Microbial Ecology in Health and Disease. 2008; 20: 74-79.
73. Timbermont L, Lanckriet A, Dewulf J, Nollet N, Schwarzer, K, Haesebrouck F *et al.* Control of *Clostridium perfringens*-induced necrotic enteritis in broilers by target-released butyric acid, fatty acids and essential oils. Avian Pathology. 2010; 39(2):117-121.
74. Timmerman HM, Veldman A, Van Den Elsen E, Rombouts FM, Beynen AC. Mortality and growth performance of broilers given drinking water supplemented with chicken specific probiotics. Poultry Science. 2006; 85(8):1383-1388.
75. Van Immerseel F, Fievez V, De Buck J, Pasmans F, Martel A, Haesebrouck F *et al.* Microencapsulated short-chain fatty acids in feed modify colonization and invasion early after infection with *Salmonella enteritidis* in young chickens. Poultry Science. 2004; 83:69-74.
76. Wierup M, Wold-Troell M, Nurmi E, Hakkinen M. Epidemiological evaluation of the *Salmonella*-controlling effect of a nationwide use of a competitive exclusion culture in poultry. Poultry Science. 1988; 67(7):1026-1033.
77. Yoruk MA, Gul M, Hayirli A, Macit M. The effects of supplementation of humate and probiotic on egg production and quality parameters during the late laying period in hens. Poultry Science. 2004; 83:84-88.
78. Yousefi M, Karkoodi K. Effect of Probiotic Thepax and *Saccharomyces cerevisiae* Supplementation on Performance and Egg Quality of Laying Hens. International Journal of Poultry Science. 2007; 6(1):52-54.
79. Zakeri A, Kashafi P. The comparative effects of five growth promoters on broiler chickens humoral immunity and performance. Journal of Animal and Veterinary

Advances. 2011; 10:1097-1101.

80. Zhang ZF, Kim IH. Effects of multistrain probiotics on growth performance, apparent ileal nutrient digestibility, blood characteristics, cecal microbial shedding, and excreta odor contents in broilers. *Poultry science*. 2014; 93(2):364-370.
81. Zhou X, Wang Y, Li W. Effect of dietary probiotic, *Bacillus coagulans*, on growth performance, chemical composition, and meat quality of Guangxi Yellow chicken. *Poultry Science*. 2010; 89: 588-593.
82. Zhu NH, Zhang RJ, Wu H, Zhang B. Effects of *Lactobacillus* cultures on growth performance, xanthophyll deposition, and color of the meat and skin of broilers. *Journal of Applied Poultry Research*. 2009; 18:570-578.