

PERFORMANCE OF PROBIOTICS: AN ALTERNATIVE TO ANTIBIOTIC IN BROILER PRODUCTION

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ABSTRACT: During the last 5 to 6 decades antibiotics have been used in an increasing amount in the field of poultry production. Antibiotics are used for treatments and as growth promoters. Now-a-days there is an increasing demand to produce antibiotic free poultry and livestock. For this purpose in my experiment the chickens were fed Probiotics at the first day before providing them any feed. In the case of Probiotics (T₁) treatment the Probiotics mixed water at a dose one gram (2x 10⁹ CFU/gm) probiotic powder with four litter water were provided to them for eight hours at the first day. Then from the 2nd day to the 8th day they were provided at the same dose in the morning water per day. In the case of antibiotic & Probiotics (T₂) treatment, 3 days after using antibiotics, Probiotics mixed water at the same dose was provided to them for three consecutive days in the morning water. In the case of antibiotic (T₀) treatment, Probiotics were not used. In the 15th, 16th and 17th days Probiotics were again provided at the same dose mentioning above in the morning water for T₁ and T₂ treatment groups. Among all the treatments Probiotics showed the best weight and feed conversion rate (FCR). FCR of the Probiotics treated broilers was 75.08 % while the FCR of the T₀ and T₂ treated broilers were 63.78 % and 67.48 % respectively. The degree of difference in the case of immunity development was also noticeable. The event of incidence of Diarrhoea was zero for Probiotics treated broilers which indicated normal gut microflora raised from the exclusion and killing of pathogens in the intestinal tract while other treatment given groups showed Diarrhoea for several times. The mortality rates were 0 % for T₁ and T₂ treatments while the mortality rate was upto 10 % in the case of T₀ group. Eventually the Probiotics treated broilers achieved the possibility of decreased human health risk.

Keywords: Antibiotic, Probiotics, FCR, Microflora, Diarrhoea, immunity.

INTRODUCTION

Probiotics are dietary supplements containing potentially beneficial microorganisms. Probiotic bacterial cultures are intended to assist the body's naturally occurring flora within the digestive tract. They execute their activities by adopting several mechanisms. The mechanisms are described as follows:

Creates gut microenvironment suitable for beneficial microorganisms:

Since there are prospective invasive microorganisms existing in our common environments the steadiness among the gut micro flora and the host in both mammals and birds can be confronted every day. Those

microorganisms might be commensals (they live in the intestinal gut without causing problems when having a normal balance among microbiological species) or nosocomial (opportunistic pathogens living outside the body). These potential pathogens are present in our drinking water, food and the air we breathe. They are always ready to confront the symbiotic relationship between the host and the gut microbiota. Since of this constant state of blockade; complex defense mechanisms have evolved to deal with the potential invaders.

It can be noticed that Probiotic organisms consisting of host adapted character are able to exclude or kill pathogenic bacteria. the immune system can also be stimulated by their non-

pathogenic characteristics. It is possible to enhance the animal performance by resisting gastric acids and bile salts by them; they can decrease carcass contamination through attaching readily to epithelium and mucus. They can improve nutrient absorption by altering microbial activity; through modulation of immune responses it is possible to decrease diarrhea by them; Through the inhibitory substances they produce against other bacteria they can increase vitamin B synthesis. All foods must be subjected to gastric pH in the range of 2.0 to 4.0 that can cause a ten to one hundred fold killing of bacteria in the food since these are digested in the upper gastrointestinal tract. The micro-ecology of the intestinal tract is the influential factor for the survival of specific microorganisms. The creation of volatile fatty acids at a pH under 6.0 is known to reduce the populations of *Salmonella* and *Enterobacteriaceae* (Maynell, 1963). Killing of the normal intestinal microbiota with antibiotics must bring to an end this protective mechanism since the concentrations of volatile fatty acids which are produced by the intestinal microorganisms will reduce and gut pH will increase. According to some literature in newly hatched chicks in the industrial hatcheries, the concentration of volatile fatty acid and the acidic condition are not adequate to suppress pathogens (Barnes *et al.*, 1979, 1980a, 1980b), and that is why, probiotic microorganism's supplementation will be very advantageous.

A well balanced microbial environment provided by supplementation of probiotic organisms prevents adaptation of ingested and momentary pathogenic microbes. It is very much necessary to apply probiotic products as soon as possible for achieving the best results in poultry (Casas *et al.*, 1993, 1998; Edens *et al.*, 1997a).

Chicken hatching environments are seriously contaminated by bacteria, viruses, and protozoans. That is why it is a must to begin to develop protective gut microflora as soon as a chicken is hatched. The gastrointestinal tract of the chicken is usually a void of beneficial bacteria at the time of hatching, and a stage of five to seven days after hatching is necessary to set up a healthy population of lactic acid

bacteria in the gut. The lactic acid bacteria are the leading bacteria throughout the gastrointestinal tract from the crop through the large intestine since they can survive both aerobic and anaerobic condition. Having the abundance of substrates, the lactic acid bacteria flourish in the gut and produce lactic acid and hydrogen peroxide as well as antibacterial products such as bacteriocins, reuterin, nisin, or lactococcins, all of those are recognized to have inhibitory effects on enterobacteriaceae genera such as *E. coli* and *Salmonella* spp., even some other bacteria such as *Staphylococci* spp., *Clostridium* spp., *Listeria* spp. both *in vitro* and *in vivo*.

According to the work of Mishra and Lambert (1996) it is known that different types of inhibitory products produced by probiotic bacteria have different mechanisms of inhibition on target organisms. Such as- Lactic, butyric, propionic, and acetic acids can disorder the metabolism of target organism, Hydrogen peroxide can inactivate the indispensable biomolecules and induce lactoperoxidase system, arginine utilization can be interefered with Diacetyl. Bacteriocin, nisin, pediocin A, reuterin, subtilisin, colicin etc. show broad and narrow spectrum activities against membrane and membrane structures, membrane lysis and distraction of receptors.

Newly hatched chicken begins to pick-up coliforms and streptococci from the food and its environment before the development of lactic acid bacterial populations in the gut. Because of the delay of development of a population of beneficial bacteria, these bacteria can be beneficial or pathogenic, the potential for colonization by pathogenic strains can be elevated, but usually, for the maternal antibodies pathogen colonization can be prevented. In the normal conditions, three to five week period is necessary for the development of a secure population of gut associated bacteria, and it is in the ceca where the greatest numbers reside (Sarra *et al.*, 1992). Inside the ceca, an anaerobic environment is developed that favors the growth of organisms such as *Bifidobacterium* spp. and *Bacteriodes* spp. which create a microenvironment that can be characterized by an acid pH resulting from

the production of volatile fatty acids (acetic, butyric, propionic, and lactic acids) and antimicrobial products that potentially exclude or kill many different harmful pathogens.

Available receptor sites abolition

Polysaccharide-containing components attached to the cell wall of bacteria are responsible for the the adhesion of microorganisms to the gut epithelium (Soerjadi *et al.*, 1982). Common bacteria can adhere each other by an acidic polysaccharide cell wall component and this component is also responsible for binding to the intestinal epithelium preventing other bacteria from attaching to the epithelium, successfully blocking all receptor sites (Fuller, 1975). Though, a large number of other mechanisms are also prevailed. Neeser *et al.* (2000) demonstrated that *Lactobacillus johnsonii* La1 had two major carbohydrate-binding specificities having O-linked oligomannosides and the gangliotriosylceramide and gangliotetraosylceramide (asialo-GM1). Several enteropathogens also express similar kind of carbohydrate-binding specificities. Thus, the binding of the pathogens to the mucosal epithelial mannan receptors *can be inhibited L. johnsonii*. Gusils *et al.* (2000) have demonstrated that chicken *L. animalis* and *L. fermentum* make use of a lectin-like structure which has glucose/mannose as specific sugars of binding. Adhesion of chicken *L. fermentum* to host specific epithelial cells can be reduced by the addition of mannose or sialic acid to culture media. Chicken *L. fermentum* reduces attachment to host-specific epithelial cells of *S. pullorum* by 77%, and *L. animalis* decreases adhesion by *S. pullorum*, *S. enteritidis*, and *S. gallinarum* by 90%, 88%, and 78%, respectively.

A research report by Lee *et al.* (2000) demonstrated that even though probiotic bacteria such as *L. rhamnosus* GG and *L. casei* Shirota have alike carbohydrate-binding specificities compared with *E. coli*, they do not stop binding of the pathogen to intestinal cells even if sufficient amount of probiotic cell numbers are exist. If sufficient numbers of probiotic bacteria are exist, the probiotic bacteria seem to inhibit *E. coli* attachment to intestinal cells. Complex and extremely

competitive competition are present among probiotic and pathogenic bacteria. If the amounts of *Lactobacilli* decrease in the intestinal lumen, the *Lactobacilli* can be replaced by pathogens. Adhesion of probiotic and the pathogenic bacteria significantly depends on the mucus layer on the intestinal cells. While some probiotic bacteria have low affinity for mucus binding sites, others have very high affinity. In addition, pathogenic bacteria have irregular affinities for binding sites on the mucus layer. Having multiple binding sites in mucus and on the intestinal cell surface, a probiotic bacterium has its ability to eliminate pathogens might be enhanced. Therefore, providing the highest number of probiotic bacteria is necessary to attain the best results for the control of pathogenic bacteria.

pH of the luminal contents influence the competition for available binding sites on the intestinal mucosa. It has been demonstrated by Fuller (1977, 1978) that the survival of acid loving bacteria such as the *Lactobacilli* is favored by an acid pH. Eventually, a huge numbers of *Lactobacilli* will bind to the intestinal mucosal epithelial cells and leave out pathogens such as *Salmonella* and *E. coli*. In addition, the media component in which the probiotic microorganisms are growing will influence the bond of the organism to the mucosal epithelium and influence its resistance to acid (Fuller, 1975).

Digestive tracts contents are not always steady. Microbes attached to the intestine or free floating always influence the transition of the intestinal contents and affect the capability of pathogens and probiotic bacteria to bind to the epithelial cells in the lumen (Savage, 1977). Many of the beneficial microorganisms can arouse lower gut motility through production of short chain fatty acids and lessening pH (Ohashi *et al.*, 2002). The carbohydrate and protein content of the mucin influence the involvement of mucus in the ability of microbe to attach to the underlying epithelial cells (Mikelsaar *et al.*, 1987). It is clear that *Lactobacilli* need the mucin for their attachment, and if the mucin content declines, the beneficial *Lactobacilli* numbers also declines (Mikelsaar *et al.*, 1987). However, there are some pathogens which have evolved to take

benefit of this reaction in the gut and even augment the rate of mucin degradation (Mikelsaar *et al.*, 1987). Furthermore, the advantageous *Lactobacilli* also metabolize both protein and sugar content of the mucin and make use of it for energy and growth.

Modulation of ucosal immunity in animals given probiotic microorganisms has been a significant amount of speculation. Marteau & Rambaud, 1993; McCracken & Gaskins, 1999; Perdígón *et al.*, 1995 have extensively reviewed the influence of probiotic microorganisms. Local stimulation of gut associated lymphoid tissues can rouse a generalized systemic response since the gastrointestinal tract contains the majority of all of the immuno-competent cells in humans and other animals, (McCracken & Gaskins, 1999). Numerous immuno-modulator events in human and animal models given probiotics are summarized by Sanders (1999). By activating macrophages, increasing cytokine production by intraepithelial lymphocytes (IEL), and increasing levels of immunoglobulins especially IgA probiotic microorganisms are capable of enhancing both specific and nonspecific immune responses. According to the research of Sanders (1999) the presence of single and combinations of probiotic bacteria demonstrate some immune responses in human and animal models. As for example *L. acidophilus* produce immune enhancement; stability intestinal microflora; decline fecal mutagenicity; reduce fecal enzyme activity, rotavirus diarrhea can be treated by *Bifidobacterium bifidum* and it can balance intestinal microflora, *Bifidobacterium lactis* demonstrate increased I_gA levels etc. Immunoglobulin IgA has the most active function in the gut and inhibits bacterial colonization. However, it is also noticeable that all probiotic microorganisms do not show the same immunological memory and that is why it necessary to select the specific strain of probiotic organism to treat diseases (Maassen *et al.*, 1998).

Produce antimicrobial metabolites

Many Probiotic organisms produce anti-microbial metabolites that help them to compete with other organism to establish colonization in the intestinal tract. Antimicrobial substances

produced and secreted by natural probiotic organisms of the intestinal tract are able to either kill or inhibit growth of pathogenic microorganisms (Rolfe, 1991). Usually, many bacteria generate agents that either kill or inhibit related species or even different strains of the same species of bacteria (Iglewski & Gerhardt, 1978).

There are some some other metabolites named as bacteriocins which can be distinguished from antibiotics. Large variety of organisms produce bacteriocins and these are mediated through plasmids (Mishra & Lambert, 1996). Bacteriocins are proteinaceous compounds come from the bacteria and that are toxic to bacteria other than the mother strain. From the study of Joerger, 2003, it has been pointed out that bacteria produce bacteriocins to have competitive advantage in the gut with other bacteria. *Lactobacilli* have shown produce significant amounts of bacterial growth inhibitory substances such as nisin and reuterin. Nisin is usually familiar as safe. The mode of action of this bacteriocin is as a targeted membrane-permeabilizing peptide that induces pore formation in bacteria (Breukink *et al.*, 2003). *L. reuteri* produce Reuterin, a product of glycerol metabolism that has broad-spectrum killing abilities in the intestinal tract of chickens (Dobrogosz *et al.*, 1989; Talarico *et al.*, 1988, 1990; Talarico & Dobrogosz, 1989, 1990). *Bacillus* has a wide range of antimicrobial actions linked with a serine protease called subtilisin. *Bacillus subtilis* is able to facilitate the growth of another probiotic organism, *L. reuteri*, by the production of catalase and subtilisin (Hosoi *et al.*, 2001).. Colicins are plasmid-encoded polypeptide toxins produced by *E. coli* and it is active against *E. coli* and intimately related bacteria. The channel-forming colicins are transmembrane proteins that can depolarize the cytoplasmic membrane, leading to indulgence of cellular energy (Parker *et al.*, 1992; Braun *et al.*, 1994).

Establish Competition for essential nutrients

There is always a competition for nutrients inside the gut among different bacteria. Rolfe (1991) mentioned in his research many environmental factors come into play that either

increases the availability of nutrients from the diet of the host or through exploitation of dietary ingredients that increases the growth of certain microbial populations. Eventually it may lead to exclusion of other bacterial species. A normal balance of bacteria in the gastrointestinal tract is able to utilize all of the possible carbon sources in the environment (Freter *et al.*, 1983). It has also been demonstrated that through manipulation of the lactose concentration in the diets of chicks and poults, it may possible to selectively offer a benefit for the augmentation of *L. reuteri* (Casas *et al.*, 1993, 1998). After providing the day old chickens an *E. coli* (O75:H10) with 2.5% dietary lactose Behling & Wong (1994) found that there was a considerable protection against *S. enteritidis*. Oyoyo *et al.* (1989a) made an *in vitro* study to see the effect of mannose on the colonization of *S. typhimurium* in chickens. To perform this they incubated intestinal sections, cut off from one-day-old chickens, with either radiolabeled-*S. typhimurium* strains ST-10 and ST-11 (mannose-sensitive), or strains Thax-1 and Thax-12 (non-yeast-agglutinating strains), or with only phosphate buffered saline in the presence of D-mannose, arabinose, methyl- α -D-mannoside, or galactose. That intestinal sections incubation with bacteria and mannose resulted in a significant reduction of *S. typhimurium* attachment. They have confirmed this result *in vivo* as well (Oyoyo *et al.*, 1989b). After providing mannose orally to chickens, they subsequently challenged the chickens with *S. typhimurium*, and reported that mannose inhibited *S. typhimurium* colonization to the intestine. Oyoyo *et al.* (1989c) tested other carbohydrates such as dextrose, sucrose, and maltose with little if any inhibition of colonization in other studies.

Stress factors that affect performance of Probiotics

Application of Probiotics for Broiler chicken production is not free from risks and boundaries. Many factors present in the environment of a newly hatched chicks, which can lower the effectiveness of the maternally derived antibody defense system and can allow the pathogenic microorganisms to colonize in the gut of chicken in the post hatch stage. The stress factors may be of various sources such as nutritional,

environmental, physical and immunological, managerial, use of antibiotics improperly and lack of relationship with mother hens.

MATERIALS:

(A) Farm oriented:

1. Day old Chicken of Broiler
2. Trial farm
3. Necessary Lights
4. Heating plates for brooding of broiler
5. Rice straw for litter preparation
6. Water pots of 2 liter and 8 liter size
7. Ready- made feeds

(B) Weighing equipment:

1. Digital balance
2. Top load balance

(C) Probiotic organisms

1. Collection of day old chicken of broiler

The day old chickens of broiler were collected from a renowned hatchery named "Gyne Hatchery" in Batiaghata upozilla under the district of Khulna, Bangladesh. The name of the strain of broiler is Huffbd classic.

2. Trial Farm

A trial farm was managed as rent basis. The farm is located at vill-Muhammad Nagor, Upozilla- Batiaghata, Dist- Khulna, Bangladesh near about Khulna University campus. The name of the owner of the farm is Abul Kasem.

3. Necessary lights, heating plates and water pots were collected from the owner.

4. Materials for litter preparation were bought from market.

5. Ready-made feeds

Ready-made feed bags were used for feeding the experimental poults.

6. Weighing equipment:

The equipments were collected from Khulna University Laboratory.

7. Probiotic organisms:

Used Probiotic product is manufactured by a reputed company of U.K. named Probiotics international Ltd and marketed by Novartis Bangladesh Ltd. The product contains 7 species of bacteria, 1 species of Mold and 1 species of Yeast. The names of the organisms are given below:

Bacteria:

Lactobacillus plantarum

Lactobacillus bulgaricus

Lactobacillus acidophilus

Lactobacillus rhamnosus

Bifidobacterium bifidum

Streptococcus thermophilus

Enterococcus faecium

Mold: *Aspergillus oryza*

Yeast: *Candida pintolopesi*

WORKING PROCEDURE:

(A) Farm Preparation The farm was prepared by using different types of cleaning process. To make the farm as possible as free from different types of pathogenic surface microorganisms, soda lime (Ca (OH)₂), KMnO₄ were used.

The farm was divided into three parts for applying three treatments. Each part was divided into four parts for considering four replications. The parts were separated by using wood and net. Schematic diagram of the farm:

Blocks	TREATMENTS		
	Treatment, T ₀ (Antibiotics)	Treatment, T ₁ (Probiotics)	Treatment, T ₂ (Antibiotics and Probiotics)
Block 1	Replication 1(10 chicken)	Replication 1(10 chicken)	Replication 1(10 chicken)
Block 2	Replication 2(10 chicken)	Replication 2(10 chicken)	Replication 2(10 chicken)
Block 3	Replication 3(10 chicken)	Replication 3(10 chicken)	Replication 3(10 chicken)
Block 4	Replication 4(10 chicken)	Replication 4(10 chicken)	Replication 4(10 chicken)

(B) Probiotics dose designing for feeding the chickens:

1.0 gram of probiotic powder used for experiment contains 2x 10⁹ colony forming unit. The dose of probiotic powder for treatment T₁(Probiotics) and T₂(Antibiotics + Probiotics) was designed as 1 gram of probiotic powder was mixed with 4 liters of water. The chickens in the case of Probiotics (T₁) treatment were fed at the first day before providing them any feed. The Probiotics mixed water was provided to them for eight hours at the first day. Then from the 2nd day to the 8th day they were provided at same dose in the morning water. In the case of T₂ treatment, 2 days after using antibiotics, Probiotics mixed water at the same dose was provided to them for three consecutive days in the morning water. In the case of T₀ treatment, Probiotics were not provided. In the 15th, 16th and 17th day Probiotics were also provide the same dose mentioning above in the morning water in case of T₁ and T₂ treatments.

Results and Discussion:

Body weight is the prime consideration during broiler production. Various factors such as incidence of diarrhoea, texture of litter, deficiency of vitamins and other diseases like cold and fever are directly related to body weight. Mortality rate is also an important factor for commercial broiler production. The day old broiler chicks were treated with Probiotics, antibiotic and antibiotic + Probiotics upto 10 days and incidence of diseases like diarrhoea, paralysis, cold and fever problem; texture of litter, mortality rate were observed and body weight was recorded at 10th day. The average body wt. were 191.0, 210.75, 194.25 g for treatment antibiotic, Probiotics, antibiotic + Probiotics respectively. Events of incidence of

diarrhoea within 1st 10 days were 2 times for antibiotic treatment and 1 time for antibiotic + Probiotics treatment. But there was no event of evidence of diarrhoea for Probiotic treatment. Texture of litter within these days were very wet, normal and medium wet for treatments of antibiotic, Probiotics and antibiotic + Probiotics respectively. The observable appearance for deficiency of vitamin B₁,B₂ was not noticed. Avian mycoplasmosis problem was also not occurred during these days. From the table 1, it is clear that the average body weight in the case of Probiotics treated chicks was the highest (210.75) among other treatments. The antibiotic treated broilers showed the lowest (191.00) average weight. The reason might be due to the fact that chicks fed Probiotics gained a better nutrient absorption rate. The improved performance of chicks fed Probiotics can be correlated with microstructures in the intestine where villus height is increased, goblet cell numbers increase, and crypt depth is decreased (Edens, 2003). Thus Probiotics improve the morphology of the intestinal tract leading to improved nutrient absorption rate Incidence of diarrhoea is directly correlated with the gut microflora. The two times diarrhoea in the case of antibiotic treated chicks indicates that there was microbial imbalance in their intestine. This assumption came as true in the fact that when the other group treated with antibiotic + Probiotics, the incidence of diarrhoea reduced to one. The quality of texture of litter also proves better result in favor of balanced microflora. A clear difference is present in the mortality rate. The zero mortality rates of the chickens fed Probiotics supports the work of Fernandez *et al.* (2000) that the increased numbers of probiotic bacteria indirectly inhibit the colonization of pathogenic organisms by preventing their attachment to the gastrointestinal epithelial cells. It can be pointed out that antibiotics are used to kill the pathogenic microorganisms in the intestinal gut to prevent the disease such as blood dysentery. But when the broad spectrum antibiotics are administered they kill the pathogenic microorganisms as well as the beneficial microorganisms which play an important role for digestion of food. Therefore a serious microbial imbalance is occurred in the intestine. Microbial imbalance is caused for

indigestions of food. Eventually the body's external immune system excludes the indigested food materials and the symptoms of diarrhoea are expressed. But when the broilers are supplemented with Probiotics after using antibiotic the microflora are reestablished and resulting in the reduction of incidence of diarrhoea. Several other reasons are responsible for diarrhoea which can be minimized by supplementation with probiotic bacteria.

Table 1: Effects of Probiotics, Antibiotic and (Antibiotic + Probiotics) on weight, disease incidence and mortality of broiler; Data recorded at day 10.

Treatment	Incidence of Diarrhoea within 1 st 10days (No.)	Avian Mycopl- asmosis due to excessive cold	Deficiency of vitamins B ₁ &B ₂ within 1 st 10 days	Texture of litter within 1 st 10 days	Mortality rate within 1 st 10 days. (%)	Average body wt.(g)
Probiotics	0	Not observed	Not observed	Normal	0	210.75
Antibiotic	2	Not observed	Not observed	Wet	5	191.00
Antibiotic + Probiotics	1	Not observed	Not observed	Medium wet	0	194.25
Significance level	-	-	-	-	-	***

N. B. - Incidence of diarrhea is caused by the imbalance of gut microflora and food poisoning.

*** Denotes- This experiment is significant at the 1% level of significance.

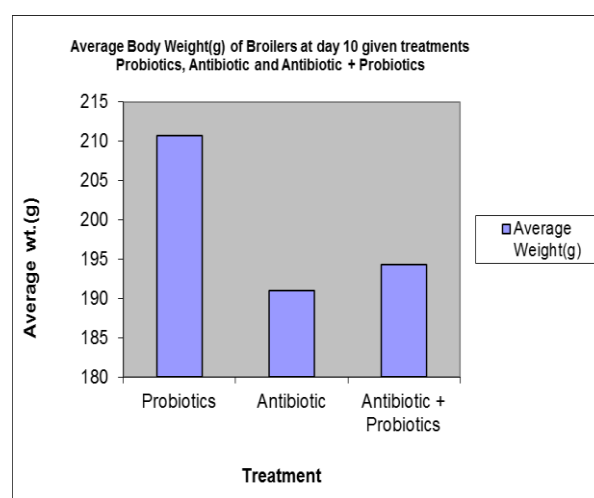


Fig-1: Graphical representation of average body wt. of broilers at day 10

Table 2 indicates the observed data collected at day 20. Body weights were measured at the appointed day but the other characteristics were observed from 11th- 20th days. It shows the average body weight from different replications under different treatments. The average body wt. were 727.5, 763.5, 736.5 g for treatment antibiotic, Probiotics, antibiotic + Probiotics respectively. Incidence of diarrhoea within 2nd 10 days was not observed for any treatment. Textures of litter within these days were normal for all treatments. The observable appearance for deficiency of vitamin B₁ and B₂ was noticed in the case of antibiotic treatment during day 13 to 16. Then they were supplied with these vitamins. Avian mycoplasmosis problem was also not occurred during these days for any treatment.

From the table 2, it is clear that the average body weight in the case of Probiotics treated chicks is the highest (763.5g) among other treatments. The lowest average weight (727.5g) was observed for the antibiotic treatment. This supports the work of Bradley et al. (1995) who observed improved body weight and ileum morphology after using Probiotics. During these

days the event of incidence of diarrhoea was zero for three treatments. It is due to the fact that as the day passes the gut microbiota of antibiotic treated chicks become near about balanced and the beneficial microbiota can stimulate lower gut motility via production of short chain fatty acids and decreasing p^H which is harmful for pathogenic organisms. *Lactobacilli* present in the intestine can metabolize both protein and carbohydrate content of the mucin and use it for energy and growth. As a result the incidence of diarrhoea was eliminated. This phenomenon was proved by the quality of texture of litter. A noticeable characteristic had been observed during these days. There was symptom of deficiency of vitamin-B₁ and B₂ in the case of antibiotic treated group but not in other treated groups. Vitamin B₁ and B₂ are necessary for the growth of broilers during these days. It is clear that probiotic bacteria produced some vit-B₁ and B₂ which supports the work of Hosoi *et al.* (2001). The mortality of two chicks of antibiotic treated group creates a decision that some organisms were resistant to antibiotics which cause for death that agree the work of Kolar et al. (2002).

Table 2: Effects of Probiotics, Antibiotic and (Antibiotic + Probiotics) on weight, disease incidence and mortality of broiler; Data recorded at day 20.

Treatment	Incidence of Diarrhoea within 1 st 10 days (No.)	Avian Mycopl- asmosis due to excessive cold	Deficiency of vitamins B ₁ &B ₂ within 1 st 10 days	Texture of litter within 1 st 10 days	Mortality rate within 1 st 10 days. (%)	Average body wt.(g)
Probiotics	0	Not observed	Not observed	Normal	0	763.5
Antibiotic	0	Not observed	Not observed	Normal	5	727.5
Antibiotic + Probiotics	0	Not observed	Not observed	Normal	0	736.5
Significance level	-	-	-	-	-	***

N. B. - Incidence of diarrhea is caused by the imbalance of gut microflora and food poisoning.

*** Denotes- This experiment is significant at the 1% level of significance.

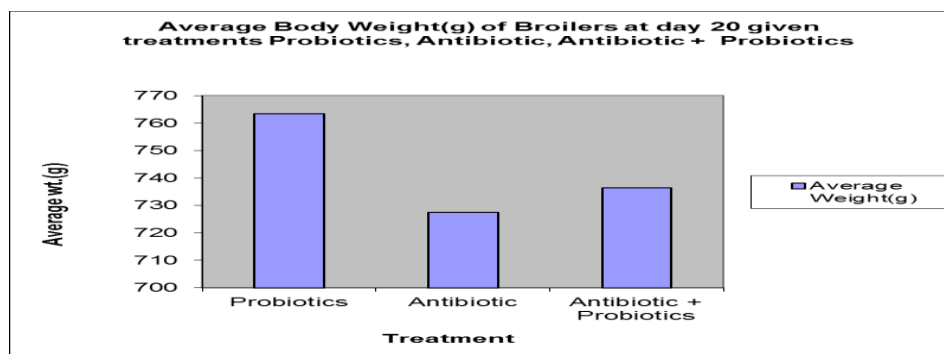


Fig-2: Graphical representation of average body wt. of broilers at day 20.

Table 3 indicates the observed data collected at day 33. Body weights were measured at the appointed day but the other characteristics were observed within 21st- 33rd days. It shows the average body weight from different replications under different treatments. The average body wt. were 1654.5, 1936.0, 1747.0 g for treatment antibiotic, Probiotics, antibiotic + Probiotics respectively. Incidence of diarrhoea within these days was not observed for any treatment. Texture of litter within these days was normal for all treatments. The observable appearance for deficiency of vitamin B₁ and B₂ was not noticed. An additional problem i.e. avian mycoplasmosis problem was noticed in the case of antibiotic treatment during day 24 to 27. Then they were supplied with antibiotic of group anrofloxacin. Overall Feed Conversion Rate (FCR) was measured on this day. The FCR were 63.78 %, 75.08 %, 67.48 % for treatment antibiotic, Probiotics, antibiotic + Probiotics respectively.

In the case of Probiotics treated broilers the average body weight was superior (1936.0g) to others viewed from table 3. As usual the lowest weight (1654.5g) was for the antibiotic treated broilers. This is because due to the fact that from the very beginning the Probiotics treated broilers didn't fall into any stress. On the other

hand antibiotic treated broilers fall into stress several times. All other characteristics of three treatments were similar. This is because that the gut microflora were similar for three treated group broilers as the 1st 20 days is very crucial for broilers life but after the 20th day the gut microflora become balanced. There was a significant sign of immunostimulation in the case Probiotics treated broilers. As the weather was very cold during that period the antibiotic treated broilers were affected by mycoplasma resulting in an avian mycoplasmosis problem within these days. But the Probiotics treated broilers were not affected. It is due to might be the fact that lactic acids produced by the probiotic bacteria react with the mycoplasma coating and with the internal components of Mycoplasma interfering with the replication of Mycoplasma. This is coupled by the birds' immune system activation results in the eventual elimination of the Mycoplasma infection. Immunostimulation by Probiotics is indicated by improved production of immunoglobulin A, M, G; improved phagocytosis, promotion of natural killer T-cells and ultimately faster shed of intestinal pathogens. The overall feed conversion rate is the highest for Probiotics treatment. This result supports the work of Pedroso *et al.* (1999).

Table 3: Effects of Probiotics, Antibiotic and (Antibiotic + Probiotics) on weight, disease incidence and mortality of broiler Data recorded at day 33.

Treatment	Incidence of Diarrhoea within 3 rd 13 days (No.)	Avian Mycoplasmosis due to excessive cold	Deficiency of vitamins B ₁ &B ₂ within 3 rd 13 days	Texture of litter within 3 rd 13 days	Mortality rate within 3 rd 13 days. (%)	Average body wt.(g)	Feed Conversion Rate (FCR) (%)
Probiotics	0	Not observed	Not observed	Normal	0	1937.0	75.08
Antibiotic	0	Observed	Not observed	Normal	5	1654.5	63.08
Antibiotic + Probiotics	0	Not observed	Not observed	Normal	0	1747.0	67.48
Significance level	-	-	-	-	-	***	-

N. B. - Incidence of diarrhea is caused by the imbalance of gut microflora and food poisoning.

***Denotes- This experiment is significant at the 1% level of significance

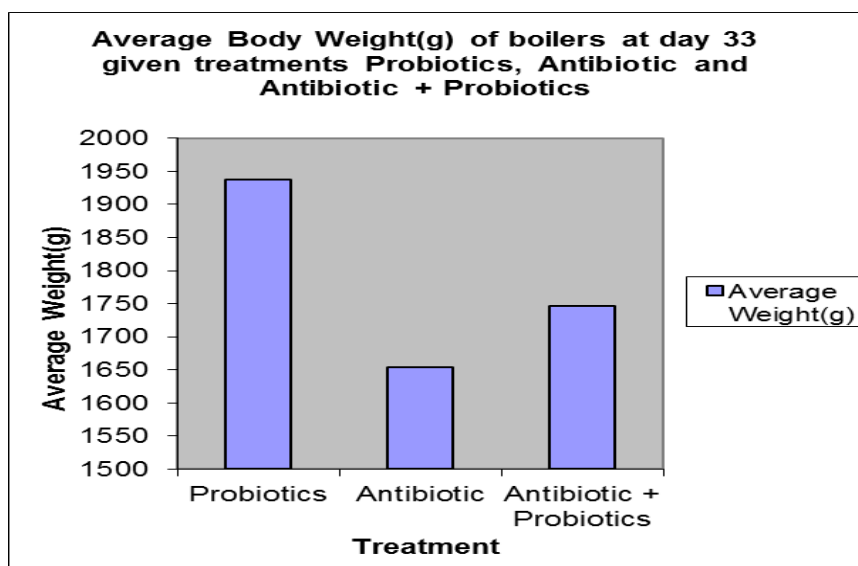


Fig-3: Graphical representation of average body wt. of broilers at day 33.

Table- 4: Average body weight at different days for Probiotics, Antibiotic and Antibiotic + Probiotics.

Treatments	Body weight (g) at different days		
	10 th day	20 th day	33 rd day
Probiotics	210.75	763.5	1937.0
Antibiotic	191.0	727.5	1654.5
Antibiotic + Probiotics	194.25	736.5	1747.0

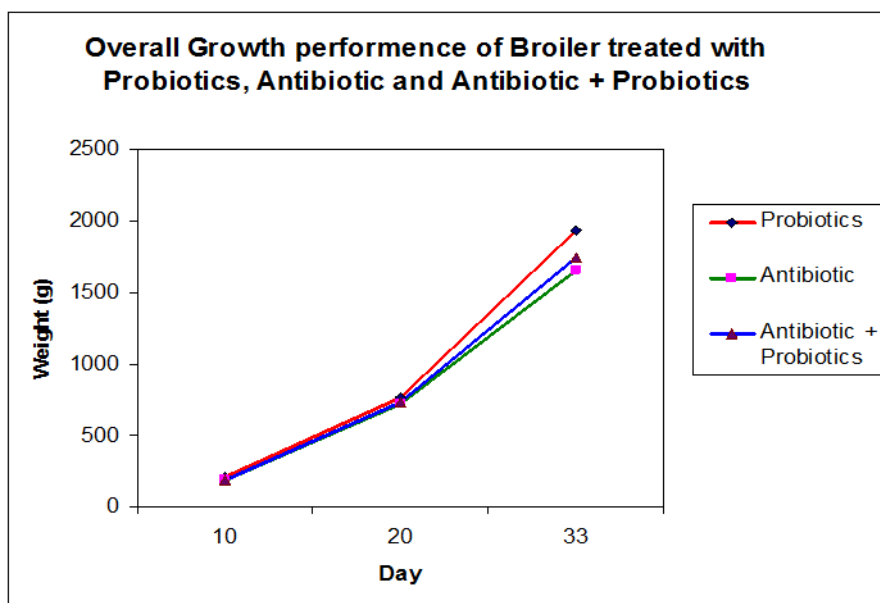


Fig- 4: Graphical representation of overall growth performance of Broilers with Probiotics, Antibiotic and Antibiotic + Probiotics.

CONCLUSION

Recently we all are acquainted with a term “Super bug”. Many of the pathogenic bacteria are becoming resistant to multiple drugs. Considering this issue sub-therapeutic administration of antibiotics to broilers is under concentrated study since they do contribute to the diffusion of antibiotic resistant bacteria into the food chain. It can also be noticed from studies that there is a link between the farming use of antibiotics and antibiotic-resistant human infections. Antibiotic-resistant organisms from broilers and human wastes enter the environment and ultimately they re-enter the human and poultry populations through a number of pathways including natural waters, drinking water, and vegetables and foods. Therefore, it has been an obvious fact to find out an alternative to antibiotic to produce broiler production.

APPENDICES

Appendix I

Symptoms and causes of Diarrhoea

Diarrhoea is the inflammation in the intestinal mucosa which is characterized by dehydration. During dehydration watery faeces is secreted from the intestines. It is caused by multiple

etiologies from infectious and parasitic cause to non-infectious causes.

Infectious causes include fowl typhoid, fowl cholera, new castle disease, Gambaro disease etc. Infectious cause indicates microbial imbalance in the intestinal gut.

Non- infectious causes include food poisoning, the presence of excessive NaCl, Na₂So₄ in the drinking water, the presence of excessive Mg ion and the low quality of food.

Appendix II

Symptoms and causes of deficiency of vitamin B₁ and B₂:

Paralysis of leg. Paralysis is the loss of feeling in or control of all or part of the body, caused by a disease of or an injury to the nerves. The paralysis of broilers is generally caused by the deficiency of vitamin B₁. Vitamin B₁ deficiency is caused by the high pH in the intestine, low presence of beneficial microorganisms in the gut, environmental stress etc.

From this research work we have been able to make out that Probiotics provide the dietary means to balance the intestinal bacteria in poultry and promote a responsive and improved immune system that can detect and eliminate

certain potential pathogens from the intestinal tract. Probiotics also stabilize the intestinal mucosa making it more difficult for pathogens to colonize and cause damage in the intestinal tract, and they also promote a condition in which less contamination occurs on processed meat and meat products thereby decreasing the risk of compromised human health status. As Probiotics show better growth rate, better nutrient absorption capability and overall decreased human health risk a trend for using Probiotics as alternative to antibiotics should be initiated by the poultry producers immediately.

Due to lack of vitamin B₂ the growth of broilers is severely hampered. vitamin B₂ deficiency is caused by the lack of riboflavin in the food, lack of beneficial bacteria in the gut.

Appendix III

Symptoms and causes of avian mycoplasmosis:

The clinical signs of this disease are mainly associated with respiratory tract which may include coryza, sneezing and breathing through the partly open beak; Mucus or catarrhal exudates in the respiratory tract and caseous exudate in the air sac. Mycoplasmosis is caused by the infection for *Mycoplasma* during excessive cold. Such as- *Mycoplasma gallisepticum*, *M. meleagridis* etc.

Appendix IV

Feed Conversion Rate (FCR):

$$\text{FCR} = \frac{(\text{Final Weight} - \text{Initial Weight}) \times 100}{\text{Total Feed Consumed}}$$

Mortality (%):

$$\text{Mortality (\%)} = \frac{\text{Numbers of dead birds} \times 100}{\text{Total numbers of bird}}$$

ACKNOWLEDGEMENTS

The author feels pleasure to express his greatest appreciation, indebtedness and deepest gratitude to the supreme of the universe almighty ALLAH, the most gracious and most merciful to whom all the praises go for enabling me to complete my thesis

The author wishes to express his heartiest indebtedness to his respected supervisor and honorable teacher, Professor Dr. Md. Raihan Ali, Biotechnology and Genetic Engineering Discipline, Khulna University, Bangladesh for his necessary supervision, active encouragement and constant effort throughout the progress of this work and preparation of thesis paper.

It is his pleasure that he avails to express his deepest and sincerest appreciation to his priest Co-supervisor Dr. Akramuzzaman, Veterinary Surgeon, Novartis, Regional sales centre, Khulna for accepting him as a research student under his supervision. The author likes to express his sincere and grateful appreciation to his friends Rakibul Islam, Nur Alam, Fakrul, Shahjahan, Ariful Islam and others for their constant assistance during performing the thesis work.

The author wishes to express his gratitude and special thanks to Md. Abul Kasem, owner of the experimental poultry farm, Muhammad Nagor, Batiaghata, Khulna for his kindness for permitting to carry out the experiment in his farm.

Finally, the author likes to pay gratitude to his parents who supported him in every possible way for successful completion of this challenging work. The author is also grateful and brothers (Shohel, Asik Imran, Swapan) and sisters (Sabina, Rokhsana, Sanjida) for their uninterrupted support.

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