PROBIOTICS AS ALTERNATIVES TOANTIBIOTICS IN POULTRY RATIONS

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SUMMARY

This review showed that probiotics can be used as alternatives to antibiotics for sustaining the growth of the poultry industry using recent developments in biotechnology. Probiotics (direct-fed microbials, DFM) have been defined as a mono or mixed culture of living micro-organisms which (applied to animal or man) beneficially affects the host by improving the properties of the indigenous microflora. The following factors are important factors to be considered in the application of probiotics: early administration, prohibition of simultaneous application of antibiotics and disinfectants and proper storage. Probiotics benefit the host bird by stimulating appetite, improve intestinal microbial balance, stimulate the immune system and improve the utilization of the nutrients due to the digestive enzymes produced by *Lactobacillus* species. Probiotics to animals has been found to maintain the beneficial intestinal microflora in two ways: by competitive exclusion and by antagonistic activity towards pathogenic bacteria. Probiotic can be combined with a prebiotic to form what is called a symbiotic which is more effective than each alone. However, some studies showed that there were no positive effects due to probiotics. The reasons for the ineffectiveness of *Lactobacillus* in some studies were attributed to the non-host-specific or that the amount of viable *Lactobacillus* was too low to be effective . It is suggested to conduct such studies, under commercial conditions, with sufficient replicates, proper controls and statistical analysis of the results. New strains should be carefully assessed and tested before their incorporation into products or poultry rations.

Keywords: *antibiotics, probiotics, poultry, performance.*

1. INTRODUCTION

The intensive nature of modern poultry production is conducive to the spread of disease causing organisms and parasites (Reece, 1988). The chickens are stressed by various factors such as transportation, overcrowding, vaccination, chilling and / or overheating. These factors tend to create an imbalance in the intestinal microflora and a lowering of body defense mechanisms. Under such circumstances, antimicrobial feed additives such as antibiotics and synthetic antimicrobial agents are often used to suppress or eliminate harmful organisms in the intestine, and to improve growth and feed efficiency. The use of antibiotics as routine feed additives has been banned in some countries because of public concern over possible antibiotic residual effects and the development of drug-resistant micro-organisms in humans (Jin *et al.* , 1997). The adverse effects of chemotherapeutic agents were also reported by Reece (1988) who indicated that toxicity episodes usually follow errors in dosage rates, dosing with undesirable

combinations of chemotherapeutic agents, and variations in bird susceptibility depending upon species, age, body condition, rate of egg production and growth rate. Other adverse effects also can occur. Prolonged oral treatment with chlorotetracycline, or other antibacterial agents, can significantly alter the enteric flora and allow the proliferation of potentially pathogenic fungi such as *Candida albicans* (Tripathy *et al.*, 1967). Residues of some chemotherapeutic agents in poultry products may contribute to human hypersensitivity reactions (Huber, 1984).

Probiotics or other natural products have been incorporated into animal feeds, as an alternative to antibiotics, to maintain efficient feed utilization (Kung, 1992). However, the effects of probiotics on poultry production are not consistent, resulting in uncertainties and skepticism for development of the products. *Lactobacillus* used and the fact that the birds were being kept under relatively ideal conditions. The reasons for the ineffectiveness of *Lactobacillus* in some studies were attributed to the non-host-specific. Another reason may be that the amount of viable *Lactobacillus* was too low to be effective (Goodling *et al.*, 1987).

Although used in humans and animals for generations, probiotics have only recently been subject to clinical research. The purpose of this paper is to define probiotics and review the most recent studies of the use of probiotics in enhancing poultry production.

2. DEFINITION OF PROBIOTIC

Jin *et al.* (1997) reported that the term 'Probiotic' was first introduced by Lilly and Stillwell (1965) to describe growth promoting factors produced by micro-organisms. 'Probiotic' is derived from Greek which means 'for life' (Gibson and Fuller, 2000). One early formal definition was that of Parker (1974) who used the term 'probiotic' for micro-organisms or substances which contribute to intestinal microbial balance. Fuller (1989) considered the definition given by Parker (1974) to be too broad because it included not only cultures, cells and metabolites but also antibiotic preparations. He redefined 'probiotics' as a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance. Havenaar *et al.* (1992) pointed out that the definition of probiotic by Fuller (1989) was restricted to feed supplements, animals and the intestinal tract. Havenaar *et al.* (1992) broaden Fuller's definition of probiotic as a mono or mixed culture of living micro-organisms which (applied to animal or man) beneficially affects the host by improving the properties of the indigenous microflora (Jin *et al.*, 1997). The US FDA required manufacturers to use the term direct-fed microbial (DFM) rather than probiotic (Miles and Bootwalla, 1991).

3. MICROORGANISMS USED AS PROBIOTICS

Many microorganisms have been used or considered for use as probiotics. Because viable and biologically active microorganisms are usually required at the target site in the host, it is essential that the probiotic be able to withstand the host's natural barriers against ingested bacteria. The most commonly used probiotics are strains of lactic acid bacteria (e.g., *Lactobacillus, Bifidobacterium* and *Streptococcus*). The beneficial effects of *Lactobacillus* and *Bifidobacterium* have been discussed for decades. Bacteria in these two genera resist gastric acid, bile salts and pancreatic enzymes, adhere to intestinal mucosa and readily colonize the

intestinal tract. They are considered important components of the gastrointestinal flora and relatively harmless. Lactic acid bacteria have been demonstrated to inhibit the in vitro growth of many enteric pathogens including *Salmonella typhimurium, Staphylococcus aureus, Escherichia coli, Clostridium perfringens* and *Clostridium difficile* and have been used in both humans and animals to treat a broad range of gastrointestinal disorders (Meurman *et al.*, 1995 and Silva *et al.*, 1987). The FDA defines direct-fed microbial (DFM) as "a source of live (viable) naturally occurring micro-organisms", and this includes bacteria, fungi and yeast (Miles and Bootwalla, 1991).

4. SAFETY OF PROBIOTICS

The use of lactic acid bacteria in foods has a long history, and most strains are considered commensal microorganisms with no pathogenic potential. Members of the genus *Lactobacillus* are most commonly given safe or generally recognized as safe status.The European Union novel foods regulation allow a set approach for safety assessment of such strains. New strains should be carefully assessed and tested, especially for the following areas, before their incorporation into products (Lee *et al.*, 1999):

1-Metabolic products of the probiotic.
2-Acute toxicity of ingestion of extremely large amounts of the probiotic.
3-The "novel" status in EU regulations.

5. SELECTION OF PROBIOTIC MICROORGANISMS

A number of selection criteria may be important for the success of probiotics. Efficacious probiotics should have as many as possible of the following characteristics Gibson and Fuller (2000):

1-Strain origin—those isolated from the same species as the intended use ought to have an enhanced chance of survival.
2-Safety—probiotics should be generally recognized as safe with minimal possibilities for the transfer of antibiotic resistance.
3-Production characteristics—able to grown in bulk culture, without genetic variation.
4-Adherence—to enhance survival in the gut.

6. MODES OF ACTION OF PROBIOTICS (DFMs)

Continuous feeding of probiotics to animals has been found to maintain the beneficial intestinal microflora in two ways: by competitive exclusion and by antagonistic activity towards pathogenic bacteria.

6.1. Competitive exclusion

Nurmi and Rantala (1973) introduced a technique based on "competitive exclusion" to increase the resistance of young chicks to salmonella infection by inoculating them orally with adult fowl intestinal contents. They demonstrated that oral inoculation of 1-2 day-old chicks with a 1:10 dilution of normal intestinal contents from healthy adult birds one day prior to oral challenge with *S. infantis* resulted in 77% of the birds being free of infection compared with a 100% infection rate in the control birds. This study laid the foundation for further development of the competitive exclusion technique. Corrier *et al.* (1995) reported that pre-treatment (on the day of hatch) of 10-day-old chicks with a partly characterized mixes culture of caecal bacteria containing 29 bacterial isolated representing 10 genera decreased the

number of *Salmonella* in the caecal contents by an average of 5.33 $\log_{10}$ units when compared with control chicks. The percentage of *Salmonella*-infected chicks was also decreased by an average of 75% compared with the controls.

The proposed mechanisms involved in exclusion of pathogenic bacteria by probiotics include aggregation of lactic acid bacteria with pathogens, competition for adhesion sites, competition for the nutrients and production of bactericidal substances (Rolfe, 1991). Sissons (1989) explained that attachment is necessary for proliferation and for reducing the rate of removal of organisms from specific sites in the gastrointestinal tract due to the movement of digesta caused by peristalsis.

6.2. Antagonistic activity

Oyarzabal and Conner (1995) found that three commercial strains (*L. acidophilus*, *L. casei* and *S. faecium*) were able to inhibit the growth of six *Salmonella* serotypes. Jin *et al.* (1996) also indicated that all 12 *Lactobacillus* isolates studied had the ability to inhibit the growth of five *Salmonella* strains and three serotypes of *E. coli*.

The antagonistic activity of lactic acid bacteria towards pathogens can be attributed to the production of bactericidal substances. Among those produced by lactobacilli are organic acids, hydrogen peroxide and bacteriocins (Jin *et al.*, 1997). Lee *et al.* (1999) revealed that all lactic acid bacteria produce organic acid. Naidu (2000) explained that fermentation involving lactic acid bacteria results in accumulation of organic acids, primarily lactic acid, as a major end product of carbohydrate metabolism, generated from pyruvate by lactic acid dehydrogenase. The accumulation of lactic acid and the concomitant reduction in pH of the milieu results in a broad-epectrum inhibitory activity against Gram-positive and Gram-negative bacteria. Acetic acid is produced by *Leuconostoc citrovorum* and has been shown to inhibit *Salmonella gallinarium* (Sorrels and Speck, 1970) and *P. fragi* (Pinheiro *et al.*, 1968). Acetic acid has been shown a more effective inhibitor of microorganisms than lactic acid (Doores, 1990). Silliker, *et al.* (1980) reported that lipophilic acids such as lactic acid and acetic acid in undissociated form can penetrate the microbial cell membrane, and at higher intracellular pH, dissociate to produce hydrogen ions that interfere with essential metabolic functions such as substrate translocation and oxidative phosphorylation.

Collins and Aramaki (1980) found that *Lactobacillus* species, *Lactococcus lactis* and *Leuconostoc cremoris* produce hydrogen peroxide when transferred from anaerobic to aerobic condition. Certain strains of *Lactobacillus* and *Pediococcus* isolated from meat were found to produce *in vitro* high amounts of hydrogen peroxide to initiate oxidation of biomolecules (Juven *et al.*, 1988). Naidu (2000) revealed that in the presence of oxygen, lactic acid bacteria produce hydrogen peroxide (H_2O_2) through electron transport via flavin enzymes. In the presence of H_2O_2, superoxide anions form destructive hydroxyl radicals. This process may lead to peroxidation of membrane lipids and increased membrane permeability. The resulting bactericidal effect of these oxygen metabolites has been attributed to their strong oxidizing effect on the bacterial cell as well as destruction of nucleic acids and cell proteins. In addition, H_2O_2 could react with other cellular and milieu components to form additional inhibitory substances.

Naidu (2000) found that lactic acid bacteria produce a wide range of antibiotic-like substances and

bactericidal proteins, collectively termed as bacteriocins. These antimicrobial agents are species specific and exert their lethal activity through adsorption to specific receptors located on the external surface of sensitive bacteria, followed by metabolic, biological and morphological changes resulting in the killing of such bacteria. Gilliland and Speck (1977) suggested that hydrogen peroxide produced by *L. acidophilus* was partially responsible for the antagonism. They concluded that the antibacterial action produced by *L. acidophilus* was probably due to a combination of factors which included acids, hydrogen peroxide and bacteriocins.

7. ENHANCEMENT OF PRO-BIOTIC ORGANISMS

It was found that colonization by an exogenous probiotic could be enhanced and extended by simultaneous administration of some food ingredients. These ingredients are called prebiotics. Gibson and Roberfroid (1995) defined prebiotic as "a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon". Prebiotics to date have been carbohydrates, ranging in size from small sugar alcohols and disaccharides, to oligosaccharides and large polysaccharides (Rolfe, 2000). A probiotic can be combined with a prebiotic to form what is called a symbiotic (Gibson and Roberfroid, 1995 and Lewis and Freedman, 1998). In this regard Mulder (1991) reported that fructooligosaccharides are not digested by intestinal enzymes and favour the growth of *Lactobacilli and Bifidobacteria* which can utilize the compounds as energy source. Edens *et al.* (1997) found that manipulating the lactose concentration in the diets of chicks and poults could selectively provide an advantage for the enhancement of *L. reuteri*.

8. APPLICATION AND THE DIFFERENT ACTIVITIES OF PROBIOTICS

Throughout the world there appear to be 42 strains of bacteria used in probiotics and there are 6 strains used in China: *Bacillus acidilactici, Streptococcus acidilactici, Streptococcus faecalis, Bacillus, Bifidobacterium* and yeast. The following factors are considered to be important factors to consider in the application of probiotics: early administration, prohibition of simultaneous application of antibiotics and disinfectants and proper storage (**Dou and Dou,** 1998).

8.1. The effect on poultry performance

A. The effect on growth performance

Nimruzi (1999) reported that lactic acid bacteria (mainly lactobacilli) found in whey proved to be natural probiotics. Four groups of 40 chicks received 2, 4, 6 and 8% solutions of whey via drinking water over an experimental period of 42 days. Results indicated that the birds receiving whey had a significantly higher average daily weight gain (30.1 g/day) and final weight (1795 g/bird). The group receiving 4% whey solution were significantly more healthy than controls. Jadamus *et al.* (2000) examined the germination rate of *Bacillus cereus var. toyoi* in different sections of the intestinal tract of broilers. They found that *B. toyoi* spores germinated quickly in the intestinal tract of broilers which is an essential precondition for the efficiency of *Toyocerin.* Finishing trials showed that the performance of broilers and turkeys could be improved by the probiotic *Toyocerin.* Pietras and Skraba (2000) used 3 groups of broilers. Group 1 received no supplement, group 2 a

supplement of 0.5 g/kg of flavomycin, and group 3 a supplement of 250 mg/kg of probiotic preparation of *Lactobacillus acidophilus* and *Streptococcus faecium*.

For the 3 groups respectively, body weight averaged 2121, 2158 and 2212 g, feed conversion 2.27, 2.22 and 2.24 kg/kg gain, and mortality 2.3, 2.4 and 1.2%. Fritts *et al.* (2000) conducted two trials to evaluate the effect of inclusion of 30 g/ton *Bacillus subtilis* C- 3102 (Calsporin) from 1 to 42 days on live performance of broiler chickens. The results indicated that inclusion of Calsporin yielded an increase ($P \leq 0.05$) in 42-day body weight and improvement in feed conversion during the 21- to 42-day period. They reported that the results of the present study and other recent reports demonstrate that inclusion of certain *Bacillus* spp. in poultry diets may improve live performance of broilers in the absence of antibiotics and may contribute to on-farm pathogen reduction. Cortes *et al.* (2000) evaluated the effect of a pr biotic (*Bacillus toyoi* 1010 spores/g) on performance of broiler chicks. Three levels of the probiotic (0, 50, 100 and 150 ppm) were used. Results for weight gain at 49 days showed a linear effect ($P \leq 0.05$) with the probiotic addition (2258, 2321, 2376 and 2433 g). For feed consumption (4648, 4802, 4782 and 4843 g), feed conversion (2.06, 2.07, 2.01 and 1.99), total mortality (10.4, 7.5, 9.6 and 5.4%) and ascites syndrome mortality (6.43, 3.20, 6.43 and 3.20%) no statistical differences were found among treatments. Results in this research showed a growth effect and a reduction of ascites syndrome mortality due to the probiotic addition. Kim *et al.* (2000) studied the effect of dietary supplementation of multiple probiotics (MS102) obtained from local soil on growth performance. A total of 240 broiler chicks (3 days old, Ross x Ross) were randomly divided into 12 groups and assigned to four dietary treatments, which were a basal diet containing 0.1% salinomycin and 0.05% Zn-bacitracin and three non-medicated diets containing 0.1, 0.3, or 0.5% MS102. The chicks fed starter diet containing 0.1 or 0.3% MS102 (3 to 21 days of age) showed improved average daily gain and feed conversion rate ($P \leq 0.05$) compared with the other groups during the first stage of the experimental period (3 to 21 days of age). During the later stage (21 to 28 days of age), average daily gain and feed conversion rate of birds fed the non-medicated probiotic diet were not significantly different from those fed the medicated basal diet. They concluded that locally obtained multiple probiotics MS102 can be effectively used as a growth promoting agent in a non-medicated broiler diet. Maiorka *et al.* (2001) tested the substitution of antibiotics with prebiotics, probiotics or symbiotics in diets of broiler chickens up to 45 days of age. Day-old chicks were divided into 5 treatments as follows: T1-no additives, T2-antibiotics, (Olaquindoxa and Nitrovina), T3-prebiotic (0.2% *Saccharomyces cerevisiae* cell wall), T4-probiotic (300 ppm *Bacillus subtilis*) and T5-symbiotic (T3+T4). The performance of broilers up to 45 days of age was influenced by treatments, with better liveweight gain in birds fed with symbiotics followed by antibiotics, prebiotics and probiotics. The worst liveweight gain was observed in broilers whose diets were not supplemented by any additive. The birds not supplemented showed the worst feed conversion values when compared to the birds in the other treatments. These findings suggest that the substitution of antibiotics by symbiotics in broiler chicken diets is an alternative for the poultry industry, since no negative effect was found on performance. However, the

total absence of additives in the diets worsened broiler chicken performance.

On the other hand Loddi *et al.* (2000) observed that there were no beneficial effects of probiotic supplementation. They assigned a total of 2400 broiler chicks to a completely randomized design in a 2 X 2 X 2 (sex, with and without probiotic, with and without antibiotic) factorial arrangement. Body weight and weight gain were higher for males fed antibiotic when compared with the unsupplemented. Use of antibiotic increased feed intake up to 42 days of age, but it did not affect the other characteristics. Probiotic supplementation negatively influenced body weight, weight gain and feed intake of broilers from either 1 to 21 or 1 to 42 days of age. Senani *et al.* (2000) offered three strains of *Lactobacillus*, NCDC-3 (*L. delbrueckii* subsp. *lactis*), NCDC-8 (*L. delbrueckii* subsp. *bulgaricus*) and NCDC-14 (*L. acidophilus*) to 3 groups of 20 chicks, 1 day old, until they were 9 weeks of age. A fourth group served as the control. No difference in 6-week body weights were observed between treatments. Mortality (0-6 weeks) was highest with NCDC-14 and lowest with NCDC-3. However, 9-week body weights were higher in NCDC-3 and NCDC-8 groups compared with the control. Kahraman *et al.* (2000) used a probiotic combination (*L. plantarum, L. delbrueckii* subsp. *bulgaricus, L. acidophilus, L. rhamnosus, Bifidobacterium bifidum, Streptococcus salivarius* subsp. *thermophilus, Enterococcus faecium, Aspergillus oryza* and *Candida pintolopesii* cultures, 3 X 1011 8 CFU/kg, starter; 2 X 1011 CFU/kg, grower) and/or antibiotic (zinc bacitracin, 75 ppm). They concluded that the addition of probiotic and antibiotic to the diet does not influence broiler performance kept under good hygienic conditions.

B. The effect on layer performance

Han *et al.* (1999) concluded that AO culture alone could be used as a probiotic supplement for layers. Panda *et al.* (2000a) studied the effect of probiotic supplementation on performance and immune response of White Leghorn layers from 48 to 64 weeks of age. WLH layers at the age of 48 weeks were randomly distributed and supplied with one of the three diets. The three diets were basal diet, basal diet with 100 mg of probiotic (a commercial preparation containing *Lactobacillus acidophilus, L. casei, Bifidobacterium bifidum, Aspergillus oryzae, Streptococcus faecium* and *Torulopsis* spp. with 27 billion CFU/100 g) and basal diet with 200 mg of probiotic per kg diet. Supplementation of probiotic at 100 mg/kg diet improved ($P \leq 0.05$) hen day egg production. Probiotic supplementation had no effect on daily feed intake, feed conversion, egg weight or on concentration of albumin and yolk. However, there was improvement ($P \leq 0.05$) in shell thickness by supplementation of 100 mg of probiotic per kg diet.

Yalcin *et al.* (2002) determined the effects of the usage of enzyme, probiotic or antibiotic alone or in combination in the rations on live weight, feed consumption, egg production, feed efficiency and egg quality of laying hens. A total of 189 Isa Brown layers aged 25 weeks were used in this experiment. They were divided into 7 groups (1 control and 6 treatment groups) each containing 27 hens. Each group was divided into 3 subgroups containing 9 hens. Diets based on wheat and barley were arranged. Experimental diets were supplemented with enzyme, probiotic and antibiotic alone or in combination.

GrindazymTM GP 5000 (hemicellulase, pentosanase, beta-glucanase (5000 unit/g), pectinase, protease, amylase) at the levels of 0.5 kg/ton as enzyme, Biocell (*Saccharomyces cerevisiae*, 2.5 x 109 CFU/g) at 0.75 kg/ton as probiotic and Stafac 20 (2% virginiamycin) at 1 kg/ton as antibiotic (20 ppm virginiamycin) were used. The experimental period lasted 20 weeks. At the end of the study, there were no statistical differences among the groups in live weight, feed efficiency, egg yolk index, egg white index and egg Haugh units. Egg production was significantly increased ($P \leq 0.01$) with the supplementation of enzyme, antibiotic, enzyme+antibiotic, antibiotic+probiotic and enzyme+probiotic to the rations. Egg weight was significantly improved ($P \leq 0.01$) with the supplementation of enzyme, antibiotic+probiotic and enzyme+probiotic to the rations. As a result, the supplementation of probiotic with enzyme or antibiotic to the rations based on wheat and barley improved egg production and egg weight without adverse effect on feed efficiency and some egg quality characteristics.

8. 2. The effect on the quality of poultry products

Giardini *et al.* (1998) concluded that metabolites from intestinal fermentation can be absorbed and can significantly affect the organoleptic characteristics of poultry meat and fat. Chantsavang *et al.* (1999) studied the influence of effective microorganisms (EM) on the quality of poultry products. Results from a 16-week experiment on Muscovy ducks showed that EM added in the feed and/or in drinking water significantly increased breast yield percentage, significantly reduced breast ash content and tended to increase protein content of breast meat and polyunsaturated fatty acid content in duck oil. Results of a 7-week experiment on Arbor Acres chickens showed that EM added in the feed and/or in drinking water had no effect on production and carcass characteristics but tended to decrease ash content of breast meat. In laying chickens, EM added in feed had no significant influence on all traits studied except for yolk colour. Layers given EM in the feed showed darker yolk colour. Similar results were obtained in a 12-week experiment with Japanese quails.

Mahajan *et al.* (2000) examined the quality of the cooked chicken meat balls prepared from Lacto-Sacc fed broilers during refrigerated storage. It was observed that the total viable counts of the meat balls were significantly ($P \leq 0.001$) lower in Lacto-Sacc fed broilers. The scores for the sensory attributes of the meat balls viz., appearance, texture, juiciness and overall acceptability were significantly ($P \leq 0.001$) higher and those for flavour were lower in the Lacto-Sacc fed group. Fritts *et al.* (2000) studied the effect of inclusion of 30 g/ton *Bacillus subtilis* C-3102 (Calsporin) from 1 to 42 days on carcass microbiological status of broiler chickens. They observed reductions ($P \leq 0.05$) in aerobic plate count, coliforms (non *E. coli*), and *Campylobacter* on processed carcasses. All 94 pre-chill carcasses of birds fed the control diet were positive for *Salmonella*, while 41 of 96 carcasses of birds fed Calsporin were positive. A total of 240 broiler chicks (3 days old, Ross x Ross) were randomly divided into 12 groups and assigned to four dietary treatments, which were a basal diet containing 0.1% salinomycin and 0.05% Zn-bacitracin and three non- medicated diets containing 0.1, 0.3, or 0.5% MS102 (multiple probiotics obtained from local soil). Abdominal fat (% BW) of the birds fed the MS102 supplemented diets was lower ($P \leq 0.05$) than that of the control group. Composition (% BW) of liver,

breast and leg muscles was not significantly different among treatments (Kim *et al.*, 2000).

Pietras (2001) determined the effect of *Lactobacillus acidophilus* and *Streptococcus faecium* bacteria on selected meat indicators of broiler chickens. The chickens received the probiotic (250 mg/kg) for either the whole rearing period (group I), from days 1 to 21 of age (group II), or from days 22 to 49 of age (group III). The meat of chickens given the probiotic for the whole period of rearing had a significantly higher protein content, while their crude fat and total cholesterol contents tended to decrease.

8. 3. The effect on nutrients utilization

Xu *et al.* (1999) gave one-day-old broiler chicks a diet supplemented with 1% K94 compound containing *Lactobacillus*, *Bifidobacterium* and 3 species of *Clostridium* (the experimental group), or an unsupplemented diet (control group). Compared with those of the control group.The apparent digestibilities of DM and CP increased by 10.31% and 0.27%, respectively (1-15 days) and 14.47% and 8.7% (16-30 days). Senani *et al.* (2000) offered three strains of *Lactobacillus*, NCDC-3 (*L. delbrueckii* subsp. *lactis*), NCDC-8 (*L. delbrueckii* subsp. *bulgaricus*) and NCDC-14 (*L. acidophilus*) to 3 groups of 20 chicks, 1 day old, until they were 9 weeks of age. A fourth group served as the control. Higher N, Ca and P balances were observed in these treatment groups. N balance was 0.93, 3.66, 3.28 and 2.70 g, Ca balance was 0.73, 1.06, 0.98 and 0.72 g, and P balance was 0.46, 0.56, 0.51 and 0.39 g, in the control, NCDC-3, NCDC-8 and NCDC-14 groups, respectively. Bilal *et al.* (2000) conducted an experiment using day-old male broiler chicks to determine the effects of competitive exclusion and/or Zn bacitracin on intestinal pH and feed digestibility.The chicks were divided into four treatments with 60 broilers in each group. The groups are control, Zn bacitracin (100 mg/kg), Broilact R (diluted in drinking water, 1 mg per chick by oral administration), and Broilact R with Zn bacitracin. The pH of the ileum and caeca at 15 d of age in the two Broilact R treatments were significantly higher than the other groups ($P \leq 0.05$). At the end of the experiment, total digestibility at 35 d of age in the group treated with only Broilact R was significantly higher than for the other groups ($P \leq 0.05$).

Jin *et al.* (2000) investigated the effects of adherent *Lactobacillus* cultures on (1) amylolytic, lipolytic, and proteolytic enzyme activities in the contents of the small intestine (from the distal end of the duodenum to the ileocaecal junction) and (2) bacterial beta-glucuronidase and beta-glucosidase activities in the intestinal contents and faeces of broiler chickens. Three dietary treatments were randomly assigned to three groups of chicks, i.e., basal diet only (control group), basal diet+0.1% dried culture of *L. acidophilus*, and basal diet+0.1% dried culture of a mixture of 12 *Lactobacillus* strains. The results showed that supplementation of the adherent *Lactobacillus* cultures to chickens, either as a single strain of *L. acidophilus* or as a mixture of 12 *Lactobacillus* strains, increased ($P \leq 0.05$) the levels of amylase in the small intestine. However, the proteolytic and lipolytic activities in the small intestine were not affected by addition of either of the adherent *Lactobacillus* cultures. Addition of either *L. acidophilus* or a mixture of 12 *Lactobacillus* strains was also found to reduce ($P \leq 0.05$) the intestinal and faecal beta-glucuronidase and faecal beta-glucosidase but not the intestinal beta-glucosidase at 40 days of feeding.

Samanya and Yamauchi (2002) fed chickens dried *Bacillus subtilis* var. *natto* for 28 days. In these birds, blood ammonia concentration was decreased ($P \leq 0.05$). The results indicated that intestinal function was activated by the depressed blood ammonia concentration in the body of the chicken.

8. 4. The effect on blood constituents

Endo *et al.* (1999) studied the effects of a probiotic (a mixture of *Bacillus, Lactobacillus, Streptococcus, Clostridium, Saccharomyces* and *Candida*) on the lipid metabolism. The cholesterol level of the liver and serum was significantly decreased in the cocks fed on the cholesterol-enriched diet containing the probiotic. The concentration of short-chain fatty acids in the caecal content of the cocks fed on the cholesterol-enriched diet supplemented with the probiotic was increased. Kim *et al.* (2000) divided a total of 240 broiler chicks (3 days old, Ross x Ross) randomly into 12 groups assigned to four dietary treatments, which were a basal diet containing 0.1% salinomycin and 0.05% Zn-bacitracin and three non-medicated diets containing 0.1, 0.3, or 0.5% MS102 (multiple probiotics obtained from local soil). Blood cholesterol levels of chicks fed MS102 diets were lower ($P \leq 0.05$) than that of the control.

8. 5. The effect on immune system

Shao *et al.* (2000) studied the effects of dietary mannan-oligosaccharide (MOS) and *Enterococcus faecium* on cell-mediated immunity. One-day old male chickens of Hy-line were divided randomly into 4 groups and fed a basal diet containing 0.2% MOS (group 1), basal diet containing 60 X 10-6 *E. faecium* (group 2), basal diet containing 0.2% MOS and 60 X 10-6 *E. faecium* (group 3) or basal diet alone (group 4). Macrophage activities in groups 1, 2 and 3 (25.30, 25.30 and 27.70, respectively) were significantly higher than that in control birds (23.60). It is concluded that cell mediated immunity is stimulated by administration of MOS and *E. faecium* treatment. Panda *et al.* (2000b) conducted an experiment on 320 broiler chicks. The chicks were placed on one of the four dietary treatments. The dietary treatments were a basal diet (control), and three other diets were the same in composition as that of the basal diet but supplemented with probiotic (Probiolac, 100, 150 or 200 mg/kg diet). There was significantly higher antibody production in the 100 mg probiotic supplementation group at 10 days and 5 days post-inoculation in response to sheep red blood cells (SRBC) antigen when injected at 14 days and 21 days of age respectively, compared to control. The birds fed probiotic were less susceptible to *E. coli* challenge than controls, however no difference was observed in the weight of bursa and spleen due to probiotic supplementation.

Panda *et al.* (2000a) studied the effect of probiotic supplementation on immune response of White Leghorn layers from 48 to 64 weeks of age. WLH layers at the age of 48 weeks were randomly distributed and supplied with one of the three diets. The three diets were basal diet, basal diet with 100 mg of probiotic (a commercial preparation containing *Lactobacillus acidophilus, L. casei, Bifidobacterium bifidum, Aspergillus oryzae, Streptococcus faecium* and *Torulopsis* spp. with 27 billion CFU/100 g) and basal diet with 200 mg of probiotic per kg diet. The antibody production in response to SRBC was higher ($P \leq 0.05$) in the 100-mg probiotic supplemented group.

8. 6. The effect on gastrointestinal bacterial infection and diseases

Ramesh *et al.* (2000) subjected 1-day-old *Salmonella* free chicks to the

following treatments: control (T1), *L. acidophilus* (108 cfu/bird) fed for 2 weeks (T2), *L. acidophilus* fed for 2 weeks followed by oral infection with *Salmonella gallinarum* (101 organisms/0.1 ml bacterial suspension; T3), and lastly birds infected orally with *S. gallinarum* (T4). Six birds from each group were killed randomly on days 1, 3, 5, 7 and 27 after infections. Liver was analysed for viable *Salmonella* and pH was recorded from various intestinal segments (duodenum, jejunum, ileum and caecum). T4 birds manifested clinical signs of dullness, inappetence, reduced growth rate and diarrhoea, while those pre-treated with lactobacilli (T3) remained normal. T1 and T2 birds were active and healthy. T3 birds showed *Salmonell*a viable counts only on day 1 and 3 post infection which were significantly ($P \leq 0.05$) low compared with viable counts on respective days in birds of T4. Birds fed *lactobacillus* showed a lowered surface pH in the duodenum, jejunum, ileum and caecum.. Kumar *et al.* (2002) studied the efficacy of supplementing probiotics such as *Lactobacillus acidophilus*, mannan oligosaccharide and native gut culture in the prevention of experimental *Salmonella gallinarum* infection in broiler chicks. The study indicated that early establishment of *L. acidophilus*, mannan oligosaccharide and native gut culture in the GIT helps increase the resistance to Salmonella colonization and may even have a possible role in the competitive exclusion by elimination of *Salmonella* organisms from the gut.

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الدافعات الحيوية (البروبيوتيك) كبدائل للمضادات الحيوية فى علائق الدواجن

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أوضحت نتائج الدراسات المتعددة امكانية استخدام الدافعات الحيوية (البروبيوتيك) كبدائل للمضادات الحيوية للحفاظ على نمو صناعة الدواجن وذلك عن طريق الاستفادة من احدث ماوصل اليه العلم فى مجال التكنولوجيا الحيوية. و تم تعريف البروبيوتيك بأنها بيئة أحادية أو مختلطة من الكائنات الحية الدقيقة التى لها تاثيرات مفيدة للعائل(فى حالة كل من الانسان والحيوان) عن طريق تحسين خصائص البيئة الميكروبية الطبيعية. كما اوضحت هذه الدراسة اهمية بعض العوامل التى يجب اخذها فى الاعتبار عند التطبيق مثل تقديم البروبيوتيك مبكرا حتى تتاح لها الفرصة لتكوين مستعمرات تكون قادرة على القيام بدورها ، التحذير من اضافة المضادات الحيوية أو المطهرات فى نفس الوقت مع البروبيوتيك وكذلك يجب مراعاة تخزين البروبيوتيك تحت الظروف المناسبة.

اظهرت نتائج هذه الدراسات ايضا التاثيرات المختلفة للبروبيوتيك واهميا زيادة شهية الطائر، تحسين الاتزان الميكروبى للقناة الهضمية ، تنشيط الجهاز المناعى وكذلك تحسين الاستفادة من العناصر الغذائية عن طريق الانزيمات الهاضمة التى يتم افرازها عن طريق انواع اللاكتوبسيلس على سبيل المثال. وقد اقترح ناحيتين يتم بهما محافظة البروبيوتيك على الاتزان الميكروبى للقناة الهضمية ، الناحية الاولى هى تنافس البروبيوتيك للميكروبات الضارة و استبعادها و الاتجاه الثانى هو عن طريق النشاط المضاد وذلك بواسطة الافرازات المختلفة للبروبيوتيك. وجد ان اضافة البريبيوتيك (الغذاء المفضل للبروبيوتيك) متحدا مع البروبيوتيك يؤدى الى زيادة كفاءة البروبيوتيك عن استخدام كل على حدى ويسمى المنتج فى هذه الحالة سيمبيوتيك. و قد تم ارجاع عدم وجود تاثيرات ايجابية نتيجة استخدام البروبيوتيك فى بعض الاحيان الى ان البروبيوتيك قد يكون معزول من نوع و قدم الى نوع اخر أو عدم كفاية الجرعة المستخدمة.ولضمان الاستنتاج الصحيح يوصى باجراء مثل هذه الابحاث تحت الظروف التطبيقية التجارية مستخدما مكررات كافية و معاملة مقارنة مناسبة واخيرا التحليل الاحصائى المناسب للنتائج.اما بالنسبة للانواع والسلالات الجديدة من البروبيوتيك فيجب ان تخضع للاختبارات الدقيقة والتاكد من سلامتيا قبل استخدامها فى علائق الدواجن.