Urmia University

Effects of Dietary Probiotics Saccharomyces cerevisiae and Bioplus 2B on Performance, Egg Quality and Yolk/Serum Cholesterol of Laying Hens

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To

My

Parents

My kind full father and mother who devoted their life for me

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Map of the thesis

Chapter1

Introduction

The concept of probiotic was in use in the early 1990, however, the term was only coined in 1965 by Lilly and Stilwell and has subsequently evolved. Numerous definitions have been proposed. Initially it was used by Lilly and Stilwell to refer to the stimulation of the growth of one microbe by another, in other words, the opposite of antibiotic. Today it is generally agreed that a probiotic is a preparation of live microorganisms which, applied to man or animal, beneficially affects the host by improving the properties of the indigenous microbiota (Havenaar, et al., 1992).

In a natural environment the establishment of a microbial population in the digestive tract of all warm blooded animals, soon after birth, is inevitable. The microbial types which first establish, in most cases, are the forerunners of the final organisms which will colonize and persist in the digestive tract throughout the adult life of animal. It is known that the various type of colonizing bacteria is sensitive to changes which may occur in the digestive tract of the host. The digestive tract must supply the factors necessary for the existence of any micro-organism that finally becomes established. These factors include a favorable temperature, a constant supply of nutrient and essential fluids. In this situation the micro-organisms benefit from the environment and the animal benefits maintaining a micro flora that does not cause any disease state. There are generally two different types of bacterial populations which can become established in the digestive tract. The first is that which exists in close association with the gut epithelium and the second is that which occur free in the gut lumen. The population which establish themselves in the digestive tract can be either beneficial or harmful to the host. Not only can certain bacteria produce specific disease known to be detrimental to the host but they can complete for essential nutrients. If beneficial bacteria establish themselves, the host animal should also benefit accordingly. The ideal situation throughout the life of any animal would be to maintain specific numbers of beneficial bacteria in the digestive tract. This would ensure that at all times the animal would have the proper microbial balance. This, of course, cannot be guaranteed under natural field conditions. However, if microorganisms and/or substances which contribute to the proper, microbial balance are added to the diet then the animal would continually receive a "boost" to establishing the proper microbial population. (Fuller. 1971)

There are sufficient data in the literature the use of bacterial cultures to control and/or promote the proper environmental conditions for establishment of an ideal microbial population in an animal digestive tract. It is thought that These organisms will promote metabolism and suppress other undesirable bacteria. The typical bacterial culture used for this purpose has been that *lactobacilli*. The term" probiotic" has been used to indicate substances or micro-organisms which contribute to an ideal microbial balance. As defined by Crawford (1979) a probiotic is a culture of specific living micro-organisms (primarily *lactobacillus*). Which implants in the animal to which it is fed and ensures the effective establishment of intestinal populations of both beneficial and pathogenic organisms; the culture must consist of specific counts of the bacteria present, be maintained in a dry form for storage purposes, be temperature dependent produce an optimum response within a specific dose range. Approximately %90 of the intestinal flora of birds is composed by facultative bacteria. The remaining %10 consists of *E.coli, Clostridiums, Staphylococcus, Pseudomonas* and others (Fox, 1988). Microorganisms used in animal feeds are mainly bacterial strain belonging to different and sometimes distant genera, e.g. *Lactobacillus, Enterococcus, Pediococcus and Bacillus*, some of which are spore-forming. Other probiotics are microscopic fungi, including *Saccharomyces* yeasts (Guillot, 1998). Direct feed microbial benefit the host animal by stimulating appetite (Nahashon et al., 1992)

Haddadin et al., (1996) reported that egg size, egg production and egg quality were improved by the addition of probiotic to diet. The supplementation of *Lactobacillus Casei* with a maizebarley (50/50) based diet improved feed conversion ratio (FCR), egg weight, egg production and albumen quality (Tortuero and Fernandez, 1995). Mahdavi et al., (2005) reported that inclusion of probiotic (Bioplus 2B) in laying hens caused no significant decrease in feed consumption, egg production and egg weight.

It is also observed that some biochemical factors are influenced by probiotics e.g., this food additives can depress cholesterol concentration in blood and egg yolk(Mohan et al..,1995.; Abdulrahim et al.;1996., Haddadin et al.., 1996; Mahdavi et al.,2005).

Sccharomyces cerevisiae, known as" baker yeast" is one of the most widely commercialized species and one of the effective absorbent which is rich in crude protein (40-45%) and its biological value is high and it is rich in vitamin-B complex. (Reed and Nagodawithana., 1991). The effect of *Sccharomyces cerevisiae*, on egg production, egg quality, and blood and yolk cholesterol concentration of laying hens has never been evaluated or is scarce. This study was conducted to determine the effect of *Saccharomyces cerevisiae* and Bioplus 2B on performance, blood and yolk cholesterol of laying hens.

Chapter2

Literature review

2-1. Broilers

Torturo (1973) worked on the addition of *L. acidophilus* to poultry feeds. This author concluded that research relative to the use of lactic acid producing bacteria as growth stimulants for broilers was limited. The majority of research regarding probiotic has been concerned with topics other than growth promotion.

Torturo (1973) reported results from a study in which broilers were fed a culture of *L. acidophilus*. Data collected were weight gain, feed conversion, fat digestibility, nitrogen retention, caeca and faeces weight and levels of the lactic acid flora and *enterococci* up15 day of age. Four hundred sexed Hubbard chicks were divided into 4 groups of 100 chicks each. Fifty male and 50 female chicks were in each group. The following treatments were used (1) control (untreated) , (2) *L. acidophilus* in the drinking water, (3) antibiotic (20gm/ton zinc bacitracin) in feed,(4) *lactobacillus* in drinking water plus antibiotic in feed. The environment in the experiment was considered "old" because the house had been used for several years. Results indicated that implantation of *lactobacilli* resulted in an effect similar to that observed in chicks fed the probiotic and antibiotic. Although not statistically significant, differences in fat digestibility and nitrogen retention were reported. The implantation of *L. acidophilus* resulted in lower caeca and excreta weighted. The distinct change in the bacterial flora in the caeca and small intestine also occurred. By nine days the population of *enterococci* had almost completely disappeared.

Dilworth and Day (1978) conducted experiments designed to evaluate two *lactobacillus* cultures as supplements in broiler diets. In experiment 1, diets were fed that contained six leveled of probiotic culture of 0, 0.0250, 0.0375, 0.0500, 0.0625, and 0.0750%. Adding the probiotic culture to the diet resulted in a significant improvement in growth and feed efficiency. A three-week battery trial was conducted in second experiment and the probiotic was added to the diet at levels of 0, 0.05, 0.1 and 0.20% in half of hens diets, the levels of Methionine plus cystine and lysine were restricted to 90%of the control. Chicks fed the probiotic in 2of 3 comparisons at suboptimal amino acid levels had growth rates equal to those chicks fed adequate levels of amino acid. Growth was not increased by the addition of the probiotic in the diet containing adequate amino acid levels. No differences in feed efficiency were reported.

Crawford, (1979) reported data from four laboratory trials with broilers. A probiotic culture was fed in each trial at a rate of 454 g per ton of feed. The studies were from hatch to market weight. The mean weight gain between birds fed the probiotic and the control were 1.88 kg, and 1.83 kg, respectively. However, this difference, favoring the probiotic, was not statistically significant. Feed conversion ratio for probiotic and control was 2.26 and 2.37, respectively.

Burkett et al. (1977) supplemented a control broiler diet with a *lactobacillus*, yeast, or a combination of *lactobacillus* and yeast. Birds were raised under commercial condition. At 8 weeks no significant differences in body weight gain between treatment groups were found. Birds fed the combination of probiotic and yeast had greater pigmentation and fat deposition.

Couch (1978) fed probiotic to commercial broilers at a rate of 454 g per ton. *Lactobacillus* supplemented birds had an increase in average weight of 47 grams, 0.04% decreased in mortality and 0.81 points improvement in feed conversion. Adler and Damassa (1980) reported that feeding a *lactobacillus* culture to chicks resulted in an improvement in body weights and reduced the occurrence of pasted vents.

In contrast to several beneficial responses of probiotic supplementation to broiler diets, Buenrostro and Kratzer (1983) found that broiler grown in battery brooders and fed a diet containing a *lactobacillus* culture did not performed as well as control birds or those fed antibiotics. The study involved feeding broiler chicks various levels of biotin to determine the effect on the biotin status of chicks. The *lactobacillus* groups were fed a diet similar or the control group, but chicks were inoculated on alternate days with a commercial *L. acidophilus* culture which contained 1×10^8 organisms. This inoculation resulted in a significant decrease in growth and liver biotin in chicks fed a diet marginally deficient in biotin. Rogosa et al. (1961) reported that *lactobacilli* respond to biotin. These authors implied that the poor performance of inoculated chicks was a result of competition for biotin between host and micro-organism. From these data it would seen advisable to ensure an adequate biotin status in the diet if probiotic are fed. Watkins and Kratzer (1983) suggested that there was possibly a proper level of *lactobacilli* required by chicken that provides the most host benefits. Dosing below or above may results in undesirable effects such as bacteria competition for biotin. Watkins and Kratzer (1983) reported that *lactobacilli* did not decrease liver biotin in broilers fed a practical diet adequate in biotin.

2-2. Layer Hens

Kruger et al. (1977) conducted a study with young leghorn hens to investigate the possibility of an interaction between gentian violet a *lactobacillus* culture in the diet. The gentian violet and *lactobacillus* culture were added to the diet separately and in combination at rates of 454 grams and 2.27kg per ton, respectively. When compared to a control diet the addition of gentian violet or the probiotic to the diet increased egg production 3.07 and 3.03%, respectively. Feed efficiency improved 3.46 and 7.41% for gentian violet and probiotic, respectively. A 9.02% improvement in egg production and 10.51%improvement in feed efficiency resulted when both gentian violet or and probiotic were fed in combination. Neither treatment influenced fertility or hatchability.

Charles and Duke (1978) fed 21-week old white leghorn hens a control and a probiotic supplemented diet for twelve 28-day periods. The probiotic was supplemented at 0.25%of the diet. Result indicated that no significant deference existed between egg production during periods 1 to 6, but in periods 7 to 12 a significant ($P<0.05$) increase in egg production, favoring the probiotic, resulted. In a second experiment the probiotic was fed at levels of 0, 32 or 64 grams per kilogram of diet. Birds were housed in floor pens and were 23 weeks old at the inhibition of the experiment. The probiotic had influence on egg production.

Crawford (1979) reported result from nine trials with commercial layers using probiotic while control hens. Overall egg production averaged 72.17% for hens fed the probiotic while control hens averaged 69.5%. The kilograms of feed required to produce a dozen eggs was improved from 1.75 to 1.69 in the control *vs.* the probiotic groups respectively.

Miles et al. (1981) incorporated a living *L. acidophilus* culture into the diets of two commercial strains of laying hens at three geographical locations within the United States. The culture was added at the rate of 0.0125, 0.0375 or 0.0625% of the diet. Feeding the probiotic resulted in increased egg production at one location (Arizona), a numerical improvement at the second (Florida) and no differences at third location (South Dakota). An across trial analysis of all egg production data provided a statistical advantage to adding probiotic to the diet. One location (Arizona) reported a significant response in feed efficiency at lower probiotic levels. The probiotic had no influence on egg quality or egg weight. At two locations hens consumed more feed during warmer months of the year. An early increase in *L. acidophilus* levels accompanied by a reduction in *coliforms* in selected gastrointestinal sections was reported at the Florida location. A later analysis of the micro flora did not substantiate this previous observation.

Cerniglia et al, (1983) conducted five trials in which they reported the response of cage and floor housed layer fed a probiotic. In trial 1, a liquid nonviable *lactobacillus* product was added to the diet of floor and caged birds at levels of 0.236, 0.473, and 0.709 liters per ton. No significant differences were observed in percent hen day egg production, daily feed intake, mortality or body weight gain. In trial 2, a dried non-viable *lactobacillus* product was fed at 227, 454 and 686 grams per ton of feed. The probiotic diets were fed without or with 25 grams zinc bacitracin per ton. The only beneficial response from the probiotic was a significant increase in the percent large and extra large eggs from birds fed the diet containing the highest probiotic level without zinc bacitracin. The effect of feeding a dried viable *lactobacillus* product in either a 14 or 17% protein diet was studied in trial 3. No difference in egg production from 27 to 43 or 27 to 70 weeks of age were increase in the number of large and extra large eggs for the same time period. In trials 4 and 5 the probiotic had no influence on performance of various age birds from day 1 through 72 weeks of age.

Haddadin et al, (1996) reported that fed for a 48- wk period with a basal diet supplemented with a selected strain of *lactobacillus acidophilus* at levels up to four million viable cells per gram of feed on laying hens showed that levels of egg production and feed conversion ratio were significantly higher (8 and 14.8% respectively) than in the control flock, and cholesterol values yolk were decreased by 18.8%. It is suggested that the effect was a reflection of lower serum cholesterol concentration in treated birds; a maximum reduction of over 55% followed incorporation of the culture into feed. The level of viable cells in the feed was confirmed as being critical to register the above effects.

Recently Mahdavi et al, (2005) studied the effect of probiotic supplements (0, 400, 100 and 2000 gr Bioplus 2B) on hens performance, egg quality on eighty white leghorn Hy-line, W-36 strain. Evaluated traits were egg production, egg weight, egg mass, feed consumption, feed conversion ratio, shell thickness, shell hardness, Hough unit, egg cholesterol levels of probiotic caused highly significant increase ($p<0.01$) in goblet cell numbers, significant increase ($p<0.05$) in destroying apical cells of villus and significant decrease (p, <0.05) in plasma cholesterol, plasma triglyceride and egg cholesterol (mg gr^{-1} of yolk), but it had no significant effects on other traits.

2-3. Turkeys

In a popular article (feedstuffs, 1977) a report summarized research conducted at south Dakota State University (USA) in which a probiotic culture was fed to turkeys until 22 weeks of age. A total of 660 poults, 330 of each of two strains, were grown in batteries until 3 weeks of age when they were moved to floor pens. The probiotic culture was fed at 156 or 1, 135 grams per ton. Results indicated that there was no beneficial effect due to the probiotic.

Fracis et al. (1978) conducted an experiment using Broad Breasted large white turkey poults to study the effect of adding a probiotic or zinc bacitracin, either individually or in combination, to the diet. Day old poults were divided into4 test groups. The *L. acidophilus* mixture was added to the diet at 0 and 750 mg per kilogram. Zing bacitracin was used added at a level of 0 and 55 mg per kilogram. A corn soybean meal basal diet was used in this study. The poults were housed in batteries for 3 weeks. The addition of either the probiotic or zinc bacitracin to the diet resulted in numerically improved body weights feed efficiency. The improvement in growth from the combination of the two supplementations was not as great as when either was fed alone. The probiotic resulted in a significant decrease in *coliform* and total aerobe counts in the feed and alimentary canal. When zinc bacitracin was added alone there was a significant decrease *coliform* level and in total aerobes in the alimentary canal.

However, this reduction was greater with the probiotic alone. Total *lactobacillus* counts were higher in the alimentary canal when the probiotic was added to the diet.

Potter (1979) conducted an experiment with Medium White turkeys from 0 to 16 weeks of age to evaluate the influence of a dry L. acidophilus culture in the diet. Levels of probiotic added were 0, 0.025, 0.050, and 0.075%. The lowest level of probiotic increased ($p<0.05$) body weight 1.6 to 2.5% at 8, 10 and 12 weeks of age. At 16 weeks average body weight were 5.55, 5.56, 5.58 and 5.65 kg and feed efficiencies (gain: feed) were 0.422, 0.422, 0.423 and 0.426 for turkeys fed 0, 0.025, 0.050 and 0.750% probiotic, respectively. However, the body weight and feed efficiencies were not significantly different.

Damron et al. (1981) reported results from two experiments which were conducted for 112 days each. Broad Breasted large White turkey hens were housed individually in wire cages in experiment 1. In experiment 2, floor pens that contained 5 hens each were used. Each diet was fed to 5 replicate pens. Treatments consisted of a control corn-soybean meal diet and a similar diet containing 625 mg of a probiotic per kilogram. Egg production, daily feed intake, body weight change, fertility and hatchability were not influenced by the addition of probiotic to the diet in either experiment

Bradley et al., (1994) worked on the effects of supplementing diets with *Saccharomyces cervisiae* var. *boulardi* on male poult performance and ileal morphology. They showed that body weight was improved during 7, 14and 21 days in groups which had used *Saccharomyces cervisiae* in their diets.

2-4. Bobwhite quail.

Miles et al. (1981) conducted two experiments in which 4800 Bobwhite Quail were fed a corn-soybean meal starter diet supplemented with a probiotic culture containing *L. acidophilus* and other *lactobacilli.* In experiment 1, there were fed in each of two trials to four replicate pens of 200 chicks per pen from hatch to 5 weeks of age. The three experimental diets contained probiotic at levels of 0, 250 and 625 mg per kilogram respectively. Diets were mixed each 2 weeks to insure a viable culture during the experimental period. In experiment 2, the same design was used as in experiment 1 except levels of 0, 125, 250 and 375 mg probiotic per kilogram were fed. Results indicated that no significant differences existed in growth, feed efficiency or mortality when quail fed the probiotic were compared those fed the unsupplemented control diet. Mortality in this study was higher than normal in all treatments but was not treatment related.

Miles et al. (1981) conducted an experiment with Bobwhite quail breeder 80 weeks of age. The experiment was conducted for 56 days and a total of 96 caged pairs of male and female birds were fed a corn-soybean meal breeder diet containing 0 or 625 mg probiotic per kilogram. No significant differences were found between treatments in egg production, feed consumption, and fertility, hatchability of fertile eggs or mortality.

2-5. Japanese quail

Probiotic have been conducted with increasing frequency in nutrition and for prophylactic purposes. in this study Strompfova et al (2005) investigated the effect of lactobacillus fermentum AD – canine isolated on selected intestinal microbial group, weight gain, organic acids, hematology, glutathione peroxides and phagocytosis of leucocytes in 2- days- old Japanese quail (*coturnix coturnix japoaica*). The results demonstrated that the 4-day application of this strain significantly increased the population of lactic acid bacteria- *lactobacilli* and *enterococci* in faeces ($P<0.05$). The daily weight gain was increased by 14%. Although intestinal pH of both groups of birds was similar, the concentration of other organic acids (acetic, acetoacetic, formic, succinic, valeric, propionic, butyric) as well as blood glutathione peroxidase was not influenced. The index of phagocytic activity of leucocytes was significantly improved.

2-6. Probiotics from the point of view of animal nutrition

The concept of probiotic was first developed by Elie Metshinkoff nearly one hundred years ago who proposed the hypothesis that special bacteria in fermented milk produced may be capable of controlling fermentation processes in the intestinal tract and furthermore of preventing arteriosclerosis and promote longevity of men. Mainly several species of the genus lactobacillus are considered to be having this health promoting effects in humans. In the last two decades the probiotic concept has also been applied to animal nutrition. For this application probiotics are defined as viable microorganisms which are used as feed additive and which lead to beneficial effects for the animal because of an improvement of the intestinal microbial balance. In farm animals beneficial effects are measurable parameters like improvements in daily weight gain, feed conversion ratio or reductions of the incidence of diarrhea especially in young animals. The goals of the use of probiotics in animal nutrition are therefore short-term effects in contrast to the use in humans were long-term effects are expected. There are even more differences between the use of probiotic in human nutrition and animal nutrition. This applies to the genera of microorganisms used, their frequency of intake and the concentration of probiotics in food/feed. Animals consume probiotic microorganism in each meal in an equal concentration which is usually in the range of 10^9 viable counts per kg of feed. (Fuller, 1977)

2-6-1. Alternatives to antimicrobials in food animals

Alternatives to antimicrobials in food animal production include management practices that reduce the likelihood and effect of infectious diseases and also increase the production efficiency. Established veterinary steps to prevent or control infectious diseases include improved husbandry practices, quarantines and other biosecurity measures, and vaccinations. Other treatments include genetic selection to enhance disease resistance, uses of antiseptics such as teat dipping to prevent mastitis, vector control, and use of probiotics or other competitive microorganisms to exclude pathogens (Dial., et al 199). Moreover, control of viral and other infections can reduce secondary bacterial infections, thus reducing the need for antimicrobial therapy (Wills et al., 2000).

2-6-2. Mode of action

Lactobacilli are capable of producing large amounts of lactate from simple carbohydrates and concomitantly can withstand a high degree of acidity which is usually fatal to other bacteria. Fuller (1977) adjusted the pH of an agar medium to 4.5 with lactic acid or HCL. The growth of *E. coli* was inhibited by the low pH. Lactic acid was shown to inhibit the growth of *E. coli* and the inhibited effect of hydrochloric acid was identical to that obtained with lactic acid. It was also reported that inhibition of *E .coli* in the crop was dependent on the presence of sufficient numbers of *lactobacilli*. Rantala and Nurmi(1973) showed that a probiotic culture had the ability to prevent the establishment of *Salmonella infantis* in the caeca of chicks although in this case *lactobacilli* were not the only organisms present. Some *lactobacilli* are part of the normal intestinal flora of warm blooded animals. Lactic acid bacteria have long been considered desirable inhabitants of the digestive tract. Mitchell and Kenworthy(1976) investigated the possibility of lactic acid bacteria interacting with the enterotoxin produced by pathogenic *E. coli*. Nine of eleven species tested inhibited the growth of *E. coli* an agar medium.

Timms (1968) was able to demonstrate that the population of *lactobacilli* increased when chicks consumed high carbohydrate diets. March (1979) published a review on the host animal and its microflora. This article explained the complex relation between the host animal and intestinal micro flora and why knowledge in this area is still fragmentary. While rare instances of an association between *lactobacilli* and pathological conditions has been noted, these bacteria are essentially non pathogenic. They are primarily of interest in the dairy and fermentation industries and more recently in the production of probiotic for use in domestic production animal.

Savage (1981) presented an excellent review concerning the mode of action and the potential of probiotic in animal feeds. He indicated that strains of certain endogenous *lactobacillus* species are known to associate with epithelial surfaces in the alimentary canal of some mammals and birds. The bacterial strains colonize the surface early in the animal's life and adhere to them by mechanisms which are relatively specific for the animals of the species from which the strain are derived. He stated further that in animals from some species, the micro-organisms colonize the epithelial habitat and multiply on the surface. The crop of fowl normally contains a micro flora in which the lactobacilli predominate over *coliforms* and *streptococci* (Smith, 1965). This population of *lactobacilli* can have an influence on the population of the small intestine. Ecological studies by Fuller and Turvey(1971) on the *lactobacillus* flora associated with the intestinal wall demonstrated that the crop was the source of *lactobacilli* for maintaining the bacterial balance in the intestines. Attachment of the lactobacilli to the crop epithelium is important because it enables a large number of these bacteria to remain after the food has left the crop. This adhering population of organisms serves to inoculate incoming food and ensures dominance for the suppression of the *E. coli* population. Fuller (1973) reported that the adhesion is species specific and *lactobacilli* isolated from animals of other species will not successfully adhere to crop cells of fowl *in vitro*. Fuller (1975) was able to show that certain host specific strains of *lactobacilli* would attach to crop epithelium. The exact mechanism of how *lactobacilli* attach to crop epithelium of fowl is discussed in an article by Brooker and fuller (1975) and Costerton et al. (1978).

Lactobacillus strain can colonize the alimentary surface if they can multiply in the prevailing environmental and nutritional condition. Lev and Briggis(1956) reported that after feeding a l*actobacillus* culture to chicks a balanced lactic acid micro flora had established in the duodenum, ileum, and caecum within 24 hours. It would therefore seem effective to use probiotic flowing antibiotic withdrawal from the diet to promote the re-establishment of a favorable microbial population in the digestive tract. When colonizing the surfaces, the *lactobacilli* prevent certain indigenous yeasts from establishing the regain, and probably also contribute to repressing the growth of *E. coli* and certain gram-negetive pathogens in the small intestine.

Recently, Watkins and Miller (1983) reported that the shedding of pathogenic *S. typhimurium* and *Staph. Aureus* in the faeces of gnotobiotic chicks was greatly reduced by consecutive treatment with *L. acidophilus* for both prophylactic and therapeutic treatment schedules. A significant (P<0.05) increase in the shedding *lactobacillus* treatments was observed with the decreased shedding of both pathogens. The *lactobacillus* treatments reduced average mortality from *S. typhimurium* from 36.7 to 8.8%. Mortality from *Staph. Aureus* was reduced from 32.6 to 11.1% by consecutive treatments with *lactobacillus acidophilus*. Many other investigators have studied the potential of this" beneficial" genus of bacteria, species of which exert inhibitory effects toward enteric micro-organism (Morishita et al., 1971; Fuller, 1977; Gilliland and Speck, 1977; Watkins, 1981 and Watkins wt al., 1982). Also, *Lactobacilli* have been reported to inhibit growth of *salmonella*.

2-6-3. Composition of gut flora

Fuller in 1977 reported that there is, in the gut, a very complex population of micro organisms which interact with each other and with the host animal. Estimates put the number of different types of micro organisms in the gut at 400 and the total number of bacterial cells at 1014; a figure which far exceeds the total number of human beings in the world. Although the composition of the gut micro flora is fairly constant and characteristic for each host species it can be affected by various factors such as:

Age: the micro flora of live young suckling mammal is different from that of the adult Diet: to some extent this will be responsible for the changes seen with age, but even between adults the composition of the diet can affect the composition of the gut micro flora.

Environment: the conditions under which farm animals are reared differ from the natural conditions under which their wild counterparts developed. The physiological responses to the artificial nature of the domestic/farm environment may in turn affect the gut micro flora.

Stress: the unnatural conditions of farm rearing product stresses which induce hormonal changes which can affect mucous secretion and flora composition of the gut.

Medication: the use of antibiotics and other chemical antibacterial compounds either as growth promoters or as therapeutic agents can change the gut micro flora in such a way as to allow the growth of pathogens.

2-6-4. Role of the gut flora

Why does the animal tolerate the presence, within its gut, of this vast number of micro organisms? It does so because it has evolved a symbiotic relationship in which the bacteria get food and a suitable environment for growth and the host animal acquires protection against some forms of disease. The evidence for this is as follows:

Comparison of germ free and conventional animals with a complete gut micro flora shows that the former are more susceptible to disease than are their normal counterparts.

Oral administration of antibiotics and other antibacterial compounds increases susceptibility to disease. The difference is that the antibacterial compounds are suppressing the organisms which normally protect against disease, allowing the pathogens to grow.

Animals with a deficient flora can have their resistance restored by administration of a faecal suspension from healthy adult animals of the same species. A good example of this effect is the faecal dosing of day old chickens hatched into a clean environment without the opportunity to acquire their protective flora from the mother hen. These chicks are more susceptible to colonization with Salmonellae but after dosing with a faecal suspension from an adult chicken they become resistant. (Fuller, 1977)

2-6-5. Composition of probiotic preparations

While dosing with a faecal suspension is very effective, it risks introducing pathogens to the animal being dosed. To avoid this risk many groups of research workers throughout the world have attempted to produce a faecal suspension which is free from pathogens. Others have attempted to identify the particular organisms involved in the protective effect and restore the resistance by supplementing the diet with these known cultures. Preparations such as these are known as probiotics, a word first used in this context by Parker in 1974. At this time Parker defined probiotics as: 'Organisms and substances which contribute to intestinal microbial balance'.

Later fuller, in 1989, modified this to read: 'A live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance. This revised definition stresses the importance of live cells as an essential component of the probiotic preparation. The most commonly used organisms in probiotic preparations are the lactic acid bacteria (*lactobacilli, streptococci* and *bifidobacteria*). These are found in large numbers in the gut of healthy animals and do not appear to affect them adversely. They are in the words of the America FDA, Generally Regarded as Safe (GRAS). Organisms other than lactic acid bacteria which are currently being used in probiotic preparations include Bacillus sp., yeasts (*Saccharomyces cerevisiae Sac. boulardii*) and filamentous fungi (*Aspergillus oryzae)*. These probiotic preparations may be presented in different ways depending on the animal receiving the supplement and the condition to be treated. Thus they may be in the form of powders, tablets, capsules, pastes or sprays

2-6-6. Probiotic Action mechanism

Approximately 90% of the intestinal flora of birds are composed by facultive bacteria that produce lactic acid (*Lactobacillus, Enterococcus,* etc.) and strict anaerobic bacteria (*Fusobacterium, Eubacterim*,erc). The remain 10% consist of *Escherichia coli, Clostridum, Staphylicoccus, Psedomonas*, and others (Fox, 1988).

The birds are submitted to several stress factors, such as, transport from the incubator to commercial farms, overpopulation in aviaries, vaccinations and temperature changes. This tends to induce a misbalance in the intestinal micro flora and damage to the birds corporal defense mechanism(Jin et al.,1997), causing a low productive performance and such intestinal infections, as intestine rotting with the formation and liberation of toxins; jeopardizing of the growth, opportunistic bacteria become pathogenic; emergence of infections, diarrheas and anemia (Fox,1988).

While research alternative to replace antibiotics in animal husbandry, specialists focused directed their attention to the natural defense mechanism of animals, the microorganisms present in the gastro-intestinal tract. Thus, the probiotic because an effective alternative to replace antibiotics, acting as growth promoters in the treatment of alimentary diarrheas and/or bacterial. The microorganisms used as probiotic are;*Lactobacillus acidophilus plantarum, Lactobacillus bulgarius, Lactobacillus married, Lactobacillus faecium,* gram positive bacteria that produce lactic acid, natural inhabitants of the gastro-intestinal tract and that act indeed as probiotic, sticking to the intestinal epithelium and colonizing the track. Other such as *Bacillus subtilis, Bacillus toyo* and *Bacillus bifidum* are used combined, isolated or some times associated with years, enzymes and other agents, with the purpose of helping the bacteria that produce lactic acid in colonization (Maruta, 1993). Among these, the most used microorganisms are *Bacillus subtilis* (classified as transitory in the gastro-intestinal tract, because it does not possess the capacity to fix itself on the intestinal epithelium, but that of helping in the multiplication and colonization of lactic acid producing bacteria); *Lactobacillus acidophilus*(bacteria that produces lactic acid from the fermentation of sugar, it is anaerobic facultive and is nutritionally demanding, needing for its growth the vitamins; niacin, riboflavin and folic acid); besides the *Enteroccoccus faecium*(a quite aggressive microorganism and a little more resistant to high temperatures than *Lactobacillus*.

The probiotic act through competitive exclusion, sticking to specific sites located in the intestinal epithelium, thus decreasing colonizing pathogenic microorganism. The mechanism of competitive exclusion is not totally explained, however several researches were made and some of the probiotic ways of action are displayed bellow (FOX, 1988 and Jin et al., 1997)

•In the intestine, probiotic microorganisms will accomplish a fast metabolization of substrata (sugar, vitamin, amino acids, proteins) making them unavailable to pathogen and, consequently, preventing their proliferation
•Through the production of lactic acid, they cause a reduction in the intestinal pH, making it inappropriate for the multiplication of pathogenic agents.
•They secrete proteins (bacteriocines) that have an inhibitory or destructive action against a specific strain of bacteria.
•The lactic acids producing bacteria can stimulate the production of antibodies and the phagocyte activity against pathogens within the intestine and in other tissues of the body
•Beneficial bacteria increase enzymatic in the gastro-intestinal tract.
•Increase of the area of absorption of the small intestine.

2-6-7. Probiotics for Farm Animals

Modern rearing methods which include unnatural rearing conditions and diets induce stress and can cause changes in the composition of the microflora which compromise the animals' resistance to infection. The aim of the probiotic approach is to repair the deficiencies in the microflora and restore the animals' resistance to disease Such a treatment does not introduce any foreign chemicals into the animal's internal environment and does not run the risk of contaminating the carcass and introducing hazardous chemicals into the food chain
Probiotics are now replacing the chemical growth promoters for farm animals and claims have also been made for increasing resistance to disease. The benefits claimed for probiotics in farm animals are as follows:
Increased growth rate
Improved feed conversation
Improved resistance to disease
Improved milk yield and quality

Improved egg production
The results obtained are sometimes variable but bearing in mind the different ways and conditions under which probiotics may be operating it is not surprising that they are sometimes not active. It should be remembered that probiotics are not a single entity; different probiotics contain different micro organisms which may behave differently. Even different strains of the same species may have different metabolic activities which affect the result when they are used as probiotics. Negative results may also be explained by the poor viability of the preparation. Although this is crucial to the outcome it is not always checked when trials are done. Other factors which may also explain variation in results include the growth phase of the animal, the type of dosing used and the hygienic condition of the housing. With all these possible variations it is not surprising that probiotics do not always give the desired result but the fact that significant results are obtained shows that using the right probiotic, under the right conditions and using the correct method of administration they do work and are an effective feed supplement for farm animals.(Fuller, 1989)

2-6-8. Commercial utilization of probiotics

Commercial preparations of probiotic can be produced if methods can be developed to growth the micro-organism in sufficient quantity and without loss of properties essential for colonizing epithelial surfaces in the alimentary canal. Many commercial companies today are engaged in the production of several forms of these probiotic preparations. Also, many research institutions have been actively involved in studies designed to test the efficiency of such preparations on animal growth and performance. A considerable part of this research has been directed to studying the mechanisms of these preparations as they are related to attachment, colonization, host specificity, nutritional requirements and interaction with other micro-organisms. Because of this importance of poultry as an economic and nutritious from of animal protein and the fast growing characteristics of this animal, research workers have devoted studies to the use of probiotic in poultry.(Miles, 1981)

2-6-9. Age and physiological state of animals

Studies have indicated that at certain ages or physiological states herbivores have increased faecal output of pathogens. For example, immediately post-partum an increase in faecal shedding in dairy cows and sheep has been observed (Mechie et al. 1997), but the most critical time for faecal shedding of E. coli O157 in cattle is immediately after weaning of the calf (Garber et al. 1995;). Garber et al. (1995) found that calves were 3 times more likely to contain E. coli O157 immediately post-weaning than pre-weaning. However, the infant calf offers a possible point of control. All dairy calves and most beef calves are fed milk replacement diets rather than milk from their mothers. Thus, the potential to manipulate the pre-ruminant gut at this time offers a mechanism for establishing a 'good' gut micro flora, by feeding either pre-fermented milk replacement powder or probiotic bacteria that establish in the gut of the ruminant. Control of enteric pathogens in the pre-ruminant will at the very least reduce environmental contamination through faeces, but may enable establishment of a gut environment at this early age that gives life-long resistance to future establishment of pathogenic populations in the gut. Establishment of probiotic bacteria including non-pathogenic E. coli and Proteus mirabilis (Zhao et al., 1998) was found in calves dosed with a