Ministry Of Higher Education And Scientific Research
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College of Veterinary Medicine

EFFECT OF PROBIOTIC SUPPLEMENTATION ON SOME BACTERIAL INFECTION IN BROILER-CHICKENS

A Research

by

Mohammed Hussain Mashai

Submitted to the council of the college of Veterinary Medicine / University of AL- Qadisiyah in partial fulfillment of the requirements for the degree of Bachelor in Veterinary Medicine

Adviser

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INTRODUCTION

Poultry is one of the fastest growing segments of agriculture and veterinary sector. Like other sector of agricultural industry, major aim of this industry is also to produce maximum with minimum input. The ration is one of the largest items of expenditure in poultry production and it alone accounts to 70% of total poultry production. The constant increase in the cost of poultry feed ingredients and compounded feed is making the profit less for poultry farmers. Therefore, balanced and effective feeding is most important requisite to superior germplasm for economic poultry production. Several feed additives (as growth promoter) like antibiotics and synthetic hormone have been extensively used for improvement poultry production but due to development of antibiotic resistant bacterial strains and residual effects of these feed additives in meat and eggs, they lead to various health hazards to consumers (Kapil Jadhav, K.S. Sharma, S. Katoch, VK Sharma and B.G. Mane, 2015). Moreover, poultry industry has been looking for enhancement of production indexes and broiler growth through promoting healthcare and providing good nutrition. Researchers worldwide are working on organic alternatives due to the ban of a wide range of drugs for animal production.

Enteric diseases are an important concern to the poultry industry because of lost productivity, increased mortality and the associated contamination of poultry products for human consumption. With increasing concerns about antibiotics resistance, the ban on subtherapeutic antibiotic usage in Europe and in the United States, there is increasing interest in finding alternative to antibiotics for poultry production (Patterson and Burkholder, 2003).

From 2006 onwards, the European Union has decided to prevent

antibiotics as feed additives (Simon, 2005). Hence, probiotics have been used for alternative to antibiotic. Since 1970, probiotics used as animal feed supplements. They increase growth of animal and improve its health by increasing its resistance to disease and stimulating the immune system (Fuller, 1992). However, the health benefits will occur when probiotic microorganisms reach the intestine in sufficient numbers and in viable form. Therefore, the survival of the probiotic is required during feed processing and storage.

The balance of microflora within the digestive tract of all animals is important to their digestive process and critical to their overall health. This bacterial population is particularly significant. Although it was Pasteur who postulated that microorganisms are necessary for normal life, it has only been in the past several decades that the microflora of the alimentary tract has generated much interest amongst investigators. With the exception of pathogens and the diseases that they cause, there was little appreciation of the normal intestinal microflora.

The aim of this study was to explain the different effects of a probiotic on broiler-chickens- performance.

LITERATURE REVIEW

Environmental degradation associated with contamination from industrial wastes, as well as the widespread use of chemicals in the plant, causing the accumulation in feed various toxic substances (heavy metals, pesticide residues, etc.), which are the cause of metabolic disorders, the occurrence of various diseases in poultry and meat quality deteriorates. Thus, in this study are important endeavor to develop and apply technology and feeding birds with various feed additives which improve the performance, but also in maintaining animal health and production of good products of high quality.

In veterinary medicine, probiotics are widely used. They are ecologically un harmful preparations, do not have a side effect if used for a long time and regularly, they can replace antibiotics in the general scheme for treatment and prevention of many diseases. Probiotics produce antibacterial components, inhibit pathogenic and opportunistic microorganisms, increase the level of antibodies, increase the activity of macrophages.

The effectiveness supplementation of various probiotics different widely and depends on many factors, including the composition of the microorganisms in the preparation. In the practice, most probiotics known that contain several kinds of bacteria. The combination of biological properties of different type of microorganisms in probiotic allows increasing the effectiveness of preparations. Lactic acid bacteria, bifidobacterium, streptococci are belong to most famous microorganisms which used in production of probiotic preparations. Bifidobacterium - and lactobacilli, that producing acids, consider antagonistic to pathogenic and opportunistic microorganisms as: Escherichia coli, Proteus, Salmonella, Staphylococcus and others.

Definition

In Greek Probiotic means «for life». Probiotics are defined as live microbial food supplements, which beneficially animals health by improving its intestinal balance **(**11, 13, 33; 38, 40, 61). In other words, probiotics include viable microbial and microbial fermentation products which are beneficial to decrease the undesirable micro flora population in the gastro-intestinal tract of chicks (13)

Their efficiency was demonstrated for the treatment of gastrointestinal disorders as well as respiratory infections. In most cases, evidence for a beneficial effect was obtained by studies using animal models (114).

According to Food and Drug Administration (FDA), probiotics come under the category as Generally Recognized as Safe (GRAS) ingredients. They have no residual and harmful effects. Probiotics regulates the microbial balance in the intestine, reduce digestive upsets and prevent pathogenic gut bacteria, thereby improve live weight gain, improve feed conversion ratio, decrease mortality, increase feed conversion ratio in layers and improve egg production.

Probiotics - consisting of live or dead organisms and spores (89) and others have emerged in the last decades as some of the tools that could be potentially useful in the near future for pathogen control and poultry performance enhancement.

The use of probiotics in farm animals is based on the concept that the balance of intestinal microorganisms in healthy animals enhances resistance to diseases and is necessary for efficient digestion and maximum absorption of nutrients (31). Microbial balance can be changed, lead to increase populations of pathogenic microorganisms, which have negative effect on animal performance (77). The purpose of supplying probiotics is to prevent disturb microbial balance or to

maintenance the ideal balance between beneficial and pathogenic microorganisms.

Probiotics are microorganisms introduced orally into gastrointestinal tract (GIT) that are able to participate positively to the activity of gut microflora and therefore, to the health of its host. Most probiotic bacteria belong to the group of lactic acid bacteria (LAB) and among them lactobacilli and bifidobacteria reportedly play important role in maintaining the intestinal ecological situation and in stimulating the immune system of the host (100). Many in vitro characteristics, such as adhesion, resistance to pH, etc., are usually investigated to determine if a specific selected strain would be suitable as a probiotic (17).

Characteristics of good probiotics

Fuller (1989) listed the following as features of a good probiotic (33): An ideal probiotic should have the following characteristics (55):

1. It should be a strain, which is capable of exerting a beneficial effect on the host animal, for example increased growth or resistance to disease.
2. Non-pathogenic and non-toxic to animals and human
3. Should be present as viable cells, preferably in large numbers although the minimum effective dose is not fully defined.
4. It should be stable and capable of remaining viable for periods under storage and field conditions.
5. Ability to withstand processing and storage
6. Ability to adhere to epithelium or mucus
7. Persistency in intestinal tract
8. Ability to modulate immune response
9. Ability to produce inhibitory compounds

10. Capability of altering microbial activity

In another words, for a strain to qualify as a probiotic it must carry out certain physiological properties, mainly survival in the digestive tract, tolerance to low pH, tolerance to bile in the form of glycocholic or taurocholic acid and sodium desoxycholate (69, 114). It must also able to adhere to the intestinal mucus and epithelial cells. This is important based on the two proposed mechanisms for lactic acid bacteria's beneficial effects in the gastro-intestinal tract:

a) production of antimicrobial substances such as lactic acid and bacteriocins; and

b) adherence to the mucus, coaggregation and autoaggregation to form a barrier which blocks colonization by pathogens (26).

Types of probiotics

For many years, different kinds of probiotics have been used in the rations of animals for stimulating production and / or feed utilization efficiency.

The difference in the probiotics comes with the strain of bacteria that was used, dosage, mode of application, time of application etc. Probiotic products may contain different genera, different species, or even different strains of the same species, and not all products should be expected to work the same. Therefore, claims of efficacy should be target specific and should be made only for products that have been presented efficacious in carefully designed studies.

However, the most commonly used probiotic contains strains of lactic acid bacteria such as Lactobacillus, Bifidobacterium and Streptococcus, which rarely produce optimum results in pelleted feed. It is most likely because the lactic acid bacteria are destroyed partly and totally by the current pelleting process. Normally, lactic acid bacteria

have the optimum viability temperature in ranging of 30-37°C. while pelleting process may raise the temperature of finished feed up to 65-85°C.

Probiotics for chicken are designed for two main reasons namely:

(a) To replace beneficial organisms that is not present in the alimentary tract.

(b) To provide the chicken with the effects of beneficial organisms. Such beneficial (28).

Mode of action:

Probiotics act by six different means (76):

(a) adherence to the binding sites of the intestinal epithelium (competition with pathogenic bacteria);

(b) direct antagonism through the production of bactericidal substances;

(c) stimulus to the immune system;

(d) facilitating the digestion and absorption of nutrients;

(e) suppression of ammonia production, which might be toxic to intestinal cells; and

(f) neutralization of enterotoxins.

Probiotics are microorganisms that are supply to animals to colonize the intestinal tract and provide a better normal flora balance (33). As well as, these microorganisms are responsible for production of vitamins of the B complex and digestive enzymes, and for stimulation of intestinal mucosa immunity, increasing protection against toxins produced by pathogenic microorganisms. Probiotics bacteria have effect host body including its immune system. One of the presumed mechanisms of the

inhibitory activity of probiotics on pathogens of alimentary tract is the receptors (20, 56, 67, 70, 103). competition for the intestinal mucosa

The inhibitory effect against intestinal pathogens is mostly due to the metabolites, such as hydrogen peroxide, organic acids and bacteriocins produced by the probiotic bacteria (33). Due to the historical belief of lactic acid bacteria are commonly used in most probiotics, that they are desirable members of the intestinal microflora and are thus generally regarded as safe . A wide range of microorganisms have been used as probiotics. However, the species currently being used in probiotic preparations are Lactobacillus acidophilus, L. bulgaricus, L. casei, L. lactis, L. helveticus, L. plantarum, L. salivarius, Streptococcus thermophilus, Enterococcus faecalis, Ent. faecium, and Bifidobacterium spp. Bacillus spp. and fungi like Sacharomyces cerevisiae, Sacharomyces boulardii and Aspergillus oryzae (33, 34, 77). However, lactic acid bacteria (LAB) have attained major attention for probiotic activity and have generally been considered as good probiotic organisms (97, 108). Among lactic acid bacteria, lactobacilli are the most important (109) but not all lactic acid bacteria have the probiotic properties, and the primary O451characteristics required for the candidate probiotics are the ability to survive in the acidic conditions and establish in the gastrointestinal tract of the host .

The crop and ileum flora are mainly composed of lactobacilli in birds (32). Many lactobacillus strains isolated from different sources are being used as probiotic preparations and it is unlikely that each species/strain possesses all of the desired properties that will make it a suitable probiotic.

Throughout the last years the application of probiotics in poultry has gained considerable interest because antibiotic growth promoters (AGPs),

supplied to animal feed to enhance growth and decrease the incidence of diseases, are leaving harmful residues in poultry product.

is On the other hand, the mode of action of probiotics preparations competitive exclusion. related to the competition for attachment sites, or probiotic attach to the mucosa of The microorganisms found in the a physical barrier that closes the attachment of intestine, thus forming by the production of pathogenic bacteria (35). Probiotics play other roles the compounds and enzymes, as well as the stimulation of antibacterial and activity. In bird's immune organs by increasing phagocyte population tonsils, Peyer's patches, and the bursa of Fabricius are sites of body, cecal that tissue accumulation. These organs capture antigens lymphoid the production of the circulate in the digestive tract and that stimulate immune system B and T cells.

Several possible mechanisms have been suggested such as altering of the gut pH, maintaining protective gut mucins, selecting beneficial intestinal organisms or ones antagonistic to pathogens, enhancing nutrient uptake, enhancing fermentation acids and increasing the humoral immune response (49).

Probiotics and prebiotics may enhance health by stimulating antibody production (100).

Importance of using:

Probiotics are one of the approaches that have a potential to reduce chances of infections in birds and subsequent contamination of poultry products. Probiotic preparations have been consumed for centuries, either as natural components of foods. Amongst the most promising targets for functional foods are the gastrointestinal functions, including those that control transit time, gut habits, and mucosal motility as well as those that modulate epithelial cell proliferation.

Promising targets are also gastrointestinal functions that are associated with a balance colonic normal flora, that are associated with control of nutrient bioavailability (ions in particular), that modify alimentary immune activity, or that are mediated by the endocrine activity of the digestive system. Finally, some systemic functions such as lipid homeostasis that are indirectly influenced by nutrient digestion or fermentation represent promising targets (15, 92).

The strain of selected microorganisms, the dosage, method of preparation, and condition of animals could be partially responsible for viable microorganisms in probiotics such discrepancies. The number of has been considered a critical factor affecting the efficacy of probiotics (87).

Addition of probiotic to broiler feed resulted in significant improvement concerning hemato-biochemical parameters (71).

In lambs fed diets supplemented with probiotics observed higher blood glucose concentration this might be due to more nutrient digestibility resulting in increased precursor availability for gluconeogenesis.

Antunovic et al. (2005) reported non-significant but slightly lower glucose concentration in lambs fed diets supplemented with probiotics. Increased Hb and MCHb values might be due to the probiotics which might have increased hematopoiesis (2).

The results of study Onifade et al. (1999) observed increased Hb and MCHb concentrations in the animals fed diets containing probiotics (84). Increased WBC might be related to the production of more immune cells (60) that play an important role in defending the biological system against various diseases.

Paryad and Mahmoudi (2008) also reported higher WBC in animals fed different levels of probiotics than those fed diets without probiotics (88).

Antunovic et al. (2005) and Masek et al. (2008) observed non change serum minerals in lambs fed diets supplemented with or without probiotics (2, 73).

Usage of feed additive had positive effect on level of minerals in blood. It has been reported that probiotics improve calcium absorption from intestinal tract. Fermentation products as a result of probiotics' activity may enhance the absorption surface by accelerating proliferation in enterocytes. Furthermore short chain fatty acids and the other products of some probiotic bacteria decrease the gut pH. Therefore, calcium solubility increases and this may be related to improved calcium absorption (101).

Calcium regulates the contraction and relaxation of muscle and regulates the passage of substance into and out of the cells. So, calcium is one of the most important nutrients for aquatic species (119).

The influence of probiotics on improving intestinal calcium absorption may be related to increased expression of calcium channels in intestinal mucosa. (116) noticed that supernatant from milk fermented by Lactobacillus helveticus R389 enhanced expression of TRPV6 channels in the duodenum. Improved expression of Ca channels indicates enhancement of dietary Ca uptake capacity.

Probiotic bacteria with active bile salt hydrolase or products containing them have been suggested to lower cholesterol levels through interaction with host bile salt metabolism (21). In several studies have shown that using of probiotic has the ability to decrease cholesterol in blood (41, 109, 118).

Kamgar et al. (2013) and Newaj-Fyzul et al. (2007) found reduce uric acid in probiotics received groups. There was a significant decrease in uric acid level in probiotic groups, indicating beneficial effect of the probiotic on the kidney function (54, 80). On the other hand, certain probiotic microorganisms can utilize urea, uric acid and creatinine and other toxins as its nutrients for growth (99).

groups, circulating cholesterol In the probiotic supplemented concentration tended to decrease (84), they are showed that the including yeast to ration supplementation of innocuous microorganisms serum cholesterol, triglycerides of broiler chickens and rabbit decreased and phospholipids. Also, blood cholesterol levels of layers fed yeast were lowered than the control (93). Similar studies supplemented diets with by (23, 117) found that cholesterol level was reduced conducted inclusion of yeast into broiler chicks ration.

serum triglycerides level may be due to an A reduction in the population of lactic acid bacteria in the gastrointestinal enhance in the supplementation of *Bacillus* tract. Santose et al. (1995) have reported that chickens, in addition to lowering the *subtilis* to the ration of broiler the triglycerides concentration in the serum, the liver carcass fat, reduce effective in the carcass and suggest that this bacterium can be and decreasing the activity of acetyl coenzyme A carboxylase (the enzyme fatty acids) (99). limiting the synthesis rate of

The probiotic supplementation reduced the serum cholesterol and experiment with broiler chickens fed triglyceride significantly (9), in this between treatments for probiotic, have found a significant difference serum lipids.

probiotic supplementation significantly reduces It is reported that the cholesterol content lipids (cholesterol and triglycerides) levels the serum of the chickens (52, 53, 86).

In another study, Mannaa, F. et al. (2005) observeed that the enhance in addition of *Saccharomyces cerevisiae* caused significant the enzymatic activity serum ALT and ALP activities. The differences in probiotic interventions. may be due to animal species and In vitro and in vivo studies have demonstrated that lactic acid producing bacteria are able to inhibit poultry pathogen like Salmonella and E. coli by reducing the pH of the alimentary tract (30, 59, 64). Experimental and commercial studies conducted in the U.S.A. have organisms are able to significant reduce shown that the probiotic (44). colonization in turkeys and broilers Salmonella Proposed production benefits of probiotics include enhanced survival of chicks, reduction or prevention of gastrointestinal disorders, promoted growth rate, improved feed efficiency, promoted immune response and ammonia gas emission in broiler house etc. The application of probiotics like Lactobacillus, yeast etc. is receiving much attention. The supplementation of these substances to the ration or their introduction to animal body exploits the potential of utilization of feed and improves the efficiency of consumption of feed (82, 93). Moreover, it has been shown that *Lactobacilli* and *E. faecalis* could colonization in the protected chickens against pathogens by gastrointestinal tract (81)

Stimulation of immune system:

Immunity resulting from gut exposure to a variety of antigens, such as pathogenic bacteria and dietary protein, is significant in the defense of young animals against enteric pathogens. Dunham et al. (1993) reported that birds treated with L. reuteri exhibited longer ileal villi and deeper crypts, which are a response associated with enhanced T cell function and promoted production of anti-Salmonella IgM antibodies (24). Nahanshon

etal. (1994) found that supplementation Lactobacillus for layers rations improved cellularity of Peyer's patches in the ileum indicating a stimulation of the mucosal immunity that responded to antigenic stimuli by secreting immunoglobulin (IgA) (79).

Havenaar and Spanhaak (1994) has reported that probiotic preparations stimulate the immune system of the chickens in two ways (a) microorganisms from probiotic migrate throughout wall of the alimentary tract and proliferate to a limited extent or (b) antigen released by the dead organisms are absorbed and thus stimulate the immune system. At present it is believed that there is some relationship between the ability of strain to translocate and the ability to be immunogenic (43).

As well as, the improvement in the immune system may be by three different ways:

(a) improved macrophage activity and disturbance and enhanced ability to phagocytose microorganism or carbon particles;

(b) promoted production of antibodies usually of IgG & IgM classes and interferon (a nonspecific antiviral agent) and;

(c) improved local antibodies at mucosal surfaces such as the gut wall (usually IgA).

Different effects of probiotics

Probiotics have protective effect for animal body systems. Some authors suggest that probiotics, in particular based on bacteria B. subtilis, able to transform mycotoxins less toxigenic metabolites and block their active sites. Moreover, B. subtilis bacteria can synthesize antibiotic substances, a number of organic acids (acetic, citric, oil) and amylolytic enzymes, which help to normalize digestive function of the gastrointestinal tract, inhibit pathogens, developing on the background of weakening resistance mycotoxicosis (111).

- Effect on content of blood:

Probiotics (*Saccharomyces cerevisiae)* supplemented chickens did (86, 103, 106) but profile not show any harmful changes on blood probiotic consist of *Enterococcus faecium*) may improve health condition by increasing level; of hemoglobin, hematocrit and red blood cell count in broilers (8, 106) and also in turkey (58).

The results of Marcela *et al.* (98) showed that *Enterococcus faecium* M-74 strain did not cause significant change in total leucocytic count in turkeys. Also, Shareef and Al-Dabbagh observed similar results to that (103).

- **Effect on lipid metabolism:**

the values of Results of lipid profile showed significant reduce in all probiotic supplied groups cholesterol, triglycerides, HDL and LDL in whole period of the experiment. compared with the control during the to reduced absorption and/or synthesis of This could be attributed (89) the alimentary tract by probiotic supplementation cholesterol in *acidophilus* decreases cholesterol in the speculated that *Lactobacillus* preventing them blood by deconjugating bile salts in the intestine, thereby from acting as precursors in cholesterol synthesis. Also, authors reported that probiotics may possess the character of reducing cholesterol in the hepatic synthesis, or by deconjugating the blood by inhibition of its biliary salts (38, 66).

Moreover, the decrease of cholesterol, triglycerides, HDL and LDL in infected birds might be due to anorexia and/or defective lipid metabolism due to hepatopathy (3).

- Effect on gut tract and its lymphoid tissue:

have less developed gut-associated lymphoid Germfree animals (GALT), but gut colonization in these animals by members of tissue diversification commensal gut microbiota results in the enhancement and of the antibody-mediated immune response (91, 112). The lamina propria of immunoglobulin A (IgA)- of the gut contains a large population germfree animals has a very small number producing plasma cells, while (51). of these cells

the chicks, commensal bacteria colonize the After hatching of changes in an gastrointestinal tract and the composition of the microbiota commensal bacterial age-dependent manner (47). The predominant of the *Lactobacillus* spp., but species found in young chicks are members *Bifidobacterium* spp. predominate (1). It is over time, members of the bacteria present in chicken gut microbiota possible that commensal the immune system and have an effect on the interact with cells in GALT, which immune response. An equivalent of the mammalians lymphocytes, natural contains various cell subsets, including B and T been described to exist in killer (NK) cells, and macrophages, has hatching, a chicken's GALT lacks chickens (65). Immediately after is gradually populated by migrating mature B and T cells but lymphocytes, and after 2 week from hatching, the GALT reaches its There is little information available on the functional maturity (4). of the immune response in the chicken gut. It process of induction antigens that enter the chicken gut are taken up by epithelial appears that cells (27). M cells or specialized intestinal cells that resemble mammalian relation to the fates of However, there have been contradictory findings in to B and T lymphocytes (7). antigens and the cells that present them antigen delivery via the gut may be the Nevertheless, the outcome of

response systemically and locally (65). induction of an antibody

- **Effect on immune organs:**

- The exact mechanisms of the enhancement of immune responsiveness conferred by probiotics remain to be discovered. However, it has been shown that probiotics stimulate different subsets of immune system cells to produce cytokines, which it play a role in the induction and regulation of the immune response (14, 61, 68). The production of the mucosal IgA response is dependent on other cytokines, such as transforming growth factor (63).

Probiotics, especially lactobacilli, could modulate the systemic the antibody response to antigens in chickens (46, 57). Moreover, cytokines supplementation of probiotics preparations lead to secrete of and changes in lymphoid cells in the chicken gut, which may lead to improve immunity to *Eimeria acervulina* (18, 19). However, little is effects of probiotics on the known about the immunomodulatory response to soluble and cellular antigens induction of a systemic antibody as well as on the antibody response in the gut. Both systemically and have locally administration of probiotic bacteria or their products may immunomodulatory effects (74). Specifically, these bacteria may increase the antibody response (22, 50).

Chickens that were fed fermented liquid feed supplemented with various lactobacilli showed enhanced IgM and IgG responses to trinitrophenyl (TNP) (57).

Researchers observed that probiotics containing L. acidophilus and L. casei improved the serum IgA response to KLH, but that the treatment did not affect the IgG response to this antigen (46). In other studies, egg layer and broiler chickens treated with probiotics responded differently to TNP, with layer chickens mounting a significantly higher antibody response than broiler chickens, indicating that the genetic background of chickens plays an important role in the mediation of immunomodulatory

activities of probiotics (57). So, these findings support the idea that the immunomodulatory activities of probiotics in increasing the antibody response are highly dependent on the antigen, immunization regimen, type and number of species of bacteria found in probiotics, and genetic background of the host.

- **Different uses:**

Supplementation of the probiotic «Gress» in the rations of horses promotes the activation of metabolic processes of organism, which is accompanied by an increase in the milk productivity of mares and the daily growth rate of young animals (36).

One of the most important factors, that provide normal flora in the organism, is the maintenance of a natural biocenosis in this organism. Researchers in recent years have confirmed that the widespread use of antibiotics and chemical preparations in practical medicine leads to a change in the properties and reduction of normal intestinal microflora (95).

PROBIOTICS FOR CHICKEN

As with other mammals, the use of probiotics for poultry has developed out of our increasing understanding of the microflora of the gastrointestinal tract although an earlier observation suggested that the host and its intestinal micro flora were interdependent. This description of the intestinal microflora in adversarial terms was perpetuated by Dubos et al. (1965) who divided the indigenous microflora into the autochthonous organisms (such as *Lactobacilli* and *Bacteroides*, which had developed an evolutionary symbiotic relationship with the host) and allochthonous organisms (such as *Eschericha* and *Clostridium* which were potential pathogens). These, together with non-enteric organisms acquired from the

environment, comprised the normal intestinal flora. These descriptions are far too simplistic and must be seen as early models attempting to describe several highly complex ecosystems. For instance, microbial opportunism and true commensalisms were largely ignored. Regarding the flora as a climax community in which every niche is occupied is also patently inaccurate. Their inadequate understanding of microbial taxa at that time presumably led to regarding *Escherichia coli* as potential pathog en although many strains may be beneficial to the host and can be used in that way (25). However, these hypotheses provided an important stimulus to studying the microecology of the alimentary tract. The early models had profound effect on the development of probiotics. Many preparations currently used for poultry and other animals are based on the assumption that the early hypotheses are correct with the result that the approach to probiosis is often too simplistic.

- **Probiotics as feed additive**

Probiotics, Yeast culture and other feed additives for poultry and pigs‘ feeds have gained more attention over antibiotics in the poultry industry. Much of this interest has been generated because of increased public awareness and objection to the utilization of antibiotics as growth promoting feed additives in those industries (10, 45).

Patterson and Burkholder (2003) have observed in numerous in vivo and in vitro studies that the commensal intestinal microbiota inhibits pathogens (88). A variety of microbial species have been used as probiotics in animal feed and are mainly bacterial strains of Gram positive bacterial including *Lactobacillus acidilactici, Lactobacillus farciminis, Lactobacillus rhamnosus, Enterococcus faecium, Enterococcus mundtii, Pedicoccus acidilactici, Bacillus cereus, Bacillus licheniformis, Bacillus subtilis, Bifidobacterium, Streptococcus* and a

variety of microscopic fungi such as strains of yeast belonging to the *Saccharomyces cereevisiae* species (42, 103).

Among these species of probiotics, *Lactobacillus*, *Bacillus*, *Bifidobacterium*, yeast and *Enterococcus* have extensively been used (88).

In assessing the value of a probiotics or direct-fed microbial, Hutcheson (1987) and Guilot (2000) enumerated characteristics necessary for a probiotics to be effective. These criteria include the following (42, 48);

- Must be a normal inhabitant of the intestine,
- Must have a short regeneration time,
- Must produce antimicrobial substance (eg lactic acid, bacteriocins, etc),
- Must be durable enough to withstand the duress of commercial manufacturing, processing and distribution so the product can be delivered alive to the intestine and
- Must be free of diffusible antibiotic resistance gene, non pathogenic and non toxigenic for target species under expected conditions for use.

The most efficient probiotic bacteria are likely to be strains that are robust enough to survive the harsh physio-chemical conditions present in the gastro intestinal tract (29). The probiotic bacteria that survive usually do not colonize the intestinal mucosa for long periods of time and are generally eliminated within few days of the cessation of their ingestion (72) necessitating continuous supplementation.

Mode of administration and timing on the efficacy of probiotics

Probiotics may be administered to the host animal in a variety of ways. It may be given as a powder, tablets, liquid suspension, capsule,

paste or spray. Moreover, the amount and interval between doses may vary (12). Probiotics may be given only once or periodically at daily or weekly intervals (110). Little is known about the minimum dose required for the probiotic effect but trials in rats, humans and pigs indicate that the effect falls off after stopping of probiotic supplementation (16, 39). It therefore seems very likely that the effect obtained will be affected by the amount and frequency of dosing.

Timmerman *et al.,* (2006) underlined the importance of way and timing in the administration as main factors affecting the efficacy of the probiotics. Administration via the feed, compared to administration in the drinking water, resulted in a higher increase of average daily gain; moreover the addition of probiotics during early life is of great importance to the host because the bacteria can modulate expression of genes in intestinal epithelial cells, thus creating a favourable habitat for themselves (110).

Some side effects of probiotic

It has been well established that the intestinal microflora plays an important role in the metabolic process and immune system of the host, and probiotics help to enhance microflora. However, it can also be argued that manipulation of the normal microflora by probiotic use may theoretically increase the risk of adverse metabolic and immunomodulatory effects. Some minor adverse effects, including thirst and constipation with S. boulardii use (McFarland *et al.,* 1994), bloating and flatulence with L. rhamnosus GG use (62) nausea, vomiting, abdominal pain, rash, diarrhoea and constipation, have been reported (75). Although serious complications from probiotic use are exceedingly rare, given that probiotics are live microorganisms, it is conceivable that they may rarely result in invasive infections. There have also been several

reported cases of S. boulardii fungemia associated with probiotic use. Most cases of invasive infections associated with the use of probiotic have occurred in patients with intravenous catheters (Hennequin *et al.*, 2000) the elderly (11) and immunocompromised population (7).

CONCLUSIONS AND RECOMMENDATION :

Conclusion

The beneficial effects of probiotic preparations in poultry production have been related to different modes of action. However, it can be concluded that dietary supplementation of probiotics to birds are that it increase the utilization of proteins, intestinal tract health, feed conversion ratio, strengthen beneficial microbial populations and inhibit harmful bacterial growth in the gastrointestinal tract, counteract harmful influence of antibiotics, nutrient synthesis, stimulate immune system, decreased diarrhea and mortality.

Further, it enhance the feed conversion ratio, feed intake, body weight, lower cholesterol in blood, serum and meat, increase the tenderness and meat quality along with carcass yield. Or in another words, probiotics promoted the metabolic processes of digestion and nutrient utilization. It is believed that the enhancement in metabolism after probiotic supplementation was due to improved development of the intestine and increased microvilli height which led to the enlargement of the microvillis' absorptive surface and enabled the optimal utilization of nutrients.

So that addition of probiotcs in broiler chicken is highly beneficial for economic production of poultry.

Recommendations:

1- It is important to attempt to isolate and purificate the different active ingredients in grape seeds extracts which consider active area for further research on the memory & learning improvement

2- Use the other methods for evaluation the learning activity of plant and needed to further studies to identify the exact mechanism of action in this aspect.

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