EVALUATION OF A COMMERCIAL "PROBIOTIC PRODUCT" IN BROILER RATIONS

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IN BROILER RATIONS

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CHAPTER I

INTRODUCTION

Antibiotics are a group of chemical compounds produced biologically by certain plants or microorganisms. Although most of these drugs are used to combat diseases in humans and animals, they are also being used to stimulate the growth and improve the efficiency of feed utilization of farm animals. They may 1) favor the growth of nutrient-synthesizing and inhibit that of nutrient-destroying microorganisms; 2) inhibit the growth of organisms that produce excessive amounts of ammonia and other toxic nitrogenous waste products in the intestines; 3) improve availability or absorption of certain nutrients; 4) improve feed or water consumption, or both, and/or 5) prevent or cure actual diseases which occur either in the intestinal tract or systemically (25).

It has been reported that the total value of the sale of drugs for use with animals in the United States during 1977 was $1.21 billion--nearly triple that of 1968 when sales totaled $411 million. The poultry industry was the primary user of these feed additives, with purchases of a little more than 60 percent of the total sales dollar value. Broilers and table egg producers each accounted for about 45 percent of the total sales, and turkey producers accounted for 10 percent. The remainder of the feed additive sales was about evenly divided between swine producers and the cattle, dairy, and sheep producers.

The subtherapeutic use of animal drugs is of vital importance because of evidence which indicates that strains of certain organisms develop resistance when antibiotics are administered continuously. For example, it has been demonstrated that strains of Pasteurella multocida and Pasteruella haemolytica have developed a resistance to certain antibiotics such as penicillin, streptomycin, sulfonamides, and tetracyclines. The development of such resistance is a serious problem, since it makes the use of these antibiotics potentially less effective in dealing with health problems in both humans and animals.

It has been suggested that "Probiotic Products" be used in animal feeding in place of antibiotics, and by so doing eliminate some of the problems and side effects caused by the use of antibiotics. A "Probiotic Product" can be defined as a mixture of beneficial viable microorganisms. "Probiotic Products" have been developed, and there has been considerable interest in recent years in the use of these products in human and animal diets. Little is known regarding the use of probiotics in animal feeds--especially in poultry feeds. Research data with poultry which have been reported at scientific meetings during the past year give some indication that the addition of certain "Probiotic Products," particularly those which contain lactobacilli, improve growth rate and efficiency of feed utilization, especially when the protein level in the diet is below recommended standards. It would appear that the deficiency of dietary methionine, expressed as percent of total protein, can be overcome to some degree when "Probiotic Products" were added to the ration.

Research data obtained under commercial feeding conditions have led to the conclusion that effects due to various forms of stress may

be alleviated to some degree through the use of "Probiotic Products." These stresses include high density of birds, exposure to disease, and high environmental temperatures, in addition to the nutritional stress when the ration is deficient in protein and amino acids.

"Probiotic Products" are being manufactured commercially in the United States, and a number are being offered for sale in Oklahoma. For this reason, a series of experiments was conducted for the purpose of obtaining data upon which to base an evaluation of a "Probiotic Product" similar to those which are being sold in Oklahoma. Experiments were designed to determine if the "Probiotic Product" would 1) improve the growth rate and efficiency of feed conversion with broilers when the nutrient levels in the broiler rations fully met recommended nutrient standards; 2) make it possible to reduce the dietary levels of fish meal and feather meal in broiler rations; 3) improve the growth rate and efficiency of feed conversion with broilers when dietary protein and methionine levels are below recommended nutrient standards; 4) reduce the adverse effects of a coccidiosis outbreak when the broilers were challenged with coccidia, and 5) bring about a reduction in feed cost per unit of broiler produced.

CHAPTER II

REVIEW OF LITERATURE

Microorganisms in the Intestinal Tract of Chickens

Normal Development of Microorganisms in the Intestinal Tract of Growing Chickens

Investigations were made on the normal development of microorganisms in the intestinal tract of normal chicks from one day to 28 days of age. The intestinal tract of the day-old chick contained a very small number of microorganisms before the chicks were fed (17, 26). The number of all groups of bacteria tested increased rapidly after the chicks were fed following the initial 21-hour starvation period. This rapid increase took place during the first 16 hours post-feeding (26). The bacteria in the duodenom of the fed day-old chick were present in very low numbers, whereas the cecum contained a considerable number of bacteria. The dominant bacteria in the cecum were enterococci, with E. coli making up most of the remaining bacteria (17). By day 2, the number of coliforms and Streptococci had increased and reached a high level (12).

At three days of age in fed chicks, S. faecalis was no longer the dominant bacteria in the cecum, but was the dominant type in the duodenum and had been replaced by E. coli in the cecum. At six days of age, the number of S. faecalis had increased in the duodenum, although

other types were present. In the cecum, enterococci were exceeded in number by other aerobic types (17). The number of enterococci decreased as the chick became older (26). At 14 days of age and through 28 days, the enterococci disappeared from the duodenum and were replaced by anaerobic types. The dominant organisms in the cecum at 14 days were anaerobes. These anaerobes increased in numbers through 28 days (17). It was suggested that "the disappearnce of S. faecalis from the intestinal tract is probably due to the effective competition of other organisms against S. faecalis after the conditions in the intestinal tract have become favorable for their development" (17). Lactobacilli were found to be the dominant group of bacteria in most areas of the intestinal tract of chicks following about two weeks of age (17, 26). The importance of lactobacilli in the intestinal tract of chicks will be discussed in another section.

Occurrence of Lactobacilli in the Crop of the Chicken

In the crop of the fowl, lactobacilli are the dominant group of organisms with the coliforms and Streptococci making up the remaining microflora. These bacteria adhere to the epithelial cells of the crop and are established immediately after hatching. The presence of these organisms persists throughout the life of the bird (10, 11, 12). The adhesion of lactobacilli is not affected by the age of the bird (10), and the different strains of lactobacilli have a specific affinity to each animal species (20). Based upon research data obtained, it was suggested that the desirable type of lactobacilli for the crop is one which is able to adhere to the crop epithelial cells and to produce

large quantities of lactic acid which lowers the pH and inhibits the growth of other microorganisms (12). When chicks were fed diets with antibiotics such as penicillin, the lactobacilli could not be detected in the content or the epithelial cells of the crop, and the number of coliforms increased (10).

Action of Microorganisms in the Intestinal Tract

The types of microorganisms which comprise the intestinal flora depend largely upon the species of animals, portion of the digestive canals, age, kind of diets, and the environmental conditions such as temperature and humidity. When the host is exposed to a sudden change in living conditions, mental or physical stresses, or is physically exhausted, exposed to X-ray radiation or takes antibiotics, the ratio among the different varieties of microorganisms in the intestinal tract may undergo a significant change (33). This change in ratio may result in an increase in the number of detrimental microorganisms in the intestinal tract and large quantities of toxic materials may be produced which could cause disorders in the liver, heart, and brain, and the acceleration of senility and shortening of the life span (33).

Research data on poultry provided evidence that lactobacilli are involved in preventing the growth of E. coli in the intestinal tract. When lactobacilli were eliminated from the intestinal tract by using antibiotics, the number of E. coli increased (10). In like manner, E. coli were inhibited by the presence of lactobacilli in the intestinal tract of gnotobiotic animals (12). This inhibition of E. coli was dependent upon the presence of large numbers of lactobacilli. In the case of the chickens as previously discussed, there are always large

numbers of lactobacilli to inoculate the feed that enters the crop, the lactobacilli are present in large numbers and, as a result, inhibit the multiplication of E. coli in the intestinal tract (12, 33).

It is postulated that the high resistance of the chicken against E. coli enteric infections, as compared with the neonatal pig and calf, is related to the presence of lactobacilli in the anterior gut (12). It is suggested that if lactobacilli are important in providing resistance to enteric disease, it may be advantageous to dose newly hatched chicks with lactobacilli either in the form of droppings or as pure cultures. This would assure the rapid establishment of lactobacilli, they would exert their regulating effect on the other microflora (10), and in so doing would promote protection against microbial infections (23, 33). Furthermore, when chicks were given lactobacilli immediately after hatching, they showed better performance even under stress conditions such as disease (11, 12, 23). Constant administration of lactobacilli through the feed helped reduce the severity of the clinical symptoms of Eimeria tenella infection (18).

It has been reported that the administration of large numbers of lactobacilli through the consumption of lactobacillus fermented milk provided a factor(s) which suppressed the synthesis of cholesterol and this, in turn, lowered the serum cholesterol level in humans. This may be related to the presence of large numbers of lactobacilli and their action on cholesterol degradation into bile acids which are excreted with the feces (27). In poultry, it has also been reported that the feeding of Lactobacillus acidophilus to laying hens resulted in a significant decrease in cholesterolemia (31).

In the intestinal tract of humans, other mammals, and poultry,

there seems to be an antagonistic action among lactobacilli and some organisms such as E. coli, Salmonella typhimurium, Staphlococcus aureus, and Clostridium perfringens(10, 11, 12, 15, 18, 21, 30), but this action is not completely understood. It was suggested that this is not completely related to a lowering of pH which is brought about by the action of lactobacilli. It seems likely that this antagonistic action is related to a combination of other factors, including the production of antibiotic-like substances produced by the lactobacilli (15).

In the growing chicken, the lactobacilli-chicken association would appear to be an example of symbiosis where the lactobacilli benefit by receiving nutrients from the diet and the host benefits from the mainenance of nongrowth-depressing microflora (11, 23). In addition, microorganisms (including lactobacilli) act in a favorable way by synthesizing vitamins and by promoting digestion and absorption of the diet (23, 26, 33).

Microorganisms enhance many enzyme reactions in the gut which are related to protein digestion and metabolism. For example, they produce proteases and peptidases, which break down intact protein, and deaminases and decarboxylases, which may bring about amino acids degradation. Conversely, they may synthesize amino acids, peptides or proteins from simpler starting materials. Microbial proteolysis may help in the breakdown of poorly digested proteins (23). Research data indicate that the combination of Aspergillus oryzae (mold) and the soybean protein enhances the release of a large quantity of free amino acids due to the presence of acid proteinase and acid carboxypeptidase IV which are present in Aspergillus oryzae (22).

The naturally occurring yeast in newborn lambs and piglets,

Torulopsis glabrata, can be found in densities of 10^6 viable cells/ml of the stomach contents. It ferments the glucose and produces up to 500 mg of ethanol/100 ml of stomach contents, but it does not ferment sucrose or lactose. A coliform-type bacterium at densities up to 10^8 viable cells/ml was regularly found in association with Torulopsis yeast. The precise role of this ethanol-producing bacterium is not clear (32).

Effect of Gut Microflora on the Nutrition of Chickens

The dietary nutrients that escape digestion and absorption by the host remain in the lumen of the intestinal tract mixed with the endogenous secretions to provide the substrates upon which the gut microflora grow. The metabolic activities of these organisms to a large degree determine the conditions that develop and are maintained in the intestine. As a result, they significantly alter some of the basic digestive mechanisms (6, 23).

In order to identify and study some of these changes in basic digestive mechanism, research data were obtained by comparing germ-free and normal or conventional chickens. In general, the pH of the intestinal contents of the conventional animals and chickens is lower than that of those which are germ-free, probably because of the acid metabolites produced by the microorganisms (6, 23). This pH is important, because it has an effect on the solubility of some minerals such as calcium, and the efficiency of the digestive enzymes that have critical pH optima (6).

The production of intestinal disaccharidases is similar in both the

conventional and germ-free chicks, with the exception of lactase, which is present in considerable quantities in conventional chicks but not in germ-free chicks (6, 23). This enzyme is produced by the organisms which have been established in the intestinal tract. Thus, the presence of microorganisms in the intestinal tract of chickens is important due to the fact that these microorganisms produce the enzyme lactase which may play a role in the digestion of lactose when present in the diet as a carbohydrate source (6).

Effects of Feeding Lactobacilli or "Probiotic Products" on Poultry

Lactobacilli for Poultry

There has been a considerable interest in determining the effect of lactobacilli when fed to broilers. Research data indicate that feeding L. acidophilus to broiler chicks during the first five days of life produced increased weight gain and improved feed efficiency when the chicks were grown in an "old" environment (30). Furthermore, when chicks were given a lactobacillus culture with the diet at a dosage level of one million cells per chick per day every other day for 15 days, the mortality rate was reduced and weight gained was increased (29).

A feeding trial was conducted under environmental conditions where the 8-week growth rate and feed utilization of broilers were below normal due to extremely adverse cold weather conditions. When lactobacilli were added to the diet, they produced a significant improvement in broiler growth and feed efficiency. In addition, the growth rate of broilers fed the lactobacillus culture in a ration substandard in amino acids was similar to that of those fed adequate amino acids. Growth rate was not increased; however, when the lactobacillus culture was

added to a diet considered to be adquate in dietary amino acids (7).

Research data indicated that the addition of a lactobacillus culture to the diet improved the performance of laying hens and turkey poults. When lactobacilli were mixed with bacitracin in the diet, the performance was not as good as that obtained when the two additives were fed singularly (8, 9). In these studies, the coliform and total aerobic counts in the feed and in the digestive tract were decreased, and the lactobacillus count of the poults was increase (8, 9).

When gentian violet or lactobacillus culture was added to the diet of Leghorn hens, they produced an increase in egg production over the control group of 3.07 percent and 3.03 percent, respectively. When gentian violet and the lactobacillus culture were fed together, the egg production and feed efficiency were increased 9.02 percent and 10.51 percent, respectively. When gentian violet and the lactobacillus culture were fed separately, the fertility and hatchability were the highest (19).

Another study was made to determine the effect of feeding a lactobacillus fermentation product on laying hens. Results of this study indicated that this product produced an increase in the percentage of large eggs laid by the young hens (24-48 weeks), and the percentage of large eggs was decreased when the hens became older. Under commercial production conditions, this lactobacillus product brought about an increase in the percentage of large eggs laid by hens 22 weeks old and during a subsequent 4-month period of egg production. This increase in the percentage of large eggs became less and less throughout the testing period (16).

It has been reported that a dry L. acidophilus culture can be used

with the diet of turkeys as a feed additive to improve their performance. In one study, the L. acidophilus culture was added to the diet of Medium White turkeys in a 0 to 16-week feeding trial. Results of this study indicated that the addition of 0.025 percent of the dry L. acidophilus culture to the diet significantly increased the body weight of the turkeys from 1.6 to 2.5 percent ($P<.05$) when they were 8, 10, and 12 weeks of age (24).

"Probiotic Products" for Poultry

In recent years, it has been reported that "Probiotic Products" can be used as feed additives in poultry production. Research data obtained from one broiler study indicated that a "Probiotic Product" when added to a ration fed to broilers in an 8-week feeding trial produced no significant differences in body weight gain. However, feed consumption and the efficiency of feed conversion were significantly lower for the broilers fed the "Probiotic Product" during the first four weeks of the growing period (3). Other results of this study indicated that the degree of pigmentation of the dressed broilers was greater when the "Probiotic Product" was fed, and that both the degree of pigmentation and the amount of fat deposition were superior when a mixture of "Probiotic Product" and live yeast culture was used. The viability and the species of microorganisms in the "Probiotic Product" were not determined.

In a study with laying hens in which fermentation products and "Probiotic Products" were used, results with the fermentation product showed that there were no significant differences in egg production during the first six periods (28 days per period) of a 12-period egg

production test. There was a significant increase during periods 7 to 12. The "Probiotic Product" results showed that there was no influence of the "Probiotic Product" on the egg production rate of laying hens (5).

In another study with female turkeys which were fed rations supplemented with a "Probiotic Product," results indicated that during the early weeks (at 4, 8, and 12 weeks) of the growing period, the "Probiotic Product" significantly increased body weight gain. This "Probiotic Product" which was said to contain live lactobacilli, produced a significant increase in body weight gain when it was fed together with zinc-bacitracin or antibiotic rather than supplemented alone (1).

CHAPTER III

EXPERIMENTAL PROCEDURE

General

Four hundred and eighty day-old chicks of a commercial broiler strain, which were color-coded, were used in each feeding trial and were obtained from a commercial hatchery. These chicks were wing-banded before they were distributed into boxes which were labeled with the numbers of the pens in the broiler house. The chicks were distributed into the boxes one chick at a time in rotation until each box contained 15 male and 15 female chicks. The initial weight of each chick was recorded in grams, and the chicks in each box were brought to the broiler house and distributed into the pens.

The broilers in each of the two feeding trials were housed in 6x12 ft pens. These pens were located on both sides of a house which was equipped with windows on the north and south walls. The pens were separated from each other by 2-ft high stem walls upon which were located wire partitions. The pens on each side were separated by a central aisle which adapted well to the randomized block design which was employed in both feeding trials. There were ridge ventilators at the ridge of the roof, and floor ventilators in the walls at floor level. The floor of each pen was covered with a sugarcane pulp litter. Each pen was equipped with an infrared brooder, a suitable size water fountain, and a feeder.

Eight pens on each side of the central aisle in the broiler house were used. Eight treatments in each trial (Tables I and II) were assigned at random to pens on each side of the broiler house (28). At least 15 and no more than 16-day old broilers from both sexes were assigned at random into each pen in both trials. The first feeding trial (Trial I) was completed during the summer of 1978 (June 13 to August 1). The second feeding trial (Trial II) was held during the fall of 1978 (October 17 to December 12). Both of these trials covered 8-week feeding periods.

Standard management practices were followed in caring for the broilers during the entire experimental feeding period in both trials. These practices included brooding (starting with 35^{o}C, then adjusting downward as the heat requirements of the broilers decreased), cleaning the water fountains and adding fresh water every day, keeping the feeder half full of feed except with the first feeding when they were full, and frequent stirring of the litter to keep it from caking. Feed and water were given *ad libitum*. The feed in the feeders was cleaned of foreign materials and dirt, and was renewed every day along with the water throughout the course of the experiment.

In order to obtain the greatest possible degree of uniformity between and among experimental rations, a basal ration was first mixed in an auger-type feed mixer (about 550 Kg capacity). Additional feed ingredients were added to the basal according to the formula for each experimental ration. Each experimental ration was thoroughly mixed in a small electric mixer (50 Kg capacity). This mixer was cleaned before and after each experimental ration was mixed. Rations that were without the "Probiotic Product" were mixed first to avoid possible

TABLE I

EXPERIMENTAL DESIGN OF FEEDING TRIAL I

Ration Number	Ration Type	Treatment Number	Treatment Type
1	commercial	1	
1	commercial	2	coccidia challenge
2	commercial plus coban	3	
2	commercial plus coban	4	coccidia challenge
3	commercial plus "Probiotic Product"	5	
3	commercial plus "Probiotic Product"	6	coccidia challenge
4	corn-soybean meal type (adequate)	7	
5	corn-soybean meal type (deficient) plus "Probiotic Product"	8	

TABLE II

EXPERIMENTAL DESIGN OF FEEDING TRIAL II

Ration and Treatment Number	Ration Type
1	commercial
2	commercial plus "Probiotic Product"
3	commercial 85%
4	commercial 85% plus "Probiotic Product"
5	corn-soybean meal type (adequate)
6	corn-soybean meal type (adequate) plus "Probiotic Product"
7	corn-soybean meal type (deficient)
8	corn-soybean meal type (deficient) plus "Probiotic Product"

contamination of the other rations with microorganisms from the "Probiotic Product." The commercial rations which were basically the same were mixed according to the recommendations of the commercial feed manufacturer from whom the formulas were obtained. After mixing, each ration was put into a separate can which was labeled with the ration and pen numbers. The cans were stored in the aisle of the broiler house just outside of each pen.

The coban that was used in the rations was a monensin sodium which was of the type used for broiler and replacement chicken rations. This product contained an equivalent of 45 g of monensin per 454 g (active drug ingredient). It was used as an aid in the prevention of coccidiosis caused by Eimeria necatrix, E. tenella, E. acervulina, E. brunetti, E. mevati, and E. maxima. The level used in the ration was 0.11 percent.

The "Probiotic Product" which was used with the experimental rations was obtained from a commercial supplier. It consisted of soybean meal as a carrier of the microorganisms and was offered as a source of a combination of L. acidophilus, L. casei, L. bifidus (Bacterium bifidum), Torulopsis (species name was not given) and Aspergillus oryzae. It was stored in bags at room temperature, according to the recommendations of the manufacturer.

Feeding Trial I

Introduction

The purposes of this trial were 1) to determine the effect of the "Probiotic Product" on broilers when they were challenged with coccidia;

2) to make a comparison between and among the commercial rations when they were fed with and without the addition of the "Probiotic Product," and 3) to determine the effect of the "Probiotic Product" when it was supplemented in a corn-soybean meal-type broiler ration in which no fish meal or feather meal was added, and where the ration was formulated to be deficient in total protein and methionine.

Experimental Design and Rations

As previously described, eight pens on each side of the central aisle in the broiler house were designated as blocks. The eight treatments which made up this feeding trial were assigned at random to the eight pens within each block. One of the five experimental rations as listed in Table III (starter), and Table IV (finisher) was assigned as appropriate to each treatment. Ration 1 was a commercial ration which was formulated without the addition of a coccidiostat (coban). Ration 2 was the commercial ration (Ration 1) with the addition of the coccidiostat coban at a level of 0.11 percent. Ration 3 was Ration 1 which did not contain coban, supplemented with 2 percent of the "Probiotic Product." Rations 1, 2, and 3 were fed to broilers in Treatments 1 and 2, 3 and 4, and 5 and 6, respectively. In Treatments 2, 4, and 6, the broilers were challenged with coccidia during the first four weeks of the growing period.

Ration 4, which was a corn-soybean meal type broiler ration did not contain fish meal or feather meal. This ration was formulated to be nutritionally adequate and was fed in Treatment 7. Ration 5, which was supplemented with 2 percent of the "Probiotic Product" was also a corn-soubean meal type broiler ration without fish meal or feather meal.

TABLE III

EXPERIMENTAL STARTER RATIONS OF BROILERS FED IN TRIAL I

Ingredients	Ration Number 1 (%)	2 (%)	3 (%)	4 (%)	5 (%)
Tallow, feed grade	5	5	5	7	4
Yellow corn, ground	54.1	53.99	54.1	41.55	48.15
Soybean meal (44%)	29	29	27	35	33
Alfalfa meal (17%)	-	-	-	3	3
Dried whey (12%)	-	-	-	3	3
Fish meal (mehnaden)	4	4	4	-	-
Feather meal	2	2	2	-	-
Live yeast culture (14%)	-	-	-	3	3
Meat and bone meal (50%)	4	4	4	5	-
dl methionine	0.15	0.15	0.15	0.1	-
Phosphorus supplement (Ca27-P18)	0.6	0.6	0.6	1	2
Calcium carbonate	0.5	0.5	0.5	0.5	1
Trace mineral mix[1]	0.1	0.1	0.1	0.1	0.1
Salt	0.3	0.3	0.3	0.5	0.5
Broiler vitamin mix[2]	0.25	0.25	0.25	0.25	0.25
"Probiotic Product"	-	-	2	-	2
Coban	-	0.11	-	-	-
Calculated analysis					
Protein (%)[3]	24.82	24.76	24.68	23.88	21.20
Methionine (% protein)	2.26	2.26	2.26	1.98	1.59
Kcal/454 g	1437	1437	1437	1346	1299
Dry matter (%)	89.20	89.10	89.70	88.90	87.60

[1]Provides in the ration: manganese, 120 ppm; zinc, 80 ppm; iron, 60 ppm; copper, 10 ppm, and iodine, 1.0 ppm.

[2]Contains per 454 g of vitamin mix: vitamin A, 1,400,000 I.U.; vitamin D, 320,000 I.U.; vitamin E, 1,400 I.U.; menadione sodium bisulfite complex, 800 mg; riboflavin, 1,400 mg; niacin, 7,000 mg; d-pantothenic acid, 2,000 mg; choline, 110,000 mg; thiamine, 200 mg; pyridoxine, 200 mg; vitamin B_{12}, 2.2 mg; d-biotin, 20 mg; folic acid, 140 mg.

[3]Kjeldahl analysis.

TABLE IV

EXPERIMENTAL FINISHER RATIONS OF BROILERS FED IN TRIAL I

Ingredients	Ration Number 1 (%)	2 (%)	3 (%)	4 (%)	5 (%)
Tallow, feed grade	5	5	5	7	7
Yellow corn, ground	59.53	59.42	59.53	49.05	45.15
Soybean meal (44%)	25	25	23	28	33
Alfalfa meal (17%)	-	-	-	3	3
Dried whey (12%)	-	-	-	3	3
Fish meal (menhaden)	4	4	4	-	-
Feather meal	2	2	2	-	-
Live yeast culture (14%)	-	-	-	3	3
Meat and bone meal (50%)	2.5	2.5	2.5	5	-
dl methionine	0.12	0.12	0.12	0.12	-
Phosphorus supplement (Ca27-P18)	0.7	0.7	0.7	1	2
Calcium carbonate	0.5	0.5	0.5	-	1
Trace mineral mix[1]	0.1	0.1	0.1	0.1	0.1
Salt	0.3	0.3	0.3	0.5	0.5
Broiler vitamin mix[2]	0.25	0.25	0.25	0.25	0.25
"Probiotic Product"	-	-	2	-	2
Coban	-	0.11	-	-	-
Calculated analysis					
Protein (%)[3]	23.31	22.75	22.56	22.28	22.26
Methionine (% protein)	2.20	2.20	2.20	2.00	1.5
Kcal/454 g	1434	1434	1434	1408	1376
Dry matter (%)	88.91	89.31	89.30	89.54	90.34

[1]Provides in the ration: manganese 120 ppm; zinc 80 ppm; iron 60 ppm; copper 10 ppm, and iodine 1.0 ppm.

[2]Contains per 454 g of vitamin mix: vitamin A, 1,400,000 I.U.; vitamin D, 320,000 I.U.; vitamin E, 1,400 I.U.; menadione sodium bisulfite complex, 800 mg; riboflavin, 1,400 mg; niacin, 7,000 mg; d-pantothenic acid, 2000 mg; choline, 110,000 mg; thiamine, 200 mg; pyridoxine, 200 mg; vitamin B_{12}, 2.2 mg; d-biotin, 20 mg; folic acid, 140 mg.

[3]Kjeldahl analysis.

However, this ration was formulated to be deficient in both total protein and methionine, and was fed to the broilers in Treatment 8.

Microbiological Analyses of the Experimental Rations and the "Probiotic Product"

Microbiological analyses were made of the "Probiotic Product" which was used in Feeding Trial I, and the experimental rations in which it was fed. The purpose of these analyses was to determine the number of viable lactobacilli which were present. These analyses were made by personnel in the Dairy Microbiology Laboratory in the Animal Science Department of the Oklahoma State University.

In general, the following procedure was used in making these analyses. One sample of feed was collected in a clean and dry plastic bottle from each ration after the ration had been thoroughly mixed. In addition, three samples of the "Probiotic Product" were collected in the same type of plastic bottle.

LBS agar was prepared in the laboratory as the enumeration medium which was selective for lactobacilli, and MRS agar was used as a non-selective plating medium following the procedures described by Gilliland and Speck (14). Results were presented as colony-forming units per gram. Five colonies from LBS agar plates prepared from each sample were isolated by transferring cells with a sterile inoculating needle from each colony into separate tubes of sterile MRS broth (10 ml per tube). The tubes were incubated at 37°C for 24 hours. The resulting cultures were subcultured into fresh broth and incubated one week at 15°C to determine if they would grow.

Measurements Made

Measurements were taken at the end of 3, 6, and 7 weeks of the growing period. These measurements included body weight and feed consumption. The body weight of each broiler was measured and recorded individually in grams. Feed consumption was recorded on the basis of the total amount of feed in grams consumed by the broilers present in each pen during each period. Mortality was recorded daily. From the body weight and feed consumption data, calculations were made for feed conversion efficiency and feed cost per 454 g of broiler produced. The broilers were killed with an electric knife, scalded in a hot water (60^{o},C) vat, and picked by a mechanical picking machine. As New York dressed poultry, they were given grades by an experienced poultry grader following the specifications of the U. S. Department of Agriculture for classes and grades of live poultry, dressed poultry, and ready-to-cook poultry, as described in Poultry Production (4).

Coccidia Challenge

Broilers from Treatments 2, 4, and 6 were challenged with coccidia at 25 days of age. The material used for challenge contained a mixture of *Eimeria tenella*, *E. necatrix*, *E. maxima*, *E. brunetti*, and *E. acervulina*. This preparation was manufactured by Sterwin Laboratories, Gainsville, Georgia. It was kept in a bottle containing dichromate solution, and stored under refrigeration conditions. The instructions for using the preparation specified that the bottle be placed in a refrigerator over night. The supernatant was then carefully poured off and discarded. The sediment was reconstituted with tap water to produce 1 ml per dose. The challenge was on a group basis so that the number of doses

given for each pen was dependent upon the number of broilers present in the pen. If the pen contained 30 broilers, 30 doses (30 ml) of the material were used. The 30 ml were diluted with the amount of water which was estimated would be consumed in an 8-hour period (7 A.M. to 3 P.M). The water consumption for this 8-hour period was measured during the day preceding the challenge. Plastic water fountains were used for the challenge in order to avoid contamination of the regular water fountains with the disease-causing protozoans and to eliminate any adverse effect that zinc in the zinc-coated fountains might have on the coccidia.

On the third day post-challenge, a coccidiostat was administered to the broilers. This coccidiostat was amprolium (water soluble powder) which is manufactured by the Chemical Division of Merck and Company, Inc., Rahway, New Jersey. Amprolium was added to the drinking water as recommended by the manufacturer at the rate of 4 ouces per 25 gallons of water (i.e., 54 g/500 ml water) for the treatment of coccidiosis, and at the rate of 4 ounces per 50 gallons of water (i.e., 45.5 g/500 ml water) for the prevention of coccidiosis. The treatment level was used for broilers which were challenged in Treatments 2, 4, and 6. The preventive level was used for broilers in Treatments 1, 3, 5, 7, and 8.

A total of 36 broilers were selected at random from Treatments 2, 4, and 6 for the post-mortem examination for coccidiosis. Four broilers per day over a 3-day period were selected from each treatment beginning on the fourth day post-challenge. The broilers were taken to the Oklahoma Animal Disease Diagnostic Laboratory, Oklahoma State University, where they were examined by an experienced veterinarian. The following

procedure was followed in making the post-morten examination.

All broilers were killed by disarticulation of the atlantal-occipital joints and separation of the spinal cord. The broilers were examined by the standard post-mortem examination procedure. Representative segments of the duodenum, jejenum, ileum, and ceca were fixed in a buffered-neutral ten percent formalin. The tissues were processed routinely, sectioned at six micrometers and stained with hematoxylin and eosin. The samples were then examined for gross and microscopic lesions. The gross and microscopic lesions were classified on a visual scoring scale. The gross scoring was based on the relative number of white or hemorrhagic foci, dilatation of the intestine, and the amount of hemorrhage into the lumens of the ceca. The intestinal mucosa was examined microscopically and scored according to the relative number and involvement of the epithelial cells with coccidial forms. Hemorrhage, necrosis, and inflammatory cells were also considered.

Microbiological Analysis of the Intestinal Contents of the Healthy Broilers

An examination of the intestinal contents was made at the end of the trial in order to determine the effect of the "Probiotic Product" on the number of lactobacilli and coliforms which were present. In order to do this, the following procedure was followed.

After the broilers had been processed at the end of the trial, six broilers each were selected from Treatment 1 (commercial ration not supplemented with "Probiotic Product") and Treatment 5 (commercial ration supplemented with "Probiotic Product"). The intestines were pulled from the abdominal cavity and samples of about 7 to 10 cm in

length were taken from both the small intestines (duodenum and the lower small intestine), and the large intestine (the rectum). These samples of intestines were placed in sterile Whirl Pak bags (Curtin Matheson Scientific, Inc., Houston, Texas) and kept in an ice bath. They were taken immediately to the Dairy Microbiology Laboratory in the Animal Science Department for analysis.

Each sample of intestine was weighed into an empty sterile blender cup and an amount four times its weight of cold sterile diluent was added (diluent was a 0.1 percent peptone in distilled water). The samples were blended on a suitable blender for two minutes and left stand for one to two minutes. The solid material (when present) was left to settle to the bottom, and the supernatant filtered through a sterile cheesecloth. About 15 ml of the liquid was transferred to a sterile screw-cap tube and placed in an ice bath. The sample filtrate was then used for the examination of the number of facultative lactobacilli and coliforms by using plating procedures, as described by Gilliland, et al. (14).

For the determination of the total solids, the sample filtrate was mixed thoroughly, and 2.5 ml was weighed into each of four pre-dried and tared pans. The pan with the sample was weighed to the nearest 0.1 milligram. The samples were dried in a forced air oven at 100^{o}C for 16 hours. Each pan with the dried sample was placed in a desiccator to cool. They were weighed, and the grams of dry matter per milliliter of sample filtrate was calculated. This was used as the basis for calculating the bacterial counts per gram of the dry weight.

Statistical Analysis

Feed data (based on broiler days) were analyzed as a split plot over time in which Treatments 1, 3, 5, 7, and 8 were the main plot in a randomized block design. The subplots were three periods of repeated measurements made at the end of 22, 42, and 49 days. The data for each period were analyzed separately. Gain per broiler per day and feed per broiler per day were calculated to find the feed conversion efficiency for those three periods.

The body weight data (based on broilers surviving 49 days) were analyzed as a split-plot design in which Treatments 1, 3, 5, 7, and 8 were the main plot in a randomized block design. The subplots were the 22, 42, and 49 days.

Based on the analysis of variance, F-value and LSD (at the 0.05 level) were used to compare differences between two treatment means for body weight, gain per broiler per day, and the efficiency of feed conversion. The experimental unit was the unweighted pen mean which was obtained by the average of 29-32 broilers. The statistical model used in the analysis of variance of the data is illustrated in Table V.

Feeding Trial II

Experimental Rations

The eight experimental rations (corresponding to eight treatments, Table II) fed in Trial II are listed in Tables VI and VII. They were assigned randomly to each of the two blocks and were fed from day 1 to eight weeks of age. The approach in this trial was to formulate and feed a series of broiler rations with different degrees of nutritional

adequacy, both with and without the addition of "Probiotic Product." The "Probiotic Product" was identical to the one used in Trial I. These rations were formulated with consideration being given to both nutritional adequacy and cost. A balance was selected in the case of each experimental ration so that nutritional adequacy was given preference at one end of the series (Tables VI and VII, Ration 1), with cost being what it had to be to meet standards for total dietary protein and dietary methionine. On the other hand, preference was given at the other end of the series (Tables VI and VII, Ration 8) to getting cost as low as possible at the expense of total dietary protein and dietary methionine.

TABLE V

EXAMPLE OF THE ANALYSIS OF VARIANCE OF THE DATA OBTAINED IN TRIAL I

Source	df	Mean Square Gain/Broiler/Day	Mean Square Feed Conversion/ Broiler/Day
Block (B)	1	12.04	3.09
Treatment (Trt)	4	11.68	13.81
Block x Treatment	4	24.26	38.68
Period (P)	2	708.62	11113.94
Trt x P	8	4.44	2.68
B x P	2	5.85	0.095
B x P x Trt	8	6.16	8.87
Error = B x P + B x P x Trt	10	6.10	7.12

TABLE VI

EXPERIMENTAL STARTER RATIONS OF BROILERS FED IN TRIAL II

Ingredients	Ration Number 1 (%)	2 (%)	3 (%)	4 (%)	5 (%)	6 (%)	7 (%)	8 (%)
Tallow, feed grade	5	5	2.5	2.5	5	5	3	3
Yellow corn, ground	53.75	53.75	59.87	59.87	45.45	45.45	49.05	49.05
Soybean meal (44%)	29	27	26.2	24.2	33	31	35	33
Fish meal (menhaden	4	4	3.4	3.4	-	-	-	-
Feather meal	2	2	1.7	1.7	-	-	-	-
Meat and bone meal (50%)	4	4	3.4	3.4	5	5	-	-
Alfalfa meal (17%)	-	-	-	-	3	3	3	3
Dried whey	-	-	-	-	3	3	3	3
Live yeast culture (14)	-	-	-	-	3	3	3	3
dl methionine	0.15	0.15	0.13	0.13	0.1	0.1	-	-
Phosphorus supplement (Ca27-P18)	0.6	0.6	1.6	1.6	1	1	2	2
Calcium carbonate	0.75	0.75	0.35	0.35	0.5	0.5	1	1
Trace mineral mix[1]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Salt	0.3	0.3	0.3	0.3	0.5	0.5	0.5	0.5
Broiler vitamin mix[2]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Coban	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
"Probiotic Product"	-	2	-	2	-	2	-	2
Calculated analysis								
Protein (%)[3]	22.80	22.60	22.20	22.62	23.75	21.83	21.15	21.01
Methionine (% of protein)	2.25	2.25	2.22	2.22	2.00	2.00	1.58	1.58
Kcal/454 g	1404	1404	1367	1367	1317	1317	1279	1279
Dry matter (%)	89.70	89.70	89.40	89.50	89.60	89.60	89.30	89.50

[1]Provides in the ration: manganese, 120 ppm; zinc, 80 ppm; iron, 60 ppm; copper, 10 ppm, and iodine, 1.0 ppm.

[2]Contains per 454 g of vitamin mix: vitamin A, 1,400,000 I.U.; vitamin D_3, 320,000 I.U.; vitamin E, 1,400 I.U.; menadione sodium bisulfite complex, 800 mg; riboflavin, 1,400 mg; niacin, 7,000 mg; d-pantothenic acid, 2,000 mg; choline, 110,000 mg; thiamine, 200 mg; pyridoxine, 200 mg; vitamin B_{12}, 2.2 mg; d-biotin, 20 mg, and folic acid, 140 mg.

[3]Kjeldahl analysis.

TABLE VII

EXPERIMENTAL FINISHER RATIONS OF BROILERS FED IN TRIAL II

Ingredients	Ration Number 1 (%)	2 (%)	3 (%)	4 (%)	5 (%)	6 (%)	7 (%)	8 (%)
Tallow, feed grade	5	5	1.5	1.5	2	2	1.5	1.5
Yellow corn, ground	59.5	59.5	67.1	67.1	56.5	56.5	56.6	56.6
Soybean meal (44%)	25	23	22	20	27	25	29	27
Fish meal (menhaden)	4	4	3.4	3.4	-	-	-	-
Feather meal	2	2	1.7	1.7	-	-	-	-
Meat and bone meal (50%)	2.5	2.5	2	2	2	2	-	-
Alfalfa meal (17%)	-	-	-	-	3	3	3	3
Dried whey (12%)	-	-	-	-	3	3	3	3
Live yeast culture (14%)	-	-	-	-	3	3	3	3
dl methionine	0.12	0.12	0.1	0.1	0.1	0.1	-	-
Phosphorus supplement (Ca27-P18)	0.7	0.7	1	1	2	2	2	2
Calcium carbonate	0.5	0.5	0.5	0.5	0.5	0.5	1	1
Trace mineral mix[1]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Salt	0.3	0.3	0.3	0.3	0.5	0.5	0.5	0.5
Broiler vitamin mix[2]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Coban	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
"Probiotic Product"	-	2	-	2	-	2	-	2
Calculated analysis								
Protein (%)[3]	22.2	18.60	20.50	16.70	18.13	19.98	18.14	18.70
Methionine (% of protein)	2.20	2.20	2.17	2.17	2.12	2.12	1.61	1.61
Kcal/454 g	1437	1437	1385	1385	1293	1293	1263	1263
Dry matter	88.70	88.70	88.30	88.14	88.60	88.60	88.63	88.70

[1]Provides in the ration: manganese, 120 ppm; zinc, 80 ppm; iron, 60 ppm; copper, 10 ppm, and iodine, 1.0 ppm.

[2]Contains per 454 g of vitamin mix: vitamin A, 1,400,000 I.U.; vitamin D_3, 320,000 I.U.; vitamin E, 1,400 I.U.; menadione sodium bisulfite complex, 800 mg; riboflavin, 1,400 mg; niacin, 7,000 mg; d-pantothenic acid, 2,000 mg; choline, 110,000 mg; thiamine, 200 mg; pyridoxine, 200 mg; vitamin B_{12}, 2.2 mg; d-biotin, 20 mg, and folic acid, 140 mg.

[3]Kjeldahl analysis.

Measurements Made

Measurements were taken at the end of 2, 4, 6, and 8 weeks of the growing period. The measurements included body weight and feed consumption. Mortality was recorded daily. The broilers were processed and given a dressed market grade for both fleshing and finish at the end of 8 weeks. The processing and grading procedures were the same as is desscribed in Feeding Trial I. From the body weight and feed consumption data, calculations were made for feed conversion efficiency and feed cost per 454 g of broiler produced.

Statistical Analysis

Feed data (based on broiler days) were analyzed as a split plot over time in which the eight treatments were the main plot in a randomized block design. The subplots were four periods of separate measurements made at the end of 14, 28, 42, and 56 days. The data for each period were analyzed separately. Gain per broiler per day and feed per broiler per day were calculated fo find the feed conversion efficiency for the periods shown above.

The body weight data (based on broilers surviving 56 days) were analyzed as a split plot design in which the eight treatments were the main plot in a randomized block design. The subplots were the same four periods indicated above.

Based on the analyses of variance, F-value and LSD were used to compare differences between two treatment means for body weight, gain per broiler per day, and the efficiency of feed conversion. The experimental unit was the unweighted pen mean. This was obtained by

the average of thirty broilers. The statistical model used in the analysis of variance of the data obtained in Trial II was similar to that made in Trial I, which is illustrated in Table V.

CHAPTER IV

RESULTS AND DISCUSSION

Feeding Trial I

Microbiological Assay of the "Probiotic Product" and Rations

The results of the enumeration of lactobacilli in the "Probiotic Product" and the five rations in Trial I are presented in Table VIII. These results show that the "Probiotic Product" contained a very small number of lactobacilli which were considered to be the main representative of the beneficial microorganisms in the "Probiotic Product." All rations contained lactobacilli, but the number of these lactobacilli was not much higher (in one case it was lower) in the rations that were supplemented with the "Probiotic Product" than in the control rations. All isolates from LBS agar grew at 15°C, which indicates that they were probably not L. acidophilus. Mold grew on all plates of MRS agar for all samples, and covered the entire plate on every dilution plated. This indicates that the mold was the most numerous organism in the probiotic samples. The samples of "Probiotic Product" fed in these experiments contained a very small number of lactobacilli.

TABLE VIII

ENUMERATION OF LACTOBACILLI IN THE FIVE DIFFERENT RATIONS AND IN THE COMMERCIAL "PROBIOTIC PRODUCT" IN TRIAL I

Ration	Treatment Number	Colony-forming Units/g LBS[1]	Colony-forming Units/g MRS[2]
1) Commercial	1	100	mold
2) Commercial + coban	3	600	mold
3) Commercial + "Probiotic Product"	5	600	mold
4) Corn-soybean meal type (adequate)	7	700	mold
5) Corn-soybean meal type (deficient) + "Probiotic Product"	8	400	mold
Samples[3]			
1) Sample		3.9×10^4	mold
2) Sample		1.4×10^4	mold
3) Sample		3.0×10^3	mold

[1]LBS was a selective agar for lactobacilli.

[2]MRS agar was non-selective.

[3]Three samples of the commercial "Probiotic Product" were taken from three bags.

Microbiological Examination of the Intestinal Contents of the Broilers

Results of the microbiological examination of the intestinal contents from the broilers in Treatments 1 and 5 (Trial I) for coliforms and lactobacilli are presented in Table IX. The overall average of the number of coliforms in the small intestine of the broilers fed the commercial ration (Treatment 1) was less than 5.81 (all counts based on $\log_{10}$/g dry weight), whereas the overall average of the number of coliforms of those broilers fed the commercial ration which had been supplemented with the "Probiotic Product" (Treatment 5) was less than 5.75. In the large intestine, the overall average of the number of coliforms in Treatments 1 and 5 was 6.63 and 6.30, respectively.

The overall average of the lactobacillus counts in the small intestine of the broilers fed in Treatment 1 was less than 7.04, and it was less than 6.84 in Treatment 5. In the large intestine, the overall average was less than 7.84 in Treatment 1, and 7.80 in Treatment 5, respectively.

The statistical analyses of these data indicate that the "Probiotic Product" had no effect on the growth of coliforms. On the other hand, the broilers which were not fed any of the "Probiotic Product" showed about the same numbers of lactobacilli in their intestines as those which were fed the "Probiotic Product." There is evidence in the literature that lactobacilli are involved in controlling the growth of *E. coli* in the intestinal tract, and this inhibition of *E. coli* is dependent upon the presence of large numbers of lactobacilli (10, 12). In our study, this effect did not appear when the "Probiotic Product" was used in feeding the broilers. Thus, it can be concluded that in

TABLE IX

MICROBIOLOGICAL EXAMINATION OF INTESTINAL CONTENTS OF BROILERS FROM TREATMENT 1 AND TREATMENT 5 IN TRIAL I

Block	Trtmt.	Bird Number	Coliforms		Lactobacilli	
			Small Intestine	Large Intestine	Small Intestine	Large Intestine
1	1	1	5.69	5.83	<6.58	<6.60
		2	6.15	7.04	7.57	8.23
		3	6.82	7.40	7.40	8.18
		Average	6.22	6.76	<7.18	<7.67
2	1	1	5.63	5.58	<6.63	7.11
		2	5.88	7.66	7.34	9.04
		3	<4.69	6.28	6.69	7.86
		Average	<5.40	6.51	<6.89	8.00
		Overall Average	<5.81	6.63	<7.04	<7.84
1	5	1	6.66	6.85	<6.54	7.82
		2	5.34	6.85	<6.64	7.87
		3	<4.65	6.30	<6.65	8.00
		Average	<5.55	6.67	<6.61	7.90
2	5	1	5.45	5.66	7.08	7.70
		2	6.78	5.95	7.52	6.75
		3	5.63	6.20	<6.63	8.66
		Average	5.95	5.94	7.08	7.70
		Overall Average	<5.75	6.30	<6.84	7.80

Note: Counts presented as $\log_{10}$/g dry weight.

addition of the "Probiotic Product" to the diet did not alter the microflora in the intestinal tract of the broilers. Since there was no increase in lactobacilli in the intestinal tract above that obtained with the unsupplemented ration, it is apparent that this "Probiotic Product" contained a very small number of viable lactobacilli.

Since a small number of viable lactobacilli were present, it could be expected that very little if any change in the number of viable lactobacilli would be apparent in the intestines of the broilers fed this "Probiotic Product." In addition, there probably would be no difference in the number of coliforms between the supplemented and unsupplemented groups.

Coccidia Challenge

The results obtained from the gross and microscopic examinations of the intestines of broilers which were challenged with coccidia are presented in Table X. The first twelve broilers submitted on day 4 from Treatments 2, 4, and 6 (these data are not shown in Table X) and the broilers submitted from Treatment 4 on days 5 and 6 post-challenge showed no symptoms of a coccidiosis infection. The broilers submitted from Treatments 2 and 6 on days 5 and 6 post-challenge showed infectious lesions. According to these results and the observations of an experienced veterinarian, there were no differences in the severity of the coccidiosis infection in broilers challenged from Treatments 2 and 6. Thus, the "Probiotic Product" was not effective in protecting the broilers from coccidiosis (Treatment 6). Broilers challenged from Treatment 4 showed no symptoms of an infection. This was due to the effect of the coccidiostat (coban) which was added to the commercial ration.

TABLE X

GROSS AND MICROSCOPIC EXAMINATION OF THE INTESTINE FOR COCCIDIOSIS OF BROILERS CHALLENGED WITH COCCIDIA IN TRIAL I[1]

Bird No.	Treat	Duodenum		Jejenum		Ileum		Cecum	
		Gross	Micro	Gross	Micro	Gross	Micro	Gross	Micro
		Second group of broilers on day 5 post-challenge							
1	2	+2	+2	0	0	0	0	0	0
2	2	+1	+1	0	+1	0	+1	+3	+2
3	2	+2	+3	0	+3	0	0	+1	+1
4	2	0	+2	0	0	0	0	0	+2
5	4	0	0	0	0	0	0	0	+1
6	4	0	0	0	0	0	0	0	0
7	4	0	0	0	0	0	0	0	0
8	4	0	0	0	0	0	0	0	+1
9	6	+1	+1	0	+1	+1	0	0	+1
10	6	0	+3	0	+2	0	+1	+3	+3
11	6	+1	+1	0	+1	0	+1	0	+2
12	6	+2	+1	+1	+1	0	0	+1	+2
		Third group of broilers on day 6 post-challenge							
1	2	+2	+2	+2	0	+1	0	+4	+4
2	2	+2	+3	+1	+3	+1	+1	+4	+4
3	2	+2	+2	+1	+1	+1	+1	+4	+4
4	2	0	+1	0	+1	0	+1	+2	+2
5	4	0	0	0	0	0	0	+2	+2
6	4	0	0	0	0	0	0	0	0
7	4	0	0	0	0	0	0	0	0
8	4	0	0	0	0	0	0	0	0
9	6	+1	+2	+1	+2	+1	+3	+4	+4
10	6	+2	+4	0	+1	0	+1	+4	+4
11	6	+2	+2	0	+2	0	+3	+3	+4
12	6	+2	+2	+1	+3	0	+2	+3	+4

[1]The first 12 broilers submitted on day 4 post-challenge were negative on gross and microscopic examination of the intestine for coccidiosis.

Note: 0 = no observed infection
+1 = slight infection
+2 = moderate infection
+3 = moderately severe infection
+4 = severe infection

It can be concluded that the "Probiotic Product" did not give additional protection against coccidiosis to broilers which had been challenged with coccidia under the conditions of this experiment. This may be due to the fact that there were very few lactobacilli present in the "Probiotic Product." It must be pointed out, however, that the challenge with coccidia was so strong and effective that the rate of mortality of the broilers was very high. The results might have been different had the challenge with coccidia been less severe.

Research data do indicate that lactobacilli promote protection against microbial infections (11, 12, 23, 33), and the constant administration of lactobacilli through the feed helped reduce the severity of the clinical symptoms of Eimeria tenella infection (18). In addition, when chicks were given lactobacilli immediately after hatching, they showed better performance when exposed to the stress of disease (11, 12, 23). No positive results such as those mentioned above were evident in this trial (Trial I). This may have been due to the fact that there were very few viable lactobacilli in the "Probiotic Product" used in this study.

Effect of the "Probiotic Product" on the Performance of the Broilers

The data on body weight, gain per broiler per day, and efficiency of feed conversion which were obtained in Trial I when the broilers were 3, 6, and 7 weeks old, and the feed cost per 454 g of broiler produced at 6 weeks of age are presented in Table XI. Emphasis was made in studying the effect of the "Probiotic Product" when it was added to a commercial broiler ration. For this purpose, a comparison was made

TABLE XI

MEAN BODY WEIGHT, GAIN PER BROILER PER DAY, FEED CONVERSION AT THE END OF 3, 6, AND 7 WEEKS OF THE GROWING PERIOD, AND FEED COST ANALYSIS AT THE END OF SIX WEEKS OF THE GROWING PERIOD OF BROILERS IN TRIAL I

Ration	Treatment No.	3-week Period			6-week Period			7-week Period			Feed Cost 6-week Period	
		Body Weight[1] (g)	Gain per Broiler[1] per Day (g)	Grams of Feed per Gram of Broiler[1]	Body Weight (g)	Gain per Broiler per Day (g)	Grams of Feed per Gram of Broiler	Body Weight (g)	Gain per Broiler per Day (g)	Grams of Feed per Gram of Broiler	Total Feed Cost[2] per Broiler (cents)	Feed Cost per 454 g of Body Weight (cents)
1) Commercial	1	565.87	23.98	1.54	1318.55	30.10	1.93	1601.45	31.5	2.03	41.80	14.39
2) Commercial + coban	3	561.74	23.60	1.52	1354.11	31.19	1.85	1631.85	32.4	1.97	41.61	13.95
3) Commercial + "Probiotic Product"	5	555.56	23.45	1.50	1275.19	29.23	1.90	1528.41	30.21	2.04	43.15	15.36
4) Corn-soybean meal type (adequate)	7	538.87	22.55	1.63	1197.88	27.48	2.05	1458.45	28.87	2.18	42.45	16.10
5) Corn-soybean meal type (deficient) + "Probiotic Product"	8	517.81	21.70	1.73	1238.93	28.53	2.08	1519.80	30.19	2.18	44.91	16.45
$LSD_{0.05}$[3]		41.620	1.983	0.118	234.811	5.86	0.1337	312.10	6.59	0.1976		

[1]Each entry is the average of two pen means.

[2]Ingredient cost only.

[3]LSD was calculated from the pooled block X treatment error mean square.

among Treatments 1, 3, and 5 (Rations 1, 2, and 3, Table XI). Even though there was no treatment X period interaction, the data were analyzed separately at the end of 3, 6, and 7 weeks of the growing period. When the broilers were 3, 6, and 7 weeks old, there were no statistically significant differences ($P<0.05$) in body weight, gain per broiler per day, and the efficiency of feed conversion among broilers fed in Treatments 1, 3, and 5. These results indicate that the addition of the "Probiotic Product" did not improve the growth performance of the broilers fed the commercial broiler ration as measured by either the body weight or grams of feed per gram of broiler produced.

If the "Probiotic Product" had contained a sufficient number of lactobacilli, it might have improved the performance of the broilers. Research data in the literature indicate that the addition of lactobacilli to the diet of broilers improved their weight gain and the efficiency of feed utilization (7, 29, 30).

Ration 5 (Table XI) was formulated and used in this trial in an attempt to measure any response that might be produced when the "Probiotic Product" was added to a deficient corn-soybean meal type ration for broilers and to compare the growth performance of broilers fed this supplemented ration with that brought about by using an adequate corn-soybean meal type ration (Ration 4). It is obvious that Rations 4 and 5 should have been fed both with and without the addition of the "Probiotic Product" in order to make a valid comparison. This situation was corrected by using a complete design and making a comparison in this regard in Trial II. However, there were numerical differences in body weight and gain per broiler per day for broilers at 6 and 7 weeks of age when they were fed a deficient corn-soybean meal type ration which was

supplemented with the "Probiotic Product" (Ration 5, Table XI) when a comparison was made with those broilers which were fed the adequate corn-soybean meal type ration (Ration 4, Table XI).

This result gives some indication that the "Probiotic Product" may have had a significant effect in raising the body weight and gain of broilers when added to a ration deficient in both the total protein and methionine. Accordingly, a further investigation was made in Trial II and the results are discussed later.

Feed Cost Analysis

The period of 6 weeks was selected for the analysis of feed cost since broilers are normally marketed at this body weight. The commercial broiler ration used in this trial with the addition of coban (Ration 2) produced 454 g of broiler at the lowest feed cost among the rations used (Table XI). In addition, the feed cost per broiler (total cost or cost per 454 g produced) with the ration which was supplemented with the "Probiotic Product" was higher than the commercial broiler ration which was unsupplemented. Therefore, it can be concluded that no economic advantage was brought about by using the "Probiotic Product" in the commercial broiler ration.

A second reason for using Rations 4 and 5 in this trial was to determine the feed cost of the corn-soybean meal type ration when this ration was used as an adequate ration without the addition of the "Probiotic Product," and to compare this cost with a ration deficient in both protein and methionine but with the addition of the "Probiotic Product." The feed cost per 454 g of broiler produced was the highest among the five rations when the deficient ration was supplemented with

the "Probiotic Product." It can be concluded that there is no economic advantage of using this "Probiotic Product" in a corn-soybean type broiler ration deficient in protein and methionine.

Market Grades for Fleshing and Finish

The results of the dressed grades for fleshing and finish of the broilers fed the five differnt rations are summarized in Table XII. These results indicate that the percentage in Finish Grades of broilers fed the commercial ration which had been supplemented with coban, and the corn-soybean meal type ration which had been supplemented with the "Probiotic Product" were about the same--62.9 percent and 63.3 percent of broilers in Finish Grade A, respectively. All other treatments were equally higher in the percentage of broilers in Finish Grade A than those in Treatments 3 and 8.

The highest percentage of broilers on Fleshing Grade A was obtained with the broilers fed Ration 2 (Treatment 3), and the lowest percentage of broilers in Fleshing Grade A was obtained with those fed the corn-soybean meal type ration (Treatment 7). It appears that the "Probiotic" Product" had no real effect on the broilers in terms of the degree of fleshing and finish.

On a pigmentation basis, it was noted that the broilers fed the commercial ration and the corn-soybean meal type ration with both of these rations supplemented with the "Probiotic Product" showed a much deeper color than those fed on the other rations which were without the "Probiotic Product." This greater degree of pigmentation which was brought about by the "Probiotic Product" may have some advantage when the broilers are brought to market. These observations would appear to

TABLE XII

SUMMARY OF THE MARKET GRADES OF BROILERS IN TRIAL I

Ration	Trtmt. No.	Finish Grade			Fleshing Grade		
		A (%)	B (%)	C (%)	A (%)	B (%)	C (%)
1) Commercial	1	76.7	21.7	1.7	56.7	33.3	10
2) Commercial + coban	3	62.9	37.1	-	69.4	27.4	3.2
3) Commercial + "Probiotic Product"	5	75.9	24.1	-	62.1	27.6	10.3
4) Corn-soybean type (adequate)	7	77.6	22.4	-	46.6	44.8	8.6
5) Corn-soybean type (deficient) + "Probiotic Product"	8	63.3	36.7	-	53.3	38.3	8.3

be in agreement with the results of an earlier study which indicated that the use of the same "Probiotic Product" resulted in a greater degree of pigmentation and fatness (3). Current data provide no explanation for this effect.

Feeding Trial II

Effect of the "Probiotic Product" on the Performance of the Broilers

The data on body weight, gain per broiler per day, and efficiency of feed conversion which were obtained when the broilers were 2, 4, 6, and 8 weeks old are presented in Table XIII. In Treatments 1 and 2, a comparison was made between nutritionally adequate commercial broiler ration, which had excellent growth response when fed under commercial production conditions, and this ration when supplemented with the "Probiotic Product." The results of this comparison show that the only significant difference in the three measurements made among broilers fed in Treatments 1 and 2 was in body weight at 6 weeks of age with the broilers which were fed the commercial supplemented ration being heaviest. This indicates that there is no advantage to be gained in adding the "Probiotic Product" to the nutritionally adequate commercial broiler ration.

The most expensive and hard to obtain ration ingredients in the commercial broiler ration (Treatment 1) were fish meal, feather meal, and meat and bone meal. The broiler rations fed in Treatments 3 and 4 were formulated to contain dietary levels of each of these three ingredients 15 percent below those used in the commercial broiler ration. At 2 and 4 weeks of age, there were no significant differences

TABLE XIII

MEAN BODY WEIGHT, GAIN PER BROILER PER DAY, AND FEED CONVERSION AT THE END OF 2, 4, 6, AND 8 WEEKS OF THE GROWING PERIOD OF BROILERS IN TRIAL II

Ration	Trtmt. No.	2-week Period			4-week Period			6-week Period			8-week Period		
		Body Weight[1] (g)	Gain per Broiler per Day[1] (g)	Grams of Feed per Gram of Broiler[1]	Body Weight (g)	Gain per Broiler per Day (g)	Grams of Feed per Gram of Broiler	Body Weight (g)	Gain per Broiler per Day (g)	Grams of Feed per Gram of Broiler	Body Weight (g)	Gain per Broiler per Day (g)	Grams of Feed per Gram of Broiler
1) Commercial	1	298.7	17.89	1.55	847.53	27.69	1.75	1546.12	34.38	1.99	2182.57	36.97	2.33
2) Commercial + "Probiotic Product"	2	294.73	17.84	1.56	832.58	27.82	1.78	1489.40	34.10	2.05	2122.86	36.86	2.37
3) Commercial 85%	3	293.71	17.82	1.49	845.10	28.39	1.77	1456.95	33.39	2.13	2083.93	36.23	2.47
4) Commercial 85% + "Probiotic Product"	4	270.10	16.19	1.66	823.32	27.90	1.81	1459.25	33.52	2.11	2104.50	36.59	2.42
5) Corn-soybean meal type (adequate)	5	287.10	17.27	1.56	826.78	27.90	1.85	1386.15	31.86	2.25	2081.90	36.26	2.58
6) Corn-soybean meal type (adequate) + "Probiotic Product"	6	287.22	17.33	1.66	810.03	27.09	1.91	1383.12	31.71	2.29	2033.74	35.32	2.62
7) Corn-soybean meal type (deficient)	7	265.90	15.69	1.66	785.37	26.10	1.92	1392.76	31.79	2.29	2030.90	35.16	2.64
8) Corn-soybean meal type (deficient) + "Probiotic Product"	8	278.02	16.34	1.53	791.82	25.64	1.92	1418.10	31.63	2.33	2044.51	34.78	2.66
$LSD^{2}_{0.05}$		16.759	1.377	0.2084	23.794	2.033	0.1065	32.537	2.0635	0.0657	77.118	2.361	0.087

[1]Each entry is the average of two pen means.

[2]LSD was calculated from the pooled block X treatment error mean square.

in body weight, gain per broiler per day, and efficiency of feed conversion among the broilers fed in Treatment 1 (commercial ration) and Treatment 3 (commercial ration with 15 percent reduction in fish meal, feather meal, and meat and bone meal), but there were statistically significant differences in body weight and efficiency of feed conversion at 6 and 8 weeks of age in favor of the commercial ration (Treatment 1). Thus, the reduction in the dietary levels of fish meal, feather meal, and meat and bone meal altered the dietary nutrient levels to the point where growth performance was decreased. The addition of the "Probiotic Product" in Treatment 4 did not compensate for this decrease in growth performance. This is shown by the fact that there were no significant differences in body weight, gain per broiler per day, and efficiency of feed conversion among the broilers fed in Treatments 3 and 4 throughout the growing period, with the exception of the first two weeks during which body weight and gain per broiler per day were significantly lower in Treatment 4.

In an attempt to reduce ration cost but to still maintain nutritional adequacy, fish meal and feather meal were eliminated entirely; the dietary level of meat and bone meal was reduced, and alfalfa meal, dried whey, and live yeast culture were used to obtain a so-called corn-soybean meal type ration (Ration 5, Treatment 5). Formulation was done to fully meet accepted nutritional standards comparable to those adhered to in the commercial ration fed in Treatment 1. In order to determine if the "Probiotic Product" would compensate for any unrecognized nutritional inadequacies in Ration 5, this product was added to Ration 6. The data in Table XIII indicate that there were no significant differences in body weight, gain per broiler per day, and efficiency

of feed conversion among the broilers in Treatments 1 and 5 starting from day one through four weeks of the growing period. However, growth performance in Treatment 5 was inferior to that obtained in Treatment 1 between four and eight weeks of the growing period. Thus, it can be concluded that Ration 5 was not the nutritional equal of Ration 1. In addition, at 2, 4, 6, and 8 weeks of the growing period, there were no significant differences in body weight, gain per broiler per day, and efficiency of feed conversion among the broilers fed in Treatment 5 (corn-soybean meal type adequate) and Treatment 6 (corn-soybean meal type adequate plus the "Probiotic Product"). These data indicate that the corn-soybean meal type broiler ration as formulated may have had unrecognized nutritional differences even when supplemented with the "Probiotic Product" is not the nutritional equal of Ration 1 (commercial broiler ration).

Ration 7 was a modification of Ration 5 in which dietary protein and methionine levels were reduced below accepted standards (Tables VI and VII). From the data obtained, it is obvious that Ration 7 is not equal to Ration 5 in terms of growth performance, especially during the first four weeks of the growing period. There is some indication that this difference is less pronounced from four through eight weeks of the growing period. When Ration 7 is supplemented with the "Probiotic Product" (Ration 8), the differences in body weight, gain per broiler per day, and efficiency of feed conversion are not significant statistically, although they are still numerically below those obtained with Ration 5. Thus, it can be concluded that the "Probiotic Product" did not compensate adequately for the total dietary protein and methionine inadequacies in Ration 8.

Feed Cost Analysis

The data for the body weight, total feed cost per broiler produced, and feed cost per 454 g of body weight when the broilers were six weeks old are presented in Table XIV. Feed cost was calculated when the broilers were six weeks old because this corresponds to the age and weight when commercial broilers are routinely marketed.

Total body weight as well as cost per 454 g of body weight must be taken into consideration in any cost analysis. A comparison of body weight among the eight treatments indicates that the heaviest broilers were produced with the commercial broiler ration fed in Treatment 1. Feed cost per 454 g of body weight was 16.83 cents in Treatment 1, compared to 16.43 and 16.77 cents, respectively, for Treatments 3 and 4 which had the lowest and next to the lowest feed cost per 454 g of broiler. The cost advantage for Treatments 3 and 4 of 0.4 cent and 0.06 cent per 454 g of body weight is not sufficient to offset the body weight advantage in Treatment 1. Assuming a return of 20 cents per 454 g, the broilers in Treatment 1 would give an additional return of 4 cents per broiler over Treatments 3 and 4, compared to a maximum additional cost of 1.4 cents.

From this comparison it can be concluded that the broilers in Treatment 1 brought the greatest monetary return over feed cost among the eight treatments studied. There appeared to be no advantage in reducing ingredients cost or in supplementing any one of the broiler rations with "Probiotic Product" to offset the nutritional inadequacies that apparently were brought about by ration simplification. Therefore, no economic advantage was brought about by using the

TABLE XIV

FEED COST ANALYSIS AT 6 WEEKS OF AGE OF BROILERS IN TRIAL II

Ration	Trtmt. No.	Body Weight at 6 Weeks (g)	Total Feed Cost[1] per Broiler (cents)	Feed Cost per 454 g Body Weight (cents)
1) Commercial	1	1546.12	57.31	16.83
2) Commercial + "Probiotic Product"	2	1489.40	58.00	17.68
3) Commercial 85%	3	1456.95	52.71	16.43
4) Commercial 85% + "Probiotic Product"	4	1459.25	53.91	16.77
5) Corn-soybean meal type (adequate)	5	1386.15	55.33	18.12
6) Corn-soybean meal type (adequate) + "Probiotic Product"	6	1383.12	57.16	18.76
7) Corn-soybean meal type (deficient)	7	1392.76	53.32	17.38
8) Corn-soybean meal type (deficient) + "Probiotic Product"	8	1418.10	56.77	18.18

[1]Ingredient cost only.

"Probiotic Product" in broiler rations.

Market Grades for Fleshing and Finish

The results of the dressed grades for fleshing and finish of the broilers fed the different rations in this feeding trial are summarized in Table XV. These results show that the highest percentage of broilers in Finish Grade A among the broilers fed the eight different rations was observed with the broilers which were fed on the corn-soybean meal type ration which was deficient in both the protein and methionine (Treatment 7). The lowest percentage of broilers in Finish Grade A was obtained with the broilers fed the corn-soybean meal type ration which was deficient, and which had been supplemented with the "Probiotic Product" (Treatment 8).

The highest percentage of broilers in Fleshing Grade A among the broilers fed the eight rations was observed with the broilers which were fed the commercial ration which had been supplemented with the "Probiotic Product." The lowest percentage of broilers in Fleshing Grade A was obtained with the broilers which were fed the commercial ration 85 percent (Ration 3, Table XV). The percentage of broilers in Fleshing Grade A for the broilers which were fed Ration 8 was intermediate, but it was similar to that for the broilers fed Ration 1. It appears that the "Probiotic Product" did not have any effect on the broilers in terms of the degree of fleshing and finish as this was observed in Trial I. The results on the pigmentation of the broilers fed the "Probiotic Product" with different rations were similar to those observed in Trial I.

TABLE XV

SUMMARY OF THE MARKET GRADES OF BROILERS FED IN TRIAL II

Ration	Trtmt. No.	Finish Grade A (%)	Finish Grade B (%)	Finish Grade C (%)	Fleshing Grade A (%)	Fleshing Grade B (%)	Fleshing Grade C (%)
1) Commercial	1	53.9	46.2	-	84.6	15.4	-
2) Commercial + "Probiotic Product"	2	57.1	41.1	1.8	91.1	7.1	1.8
3) Commercial 85%	3	48.2	51.8	-	64.3	33.9	1.8
4) Commercial 85% + "Probiotic Product"	4	54.4	45.6	-	82.5	17.5	-
5) Corn-soybean meal type (adequate)	5	41.1	58.9	-	82.1	17.9	-
6) Corn-soybean meal type (adequate) + "Probiotic Product"	6	52.7	47.3	-	83.6	16.4	-
7) Corn-soybean meal type (deficient)	7	60.7	39.3	-	75.0	25.0	-
8) Corn-soybean meal type (deficient) + "Probiotic Product"	8	35.3	62.8	2.0	82.4	15.7	2.0

Quality control procedures need to be followed in order to monitor the viability of the microorganisms in the "Probiotic Products" being sold for use in poultry rations. Research data indicate that viable microorganisms (lactobacilli) do produce beneficial results insofar as increased growth and feed efficiency are concerned (1, 7, 8, 9, 30). Thus, it can be concluded that "Probiotic Products" which contain viable microorganisms could be expected to be of real value from both nutritional and economic standpoints. For this reason, not all products of this kind should be condemned as ineffective.

CHAPTER V

SUMMARY AND CONCLUSIONS

Two 8-week feeding trials were conducted using a commercial broiler strain. The first feeding trial was completed during the summer of 1978, and the second feeding trial was held during the fall of 1978.

The broiler rations fed included a standard commercial ration currently being used under practical production conditions, as well as corn-soybean meal type broiler rations. These rations were fed with and without the addition of a commercial "Probiotic Product."

Microbiological analyses were made of the "Probiotic Product" which has been sold and offered as a source of lactobacilli, among other beneficial microorganisms, as well as the experimental rations which were used and the intestinal contents of broilers for the determination of the number of viable lactobacilli and coliforms which were present.

Broilers from Treatments 2, 4, and 6 were challenged with coccidia at 25 days of age. A total of 36 broilers were selected at random from the above treatments for the post-mortem examination for coccidiosis. Four broilers per day over a 3-day time period were selected from each treatment beginning on the fourth day post-challenge.

Body weight and efficiency of feed conversion at intervals during the growing period, feed cost per 454 g of broiler produced at market age, and the dressed market grade for fleshing and finish were measured, followed by a statistical analysis of variance of the data obtained to

determine if the "Probiotic Product" would 1) improve the growth rate and efficiency of feed conversion with broilers when the nutrient levels in the broiler rations fully met recommended nutrient standards; 2) make it possible to reduce the dietary levels of fish meal and feather meal in broiler rations; 3) improve the growth rate and efficiency of feed conversion with broilers when dietary protein and methionine levels are below recommended nutrient standars; 4) reduce the adverse effects of a coccidiosis outbreak when the broilers were challenged with coccidia, and 5) bring about a reduction in feed cost per unit of broiler produced.

The commercial "Probiotic Product" which was used in this study contained a very small number of lactobacilli which was not high enough to be effective when supplemented with broiler rations. This product did not alter the microflora in the intestinal tract of the broilers. In addition, the "Probiotic Product" did not give additional protection against coccidiosis to broilers.

There was no advantage to be gained in adding the "Probiotic Product" to the nutritionally adequate commercial broiler ration. The addition of the "Probiotic Product" to the broiler rations did not help reducing the dietary levels of fish meal, feather meal, and meat and bone meal. In addition, this product did not compensate adequately for the dietary protein and methionine inadequacies in broiler rations. From a feed cost standpoint, there was no economic advantage brought about by using the "Probiotic Product" in broiler rations. The "Probiotic Product" did not have any real effect on the broilers in terms of the degree of fleshing and finish, but it did have effect on the degree of pigmentation for broilers which were fed this product with their rations, producing deep yellow skin color.

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