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The Effect Of Probiotic On The Poultry Industry

A Graduation Project Submitted To The College Of Veterinary Medicine/ University Of Al-Qadisiyah In A Partial Fulfillment Of The Requirements For The Degree Of Bachelor Of Science In Veterinary Medicine And Surgery.

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بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

فَتَعَٰلَى ٱللَّهُ ٱلْمَلِكُ ٱلْحَقُّ ۗ وَلَا تَعْجَلْ بِٱلْقُرْءَانِ مِن قَبْلِ أَن يُقْضَىٰٓ
إِلَيْكَ وَحْيُهُۥ ۖ وَقُل رَّبِّ زِدْنِى عِلْمًا ﴿١١٤﴾

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Certificate of Supervisor

I certify that the project entitled (**The Effect Of Probiotic On The Poultry Industry**) was prepared by Rusul Haider Shahid under my supervision at the College of Veterinary Medicine / University of Al-Qadisiyah.

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Certificate of Department

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DEDICATION

To those with their blood, our life continued, to the martyrs of the holy jihad fatwa, to its owner ... to my dear mother and dear father, and to everyone who helped me one day.

Summary

The increase of productivity in the poultry industry has been accompanied by various impacts, including emergence of a large variety of pathogens and bacterial resistance. These impacts are in part due to the indiscriminate use of chemotherapeutic agents as a result of management practices in rearing cycles. This review provides a summary of the use of probiotics for prevention of bacterial diseases in poultry, as well as demonstrating the potential role of probiotics in the growth performance and immune response of poultry, safety and wholesomeness of dressed poultry meat evidencing consumer's protection, with a critical evaluation of results obtained to date.

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CHAPTER ONE

INTRODUCTION

Introduction

Lilly and Still Weel (1965) defined a biological enhancer as growth-stimulating agents produced from micro-organisms, consisting of two segments: pro, meaning for, and biotic, meaning life, which is the opposite of the term antibiotic **(Simmering and Blaut, 2001)** and **(Fuller, 1989)** is the first of The promoter was known to be a live microbial feed that positively affects the host, improves health and creates microbial balance inside the body. **Havenar and Huis (1992)** also defined the probiotic as a single or mixed microorganism. When used for animals, it affects the host and the original intestinal bacteria and improves health and all mucous membranes in the mouth and gastro-intestinal tract. The definition of a probiotic is very broad and provides a basic base of information on bacteria and yeasts that improve the health and growth of animals **(Edens, 2003),** but there are some terms that give a similar concept such as direct fed microbials (DFM), competitive exclusion **(Fuller, 1993)**.

A probiotic is described as a source from naturally occurring microorganisms **(Scott Weese and Anderson, 2002 and Edens, 2003) .** And the presence of Bacillus subtilis bacteria has a great role in getting rid of pathological bacteria and restoring the bacterial balance, and these bacteria are widely found in food and have a great ability to produce enzymes such as amylase and protenase that break down starchy and protein compounds in food, as well as decompose sugars and produce Acids and organic compounds **(Al-Sharabi and Munir, 2004)**, and its spores are distinguished by their resistance to high temperatures and they are transitional organisms in the gastrointestinal tract, with their high ability to combine with the intestinal flora **(Jiraphocakul et al, 1990)**.

CHAPTER TWO

Review of literatures

2-1 What is a probiotic?

Over the years the word probiotic has been used in several different ways. It was originally used to describe substances produced by one protozoan which stimulated by another **(Lilly and Stillwell, 1965)** but it was later used to describe animal feed supplements which had a beneficial effect on the host animal by affecting its gut flora **(Parker, 1974)**. Crawford **(Crawford, 1979)** defined probiotics as “a culture of specific living micro-organisms (primarily Lactobacillus spp.) which implants in the animal to ensure the effective establishment of intestinal populations of both beneficial and pathogenic organisms”. **Fuller** in **(2001)** later gave a unique definition of probiotics as “a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance”. The US National Food Ingredient Association presented, probiotic (direct fed microbial) as a source of live naturally occurring microorganisms and this includes bacteria, fungi and yeast **(Miles and Bootwalla, 1991)**. According to the currently adopted definition by FAO/WHO, probiotics are: “live microorganisms which when administered in adequate amounts confer a health benefit on the host” **(Hotel and Cordoba, 2001).** More precisely, probiotics are live microorganisms of nonpathogenic and nontoxic in nature, which when administered through the digestive route, are favorable to the host’s health **(Guillot, 1998).**

It is believed by most investigators that there is an unsteady balance of beneficial and non-beneficial bacteria in the tract of normal, healthy, non-stressed poultry. When a balance exists, the bird performs to its maximum efficiency, but if stress is imposed, the beneficial flora, especially lactobacilli, have a tendency to decrease in numbers and an overgrowth of the non-beneficial ones seems to occur. This occurrence may predispose frank disease, i.e., diarrhea, or be subclinical and reduce production parameters of growth, feed efficiency, etc. The protective flora which establishes itself in the gut is very stable, but it can be influenced by some dietary and environmental factors. The three most important are excessive hygiene, antibiotic therapy and stress. In the wild, the chicken would receive a complete gut flora from its mother's faeces and would consequently be protected against infection. However, commercially reared chickens are hatched in incubators which are clean and do not usually contain organisms commonly found in the chicken gut. There is an effect of shell microbiological contamination which may influence gut microflora characteristics. Moreover, also HCl gastric secretion, which starts at 18 days of incubation, has a deep impact on microflora selection. Therefore, an immediate use of probiotics supplementation at birth is more important and useful in avian species than in other animals. The chicken is an extreme example of a young animal which is deprived of contact with its mother or other adults and which is, therefore, likely to benefit from supplementation with microbial preparations designed to restore the protective gut microflora (**Fuller, 2001)**.

Schematic representation of the concept of probiotics **(modified from Fuller, 2001)**. The species currently being used in probiotic preparations are varied and many. These are mostly Lactobacillus bulgaricus, Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus helveticus, Lactobacillus lactis, Lactobacillus salivarius, Lactobacillus plantarum, Streptococcus thermophilus, Enterococcus faecium, Enterococcus faecalis, Bifidobacterium spp. and Escherichia coli. With two exceptions, these are all intestinal strains. The two exceptions, Lactobacillus bulgaricus and Streptococcus thermophilus, are yoghurt starter organisms **(Fuller, 1989)**. Some other probiotics are microscopic fungi such as strains of yeasts belonging to Saccharomyces cerevisiae species **(Guillot, 1998 ; Thomke and Elwinger, 1998).**

2-2 Mechanisms of Action

Enhancement of colonization resistance and/or direct inhibitory effects against pathogens are important factors where probiotics have reduced the incidence and duration of diseases. Probiotic strains have been shown to inhibit pathogenic bacteria both in vitro and in vivo through several different mechanisms.

The mode of action of probiotics in poultry includes: (i) maintaining normal intestinal microflora by competitive exclusion and antagonism **(Nurmi *et al.*, 1973 ; Jin, *et al*., 1998 ; Line, *et. al*. 1998; Kabir *et al*., 2005; Fuller, 2001; Rantala and Nurm, 1973; Kizerwetter-Swida and Binek, 2009) ;** (ii) altering metabolism by increasing digestive enzyme activity and decreasing bacterial enzyme activity and ammonia production **(Cole, *et. al*. 1987; Yoon *et al*., 2004)** ; (iii) improving feed intake and digestion **(Dierick, 1989; Awad, *et. al*. 2006)** ; and (iv) stimulating the immune system **(Lutful Kabir, *et. al*. 2004; Nayebpor, *et. al*., 2007; Apata, 2008; Haghighi, 2005; Mathivanan R, *et al*, 2007; McCracken and Gaskins, 1999; Brisbin, *et al*, 2008).**

Probiotic and competitive exclusion approaches have been used as one method to control endemic and zoonotic agents in poultry. In traditional terms, competitive exclusion in poultry has implied the use of naturally occurring intestinal microorganisms in chicks and poults that were ready to be placed in brooder house. Nurmi and Rantala (Nurmi, Rantala. 1973) and Rantala and Nurmi (Rantala, and Nurmi, 1973) first applied the concept when they attempted to control a severe outbreak of S. infantis in Finnish broiler flocks. In their studies, it was determined that very low challenge doses of Salmonella (1 to 10 cells into the crop) were sufficient to initiate salmonellosis in chickens. Additionally, they determined that it was during the 1st week post-hatch that the chick was most susceptible to Salmonella infections. Use of a Lactobacillus strain did not produce protection, and this forced them to evaluate an unmanipulated population of intestinal bacteria from adult chickens that were resistant to S. infantis. On oral administration of this undefined mixed culture, adult-type resistance to Salmonella was achieved. This procedure later became known as the Nurmi or competitive exclusion concept. The competitive exclusion approach of inoculating day-old chicks with an adult microflora successfully demonstrates the impact of the intestinal microbiota on intestinal function and disease resistance **(Nisbet, *et. al*., 1998 ; Stern, *et. al*., 2001)**. Although competitive exclusion fits the definition of probiotics, the competitive exclusion approach instantaneously provides the chick with an adult intestinal microbiota instead of adding one or a few bacterial species to an established microbial population. Inoculating day-old chicks with competitive exclusion cultures or more classical probiotics serves as a nice model for determining the modes of action and efficacy of these microorganisms. Because of the susceptibility of day-old chicks to infection, this practice is also of commercial importance. By using this model, a number of probiotics (Jin, 1998) have been shown to reduce colonization and shedding of Salmonella and Campylobacter. Competitive exclusion is a very effective measure to protect newly hatched chicks, turkey poults, quails and pheasants and possibly other game birds, too, against Salmonella and other enteropathogens (Schneitz, 2005).

Upon consumption, probiotics deliver many lactic acid bacteria into the gastrointestinal tract. These microorganisms have been reputed to modify the intestinal milieu and to deliver enzymes and other beneficial substances into the intestines **(Marteau, Rambaud 1993).** Supplementation of L. acidophilus or a mixture of Lactobacillus cultures to chickens significantly increased ($P<0.05$) the levels of amylase after 40 d of feeding **(Jin, 2000).** This result is similar to the finding of Collington *et al.* (Collington, and Parker 1990), who reported that inclusion of a probiotic (a mixture of multiple strains of Lactobacillus spp. and Streptococcus faecium) resulted in significantly higher carbohydrase enzyme activities in the small intestine of piglets. The lactobacilli colonizing the intestine may secrete the enzyme, thus increasing the intestinal amylase activity **(Duke, 1977; Sissons, 1989)**. It is well established that probiotics alter gastrointestinal pH and flora to favor an increased activity of intestinal enzymes and digestibility of nutrients **(Dierck, 1989).** The effect of Aspergillus oryzae on macronutrients metabolism in laying hens was observed **(Schneitz, 2005),** of which findings might be of practical relevance. They postulated that active amylolytic and proteolytic enzymes residing in Aspergillus oryzae may influence the digested nutrients. Similarly, it was reported that an increase in the digestibility of dry matter was closely related to the enzymes released by yeast **(Han, *et al*, 1999)** In addition, probiotics may contribute to the improvement of health status of birds by reducing ammonia production in the intestines **(Chiang and Hsieh, 1995**).

Probiotic is a generic term, and products can contain yeast cells, bacterial cultures, or both that stimulate microorganisms capable of modifying the gastrointestinal environment to favor health status and improve feed efficiency **(Dierck, 1989).** Mechanisms by which probiotics improve feed conversion efficiency include alteration in intestinal flora, enhancement of growth of nonpathogenic facultative anaerobic and gram positive bacteria forming lactic acid and hydrogen peroxide, suppression of growth of intestinal pathogens, and enhancement of digestion and utilization of nutrients (Yeo, and Kim, 1997). Therefore, the major outcomes from using probiotics include improvement in growth (**Yeo, and Kim, 1997**), reduction in mortality **(Kumprecht, and Zobac, 1998)**, and improvement in feed conversion efficiency **(Yeo, and Kim, 1997)**. These results are consistent with previous experiment of Tortuero and Fernandez (Tortuero, and Fernandez, 1995), who observed improved feed conversion efficiency with the supplementation of probiotic to the diet.

The manipulation of gut microbiota via the administration of probiotics influences the development of the immune response (McCracke, and Gaskins, 1999). The exact mechanisms that mediate the immunomodulatory activities of probiotics are not clear. However, it has been shown that probiotics stimulate different subsets of immune system cells to produce cytokines, which in turn play a role in the induction and regulation of the immune response (Christensen, et al., 2002 ; Maassen, et al., 2000). Stimulation of human peripheral blood mononuclear cells with Lactobacillus rhamnosus strain GG in vitro resulted in the production of interleukin 4 (IL-4), IL-6, IL-10, tumor necrosis factor alpha, and gamma interferon (Schultz, et al., 2003). Other studies have provided confirmatory evidence that Th2 cytokines, such as IL-4 and IL-10, are induced by lactobacilli (Christensen, et al., 2002 ; Lammers, et al.2003 ; Rakoff-Nahoum, et al., 2004). The outcome of the production of Th2 cytokines is the development of B cells and the immunoglobulin isotype switching required for the production of antibodies. The production of the mucosal IgA response is dependent on other cytokines, such as transforming growth factor β (Lebman, et al., 1999). Importantly, various species and strains of lactobacilli are able to induce the production of transforming growth factor β, albeit to various degrees (Blum, et al., 2002). Probiotics, especially lactobacilli, could modulate the systemic antibody response to antigens in chickens (Kabir, *et al.*, 2004 ; Apata, 2008 ; Haghighi, *et al*. 2005 ; Mathivanan, *et al.* 2007 ; Huang, *et al.* 2004 ; Koenen, *et al.* 2004).

2-3 Criteria for Selection of Probiotics in the Poultry Industry

The perceived desirable traits for selection of functional probiotics are many. The probiotic bacteria must fulfill the following conditions: it must be a normal inhabitant of the gut, and it must be able to adhere to the intestinal epithelium to overcome potential hurdles, such as the low pH of the stomach, the presence of bile acids in the intestines, and the competition against other micro-organisms in the gastro-intestinal tract (Nurmi, 1983 ; Chateau, 1993). The tentative ways for selection of probiotics as biocontrol agents in the poultry industry are illustrated in Figure 2. Many in vitro assays have been developed for the pre-selection of probiotic strains (Ehrmann, 2002 ; Koenen, 2004). The competitiveness of the most promising strains selected by in vitro assays was evaluated in vivo for monitoring of their persistence in chickens (Garriga, 1998). In addition, potential probiotics must exert its beneficial effects (e.g., enhanced nutrition and increased immune response) in the host. Finally, the probiotic must be viable under normal storage conditions and technologically suitable for industrial processes (e.g., lyophilized).

2-4 Evaluating Probiotic Effects on the Intestinal Microbiota and Intestinal Morphology

Kabir *et al.* (2005) attempted to evaluate the effect of probiotics with regard to clearing bacterial infections and regulating intestinal flora by determining the total viable count (TVC) and total lactobacillus count (TLC) of the crop and cecum samples of probiotics and conventional fed groups at the 2nd, 4th and 6th week of age. Their result revealed competitive antagonism. The result of their study also evidenced that probiotic organisms inhibited some nonbeneficial pathogens by occupying intestinal wall space. They also demonstrated that broilers fed with probiotics had a tendency to display pronounced intestinal histological changes such as active impetus in cell mitosis and increased nuclear size of cells, than the controls. This results of histological changes support the findings of Samanya and Yamauchi (Samanya, 2002) and they indicated that birds who were fed dietary B. subtilis var. natto for 28 days had a tendency to display greater growth performance and pronounced intestinal histologies, such as prominent villus height, extended cell area and consistent cell mitosis, than the controls. On the other hand, Chichlowski *et al.* (2007) compared the effects of providing a direct-fed microbials (DFM) with the feeding of salinomycin on intestinal histomorphometrics, and microarchitecture and they found less mucous thickness in DFM-treated chickens and the density of bacteria embedded in the mucous blanket appeared to be lower in DFM-treated chickens than in the control in all intestinal segments. Watkins and Kratzer in (1983) reported that chicks dosed with Lactobacillus strains had lower numbers of coliforms in cecal macerates than the control. Francis et al. Francis in (1978) also reported that the addition of Lactobacillus product at 75 mg/kg of feed significantly decreased the coliform counts in the ceca and small intestine of turkeys. Using gnotobiotic chicks, Fuller in (1977) found that host-specific Lactobacillus strains were able to decrease Escherichia coli in the crop and small intestine. Kizerwetter-Swida and Binek in (2009) demonstrated that L. salivarius 3d strain reduced the number of Salmonella enteritidis and Clostridium perfringens in the group of chickens treated with Lactobacillus. Watkins *et al*. in (1982) similarly observed that competitive exclusion of pathogenic E. coli occurred in the gastrointestinal tract of gnotobiotic chicks dosed with L. acidophilus. Recently Yaman *et al*. (2006) ; Mountzouris *et al*. (2007) and Higgins *et al.* in (2007) demonstrated that probiotic species belonging to Lactobacillus, Streptococcus, Bacillus, Bifidobacterium, Enterococcus, Aspergillus, Candida, and Saccharomyces have a potential effect on modulation of intestinal microflora and pathogen inhibition.

2-5 Evaluating Probiotic Effects on Immune Response

Probiotics stimulate the immune system, especially humoral and cellular immunity, by acting as immune regulators (Stern *et al,* 2001, Perdigon *et al*., 2002), where the microorganisms that make up the probiotic support the immunity of the gastrointestinal tract, as confirmed by the custom probiotic (2002) There are three divisions of the natural defense systems present in the intestinal tract: the normal flora, which includes the first line, the epithelial layer, and the mucous lining of the intestine (Gut Mucosa Epithilum), and the intestinal-associated lymphoid tissues (GALT), which include Microfold cells and Peyer's macula patches), which are active areas in addition to the presence of Dentritic cells that attract (germs and viruses) and expose them to the B and T lymphocytes, as (Havenoar and Spanhaak, 1994) proved that the germs that die by the probiotic are considered as antigens that absorb and stimulate the system. Immunostaining, as (Edens and Doerfler (1998) found that administration of biological reinforcer preparations that include beneficial microorganisms isolated from the Intestinal tract absorbs and stimulates immunity.

Usually, the biological enhancer used should be balanced, healthy and not stressing the bird, but if it has a negative effect on the bird, several side effects such as diarrhea or subclinical signs such as decreased production and dietary conversion appear, and the presence of natural flora in a stable intestine can be affected by several factors. Among them are environmental or nutritional factors, and the most important of these factors are animal health, treatments and stress. In normal life, birds get natural flora from their mothers ’feces and thus have acquired immunity (Zhu et al., 2009).

Competition between pathological and natural bacteria takes place in a process of elimination of pathogenic bacteria (Ohashi and Ushida, 2009). Competitive exclusion works to screen out pathological colonies by biotic promoters in preferred locations such as villi and macrophages (Chichlowski et al., 2007) through a change in the physical environment. To prevent the growth of bacteria, they begin to compete for food and energy sources and prevent them from gaining the energy they need for growth and reproduction in the intestinal environment (Unmmings and Macfarlane, 1997). The bio-boosters also produce a number of organic acids and volatile fatty acids (VFA), as it happens. Decrease in the level of intestinal pH, which is important to eliminate pathological bacteria such as E. coli (Chichlowski et al., 2007).

As well as the presence of intestinal cells in the intestine as a barrier to prevent food loss and to prevent pathological bacteria from invading the body, and the layer (Lamina Properia) of the intestine is rich in lymphocytes, phagocytes and stem cells, all these types of cells fight against the pathological bacteria, thus improving immunity and its effect on immunity from During activation of lymphocytes and production of antibodies (Ng et al., 2009).

The dosage and administration of the probiotic plays an important role in its effectiveness (Carita, 1992).

A number of biological promoters were marketed and produced without previous studies, which led to the emergence of conflicting problems. In 2001 the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) published guidelines and evaluation of biological promoters in food which recommended Each strain of organisms must be tested for efficacy (Vasiece *et al.*, 2014), and the bacteria that make up the probiotic are required to be able to withstand the level of acidity and bile secretions to remain in the digestive system (Chou and Weimer 1999; Tuomola et al., 2001). Digestion An acidic environment, it is important that these organisms be able to tolerate (Conway et al., 1987; Brashears *et al.*, 2003) in addition to the ability of bile secretions to disrupt the cell wall and thus cell death (Gilliland et al., 1984). Therefore, among the most important characteristics that must be present in a biological reinforcer are:

1- It should be resistant to acids and bile secretions.

2- That the strain used has the ability to rapidly divide in the intestinal tract.

3- It should have little side effect on the body.

4- It has the ability to stick to the gastrointestinal tract.

5- Its ability to reduce pathogenic microbes.

There are many bacteria that are used as a bio-promoter, such as Lactobacillus Bifado Bacteriua, these species have the ability to tolerate intestinal acids and bile salts (Jin et al., 1998), and types of yeasts such as Saccharomyces Cervisiae are used as a biological promoter due to their inhibitory properties for the growth of a number of pathogenic microbes. It remains for a long time in the intestine (Saegusa et al., 2004).

The best way to give the probiotic supplement is by drinking water, but there are problems that may arise when the bird rejects its unpleasant flavor (Schneit, 1993).

2-6 Evaluating Probiotic Effects on Meat Quality

Kabir (2009) and Kabir *et al.* (2005) evaluated the effects of probiotics on the sensory characteristics and microbiological quality of dressed broiler meat and reported that supplementation of probiotics in broiler ration improved the meat quality both at prefreezing and postfreezing storage. Mahajan *et al.* (2000) stated that the scores for the sensory attributes of the meat balls appearance, texture, juiciness and overall acceptability were significantly (p60.001) higher and those for flavour were lower in the probiotic (Lacto-Sacc) fed group. Simultaneously, Mahajan *et al.* (2000) reported that meat from probiotic (Lacto-Sacc) fed birds showed lower total viable count as compared to the meat obtained from control birds. On the other hand, Loddi *et al.* (2000) reported that neither probiotic nor antibiotic affected sensory characteristics (intensity of aroma, strange aroma, flavour, strange flavour, tenderness, juiciness, acceptability, characteristic colour and overall aspects) of breast and leg meats. On the other hand, Zhang *et al.* (2005) conducted an experiment with 240, day-old, male broilers to investigate the effects of Saccharomyces cerevisiae (SC) cell components on the meat quality and they reported that meat tenderness could be improved by the whole yeast (WY) or Saccharomyces cerevisiae extract (YE).

Conclusions and recommendations

1. Conclusion

The concept of probiotics in recent year is no more confusing as was earlier thought. It now constitutes an important aspect of applied biotechnological research and therefore as opposed to antibiotics and chemotherapeutic agents can be employed for growth promotion in poultry. In past years, men considered all bacteria as harmful, forgetting about the use of the organisms in food preparation and preservation, thus making probiotic concept somewhat difficult to accept.

Scientists now are triggering effort to establish the delicate symbiotic relationship of poultry with their bacteria, especially in the digestive tract, where they are very important to the well being of man and poultry. Since probiotics do not result in the development and spread of microbial resistance, they offer immense potential to become an alternative to antibiotics. The present review reveals that probiotics could be successfully used as nutritional tools in poultry feeds for promotion of growth, modulation of intestinal microflora and pathogen inhibition, immunomodulation and promoting meat quality of poultry.

2. Recommendations

1- Reducing the use of antibiotics by preventing the acquisition of immunity to the pathological bacteria, as the long use of the probiotic is safe and has fewer side effects compared to the antibiotic such as diarrhea and liver damage.

2- It is recommended to use it for being supports health and productive qualities as it acts as a regulator of intestinal bacteria and reduces the secretion of ammonia and urea and improves the level of production and growth.

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