EVALUATION OF COCCIVAC-B® AND SACOX 60® (SALINOMYCIN) FOR CONTROL OF 3 STRAINS OF EIMERIA IN BROILERS

By

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NOMENCLATURE

APF	Animal protein factor
C+	Control, challenged treatment
C-	Control, unchallenged treatment
E.	*Eimeria*
FCR	Feed Conversion Ratio
FDA	Food and Drug Administration
NO	Nitric oxide
S+	Salinomycin (Sacox 60®), challenged treatment
S-	Salinomycin (Sacox 60®), unchallenged treatment
V+	Vaccinated (Coccivac-B®), challenged treatment
VE-AC	DL-ά-tocopheryl acetate
V-	Vaccinated (Coccivac-B®), unchallenged treatment

CHAPTER I

INTRODUCTION

BACKGROUND

The agricultural industry has exponentially expanded within the last 100 years. This advancement has been made possible through the work of scientists, researchers, engineers, mechanics, corporations, and government officials. The list of contributions is very large. Crops are now produced in massive quantities to meet the increasing demand of the world's population. Currently, food production surpasses the world population, but due to uneven distribution, the need still remains for more efficient technologies (USDA, 2005). A large factor involved in the uneven food-population distribution is the quantity and quality of the inputs used for the food production industry. These inputs include land, natural resources (water, etc.), industrial resources such as mechanization and facility, and in the case of food animal production, grain production is vital to establish success (USDA, 2002). Agriculture has been able to grow and expand due to the necessity of a larger industry. The days of small scale production are now almost exclusively part of the world's (especially the US) past. Large-scale production is the forefront for the future of the agricultural industry.

GLOBAL PRODUCTION

On the global scale, the agricultural production industry relies on the inputs needed to achieve success. The animal production industry especially relies on available nutrient input. The most important inputs for meat production include capital, feed, and labor. The regions of the world that can most efficiently supply these resources will be able to generate the most product (Dyck et al., 2003). As these resources grow and become more available throughout the world, meat consumption will increase on a global scale.

The poultry industry can be a direct model for the increase of production with increased resources. The poultry industry has grown and become very successful because of both low-cost labor and the availability of feed products from close proximity production. The industry has also flourished globally because the meat is produced and available at a lower cost than pork or grain-fed beef. This fact means less capital is required to produce a valuable protein source (Dyck et al., 2003).

Because poultry meat is cheaper to produce compared with pork and grain-fed beef, the global consumption of poultry has increased in recent years. Poultry meat consumption per capita grew faster in all three classes of countries (high-, middle-, and low-income) than the consumption of all other meats between 1961 and 2000. This increase was 370, 635, and 201 percent for high-, middle-, and low-income countries, respectively. Although total meat consumption per capita increased worldwide, it is clear that poultry meat consumption was significantly higher than the other meat products (Taha, 2001).

POULTRY INDUSTRY ADVANCEMENT

The 20th century has proven to be an era of incredible growth for the poultry industry. The most growth and prosperity has occurred over the past 50-75 years (Etches, 1998, Hammerstedt, 1999, and Rishell, 1997). The success of the industry has come from the efforts of the scientific community to incorporate biological knowledge and discovery into the production of a fast-growing broiler chicken. Particular credit can be given to the focus of scientists on the metabolic processes occurring at a molecular level in animals. The discoveries resulting from such focus also resulted in the development of a massive biochemical and pharmaceutical industry. The advancements made by all of these contributors have allowed the industry to develop from the small, "backyard" farm into the current production schemes that incorporate large amounts of both mechanical and biological technology to run large, commercial farms capable of producing thousands of broilers every year. Because of the expansion, poultry meat is now the most commonly consumed meat in many diets the world over (Etches, 1998).

The advancements seen in biology have also led to dramatic improvement through genetic study. The industry has benefited from improved genetic selection through the last 50 years (Rishell, 1997). Geneticists are able to study the chickens based on discoveries in biology to select birds that will produce offspring able to develop into the desired end product. Although much of the industry's success has been attributed to genetic development, the chickens could not perform to their highest potential without proper environmental conditions.

The role of the nutritionist, farm manager, and veterinarian is to assure that the genetic potential of the chicken can be achieved through proper management. It is quite

clear that these workers in the industry have also helped the industry progress. Using the discoveries of others within the industry, these contributors will be able to provide the proper diet to achieve the metabolic potential of the bird or provide the proper protection against disease in the environment. The scientists within the industry have performed quite well in terms of advancement. One study by Havenstein et al. (2003) compared the carcass compositions of birds eating a typical diet from 1957 to one from 2001. The study used two separate lines of birds common to each time period. The birds consuming the 2001 diet were superior to the 1957 birds in terms of carcass weights, hot carcass yield, breast meat yield, saddle and leg yield, and whole carcass fat yield. The study shows that the typical broiler has increased in size over time yielding more end product to be sold (Havenstein et al., 2003). This fact has been achieved thanks to the combined efforts of the geneticists, nutritionists, biochemists, veterinarians, etc.

POULTRY MEAT PRODUCTION IN THE U.S.

Poultry production in the U.S. is higher than any other area in the world. The total farm value is greater than $20 billion. The U.S. is second to Brazil in broiler export (USDA, 2006). This fact demonstrates the need and use of broiler products within the U.S. The annual production of broilers has steadily increased over the years. Between 2004 and 2006, the production of broilers rose from 34,063 million pounds in 2004 to 35,799 million pounds in 2006. The annual production of both beef and pork has also risen over the past 3 years, but poultry meat still far exceeds those industries. In 2006, beef production reached 26,075 million pounds, and pork production reached 21,010 million pounds. In 2006, the USDA recorded an annual per capita consumption of broiler

meat to be 87.4 pounds while pork slightly decreased from 50 pounds in 2005 to 49.2 in 2006. Beef per capita consumption was 65.6 pounds in 2006. The projected total for all three meats shows a suspected increase in 2007 for both categories with the exception that broiler per capita consumption will decrease slightly to 86.4 pounds. Broilers will still remain the highest in both categories (USDA, 2007). One can clearly see from these numbers that broiler meat is in high demand in the U.S. Broiler meat is in high demand because of the lower cost of it compared with beef (USDA, 2007). Also, broiler meat is readily available almost anywhere in the country.

The industry must strive to meet the increasing demand for poultry products. The importance of healthy birds becomes clear after viewing the statistics. Without the ability to produce a valuable end product, the industry would surely fail. To obtain that product, the industry must have the proper growth and development of the chickens. The birds must be healthy through proper management to achieve the required growth. From the scientific community, proper management techniques can be tested to provide the best environment for the chickens to reach their potential. One area of major concern that may retard the growth of chickens is disease. Scientists have studied and developed medications and vaccines to fight diseases of all kinds. Today, techniques to fight disease are employed all over the world to aid the industry's effort to produce healthy chickens ensuring the continuation of the success of the industry. New techniques and new methodologies to use the current techniques are constantly developing to further improve industry products.

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CHAPTER II

REVIEW OF LITERATURE

COCCIDIOSIS

Coccidiosis is a disease that is commonly seen in many different species of animal. It is a general term given to a set of diseases caused by parasites that affects the intestinal tract causing the same symptoms and results. In the poultry industry, it is among the top reported diseases making it of high importance (Williams, 2002b). In chickens the disease is characterized by a quick onset of bloody diarrhea with high mortality rate. It is caused by an infection of the intestinal tract with the protozoan parasites of the genus *Eimeria* (Allen et al., 1997, Chapman, 2000, Chapman, 2001, Dalloul et al., 2005, Korver et al., 1997, Lillehoj et al., 2000, McDougland et al., 1991, Medarova et al., 2003, Williams, 2002b, and Yaissle et al., 1999). The three potentially pathogenic species of high concern are *E. acervulina, E. maxima, and E. tenella* (Chapman, 2000). A total of nine species have been discovered from chickens with seven of those nine possessing the confirmed ability to be a parasite to chickens (Allen et al., 2002, Chapman, 2000, Dalloul et al., 2005, Lillehoj et al., 2000, and McDougland et al., 1991). These protozoa are species specific. They are so specific that if one wished to observe coccidiosis in turkeys, the parasites from infected chickens could not be used to induce the disease in turkeys. The oocysts that result from chicken infection cannot infect turkeys. Therefore, the disease cannot be brought into a house from wild fowl.

Most often, transportation of the parasites results from the interaction of people with infected chickens. The people then transport the parasites between chicken houses within a farm (C. Broussard, Schering Plough Animal Health, personal communication and Lillehoj et al., 2000).

The different strains of *Eimeria* affect different regions of the intestinal tract. Some of the species will attack the upper small intestine while others may attack the mid small intestine and the ceca. *Eimeria acervulina, E. mivati, E. praecox, and E. hagani* infect the upper small intestine. *Eimeria maxima* and *E. necatrix* infect the mid portion of the small intestine with some spillage into both the upper and lower portions with the most prominent infection remaining in the mid small intestine. *Eimeria brunetti, E. mitis, and E. tenella* infect the lower small intestine/ceca region (Allen et al., 2002, Dalloul et al., 2005, Lillehoj et al., 2000, and McDougland et al., 1991). Once in the host, the protozoa quickly multiply in the intestinal tract. The infection causes tissue damage which will affect digestion and absorption within the intestine, and hence, decrease performance. Eventually, the disease will lead to dehydration and blood loss. The disease typically occurs in young animals because exposure aids in immune development against further infection (McDougland et al., 1991).

Coccidiosis is a significant disease because of the rapid turnaround of the infection. The parasites that cause coccidiosis undergo a very short life cycle. Because of the short life cycle, an entire house can become infected in a very short period of time. It is also significant because every year it costs the industry an estimated $800 million in losses (Dalloul et al., 2005). This estimated cost considers the prevention medication, treatment medication, and losses due to mortality, inefficient feed utilization, temporary

reduction of egg production, malabsorption, and impaired growth rate (Dalloul et al., 2005).

LESION SCORING

Coccidiosis in the field must be determined by observation of malabsorption, reduced growth, bloody diarrhea, and litter sampling to determine oocyst numbers. Unfortunately, when the symptoms associated with coccidiosis are severe enough to be observed, the entire crop may be infected. A useful tool used in research areas to assess the severity of coccidial infection is lesion scoring. Gross lesion scores are used in many trials involving coccidiosis to obtain an understanding of the severity of coccidial infection. The typical lesion scoring techniques were reviewed by Johnson and Reid (1970) and this review set the standard for future scoring within the poultry industry.

Because of the organ specificity of the different species of *Eimeria* discussed earlier, the gastrointestinal tract of the supposedly infected bird must be examined at multiple points. There are four suggested regions of the small intestine that are of major concern. These regions include the upper section of the small intestine including the duodenal loop, the mid small intestine that is located above and below the yolk sac diverticulum, the lower intestine including the rectum, and cecal tonsil, and the ceca itself. The gross lesion scoring system was based on a scale ranging from 0 to +4 with the level of infection increasing with number. The system also varied from species to species in terms of visual observations because of the varying reactions to the different species (Johnson et al., 1970).

The study (Johnson et al., 1970) reviewed and established scoring procedures for the species *E. tenella, E. necatrix, E. acervulina, E. mivati, E. maxima, and E. brunetti.* The study reviewed former systems and establishes its own based on studies performed by Johnson and Reid (1970), although the studies were not described in high detail. Because the only microorganisms considered for my study are *E. tenella, E. acervulina,* and *E. maxima* only the scoring for those species will be described in detail, but the other species will be briefly mentioned in terms of first score recording. A study performed by Herrick et al. (1942) was the first recorded scoring for *E. tenella* using a numerical system from 0 to +4. The study defined the different levels as follows: 0 = no lesions; +1 = very few lesions; +2 = "slight" lesions; +3 = considerable blood present with many lesions; and +4 = severe lesions and large quantity of blood. That system was followed by many researchers with some altering the system slightly by adding a +5 score, removing the +4 score, or more specifically, defining the scores (Johnson et al., 1970). Because *E. tenella* is specific to the ceca of chickens, this region is the region scored for infection with the species. Johnson and Reid (1970) determined scoring specifications to be the following for *E. tenella*:

"0 No gross lesions.

+1 Very few scattered petechiae on the cecal wall; no thickening of the cecal walls; normal cecal contents present.

+2 Lesions more numerous with noticeable blood in the cecal contents; cecal wall is somewhat thickened; normal cecal contents present.

+3 Large amounts of blood or cecal cores present; cecal walls greatly thickened; little, if any, fecal contents in the ceca.

+4 Cecal wall greatly distended with blood or large caseous cores; fecal debris lacking or included in cores. Dead birds scored as +4."

Johnson and Reid (1970) also detailed the various studies that led to the development of the scoring systems for the various species of *Eimeria.* The first time scores for these various species were defined and recorded vary over time. *Eimeria necatrix* was first scored by Cuckler et al. in 1956, but the most detailed scoring before Johnson and Reid (1970) was performed in 1968 by Dunkley. *Eimeria mivati* is very similar to *E. acervulina,* so the scoring system to be described for *E. acervulina* may be used with the following exceptions: late infections may move toward the posterior portion of the small intestine, the shape is round contrasting the ladder shape of *E. acervulina,* and the severe congestion typical of *E. acervulina* has not been seen. *E. acervulina* scoring was first recorded by Cuckler (1957). Johnson and Reid (1970) devised a system considering the following criteria for each score:

"0 No gross lesions.

+1 Scattered, white plaque-like lesions containing developing oocysts are confined to the duodenum. These lesions are elongated with the longer axis transversely oriented on the intestinal walls like the rungs of a ladder. They may be seen from either the serosal or mucosal intestinal surfaces. They may range up to maximum of 5 lesions per square centimeter.

+2 Lesions are much closer together, but not coalescent; lesions may extend as far posterior as 20 cm below the duodenum in 3-week-old birds. The intestinal walls show no thickening. Digestive tract contents are normal.

+3 Lesions are numerous enough to cause coalescence with reduction in lesion

size and give the intestine a coated appearance. The intestinal wall is thickened and the contents are watery. Lesions may extend as far posterior as the yolk sac diverticulum.

+4 The mucosal wall is grayish with colonies completely coalescent. Congestion may be confined to small petechiae or, in extremely heavy infection, the entire mucosa may be bright red in color (Morehouse and McGuire 1958). Individual lesions may be indistinguishable in the upper intestine. Typical ladder-like lesions appear in the middle part of the intestine. The intestinal wall is very much thickened, and the intestine is filled with a creamy exudate which may bear large numbers of oocysts. Birds dying of coccidiosis are scored as +4."

Rose (1967) was the first to record scores for *E. brunetti,* but she provided no descriptions of the scores or criteria for scores. Before her study, *E. brunetti* had been described and studied, but no scored. Johnson and Reid (1970) used Levine's (1942) descriptions to establish scoring criteria for *E. brunetti.* *Eimeria maxima* proved to be a difficult species to score due to the fact that not much work existed before Johnson and Reid's in 1970 and the fact that the few records available provided little details and varying results. Their work concluded the following system:

"0 No gross lesions.

+1 Small red petechiae may appear on the serosal side of the mid-intestine. There is no ballooning or thickening of the intestine, though small amounts of orange mucus may be present.

+2 Serosal surface may be speckled with numerous red petechiae; intestine may

be filled with orange mucus; little or no ballooning of the intestine; thickening of the wall.

+3 Intestinal wall is ballooned and thickened. The mucosal surface is roughened; intestinal contents filled with pinpoint blood clots and mucus.

+4 The intestinal wall may be ballooned for most of its length; contains numerous blood clots and digested red blood cells giving a characteristic color and putrid odor; the wall is greatly thickened; dead birds are recorded with this score."

Johnson and Reid (1970) decided that scoring individual species was too difficult. Because of the fact that *Eimeria* species are specific to certain regions of the GI tract, they concluded that species could be narrowed between a few likely to inhabit the area of study. They also mentioned the importance of microscopic scoring to better determine species and to assure that the visual signs are resulting from *Eimeria* induced coccidiosis instead of some other parasitic affliction. The authors proceeded to describe their recommendations for proper scoring involving slide smearing for microscopic scores, guidelines to ensure accurate data collection (blind scorer ignorant to treatments, etc.), and proper representative numbers to be scored.

NUTRITIONAL APPROACHES

Since the discovery of coccidiosis in poultry, the industry has spent large sums of money to treat and prevent it with the hope of one day completely eradicating the disease. One area of interest in the fight against coccidiosis is nutrition. Studies have been performed changing various aspects of nutrition to hopefully discourage the disease.

Nutrition is an area of high interest because it has the ability to impact the animals' performance in so many ways.

Because coccidiosis attacks the intestinal tract where nutrients are absorbed, nutrition should be a primary route for the prevention of the disease. The organisms that cause the disease live in the intestinal tract. They directly interact with the feed ingested by the birds. Utilizing this fact, scientists have attempted to alter nutrition to fight the disease. Even though no miracle nutritional additive (antimicrobials excluded) has been discovered to combat coccidiosis, researchers will always be searching for the ingredient to destroy coccidiosis without the risk of any adverse effects on feed consumption or nutrient absorption.

Vitamin E is an essential nutrient for chicken growth and development. It is commonly known to be an antioxidant. It protects against free radical oxidative processes such as the formation of hydrogen peroxide which is fatal to many animals. Vitamin E is also known to be an immunomodulator in chickens. Vitamin E has been reported to be able to boost humoral responses to *E. coli* infection when supplied in high amounts, and it increased body weight gains and reduced lesion scores in chickens that were challenged with *E. tenella* (Allen et al., 2002).

Allen et al. (2002) conducted a study to investigate if supplementation with high levels of dietary vitamin E (DL-ά-tocopheryl acetate; VE-AC) would counteract the pathological effects of infection with *E. maxima*. The study was conducted on Ross-based roaster chickens supplied with varying levels of VE-AC that provided the experiment with five treatments ranging from 13.2 ppm vitamin E to 200 ppm vitamin E in 2 trials (Trial 1: 13.2 ppm, 27.4 ppm, 39.8 ppm, 76 ppm, and 153 ppm; Trial 2: 13.2

ppm, 25 ppm, 50 ppm, 100 ppm, and 200 ppm). Two strains of *E. maxima* were used as challenge material. During Trial 1, 15 birds were randomly assigned to infected group and 15 were assigned to control group on day 22 of the trial. Chickens in the infected group were inoculated with 175,000 sporulated oocyst/Ml of lab Strain ESS. Chickens used in Trial 2 were randomly assigned to receive one of the five levels of VE-AC. On day 14, chickens were assigned to infected or control groups. The infected group received a dose of 40,000 sporulated oocysts of the Guelph strain of *E. maxima* (Allen et al., 2002).

Trial 1 showed no differences between the birds receiving various levels of VE-AC in terms of mean body weights. The increased levels of VE-AC did not effect weight gain or feed conversion of the uninfected chickens. Only the mean gain 6 days post infection for the birds receiving 76 ppm VE-AC was significantly decreased compared to the controls. The lesion scores decreased numerically with increasing VE-AC levels, but the only statistical difference noted was between the 0 ppm level and the 153 ppm level (Allen et al., 2002).

During Trial 2 (a more severe *E. maxima* infection), the uninfected control birds showed no differences in weight gain at different VE-AC levels. The infection caused decreased weight gain in all levels of VE-AC supplementation. The chickens that were given 100 and 200 ppm VE-AC and infected with the Guelph strain had significantly lower weight gains than those given 13.2 ppm and infected with Guelph strain. Varying levels of VE-AC did not have any effect on lesion scores. They concluded that supplementation with up to 200 ppm of VE-AC did not produce the hypothesized result of reducing the negative effects of *E. maxima* infection. The

infection may have led to malabsorption of supplements leading to the lack of VE-AC effect. They also believed that an issue involving lipid absorption may have interfered with the VE-AC effect, and increased intestinal mucosal cell turnover commonly seen during coccidian infection may have attributed to the VE-AC ineffectiveness. The final conclusion they reached was that increasing amounts of VE-AC supplementation did not increase antioxidant protection in chickens infected with *E. maxima*. Their most reasonable explanation for this fact was that lipid malabsorption occurred during the infection. This led to the inability of the dietary vitamin E to access the infected tissue and provide aid.

Nitric oxide (NO) is known to have antimicrobial activity. The amino acid, L-arginine, is the substrate for biosynthesis of NO (Allen, 1999). During an immunological response to some infection, induced NO synthase (Inos) may be stimulated and will lead to biosynthesis of NO. NO levels in plasma (ions NO_2^- and NO_3^-) were previously found to increase during infection with *E. tenella, E. acervulina,* and *E. maxima.* Because of this, Allen (1999) has proposed that dietary levels of L-arginine should influence the production of NO as a response to immunological infection, specifically, coccidiosis. She conducted two experiments to determine if L-arginine was able to influence the development or pathology of the coccidian parasites.

During experiment 1, Allen (1999) divided the chickens into six groups at three weeks of age. Three of the six groups of chickens were given a daily dose of L-arginine (500 mg/kg) beginning 1 day prior to infection through 8 days post infection. The other three groups were not dosed with L-arginine. Each set of three groups contained one group inoculated with 30,000 sporulated oocysts of *E. maxima*, one group inoculated

with 50,000 sporulated oocysts of *E. tenella*, and one group not inoculated. This procedure was repeated for experiment 2 with a few alterations. The birds dosed with L-arginine were given two daily doses of L-arginine (500 mg/kg) instead of just one. The birds that were inoculated with oocysts were inoculated with either 500,000 sporulated oocysts of *E. acervulina* or 25,000 sporulated oocysts of *E. tenella* strain MS (Allen, 1999).

During experiment 1, the infection with *E. tenella* significantly reduced weight gain whether or not the birds had been treated with L-arginine. The infection with either of the oocysts increased plasma $NO_2^- + NO_3^-$. L-arginine treatment had no effect on weight gain, lesion scores, or plasma $NO_2^- + NO_3^-$. In the birds infected with *E. maxima*, arginine did not affect oocyst shedding, but those infected with *E. tenella* experienced a 26% reduction in oocyst shedding when treated with L-arginine. In experiment 2, infection with each organism significantly reduced weight gain and increased plasma $NO_2^- + NO_3^-$ levels regardless of treatment with L-arginine (dosed or not). The results for experiment 2 resemble those of experiment 1 with the only advantage of L-arginine being that it reduced *E. tenella* oocyst shedding by 39%. L-arginine had no effect on lesion scores, weight gain, *E. acervulina* oocyst shedding, or plasma $NO_2^- + NO_3^-$ levels.

The inhibition of *E. tenella* suggests that the arginine acts specifically on sites where *E. tenella* is likely to develop instead of those containing *E. maxima* and *E. acervulina*. Because the arginine did not affect weight gain, she suggested that the L-arginine "was not a rate-limiting factor for growth in infected chickens". She concluded that the supplementation with additional L-arginine provided no benefit during a

coccidial infection when it was already provided in the diet besides the inhibition of *E. tenella* development (Allen, 1999).

Yaissle et al. (1999) designed a study to investigate the interaction of dietary protein levels with coccidiosis infection in feed-restricted pullets. Literature reported before this study had conflicting conclusions regarding the effects of dietary protein on chickens during coccidial infection. A few of the reported studies exhibited contradictory results where added dietary protein acted to aid the birds to better cope with the coccidiosis infection. Other investigators suggested that higher dietary protein exacerbated the severity of the coccidial infection.

Yaissle et al. (1999) conducted two experiments to investigate dietary protein level effect on coccidial infection lasting 28 days. During experiment 1, two dietary levels of protein (15% CP and 19% CP) and three levels of vaccine dose (H_2O; 1X; 4X) were used creating a 2 x 3 factorial arrangement of treatments. The vaccine was administered as control (H_2O only), 1X (1000 Ml 4X mixed with 3000 Ml H_2O), or 4X (one vial Coccivac-D® mixed with 3000 Ml H_2O). The vaccine was administered to all birds on day 7. On day 7 the birds were started on an every-other-day (EOD) feeding schedule receiving 45 g of feed EOD until 21 days. The birds were fed 68 g EOD from 21 to 28 days. The same procedure was used for experiment 2 except birds were placed on the EOD feeding schedule on day 10. They were given 55 g of feed EOD until 21 days when the feed was increased to 78 g thereafter. The vaccine was administered on day 4 instead of day 7.

During experiment 1, no significant differences were observed between the varying levels of vaccination. Body weight and pectoralis major muscle weights were

significantly increased at 14 and 21 d post vaccination in the birds that received the 19% CP diet compared to those given the 15% CP diet. No clinical signs of coccidiosis were present in experiment 1 or experiment 2. The birds in experiment 2 that received 1X and 4X vaccine doses, regardless of protein level, both exhibited mucosal lesions present in the duodenum. The mucosal cells of the villi of both vaccine groups (1X and 4X) fed both diets were infiltrated by oocysts, macrogametes, and microgametes. The infiltration was more severe in the birds that had received the 4X dosage. The pullets given the 1X dosage were significantly heavier than the 4X birds at 28 d of age. Protein level at this stage had no effect on body weight. The results reported here reveal that increasing dietary protein level may enhance the birds' ability to cope with a coccidial infection, but it will most likely have no effect on the intestinal health of the infected birds (Yaissle et al., 1999).

Another approach taken by Korver et al. (1997) involved supplying broilers with identical diets except for the use of either corn oil or fish oil as ingredients. In addition to the two oils, the inclusion or exclusion of Lofrin and infection with *Eimeria tenella* oocysts were used as treatments. The use of fish oil numerically increased the growth of the broilers throughout the trial compared to corn oil. Infection of the broilers with *Eimeria* did not significantly affect the body weight gain over diet and Lofrin treatments. The challenge was either not severe enough or long enough to affect the broilers. However, there was a diet by infection interaction observed because the birds receiving the corn oil diet had decreased body weight when infected compared to the broilers given the fish oil diet. Within the corn oil diet groups, Lofrin decreased the negative effects of

the infection. The corn oil diets were the only groups that differed in body weight gain due to infection and Lofrin inclusion (Korver et al., 1997).

The broilers consuming the fish oil diet had a higher feed intake than those eating the corn oil diet. The birds consuming corn oil had decreased feed intake from day 15 to 23 when given Lofrin. The fish oil birds did not decrease feed intake when given Lofrin. Lofrin was shown to increase feed conversion efficiency (FCE) of the chickens fed the corn oil diet, but the ones eating the fish oil diet had no change when fed the Lofrin supplemented diet. The infected birds fed corn oil exhibited a decreased FCE, but no decrease was present in the birds fed fish oil and infected. Lofrin also acted to inhibit the negative effect of the coccidial infection on FCE. When it was present FCE did not decrease, but when it was not present, the FCE was decreased by the *Eimeria* infection (Korver et al., 1997).

Overall, substituting fish oil for corn oil or using Lofrin with corn oil decreased the negative effects of a coccidial challenge. The use of Lofrin or fish oil could aid the broiler in defense against the possibly disastrous effects seen during a coccidial challenge. Using fish oil and Lofrin together had no additive effect in the challenged broilers. The mechanisms that produced the increased performance associated with using either product are not yet known. The PUFA from fish oil is believed to decrease the coccidial infection effects on growth, and the Lofrin acts to block the 5-LO pathway possibly aiding the birds' performance (Korver et al., 1997).

New techniques and substances are being developed and tested constantly to control the instances of coccidial infections in the poultry industry. Many efforts are being made to use "natural" solutions to avoid the use of pharmaceutical controls.

Because coccidiosis mainly affects the digestive tract, the most reasonable course of action is through dietary manipulation. The substitution of typical ingredients with those that may inhibit the development of the pathogens or increase the bird's ability to cope with the challenge is commonly undergone to reduce the loss associated with coccidial infections. All of the research involved in discovering these new ingredients and techniques are aimed at realizing a cost-effective and safe means of completely controlling or even eliminated the disease. The approach taken to use the dietary manipulation using the "natural" sources comes from the belief that the process of using drugs and vaccines will eventually become outlawed.

ANTICOCCIDIALS

The development of chemotherapy against disease is believed to have begun with Paul Ehrlich. He developed a compound that was used to treat syphilis. After his success in 1909, many other researchers began the quest to develop new treatments against many diseases that had plagued humans since the beginning of time. Alexander Fleming was the man who led the effort to kill harmful microorganisms within the human body. He was successful when he discovered penicillin to kill staphylococci in 1928. Fleming's work was dismissed by many of his colleagues because the penicillin was derived from mold, but others looked to his studies as inspiration to join the fight against harmful microorganisms (Jones et al., 2003).

The development of many more antibiotics followed the early research, and now antibiotics are used all over the world for humans and animals alike. In fact, today antibiotics are often abused. Patients visiting their family doctors demand antibiotics

because the antibiotics have made them feel better in the past when they experienced a bacterial infection, but if they are infected with viruses, then they are only hurting themselves.

During the 1940s, the use of animal protein to feed poultry declined. The animal protein was not available to supply the poultry industry, so vegetable protein was used. The industry was expanding, but the supply was lacking. The performance observed in poultry and swine declined when vegetable protein was used in place of animal protein. The industries determined that an unknown factor was present in animal protein that was necessary to provide the poultry and swine with proper nutrition. It was aptly named animal protein factor (APF). Research began to determine the specifics behind the factor to aid the industries in the time of short supply (Jones et al., 2003).

In 1948, Rickes et al. and Ott et al. published reports that confirmed that vitamin B_{12} was the APF that was needed in those diets. The research performed when determining APF led to the discovery that other feed ingredients acted as the B_{12} in terms of growth promotion. Some of the other ingredients also had improved growth promotion above that of B_{12}. One such constituent was fungal mycelia, more specifically, antibiotics found in the mycelia. Many researchers performing studies on pigs and chickens around this time were able to show that the inclusion of antibiotics in feed promoted increased growth. The research of these individuals and groups led to the approval of the routine use of antibiotics in animal feed by the FDA in 1951 (Jones et al., 2003).

In 2002, the Feed Additive Compendium listed 32 antimicrobial compounds that are allowed for use in the feeds given to broilers in the United States without veterinary

prescription (Jones et al., 2003; Miller Publishing Company, 2001). Many of the compounds are used from early in the production cycle until a predetermined withdrawal time to remove any residue that would possibly remain after slaughter. They are used even when the producer has observed no signs of the microbial infection in his birds. Eleven of the antibiotics are used and classified as growth promoters to help the birds cope with any possible infection that may occur and to reduce the chance of an infection. The growth promoters are aptly named because they typically improve the birds' performance in terms of weight gain and feed efficiency by the prevention of microbial challenge when the microbe is present.

Fifteen of the 32 compounds on the list are anticoccidials (Jones et al., 2003). They are produced to fight coccidiosis in the commercial poultry industry. Anticoccidials make up the largest class of antimicrobial compound on the list. This demonstrates how important of a disease coccidiosis has become, and it shows the tremendous amount of time and effort that has been devoted to the control of coccidiosis throughout the years.

Today, most of the broilers raised in the U.S. in the commercial industry are done so with an anticoccidal drug administered in the feed. In fact, a study by Chapman (2001) showed that from 1995 though 1999, 99% of the poultry plants (comprised of a reporting unit that "represents a broiler complex comprising a group of farms in a common geographical area, which are served by a single feed mill.") used for the study employed the service of starter and grower feeds containing anticoccidial drugs. Some of those drugs have been available for over 50 years. The drugs are classified as either an ionophore produced by fermentation or a chemical produced through chemical synthesis.

These two types of drugs are commonly used together in shuttle programs, or different drugs within each category are used intermittently (Chapman, 2001).

Salinomycin (an ionophore) acts on coccidiosis by attacking the protozoan cell directly. It increases the cation concentration inside the cell. The cation concentration increase causes increased osmotic pressure leading to the lysing or inhibition of the protozoan cells (Bolder et al., 1999).

The use of anticoccidal drugs has proven to be successful in the past. Many studies have examined the performance of birds treated with anticoccidials when they were infected early in life (Chapman et al., 2004). Chapman et al. (2004) investigated the performance of broilers given salinomycin and roxarsone and infected at older ages. The study involved infecting the birds at 18 and 35 days of age. The birds were also treated with either salinomycin, roxarsone, or salinomycin and roxarsone from 0 to 4, 0 to 5, or 0 to 6 weeks. The birds were infected with field isolates of *E. acervulina, E. maxima,* and *E. tenella* at 100,000; 30,000; and 50,000 oocysts per bird, respectively. The study showed that the inclusion of anticoccidials decreases the number of oocysts that will be shed by the birds. The differing lengths of the anticoccidial use in the study showed that using anticoccidials beyond 5 weeks will be advantageous to the birds to decrease oocyst numbers. Longer use of anticoccidials decreased the feed:gain ratio but did not affect the body weights, mortality, or feed intake. The birds infected later (35 days) and medicated until 5 or 6 weeks experienced decreased weight gain compared to the nonchallenged controls. This report shows that the birds besides those medicated for 5 or 6 weeks and infected at 35 days had developed immunity. Adequate time to develop immunity had not been achieved by those particular birds. The study also showed that the use of

anticoccidial drugs is beneficial if used properly and under the correct circumstances, but if the withdrawal of the anticoccidial is required before slaughter, the birds may not be able to fight an infection with their own natural immunity. The drug would have suppressed the bird's ability to fight the infection on its own. In fact, the author recommends that the producer should weigh the economic benefits associated with the improved feed conversion against the possible detrimental effects associated with the chance that the birds will not have developed proper immunity to fight late infection.

Conway et al. (2002a and 2002b) further studied the effects of anticoccidial withdrawal. More specifically, the study dealt with the organism *E. tenella* because earlier studies have shown that immunity to *E. tenella* is the slowest to develop out of the *Eimeria* species. The first study (2002a) was performed to evaluate the withdrawal of diclazuril on days 28, 35, and 42 compared with salinomycin withdrawal on day 28. The second study was performed to determine the level of immunity that diclazuril allowed at different withdrawal times and used the birds from the first study.

In the first study, five treatment groups were used: unmedicated (UNM); salinomycin + roxarsone in starter and grower (SAL 28); salinomycin + roxarsone in starter and diclazuril in grower until 28 days (DIC 28), 35 days (DIC 35) or 42 days (DIC 42). The birds were grown on litter naturally contaminated with *E. acervulina, E. maxima,* and *E. tenella.* DIC treatments exhibited improved means for weight gain and feed conversion compared to SAL 28 and UNM treatments. Feed conversion was better for the DIC 35 and DIC 42 compared to DIC 28. UNM birds experienced the highest number of oocysts due to absence of coccidial treatment. Both SAL and DIC treatment groups demonstrated efficacy by maintaining low oocyst counts. By day 49, DIC

treatments had improved performance over UNM and SAL groups. Adverse effects resulted from the withdrawal of both DIC and SAL on day 28. Those two groups (DIC 28 and SAL 28) suffered decreased performance compared to DIC 35 and DIC 42 (Conway et al., 2002a).

The birds used in the first experiment were used in the second experiment along with other birds raised in different conditions. The treatment groups were carried over to this new study with two new groups: nonexposed, nonchallenged (NENC) and nonexposed, challenged (NEC). All birds, except the NENC birds were inoculated with 1 X 10^5 *E. tenella* oocysts per bird on day 1 of the new study (day 56 of life). They determined that birds that had diclazuril withdrawn on days 35 and 42 were highly susceptible to infection with *E. tenella*. UNM and SAL 28 treatments exhibited higher weight gain and lower lesion scores than the NEC and DIC treatments. Conway et al. (2002b) determined that this was true because the UNM and SAL 28 treatments had developed immunity to *E. tenella* in the earlier study. The DIC treatment group exhibited partial immunity to *E. tenella* because weight gain, feed conversion, and lesion scores improved compared to NEC treatment group. The same level of immunity was not seen in DIC 35 or DIC 42. These two studies reinforce the theory that late withdrawal of anticoccidials can lead to increased production costs if the birds experience a significant coccidiosis challenge after the withdrawal.

Bird inability to cope with coccidiosis challenge after anticoccidial withdrawal is not the only concern involved with using the anticoccidial programs. Another very important issue currently facing the industry is antimicrobial resistance.

ANTIMICROBIAL RESISTANCE

A major concern associated with antimicrobial resistance in animal medicine is the development of resistant strains of microbes in animals that may eventually pass to humans. Human medicine faces new challenges constantly. Introducing resistant pathogens from animals on top of the resistant strains introduced by overuse of human antimicrobials could be very detrimental to human medicine.

Currently, the resistant microbes involving humans exist mainly due to overuse of drugs in humans, but the concern associated with animal antimicrobials is rising (Bywater, 2005). Another concern involving resistance is the possible failure to control disease in food animals. If too many resistant strains infest food production, a catastrophic loss of animal products could result. With so many people relying on animals for food, there is much at stake.

With the ban of growth-promoting antibiotics taking place in Europe in 2006, the common practice of antimicrobial feed inclusion is being increasingly evaluated in the U.S. This evaluation includes investigation into antimicrobial resistance.

During the 1960s, the occurrences of a resistant strain of *Salmonella typhimurium* increased dramatically causing much concern. The observation sparked the antimicrobial resistance exploration. Since the first concern, many other resistant microorganisms have been discovered and studied. The debate among the animal production community has inflated to determining the best course of action to inhibit the further development of antimicrobial resistant microorganisms. The ban of the use of antimicrobials as growth-promoters in Europe has had mixed results. The removal of tetracycline as a growth-promoter has not achieved the initial purpose. The percentage of tetracycline resistant

strains of *Salmonella* spp. found in Europe remains slightly higher than that of the U.S. where it has not been banned. The strains still exist in Europe because tetracycline is used to treat disease. The treatment still allows for the development of the resistant strains (Bywater, 2005).

The ban in Europe has also had adverse effects on the animal welfare and health. Clearly, the removal of proven growth-promoters, designed and used to increase the performance of animals and prevent disease outbreak, caused an increase in the consumption of antibiotics for therapeutic use to treat disease rather than prevent it (Bywater, 2005). More information is needed to properly evaluate antimicrobial resistance, more specifically, resistance in pathogens causing coccidiosis.

Because coccidiosis is one of the most devastating diseases in the poultry industry, the routine use of anticoccidials has become almost a necessity as 99% of the poultry plants studied used an anticoccidial in both starter and grower feeds (Chapman, 2001). With the high incidence of use associated with anticoccidials, the likelihood of anticoccidial resistant microbes is very high. Reports from the early 1950s indicated no incidents of resistance among field isolates for coccidiosis (Cucker et al., 1955). Cuckler et al. (1955) went on to perform a study on resistance of field strains of coccidian and of laboratory strains of *E. acervulina* and *E. tenella.* The study found that 43% of the allegedly resistant field strains were sensitive to various anticoccidials. Nitrophenide resistance was encountered in 43% (13) of the field strains. Nine of those 13 were also resistant to nitrofuazone and sulfaquinoxaline. Nitrofurazone had the highest occurrence of resistance (57%). Both *E. acervulina* and *E. tenella* developed tolerance for sulfaquinoxaline after many passages through the birds and recycling (Cucker et al.,

1955). This study shows how long the incidence of anticoccidial resistance has been present in the poultry industry.

In 1974, Jeffers reported the drug resistance of *E. tenella* by obtaining field samples from over 1,000 poultry houses for 42 months (January 1970-June 1973). The field strains were fed to poultry under treatment of Bonaid, Coban, Deccox, Coyden or Amprol Plus to evaluate the resistance of the field strains. Any clinical signs of cecal coccidiosis exhibited by the birds were determined to be caused by drug-resistant strains of *E. tenella.*

The study sampled 1,145 farms with 1,052 (91.9%) of them producing coccidian from the litter samples. Out of the single anticoccidial programs investigated, birds on Nidrafur experienced the highest incidence of *E. tenella* infection (88.2%) with Zoamix having the second highest incidence (72.6%). Jeffers does, however, state that the ability to distinguish between drug failure and resistance is limited. The inability of Nidrafur, Coban, and Zoamix to control the detrimental effects of *E. tenella* is attributed to the possible limited anticoccidial activity of the drugs, not to resistance in *E. tenella*. Jeffers reported that Amprol Plus, Bonaid, Coyden, and Deccox use resulting in perpetuation of *E. tenella* is due to the development of resistance. Observations in the study also supported the incidence of cross-resistance observed in chemically similar compounds. In the present study, Bonaid and Deccox were observed to cause cross-resistant strains of *E. tenella*. Overall Jeffers observed resistance in field isolates of *E. tenella* to anticoccidials commonly used at the time (Jeffers, 1974).

While Jeffers (1974) used field strains of *E. tenella* that had already been exposed to various medications, McLoughlin et al. (1975) used a parental strain that had never

been exposed to anticoccidials. The *E. tenella* was exposed to several passages through poultry medicated with amprolium, nicarbazin, Unistat and zoalene to mimic shuttle programs. By the 40th and last passage, the coccidia had become resistant to all the anticoccidials except nicarbazin. The study showed that shuttle programs are not capable of completely preventing the incidence of resistance. In fact, this particular study produced a strain of coccidian that was resistant to three of the four anticoccidials to which it was exposed.

McManus et al. (1968) studied field isolates of coccidia from commercial farms where buquinolate was unsuccessful in controlling coccidiosis. Twelve strains were used from the commercial farms to be tested against other quinolate compounds. The isolates were tested using amquinolate, buquinolate, M&B 15497 and methyl benzoquate with no response. The field isolates were not susceptible to the four anticoccidials. The field isolates exhibited a remarkable amount of quinolate resistance. In fact, 3 of the strains were subjected to 10 times the recommended level of amquinolate and buquinolate without any anticoccidial effect. Amquinolate became ineffective to laboratory strains of *E. tenella, E. bruneti,* and *E. maxima* after only four cycles of exposure. This study demonstrated that resistance to amquionolate can develop quite rapidly leading to the question of its efficacy as an effective anticoccidial.

As the aforementioned studies have demonstrated, anticoccidial resistant strains of *Eimeria* are found in the industry. The studies have also shown that resistant strains have rendered some anticoccidials useless when used against them. The resistant strains must be controlled. The common use of anticoccidials has produced them, but they can be fought using strategies that support the prevalence of susceptible strains. Previously,

using shuttle programs was thought to prevent the emergence of resistant strains by not allowing the protozoa sufficient exposure to one anticoccidial to develop immunity. McLoughlin et al. (1975) showed that resistance during a shuttle program is possible. With the emergence of resistant strains commonplace within the industry, how can the disease be controlled?

One route of control exists with the introduction of drug sensitive strains of the coccidial organism into a production center. Jeffers (1976) found that the introduction of a drug sensitive strain of *E. tenella* into a floor pen that was highly contaminated with a resistant strain led to the reduction of the resistant portion of the *E. tenella* population within the floor pen. The resistant portion was reduced but not eliminated. The fact that these resistant microbes were still present may suggest that using an anticoccidial to which the coccidia are resistant will promote the survival of the resistant strain. This will transition the population to one that has a higher proportion of resistant strains. A possible solution to anticoccidial resistance lies in the use of vaccination.

COCCIDIOSIS VACCINES

The increasing concern with the routine use of antibiotics has elevated research involving vaccines. The possibility of using the immune response in the chicken itself through vaccination has become a realistic alternative to anticoccidial compounds (Medarova et al., 2003).

Coccidiosis vaccines first emerged over 50 years ago. CocciVac® was developed by Samuel Allen Edgar. During the last 20 years of the 19th century, coccidiosis was studied intensely and steps were taken towards controlling the disease. In 1925, it was

discovered that exposure to coccidiosis could cause immunity in chickens to the disease. This, and later studies laid the groundwork for the development of coccidiosis vaccines, but it would not occur until much later. The scientists of this era concentrated on the chemotherapy aspect of coccidiosis control. It was not until the 1940s that Edgar began contemplating the development of a coccidiosis vaccine. W.T. Johnson began work involving immunity development to coccidiosis. His untimely death in 1937 prevented his work to be finished. Had he not died, he may have led the effort for control of coccidiosis through immunity development and later, vaccination (Williams, 2002a).

Johnson's work was carried on by other scientists interested in using immunity to combat coccidiosis such as E.E. Tyzzer, E.R. Becker, R.L. Mayhew, M.M. Farr, E.M. Dickinson, and S.A. Edgar. These scientists spent their time using dosages of *Eimeria* oocysts to elicit immunity in poultry. Administration of the oocysts seemed to be the problem. The oocysts were being killed before they could be used by the poultry. Also, the doses of viable, virulent oocysts were not consistent from bird to bird. Edgar began his first study in June 1942. He confirmed that administering small doses of *E. tenella* oocysts would lead to the development of immunity without the consequence of mortality (Williams, 2002a).

Edgar realized early in his studies that uniform uptake of the viable vaccine must be achieved for success. He also realized that birds remaining susceptible after vaccination needed chemotherapeutic intervention for protection. He also realized that the administration of the vaccine should be done so at the earliest age possible to provide the chicks with early immunity. The first vaccine (CocciVac®) was developed using only *E. tenella* oocysts and therefore criticized, but this work by Edgar laid the foundation for

the development of new and improved vaccines to provide today's industry with the vaccines to fight the different *Eimeria* species causing coccidiosis (Williams, 2002a).

Currently, there are two types of coccidiosis vaccines available to the poultry industry: non-attenuated and attenuated. The non-attenuated vaccines are those that are made up of mixtures of wild type strains of *Eimeria* designed to provide the chicken with immunity without any pathogenic effects. Attenuated vaccines contain mixtures of strains that were selected for reduced or no pathogenocity. Coccidiosis vaccines are usually administered with the intention that the oocysts will be recycled in the litter and passed through the intestinal tract after the initial vaccination has occurred. This necessary event provides the birds with the solid immunity available from proper vaccination procedure (Dalloul, 2004).

Vaccines can be administered by eye spray, spray cabinet, drinking water inclusion, feed inclusion, or edible gel. Feed and water inclusion methods may need to be given during the first week of life, but the other methods can be used in the hatchery to achieve earlier immunity development. Vaccines applied to drinking water must be injected into the drinking water system. Typically, water is withheld for some time to make the birds thirsty. The water-vaccine mixture is then administered allowing the birds to consume an adequate amount of vaccine. The feed inclusion method can be done by simply spraying the vaccine evenly over the food. The edible gel method involves giving the birds a brightly colored gel either in the crates in the hatchery or on flats in the barn shortly after placement. The bright color appeals to the birds. Careful watch must be employed to ensure that all birds are able to consume the gel. The eye spray technique involves spraying the vaccine into the eyes of the birds to obtain absorption through the

nasolacrymal duct connecting the conjunctiva with the nasal cavity and oropharynx. Breeding companies prefer the use of spray cabinets. It is believed to be administered evenly throughout the group of birds being vaccinated. The method employs a cabinet to confine a group of birds. The vaccine, mixed with a colored dye, is sprayed onto the birds. The birds obtain the dose of vaccine by pecking and preening the colored vaccine (Chapman, 2000).

When using vaccines for control of coccidiosis, the species of coccidiosis used in the vaccine is of major concern. The species of *Eimeria* vaccinated against varies between vaccines. When considering broilers, all seven ubiquitous species do not need to be considered because *E. brunetti* and *E. necatrix* are very rarely encountered. On the other hand, *E. acervulina, E. maxima,* and *E. tenella* are incorporated in all broiler vaccines (Williams, 2002b).

Vaccines to combat coccidiosis have now become readily available. They have been employed in limited use since the 1950s. The U.S. poultry industry has yet to readily accept the vaccines as a feasible alternative to anticoccidials despite their proven success in providing birds with protection against coccidiosis infection. The vaccines have not been accepted because their use has been associated with poor weight gain and feed efficiency when compared to anticoccidial programs (Danforth, 1998).

Danforth (1998) performed several studies to determine efficacy of vaccination and vaccination-type programs to control coccidiosis. His studies led to the conclusion that male and female birds are able to develop immunity against various coccidial species if vaccinated on the first day of life. He determined that the method of delivery for the vaccine is very important. The vaccine must be delivered to allow the uniform

distribution of a small number of parasites. He also determined that the gel-delivery was the most successful in providing the chickens with the best protection from *E. maxima*. His studies showed that using immunovariant *Eimeria* (immunological variants of *Eimeria*) in live vaccines may cause a drop in initial bird performance. The broilers that experienced the initial performance decrease made up the losses by week 7. At the time of slaughter, the vaccinated birds and medicated birds had achieved similar feed conversions and weight gain. He also found that in floor pen trials, the use of a combination program involving vaccination on day 1 with drug-resistant *Eimeria* and use of an anticoccidial throughout the growth curve provided the broilers with protection almost completely. The performance of the broilers was not reduced during the first 3 weeks of life, but he found that in field trials, the birds that were vaccinated and given anticoccidials had reduced feed conversion values compared to the unvaccinated birds given anticoccidials. While the results of his studies are not overly decisive, Danforth believes that interest in coccidiosis vaccines will only increase with time.

Mathis (1999) reported results of his floor pen study investigating coccidiosis vaccination effect on compensatory gain of broilers compared to unvaccinated broilers receiving salinomycin. The study was performed on the broilers over 7 weeks beginning on the first day of life. The vaccinated group was sprayed with Coccivac-B® at the hatchery. The salinomycin group received salinomycin in the starter and grower feeds at a concentration of 66 ppm lasting for 5 weeks. The vaccine-associated coccidiosis affected the performance of the vaccinated birds by 3 weeks of age. The vaccinated birds experienced poor feed conversions and reduced weight gain compared to the salinomycin birds. Week 3 data was the only data recorded throughout the study exhibiting

significant differences between the vaccinated and salinomycin groups. The salinomycin treatment group was numerically superior to the vaccinated after week 3, but the slight differences between the two groups decreased with each successive week. Mathis concludes that the vaccinated birds experienced a compensatory gain after facing a coccidial immune challenge.

A large-scale study was performed to determine the efficacy of Paracox® vaccine compared to nicarbazin-monensin shuttle program in broilers in Italy. The study involved using three consecutive crops totaling 290,405 male and female broilers from winter 1997 until the summer of 1998. The birds receiving anticoccidials were fed nicarbazin during the starter phase and monensin during both the grower and finisher phases. The monensin was withdrawn from the diet 4 days prior to slaughter. The vaccinated birds were vaccinated with Paracox® vaccine which was administered through the drinking water of the birds beginning at 5 days of age. The trial had no subclinical or clinical signs of coccidiosis infection. No lesions were observed throughout. Two of the 3 vaccinated flocks had oocysts detected in the litter beginning on day 14, and the third flock produced oocysts beginning on day 21. The oocyst counts of the vaccinated flocks peaked at 27 days (22 days post vaccination). No oocysts were detected after day 49 in the 3 vaccinated flocks. The 3 anticoccidial flocks experienced detectable oocysts beginning on day 34 and continuing consistently until day 49 and reducing to undetectable level on day 56. Total oocyst count was higher for the vaccinated flocks than it was for the anticoccidial flocks. Up to day 21, no significant differences existed between mean body weights of the anticoccidial birds compared to the vaccinated birds. After day 21, the vaccinated birds were heavier than the anticoccidial birds. They were

significantly so after from day 27 until trial conclusion. The FCR for the vaccinated and anticoccidial birds were not significantly different. At the processing plant, fewer vaccinated males were rejected than anticoccidial males, but there was no significant difference when considering the females. This study demonstrates that the use of coccidiosis vaccines can produce comparable performance in broilers with anticoccidial shuttle programs. One advantage of the vaccine programs over anticoccidials is the fact that vaccines do not require withdrawal before slaughter, and the vaccination may provide the broilers with added protection during the time just before slaughter (Williams et al., 2002).

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CHAPTER III

EVALUATION OF COCCIVAC-B® AND SACOX 60® (SALINOMYCIN) FOR CONTROL OF 3 STRAINS OF EIMERIA IN BROILERS

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INTRODUCTION

The commercial poultry industry has been very successful throughout the years. Even with the advancements that have arisen within the industry, companies continue to struggle with disease. These diseases are caused by infectious organisms including viruses, protozoa, bacteria, etc. Because the birds are housed in such ways that cause constant interaction, communicable diseases are spread rapidly throughout an entire housing facility. The ability to control disease has become a necessity for success in the industry.

Coccidiosis is one of the most reported diseases in the industry (Williams, 2002b). Coccidiosis is a significant disease because of the rapid turnaround of the infection. Because of the short life cycle, an entire house can become infected in a very short period of time. It costs the industry an estimated $800 million in losses. This estimated cost considers the prevention medication, treatment medication, and losses due to mortality, inefficient feed utilization, temporary reduction of egg production, malabsorption, and impaired growth rate (Dalloul et al., 2005).

The term antimicrobial is a general term given to the very diverse group of biologically active compounds that act to combat infectious disease by attacking the harmful bacteria that might infect an animal or a human. Historically, the coccidiosis problem has been minimized by the inclusion of antibiotics such as nicarbazin (Yoder et. al, 2006), salinomycin (Chapman et al., 2004), etc. in the bird's diet. Such drugs are only provided to the bird for a limited period of time and in concentrations so as to partially protect against coccidiosis with the bird eventually developing its own immunity. The poultry industry refers to this as "slippage" (Chapman et al., 2004). The deleterious consequence of coccidiosis challenge is dependent upon the time the chickens have their challenge with this being dependent upon the feed drugs and immunity level. Some countries are considering the restriction of antibiotic application for the control of coccidiosis in an effort to protect humans from "super bugs". As of January 1, 2006, all growth-promotants such as virginiamycin have been prohibited from routine application in livestock by the European Union (Bywater, 2005). If the restrictions for anticoccidial drugs are expanded to the U.S., the American poultry industry will face severe production consequences.

It is critical that new approaches such as vaccines be developed with efficacy to eliminate coccidiosis as an environmental stressor. The development of vaccines to elicit immunity within the bird for the various coccidiosis lines offers the potential to eliminate the application of antibiotics in poultry diets for coccidiosis control (Medarova et. al, 2003). With vaccination, the birds start the immunization process on day 1 and would have maximal immunological protection by week 4 (Allen et. al, 1997).

Schering-Plough has developed a theory involving coccidiosis exposure and immunity called "Quadrants Performance". The quadrants are numbered 1-4. The quadrants are divided by the performance and economic thresholds. Increased oocyst numbers in a house above the performance threshold will severely impact the birds' performance causing decreased weight gain and feed conversion. The birds will show clinical signs of coccidiosis. The economic threshold marks the point in terms of age after which experiencing a coccidiosis infection will dramatically impact performance to the point of devastating the economic achievement of the flock. The quadrant designations can be seen in figures 45 and 46. Schering-Plough has determined that experiencing a low coccidiosis challenge early in life will allow the birds to recover performance and develop immunity in the case of any future infection. Their research has determined that the vaccination of the birds in the hatchery allows the birds to undergo a minimal immune challenge with low levels of oocysts in the litter (Figure 45) at an early age. The birds then develop immunity thanks to the vaccine preventing any further harm due to coccidiosis later in the growth curve. Their research (along with others) has shown that using ionophore programs (Figure 46) prevents the birds from undergoing an early, minimal challenge. The birds are protected from coccidiosis until the period between ionophore withdrawal and slaughter. The birds have crossed the performance threshold if they experience a coccidial infection during this time. The challenge has come too late in life for the birds to recover from performance losses due to challenge. Also, unless the housing facility is thoroughly cleaned specifically for coccidosis, the subsequent flocks will experience increased coccidosis challenge throughout the growth curve (Figure 47).

The purpose of this study is to contrast Coccivac-B® with Sacox 60® throughout the broiler's growth curve. It is important to examine efficacy throughout the growth curve as coccidiosis consequences are impacated by bird age and the bird will also eventually develop its own immunity. Also, this research experiment seeks to establish that the Coccivac-B® vaccine is efficacious throughout the broiler's growth curve to combat coccidiosis and is an alternative to the feed delivered antibiotics combating this problem. Further, the study seeks to establish coccidiosis consequence as mediated by antibiotic slippage vs. Coccivac-B® vaccination throughout the growth curve by exposing broilers to typical field isolates on days 14, 21, 28, 35, and 42. The vaccine offers the potential to reduce the undetermined rate of immunity development created by feeding low levels of antibiotics. Immunity development by vaccination also has a production cost, and there may be times during the bird's growth curve that the antibiotics will be superior. It is important that the two methods be contrasted throughout the growth curve as coccidiosis consequences and therapeutic advantage is dependent upon bird age, challenge history, and immunity development at the time of coccidiosis exposure.

MATERIALS AND METHODS

TRIAL INITIATION

Eleven hundred male Cobb x Cobb chicks were obtained from a commercial hatchery in Siloam Springs, AR. Four hundred of the chicks were vaccinated with Coccivac-B®. The vaccine was administered as a spray vaccine for absorption through the mucosal membranes of the eyes and nasal cavity. All the chicks were placed in boxes

of 100 for transport. Two vehicles were used to transport the chicks. The 400 vaccinated birds were transported in their own vehicle to ensure that the spray vaccine was not sloughed to the other 700 non-vaccinated chicks. Upon arrival at Oklahoma State University, the unvaccinated chicks were randomly placed into floor pens. To ensure biosecurity, the control group receiving no treatment for coccidiosis prevention was assigned first in a separate facility. At least one bird was taken from every box of 100 unvaccinated birds for each pen. The birds to receive 60g/ton Sacox 60® (salinomycin) feed were placed after the control birds using the same technique and the remaining unvaccinated birds. The vaccinated birds were placed last to ensure that no vaccine residue was passed to the other birds. Thirteen birds were placed in each of 72 total pens with 24 pens per treatment group (vaccinated: Coccivac-B®; salinomycin: 60g/ton Sacox 60®; and control: no treatment) after a group weight for each pen was recorded. All birds were fed a starter ration containing 22.1% CP and 3,053 Kcal/kg ME until day 21. The salinomycin birds were fed this ration with an addition of 60g/ton Sacox 60® until day 35 to provide them with coccidiosis protection. All the birds were taught how to drink water from nipples and given feed and water for ad lib consumption throughout the trial.

The birds were wing banded on day 5 and individual weights were recorded on day 7. Also, one bird from each pen was removed and humanely sacrificed to obtain day 7 body composition using the DEXA x-ray densitometer. The birds were monitored multiple times per day to ensure that proper conditions were utilized to promote bird health. Throughout the study, disposable plastic boots and foot baths containing ammonia were utilized by each worker before entering either housing facility to minimize

the risk of contaminating either facility with coccidiosis. Different coveralls were also used for each house to aid with biosecurity.

CHALLENGE PERIODS

On day 14 challenge period 1 was initiated. Birds were randomly and blindly selected using only wing band records. Mortality was taken into consideration when selecting birds for removal. Birds were removed so that the number of birds per pen was reduced to nine. All birds were then individually weighed and predetermined birds removed. The feed left in the feeders was weighed before adding more feed so that feed consumption could be determined. Of all the birds removed, 168 were moved to the metabolic chambers. A total of 56 birds were taken from each of the three treatment groups. Exactly one half of the birds from each treatment group (28) were predetermined to be either challenged or unchallenged. The unchallenged birds were given an oral dose of 1 ml sterile water. The challenged birds were given an oral dose of 1 ml coccidiosis solution containing oocysts of *Eimeria maxima*, *Eimeria acervulina*, and *Eimeria tenella* at concentrations of 20,000; 50,000; and 30,000 oocysts per bird, respectively**.** The chambers were randomly assigned with one of the six treatments (vaccinated no challenge: V-; vaccinated challenged: V+; control no challenge: C-; control challenged: C+; salinomycin no challenge: S-; salinomycin challenged: S+). Two birds were placed in each of the smaller chambers (termed "broiler" chambers), and four were placed in the larger chambers (termed "turkey" chambers). The chamber methodology and characteristics have been described by Weirnusz and Teeter (1993). In this particular study, the grating on the bottom of the chambers was covered with large sheets of heavy

duty paper to help the birds adapt quicker to the new environment in the chambers. A small amount of feed was also placed on the paper to aid with accommodation to the chambers. Oxygen consumption and carbon dioxide were recorded hourly for six days to determine heat production using the equation provided by Brouwer (1965). Feed and water were still available while the birds were in chambers with feed being added to feeders as needed to maintain ad lib consumption.

On day 20, the birds in the metabolic chambers were removed, body weight recorded, and the feed left in the feeder was weighed as feed weighback to determine feed consumption per chamber. Next, the birds were humanely sacrificed using cervical dislocation. The birds were surgically eviscerated by a coccidiosis expert from Schering-Plough to perform lesion scoring on each bird. The GI tract was examined at three critical points for gross lesion scoring. The upper small intestine, mid small intestine, and the ceca were all surgically opened and scored for lesions associated with coccidiosis infection. The scores ranged from 0 (no lesions) to 4 (severe lesions and hemorrhaging). While scoring, scrapings were taken from each of the previously mentioned areas and smeared on microscope slides to obtain microscopic lesion scores based on the number and type of oocysts. The microscopic lesion scores were also given values ranging from 0 to 4 based on the number of oocysts seen per view. After all the birds were removed from chambers, the chambers were thoroughly cleaned with ammonia to kill any remaining oocysts and paper was replaced in the bottom to prepare for the next challenge period.

The procedure outlined for day 14 was repeated on days 21 (challenge 2), 28 (challenge 3), 35 (challenge 4), and 42 (challenge 5) with the only differences being the

number of birds used each challenge period and the dosages of oocysts used for the challenge. On day 21, the number of birds per floor pen was reduced to 7. On days 28, 35, and 42, the number of birds in the floor pens was reduced to four, two, and zero, respectively. Forty-eight birds were taken from each treatment group to be moved to the chambers for a total of 144 birds moving to the metabolic chambers on days 21 and 28. On day 35, 36 birds from each treatment group were moved to the chambers. On day 42, the number decreased to 28. The birds from each treatment were evenly divided between challenged and unchallenged for each challenge period. For challenges 2 and 3, the broiler chambers housed two birds, and the turkey chambers housed three. For challenge 4, the broiler chambers held 1 bird and the turkey held 3. During the fifth challenge period, the broiler chambers held 1 bird and the turkey held 2. The dosage for the challenged birds on day 21 was 30,000; 60,000; and 37,000 oocysts per bird for *E. maxima*, *E. acervulina*, and *E. tenella*, respectively. On day 28 the challenge dosages for *E. maxima*, *E. acervulina*, and *E. tenella* were as follows: 30,000; 80,000; and 40,000 oocysts per bird, respectively. On day 35 the challenge dosages for *E. maxima*, *E. acervulina*, and *E. tenella* were 40,000; 90,000; and 50,000 oocysts per bird, respectively; and on day 42 the dosages were 55,000; 105,000; and 50,000 oocysts per bird, respectively. The oocysts were increased throughout to coincide with increasing body weights of the birds. Also the dosages were increased to mimic the increase of oocysts that would occur in a typical house without proper control. As birds are exposed to the oocysts, they will be ingested and recycled to litter. The numbers would increase rapidly, so the oocysts per bird dosages were increased to mimic the increasing number

experienced in an infected house. On days 27, 34, 41, and 48 (six days post challenge), the procedure for day 20 was repeated exactly as described.

FEED

All birds were reared on a typical broiler diet containing 22.1% CP and 3,053 Kcal/kg ME during the starter phase determined to be from initiation until day 21 for this particular study. This starter diet was provided as mash with the salinomycin birds receiving an addition of 60g/ton Sacox 60® to their feed.

Beginning on day 21, all groups were fed the grower feed containing 19.8% CP and 3,131 Kcal/kg ME and fed as pellet feed. The salinomycin birds continued to receive 60g/ton Sacox 60® through day 35 when they were fed the same feed as the other treatment groups until the trial conclusion on day 48.

Both feeds (starter and grower) were prepared as one large basal ration. After the basal ration was prepared, the required amount for the vaccinated and control birds was bagged, and labeled. The remaining feed was mixed with a premix containing the required amount to achieve 60g/ton Sacox 60® activity. After sufficient mixing, the feed was bagged and labeled.

VARIABLES

Weekly body weights were taken on all birds in the floor pens. This coordinated with the removal of birds from floor pens to be moved to metabolic chambers. The weights were taken on individual birds using wing bands to clearly identify the bird. Feed added to each floor pen was also recorded. On the days that birds were weighed

and removed from the floor pens (days 14, 21, 28, 35, and 42), the feed left in each pen was weighed and recorded as feed "weighback" so that feed consumption for each pen could be determined on a weekly basis and for the correct number of birds. The birds that were moved to the metabolic chambers for challenges were weighed before going into the chambers recorded as the weekly weight for the particular bird. Six days post challenge, the birds were weighed as they were removed from the chambers. The feed added to each chamber was recorded, and the feed removed after the challenge period was weighed as feed "weighback" to determine feed consumption while in the chamber. While the birds were in the chambers, oxygen consumption and carbon dioxide production were measured at least once per hour to later determine heat. Birds were eviscerated by an expert from Schering-Plough Animal Health who was held blind to bird identity. The birds were examined by the expert as described above to determine lesion scores for the intestinal. The birds were frozen until DEXA analysis to determine body composition. From the DEXA scan body protein, fat, water, and ash were determined. Also, percent protein, fat, water, and ash were determined by dividing the grams of each divided by body weight. The protein, fat, water, and ash gains were determined using the scan data of unchallenged birds to develop regression equations. The regression equations used live weight. The initial weights of the birds entering the chambers were used with the equations to determine initial composition. The initial composition data was subtracted from the DEXA data to determine the gains of protein, fat, water, and ash.

LIGHTING SCHEDULE

The birds were placed on a lighting schedule throughout the trial. To allow for maximum environment adaptability, the birds were given 24 hours of light from day 1 through day 7. From day 8 through day 14, the birds were placed on 18 hours of light and 6 hours dark. From day 15 to day 35, the birds were given 14 hours of light and 10 hours of dark. From day 36 until the conclusion of the trial on day 48, the birds were given 22 hours of light with 2 hours of dark.

STATISTICAL ANALYSIS

Data was analyzed using statistical analysis software (SAS). The trial was arranged using a 2 x 3 factorial arrangement. The trial used 2 groups for Challenge (+ or -) and 3 for treatment (Control, Salinomycin, or Vaccinated). The 2 x 3 factorial arranged the treatments into 6 groups: C+, C-, S+, S-, V+, and V-. Treatments were separated using the Least square means procedure when a significant F statistic was detected. All comparisons of data were considered significant when $P<0.05$. Performance data was analyzed using each respiratory chamber as an experimental unit. Composition data was analyzed using each individual bird as an experimental unit.

At the experiment initiation, the social interaction of the birds on the same treatment was deemed more important than the treatment housing due to possible vaccine particle migration to one of the other two treatment groups in the parent population. Consequently, it is not possible to statistically contrast population performance ($df = 0$) due to spatial housing differences between treatments. It was thereby decided that small

live weight differences between treatment groups being allocated to the metabolic chambers would be used as a covariant to evaluate the metabolic chamber data.

RESULTS

FLOOR PENS

It cannot be determined whether the differences seen in the floor pen data ("backgrounding period") were resultant of the treatment groups or the physical placement of the birds in separate areas. In this study, bird location effects were confounded with treatment and/or not possible to statistically represent. Regardless, the results for the "backgrounding period" are shown in Table 2.

CHALLENGE PERIODS

Being challenged with the coccidial mixture depressed feed consumption throughout the trial. The birds in the + group did not consume as much feed as the – group during each challenge period. V+ birds exhibited significantly higher feed consumption to C+ birds after day 20 ($P<0.01$). V+ birds consumed significantly less feed than the S+ birds ($P<0.01$) and similar feed compared to the C+ group ($P=0.29$) on day 20. The V+ birds ate more feed than S+ birds on days 41 ($P<0.01$) and 48 ($P<0.01$). On the other days, V+ and S+ had similar feed consumption ($P>0.14$). V- birds consumed significantly less feed than the C- birds ($P<0.01$) on day 20 and not different feed when compared to both S- and C- birds after day 20 (Table 3).

Challenge impacted the body weight, live weight gain, Average Daily Gain (ADG), and feed efficiency. The challenged (+) birds had depressed values for all those performance criteria throughout the trial compared with the unchallenged (-) birds. V+ birds were superior to the C+ birds in terms of body weight, live weight gain, ADG, and feed efficiency ($P<0.02$). The C+ birds were also inferior for body weight, live weight gain, ADG, and feed efficiency compared with the S+ birds ($P<0.01$) throughout except day 48 ($P<0.21$) where the two groups were similar for those variables. The V+ birds had significantly lower live weights, less live weight gain, and lower ADG than the S+ birds ($P<0.02$) until day 34 when they were not significantly different ($P=0.10$). On days 41 and 48, the V+ birds were significantly heavier and had significantly more live weight gain and ADG than the S+ birds ($P<0.01$). The V+ birds also had better feed efficiency than the S+ birds after day 34. V+ birds were inferior to S+ in terms of feed efficiency on days 20 ($P<0.01$) and 34 ($P<0.01$). On day 27, the two groups were similar ($P=0.13$). Only on day 20 did any differences exist between the – groups for body weight, live weight gain, and ADG. On day 20, the V- birds were significantly lighter, had lower ADG, and less body weight gain than the C- birds ($P=0.02$), but they were equal to the S- birds. C- birds exhibited increased feed efficiency to the V- birds on day 27 ($P<0.01$) with all other comparisons for feed efficiency of – birds exhibiting no differences (Table 3).

Challenge was very important for the development of gross lesion scores (upper small intestine, middle small intestine, and ceca). As expected, the challenged (+) birds had dramatically increased gross lesion scores throughout the trial compared with the unchallenged (-) birds. V+ birds exhibited fewer upper and middle SI lesions throughout

versus C+ birds (P<0.03) and after day 34 versus S+ birds (P<0.01). S+ birds exhibited fewer upper and middle SI lesions than C+ birds (P<0.01) through day 34. On day 41, the S+ birds had similar upper and middle SI lesion scores to C+ birds (P<0.15). S+ birds exhibited indifferent upper SI lesion scores and fewer middle lesion scores on day 48 to the C+ birds (P=0.17 and P=0.04, respectively). The S+ group had fewer upper SI and similar middle SI lesions compared with the V+ group on day 20 (P<0.01 and P=0.08, respectively). V- birds exhibited similar amounts of middle SI lesions throughout versus C- birds (P>0.05) and S- birds (P>0.07). V- birds exhibited fewer upper SI lesions on day 20 versus C- birds and S- birds (P<0.01) with all other days being statistically similar (Table 4).

V+ birds exhibited fewer cecal lesions after day 20 versus C+ birds (P<0.01) and after day 34 versus S+ birds (P<0.01). The S+ birds had fewer cecal lesions than V+ birds on days 20 (P=0.01) and 27 (P=0.01). The two groups were statistically not different for cecal lesion scores on day 34 (P=0.55). The S+ birds had fewer cecal lesions than C+ birds (P<0.01) throughout except on day 41 where they are not different (P=0.36). V- birds exhibited similar amounts of cecal lesions throughout versus C- birds and S- birds (Table 4).

All – groups experienced similar amounts of *E. acervulina, E. maxima,* and *E. tenella* lesions throughout the trial. No significant differences were observed amongst these three treatments for *E. acervulina, E. maxima,* and *E. tenella* lesion scores. The microscopic lesion scores had a significant reliance on challenge throughout the study. The + groups experienced higher lesion scores throughout compared with the – groups with only one exception. On day 27, the + and – groups did not experience significantly

different lesion scores for *E. acervulina*. The + birds did not experience a significant challenge from the *E. acervulina* on that day as reflected in Table 5.

V+ birds exhibited fewer *E. acervulina, E. maxima,* and *E. tenella* lesions throughout versus C+ ($P<0.02$). Throughout the trial, V+ birds exhibited fewer *E. maxima* lesions compared to the S+ birds ($P<0.01$). Lesions scores for *E. acervulina* of V+ birds and S+ birds were not different through day 34. The S+ group had a lower *E. tenella* lesion score than the V+ group on day 27 ($P<0.01$). S+ birds and V+ birds had similar *E. tenella* lesion scores on days 20 ($P=0.24$) and 34 ($P=0.06$). The V+ birds had lower *E. acervulina* and *E. tenella* scores than S+ birds on days 41 ($P<0.01$) and 48 ($P<0.01$). S+ birds had lower *E. acervulina* and *E. tenella* lesion scores throughout versus C+ birds ($P<0.03$) except on day 41 ($P<0.17$) when they were not different. S+ birds had similar amounts of *E. maxima* lesions compared to C+ on days 27 ($P=0.50$) and 41 ($P=0.52$). The S+ birds had fewer *E. maxima* lesions versus the C+ group ($P<0.02$) on all other days (Table 5).

No differences in total challenge period heat production were observed within the – treatment groups. V-, S-, and C- birds were not different throughout the trial in terms of total challenge period heat production. The V+, S+, and C+ birds were also similar throughout the trial with only one exception. The C+ birds had a decreased total challenge period heat production on day 41 ($P=0.05$) compared to the S+ birds (Table 6).

Birds in the – treatment groups maintained similar retained energy (RE) values throughout the trial except that V- birds had a lower RE on day 20 compared to the S- ($P=0.03$) and C- birds ($P=0.01$). V+ birds had higher RE than C+ birds throughout the trial ($P<0.02$). The V+ birds had lower RE than S+ on days 20 ($P<0.01$) and 27 ($P=0.04$).

V+ had higher RE than S+ on days 41 ($P<0.01$) and 48 ($P<0.01$). The V+ and S+ groups were not different on day 34 ($P=0.42$). S+ birds had similar RE compared to C+ on day 48 ($P=0.76$). The C+ birds had lower REs on days 20 ($P<0.01$), 27 ($P<0.01$), 34 ($P<0.01$), and 41 ($P<0.01$) compared to S+ birds. Challenge significantly affected retained energy. Throughout the trial, the challenged birds (+) had significantly lower RE compared with the unchallenged (-) birds (Table 6).

Retained energy efficiency (REE) was determined by dividing the RE by metabolizable energy consumption during the challenge period. Challenge impacted REE on all days but 48. Before day 48, the + birds experienced a decreased REE compared with the – group. Birds in the – treatment groups had similar REE except on days 27 where the V- birds had decreased REE compared to both C- ($P=0.01$) and S- ($P=0.01$) birds. The C+ treatment group had an indifferent REE to S+ on day 48 ($P=0.65$). On all other days, C+ REE was lower than REE of S+ ($P<0.02$). The C+ group had similar REE to V+ on day 48 ($P=0.19$). On all the other days, the V+ group had higher REE than C+ ($P<0.01$). V+ birds exhibited higher REE compared to S+ birds on day 41 ($P<0.01$). Because the V+ birds performed better than the S+ birds after day 34 in most categories, the V+ birds would be expected to have a higher REE than S+ on day 48, but they did not. The two groups were statistically similar ($P=0.05$), but V+ birds were numerically superior. The two groups were not different on days 27 ($P=0.25$) and 34 ($P=0.07$). The S+ birds were superior to V+ birds on day 20 ($P<0.01$) in terms of REE (Table 6).

The results for body composition content are very similar between the different constituents (protein, fat, water, and ash). The challenged birds had less protein, fat,

water, and ash throughout the trial due to their reduced body weights versus the unchallenged birds. The trends seen in each are very similar to each other when comparing the same ages and treatments. Birds in – treatment groups had similar protein, water, and ash content after day 27 and similar fat content after day 20. The C- birds had more protein, fat, water, and ash content than the V- birds on day 20 ($P=0.01$) and more protein on day 27 ($P=0.04$). S- and V- birds were not different throughout the entire trial for fat and protein content. S- and V- birds were not different throughout the entire trial concerning water and ash content except on day 27 where S- birds had higher ash and water content ($P=0.05$). S- birds had less fat, water, ash, and protein content than C- birds on day 20 ($P=0.03$) and they were not different on day 27 ($P>0.89$). V+ birds had less protein, fat, water, and ash content than S+ birds on day 20 ($P<0.01$) and more on days 41 and 48 ($P<0.01$). The V+ and S+ groups had similar protein, fat, water, and ash content on days 27 ($P<0.05$) and 34 ($P<0.25$). The V+ birds had more protein, water, ash, and fat content throughout versus the C+ birds. The S+ birds had similar fat, ash, water, and protein content to C+ birds on day 48 ($P<0.77$). On all other days, the S+ birds had more protein, fat, water, and ash than the C+ birds (Table 7).

The protein, fat, water, and ash gains were severely impacted by challenge. The + birds had significantly less protein, fat, water, and ash gain compared to the – birds throughout the trial ($P<0.01$). Birds in + groups experienced the same trends for protein and fat gain during the challenge periods. On days 20 and 27, the V+, S+, and C+ birds were all significantly different. The S+ birds experienced the most protein and fat gain on these days with the V+ birds second and C+ birds last. On day 34, the S+ birds and V+ birds had similar protein ($P=0.43$) and fat ($P=0.42$) gain. On days 41 and 48, the C+

and S+ birds are similar for both protein and fat gain. They both had significantly reduced protein and fat gain compared to V+ birds. On days 20 (P=0.01) and 27 (P=0.02), the V- birds had reduced protein and fat gain compared with C- birds. After day 27, all the – treatment groups had the same fat and protein gain. The V- birds also exhibited similar protein gain to the S- birds on day 20 (P=0.05), but reduced fat gain (P=0.02). The V- birds also had a lower fat and protein gain than S- (P<0.05) birds on day 27. C- birds were similar to the S- birds throughout the trial for protein and fat gain (Table 8).

The + treatment groups experienced similar patterns for water and ash gain. The V+ treatment group had increased water and ash gain after day 34 compared with S+ birds. They had the same water (P=0.47) and ash (P=0.42) gain as the S+ birds on day 34. The S+ birds had higher water and ash gain compared with V+ prior to day 34. The V+ birds had increased water and ash gain versus the C+ birds throughout the trial. The C+ birds and the S+ had similar water (P=0.77) and ash (P=0.73) gain on day 48. On day 20, the V- birds exhibited similar water and ash gain to the S- birds (P<0.10). On day 27, the V- birds achieved the same water gain as the S- birds (P=0.05). The V- birds had reduced ash and water gain compared to the C- birds on days 20 (P=0.01) and 27 (P=0.02). After day 27, the water and ash gain of V-, C- and S- were not different. The water and ash gain of S- is indifferent to that of C- throughout the trial (Table 8).

Protein percentage was highly dependent on challenge except on days 34 and 41. On those two days, the protein percentage of the + group was similar to that of the – group. On days 20 and 27, the + birds experienced decreased protein percentage compared with the – birds, but on day 48, the + birds experienced increased protein

percentage. The – treatments had similar protein percentage on days 20, 41, and 48. V- had the same percentage of body weight as protein as C- and S- throughout the trial. S- had a higher protein percentage of body weight than C- on days 27 (P=0.02) and 34 (P=0.02). Those two treatments were similar on all other days. The V+ treatment group experienced higher protein percent than the C+ birds on day 27 (P=0.01). On all other days C+ and V+ had the same protein percent. S+ birds had a higher protein percent than the V+ birds on day 48 (P=0.04). These two treatments were not different on all other days. The S+ birds had similar protein percentage compared with C+ birds except on day 27 where the S+ birds achieved a higher protein percent than the C+ birds (0.03; Table 9).

The challenged birds experienced higher fat percents throughout versus the unchallenged birds. The fat percentage of – treatments were not different throughout the trial except on day 34. On day 34, the S- had an increased fat percent compared to the C- birds (P=0.04). The V- birds had the same fat percent as both the S- (P=0.31) and C- (P=0.28) birds on day 34. V+ birds and S+ birds had similar fat percentages on days 27 (P=0.52) and 34 (P=0.98). On day 20, S+ had a higher fat percentage compared to the V+ (P=0.03) and C+ (P<0.01) birds. On days 34 and 41, C+ birds had lower fat percents than both V+ and S+ birds. V+ birds had increased fat percent compared with S+ birds on days 41 (P=0.01) and 48 (P=0.01) and compared to C+ birds throughout. S+ birds had higher fat percent than the C+ birds on days 20 (P<0.01), 27 (P<0.01), 34 (P<0.01), and 41 (P=0.01). The S+ group had the same fat percent as the C+ group (P=0.84) on day 48 (Table 9).

The – birds had decreased water percent on days 20, 41, and 48 compared with the + birds. On days 27 and 34, the two groups were similar. V+ birds had an increased water percentage versus S+ on day 20 (P=0.05). V+ had a decreased water percentage compared to C+ on days 41(P=0.01) and 48 (P=0.04). They were similar to C+ in terms of water percent on days 20 (P=0.89), 27 (P=0.67), and 34 (P=0.05). The V+ birds had similar water percent compared to S+ birds on days 27 (P=0.19) and 34 (P=0.18). They had decreased water percent compared with S+ birds on days 41 (P=0.01) and 48 (P=0.01). The – treatments were not different on days 20, 41, and 48 for water percent. On day 27, the V- birds had similar water percentages compared to both C- (P=0.10) and S- (P=0.33) birds. The S- birds had increased water percentage compared with C- birds (P=0.01). On day 34, the S- birds reached similar water percent with both V- (P=0.91) and C- (P=0.06) groups. The V- birds had increased water percent compared with the C- treatment group (P=0.04) on day 34 (Table 9).

Ash percentage was highly dependent on challenge on days 20, 27, and 48. On days 20 and 27, the + birds experienced decreased ash percentage compared with the – birds, but on day 48, the + birds experienced increased ash percentage. On days 34 and 41, the ash percentage of the + group was similar to that of the – group. The ash percentage of the – birds is not different on days 20, 41, and 48. The V- birds had similar ash percentage with the S- and C- birds on day 27 and 34. The S- birds had increased ash percents on day 27 (P= 0.02) and 34 (P=0.03) compared to C- birds. The S+ and V+ birds had similar ash percents throughout the trial. The C+, V+ and S+ birds all had similar ash percentages on days 34, 41 and 48. The S+ birds had higher ash percents than

the C+ birds on days 20 (P=0.01) and 27 (P=0.03). The V+ birds had increased ash percentage on days 20 (P=0.04) and 27 (P=0.01) compared to C+ birds (Table 9).

Heat production was calculated using composition data (HPc). It was calculated using the following equation: HPc= Metabolizable Energy consumed- Retained Energy. The retained energy was calculated using the protein and fat gain from the composition of the birds. Both protein gain and fat gain were multiplied by their respective energy values of 5.65 kcal/gram and 9.3 kcal/gram. Those values were added to quantify the energy retained as tissue in the birds. The challenge group was evaluated against the unchallenged birds. The HPc of the challenged birds was higher than that of the unchallenged birds except on days 27 and 48 when they were statistically indifferent. C+ birds had an indifferent HPc compared to the V+ birds on day 20 (P=0.06). The C+ group had increased HPc compared to V+ group on all other days. The C+ birds exhibited higher HPc than the S+ birds on all days except day 48 where the two groups were not different (P=0.86). The V+ birds had a lower HPc compared to the S+ birds on days 41 (P=0.01) and 48 (P=0.01). All other days, the two were similar. The V- birds had an increased HPc compared to both the S- (P=0.03) and C- (P=0.03) birds on day 27. On all other days, the – treatment groups were similar to each other (Table 6).

Heat production from composition (HPc) was further investigated by dividing it by the metabolic body weight giving the heat production per unit metabolic body weight (HPMB). The metabolic body weight was calculated by raising the body weight to the 0.75 power. The + group and the – group were analyzed to determine the general effects of a coccidial challenge. The challenged birds experienced an increased HPMB throughout the trial compared with the unchallenged birds indicative of the coccidial

challenge they were experiencing. The C+ birds had a higher HPMB compared to the V+ birds throughout the trial. The S+ and C+ groups had an indifferent HPMB on day 48 (P=0.89). On all other days, C+ had a higher HPMB. The C+ birds had a higher HPMB on all days compared to the V+ birds. V+ birds exhibited a higher HPMB on day 20 compared to the S+ birds (P=0.0366). These two treatment groups were not different on days 27 (P=0.76) and 34 (P=0.06). The V+ birds exhibited less HPMB on days 41 (P=0.01) and 48 (P=0.02) compared to the S+ birds. The V- birds exhibited an elevated HPMB compared to both the C- (P=0.02) and S- (P=0.03) birds on day 27. All other comparisons between the – groups, on all other days yielded no differences (Table 10).

Metabolizable Energy (ME) consumption was calculated by multiplying the feed consumption by the ME content of the diet. The ME of the birds was highly dependent upon challenge. The + group consumed significantly less ME than the – group throughout the trial. The C+ birds and V+ birds exhibited similar ME consumption on day 20 (P=0.10). The V+ birds consumed more ME than the C+ birds throughout the rest of the trial. The S+ birds displayed an increased ME consumption compared to the C+ birds on days 20 (P<0.01), 27 (P<0.01), 34 (P<0.01), and 41 (P=0.01). They had similar ME consumption on day 48 (P=0.90). The V+ birds consumed an similar amount of ME versus S+ birds on days 27 (P=0.06) and 34 (P=0.45). The V+ birds consumed less ME than S+ birds on day 20 (P<0.01) and more on days 41 (P<0.01) and 48 (P<0.01). The only differences existing for ME consumption of – treatment groups were found on day 20. The V- birds exhibited decreased ME consumption compared to the C- (P<0.01) and S- (P<0.01) birds. No other differences existed for – treatment groups for ME consumption throughout the trial (Table 10).

HPc was divided by ME consumption to determine the heat production per kilocalorie of ME consumed (HPMEc) with lower values defining better performance. The challenged birds experienced a higher HPMEc than the unchallenged birds throughout the trial. The V- birds exhibited a higher value on day 27 compared to the C- (P=0.01) and S- (P=0.03) birds. All other comparisons between – treatment groups showed no differences. S+ exhibited a decreased HPMEc compared to the V+ birds on day 20 (P<0.01). These two groups exhibited similar HPMEc values on days 27 (P=0.35) and 34 (P=0.13). The V+ birds had lower HPMEc values on days 41 (P<0.01) and 48 (P=0.01). The C+ birds exhibited increased HPMEc versus S+ on all days but 48. On day 48, the C+ and S+ groups were not different in terms of HPMEc (P=0.93). The V+ birds exhibited lower HPMEc compared with C+ throughout the trial (Table 10).

Metabolizable energy consumption depression associated with challenge (MEcr) was determined by subtracting the ME consumption of the unchallenged birds (-) birds from the ME consumption of challenged birds (+); therefore, the results presented compare the treatment groups at different ages without challenge consideration. A lower value indicates better performance and protection during challenge. The V birds had a decreased value compared to C birds on days 34 (P=0.01), 41 (P<0.01), and 48 (P=0.01). The V birds were not different to the C birds on days 20 (P=0.27) and 27 (P=0.11). The S birds exhibited decreased MEcr on days 27 (P=0.01) and 34 (P=0.01) compared with C birds. The C group and S group were similar on days 20 (P=0.13), 41 (P=0.39), and 48 (P=0.09). S and V groups exhibited similar MEcr on days 20 (P=0.68), 27 (P=0.38), and 34 (P=0.45). The V birds had a decreased MEcr on days 41 (P=0.01) and 48 (P=0.02) compared to the S birds (Table 11).

Maintenance energy elevation due to challenge (MC) was determined by the following equation: (ME consumption – accretion energy) – body weight$^{0.75}$ X 110 X 6 for challenged birds - (ME consumption – accretion energy) – body weight$^{0.75}$ X 110 X 6 for unchallenged birds. The accretion energy is the energy from protein and fat gain. As for MEcr, the results compare treatment groups at different ages without challenge consideration. The C birds had a higher maintenance energy cost due to challenge (MC) than the S birds on days 27 ($P=0.01$), 34 ($P<0.01$), and 41 ($P=0.01$). The S and C groups exhibited similar MC values on days 20 ($P=0.16$) and 48 ($P=0.12$). The V birds had decreased MC compared with S birds on days 41 ($P<0.01$) and 48 ($P=0.01$). On all other days, these two groups were not different for MC. The V birds exhibited depressed MC compared with C birds on days 27 ($P=0.01$), 34 ($P<0.01$), and 41 ($P<0.01$). On days 20 ($P=0.40$) and 48 ($P=0.29$), the C and V groups were not different (Table 11).

Total caloric cost of challenge (TCC) is determined by the addition of MEcr and MC to quantify the total energy cost of a coccidiosis challenge. S birds had decreased TCC compared to C birds on days 27 ($P=0.01$), 34 ($P<0.01$), and 41 ($P=0.01$). On days 20 ($P=0.06$) and 48 ($P=0.94$), the C and S groups were not different. The S birds had increased TCC values compared to V birds on days 41 ($P<0.01$) and 48 ($P=0.01$). The V birds exhibited similar TCC to C birds ($P=0.21$) on day 20. On all other days, the C birds were similar to the V birds (Table 11).

DISCUSSION

CHALLENGE PERIODS

The results suggest that the V birds (V+ and V-) experienced an early immune challenge due to the vaccination. The early performance of the V birds was slightly decreased compared with the S birds. The performance of the V- birds was somewhat decreased compared with the other – treatments, but the V- birds caught up to the other – treatments at the end of the trial (Odd-numbered figures 1-9 and 23-43). This fact alone is not enough to determine the presence of a challenge, but coupled with the elevated lesion scores early in the trial supports an early challenge due to vaccination. The challenged and the unchallenged V birds both had elevated lesion scores (gross and microscopic) early in the trial. Figures 11 through 22 emphasize this presence of an early challenge in the vaccinated birds. The odd-numbered plots especially support the early challenge because those represent the different lesion scores of the – birds. In every case involving the unchallenged birds, the V birds have at least slightly elevated lesion scores (gross and microscopic) in the early part of the trial above both of the other groups (C and S). Earlier studies have supported the fact that vaccination causes an early immune challenge in the chickens to elicit immunity allowing them to better cope with the introduction of the microorganisms later in life. The results reported from this study support the formation of immunity. The V+ birds performed poorer than or similar to the S+ birds through day 34 (Tables 3, 4, and 5). After the withdrawal of the Sacox 60® on day 35, the S+ birds were no longer able to cope with a coccidiosis challenge. The S birds did not develop immunity during the trial because the Sacox 60® protected them

from most exposure to coccidiosis. The natural immunity of the chickens was not utilized by the S birds to fight coccidiosis, and it had very detrimental consequences seen through the heavy losses occurring in the S+ birds' performance after day 34 compared with the V+ birds (Tables 3, 4, 5 and even-numbered Figures). Conway et al. (2002a and 2002b) and Chapman et al. (2004) had similar conclusions concerning the withdrawal of anticoccidials. The birds that were medicated with anticoccidials performed poorly when challenged with coccidiosis after withdrawal of the anticoccidial.

The performance results here are similar to those of Mathis (1999). The vaccinated birds in his study experienced poor performance compared to birds given salinomycin in the beginning of the trial. After week 3, the vaccinated birds became similar to the salinomycin birds. We experienced similar results in that the vaccinated birds in this study also had reduced performance at the start of trial, but they became similar to, and in our study, surpassed the salinomycin birds.

Performance of – birds is related to the microscopic and gross lesion scores. The V- birds experience increased lesion scores during the first challenge period. They also experienced decreased performance in terms of live weight, body weight gain, and feed efficiency. The poor performance was most likely caused by the challenge involved in giving the live vaccine clearly visible by the increased lesion scores. The C- and S- birds experienced virtually no lesion scores throughout and their performance results showed no coccidiosis challenge, as it should not have. During the final two challenge periods, the V+ birds had lower, almost nonexistent, microscopic and gross lesion scores. The S+ birds had higher incidences of lesions which were reflected in their decreased performances during the last two challenge periods. The C+ birds had increased lesion

scores throughout and they also had poor performance throughout. The results for the lesion scores correlate well with performance. The lesion scores tended to be higher when birds were also performing poorly. Clearly, the increased lesion scores resulting from coccidiosis had detrimental effects on bird performance. The only exception to this is that the S+ birds had comparable lesion scores to the V+ and C+ birds during the early stages of the trial, but they had better performance. It appears that the salinomycin prevented the coccidiosis from stunting the birds' performance, but it did not prevent the occurrence of intestinal lesions.

The total challenge period heat production calculated from the gas data (HPg) is very interesting. HPg may be expected to change based on the challenge involved with the birds. Increasing the stress of the bird through challenge is typically cause for an increase in heat production. In this study, heat production from gas was shown to remain fairly static. The challenged birds were similar to the unchallenged birds throughout the trial. There were small differences found in some treatment x challenge interaction instances, but as figures 23 and 24 show, the HPg of the different treatment groups remains similar. It is possible that a deeper issue is involved. In this case, the heat production was mediated by immunological response or nutrient utilization. A further investigation into heat production was performed using the composition data.

Retained energy is correlated with the performance data. In the beginning of the trial, the V+ birds have a much lower RE compared with the S+ birds (Figure 26). As the birds grow and recover, they are able to utilize more of the feed energy as accrued protein and fat. The S+ birds begin to lose the ability to retain energy as they become susceptible to the coccidiosis challenge. This is also reflected in the retained energy efficiency data.

Figure 28 shows the dramatic shift in S+ REE as the birds have the Sacox 60® withdrawn from the feed and are unable to remain efficient when they experience a coccidiosis challenge during the withdrawal period. Figure 27 shows how the V- birds have reduced efficiency in the beginning of the experiment because they are facing a challenge from the vaccine and the oocyst mixture. By the end of the experiment, the vaccine has provided them with immunity allowing them to become more closely efficient with the S- and C- groups.

The even-numbered figures from 30 through 36 illustrate the increase of body substrates seen in the V+ birds over time as they develop immunity. The figures here illustrate that the amount of each constituent will increase as the body weights increase. If the total body has a higher mass, the composition making up that body will also increase in mass. The comparison of figure 4 with any of these clearly illustrates the correlation of the composition masses with the live mass of the bird.

Figures 38 and 40 illustrate the effects of the withdrawal of Sacox 60®. Figure 38 shows an increase in the protein percentage of the S+ birds. In figure 40, we can see the increase in the fat percentage of the V+ birds. Typical growth curves show that after maturity, the birds should begin to decrease protein accretion and increase fat accretion. After growth is completed, the buildup of fat begins. These two figures illustrate that the V+ birds have grown to the point of accruing fat. The S+ birds are suffering the ill effects of the post-withdrawal coccidiosis challenges. The birds are not gaining sufficient weight for their bodies to be accruing a higher percentage of fat tissue compared with lean tissue. Leanness is a common sign of disease and general lack of health.

Figure 42 explains the poor efficiency of the S+ birds described earlier. The birds are displaying a much higher percentage of water in their body than the other groups. Increasing the water percentage in the body does nothing for the bird energetically speaking. The increase of water in the body will reduce the energetic efficiency of the bird because the bird gets no energetic use out of water. The increased water on days 41 and 48 is correlated with the S+ birds experiencing a significant coccidiosis challenge. The birds are either unable to accrue protein and fat with efficiency at this point, or they experienced increased water percentage due to edema caused by the coccidiosis infection. Either way, it is clear that the inability of the birds to defend against coccidiosis after salinomycin withdrawal caused the birds to have higher percent water.

The heat production values obtained from the composition data (HPc) indicate that the S+ birds were protected from the coccidiosis challenge until day 41. After the withdrawal of the salinomycin, those birds were unable to cope adequately with a challenge. They experienced increased heat production after the withdrawal on day 35 compared with the V+ birds. They experience HPc that was similar to the C+ birds on day 48. That fact implies that the S+ birds had no advantage over the C+ birds in terms of HPc. The V+ birds had a numerically elevated HPc compared with the S+ birds indicating that they were experiencing a slightly increased challenge over S+ birds. The V+ birds exhibited the same HPc as the S+ birds until day 41 where they had decreased HPc indicating that they had developed and maintained immunity. They were able to cope with the challenge as indicated by the fact that their HPc was not elevated significantly above the three – groups. The challenge they received caused no increase to HPc compared with unchallenged birds.

Heat production per metabolic body weight (HPMB) was investigated to . The results for HPMB support the Schering-Plough Quadrants of Performance theory. The V- birds had elevated HPMB on day 20 compared to the C- and S- birds. This indicates that the V- birds were most likely experiencing an immune challenge from the vaccination. This challenge aided the birds in building up immunity to coccidiosis, as seen with the reduction of the V+ birds' HPMB at the end of the trial. During the final 2 challenge periods, the V+ birds had HPMBs that were lower than the C+ and S+ birds, and statistically similar to the unchallenged treatment groups. Again, this is indicative of the protection that the V+ birds are given by the early immune challenge due to vaccination. It is clear with these results that the S+ birds experienced negative consequences after the salinomycin withdrawal on day 35. They had increased HPMB compared to the V+ birds. The C+ birds experienced detrimental effects of coccidiosis challenge throughout the trial. They had significantly higher HPMB values compared to V+ birds throughout, indicating that they were undergoing significant immune challenge due to the lack of any protection against coccidiosis. Figure 52 shows the progression of the HPMB through the growth curve of the challenged birds. The C+ birds are producing more heat throughout, and the S+ birds catch up to them at the end of the trial. Figure 51 shows the elevation of the V- birds due to vaccine challenge early in the trial, but they become similar at the end of the trial.

The heat production per kilocalorie of ME consumed (HPMEc) was determined by dividing the HPc by MEc. This value is a simple way to quantify the percentage of MEc that was lost as heat, in other words, the percentage of MEc that was wasted. The results are very conclusive. The S+ birds have the lowest value in the beginning showing

that they are able to utilize the MEc more efficiently. They are protected against the challenge better than the V+ birds during the first challenge. There was less heat production for the S+ birds at that time most likely due to the lack of immune challenge from the coccidiosis dosage. The C+ group had increased HPMEc because they were not protected. More of the ME they consumed was lost as heat due to the largely detrimental effects of the coccidial challenge. The V+ birds were also numerically elevated compared to the S+ birds during the first chalenge because they were experiencing an immune challenge from the coccidiosis challenge and vaccine challenge. The S+ birds had elevated HPMEc compared to the V+ birds on days 41 and 48. The S+ birds were unable to retain efficiency during this time because of the withdrawal of the salinomycin. The V+ birds were able to utilize the MEc for accretion instead of wasting the energy for the immune challenge. The V+ birds were protected throughout. The unchallenged birds were had similar HPMEc throughout with the exception of day 27. The V- birds were elevated on this day compared to the S- and C- birds. They were likely still dealing with an immune challenge from the vaccination.

The amount of MEc depression associated with a coccidiosis challenge (MEcr) was quantified. The value was determined by subtracting the MEc of the challenged birds from the MEc of the unchallenged birds. This allowed us to determine the extra amount of MEc that was consumed by the unchallenged birds compared to the challenged birds. Lower valued indicated better performance of challenged birds. When a group had a lower value, the group was able to cope with the challenge better because they continued to eat similar to if they had not been challenged. The V birds had decreased MEcr after day 34 compared to S birds illustrating that the vaccine was able to protect

longer in the growth curve after the salinomycin had been withdrawn. The S birds had lower MEcr before day 41 compared with the C group, but on days 41 and 48, the two were not different. This indicates that the S birds were no more protected than the C birds after salinomycin withdrawal. The V+ birds were able to consume more ME relative to the V- birds than the S+ birds were able to relative to the S- birds. The coccidial challenge impacted the MEc of the S birds more than it did the V birds after the salinomycin withdrawal. The C birds' MEc was significantly impacted by coccidial challenge throughout.

Maintenance energy elevation due to challenge (MC) was determined using the following equation: (ME consumption – accretion energy) – body weight$^{0.75}$ X 110 X 6 (maintenance energy) for challenged birds – (ME consumption – accretion energy) – body weight$^{0.75}$ X 110 X 6 (maintenance energy) for unchallenged birds. The accretion energy is found by adding the energy values for protein and fat gain: accretion energy = (protein gain X 5.65 kcal/gram)/Kp + (fat gain X 9.3 kcal/gram)/Kf. The Kp and Kf values are standards commonly used. Maintenance energy is determined using metabolic body weight (body weight$^{0.75}$) and a value derived from previous studies performed at the OSU poultry farm defining the maintenance energy requirement per day (110). The 6 used in the equation is the number of days considered, in this case 6 days for each challenge period. The increase in maintenance energy because of challenge will come from energy typically used for accretion. The V birds displayed reduced MC compared to the S birds after salinomycin withdrawal. The increase in immune challenge of S birds pulls energy from the accretion portion and uses it to maintain the bird. The S birds fall in performance because they are not able to cope with the challenge, but the V birds have

developed immunity thanks to vaccination. They are able to deal with the challenge and do not require as much of the total energy for maintenance; therefore, they are able to use more energy for accretion. The C birds had to use more of their energy for maintenance throughout most of the trial compared to the S and V birds. They were not protected at all, and the effects were obvious.

The total energy cost of coccidial challenge is quantified by adding the MC and MEcr. The V birds developed less of an energy loss due to challenge at the end of the trial. On days 41 and 48, they have the least cost of challenge. The V+ birds were able to consume more ME relative to V- birds, and they were able to utilize a similar amount of that energy for accretion instead of maintenance. The S+ birds were able to consume and utilize energy early in the trial, but they failed after withdrawal. The C+ birds were not able to consume and utilize energy throughout the trial.

The predicted ME consumption was calculated using the derived value for maintenance energy requirement per day per metabolic body weight and the protein and fat energy values. The equation to calculate the predicted ME consumption is as follows: 110 X body weight$^{0.75}$ X 6 days + (protein gain X 5.65)/Kp + (fat gain X 9.3)/Kf. The predicted ME consumption was plotted against the actual ME consumption of the birds in Figures 48, 49, and 50 for the Control, Salinomycin, and Vaccinated birds, respectively. Every plot shows the challenged vs. the unchallenged birds. Figure 48 illustrates the impact of the challenge on the control birds receiving no protection. The unchallenged birds' ME consumption follows the predicted value almost perfectly, but the challenged birds fall below the unchallenged birds. They cannot keep up with the unchallenged. They are not accruing the protein and fat that they should be accruing. The salinomycin

birds (Figure 49) follow the proper trend much better than the control birds. It is clear in the plot that some of the S+ birds have fallen below where they should, but the S birds are clearly performing better than the control birds. The vaccinated birds follow the correct path like the S birds. They have a more even distribution of unchallenged and challenged birds throughout the ME consumption. The challenged birds performing poor on the S plot are most likely the ones that were challenged after withdrawal. They are performing poorly due to immune challenge.

CONCLUSION

It is clear from the data that the challenged groups did undergo immune challenges in the form of coccidiosis infection. Salinomycin is an effective means of controlling coccidiosis caused by *E. acervulina, E. maxima,* and *E. tenella*, especially in the early stages of the broiler's growth curve. Salinomycin can significantly improve performance and reduce lesion scores associated with coccidiosis infection. After withdrawal, the birds previously given salinomycin will be severely impacted by a significant coccidiosis infection. The birds are not able to develop any natural immunity against coccidiosis and will be defenseless when the salinomycin is withdrawn. The cost of challenge is outlined previously as the total cost of challenge (TCC). It is clear that the vaccinated birds did not lose as much energy as reduced ME consumption or as increased maintenance energy due to coccidial challenge compared to the salinomycin birds after withdrawal of salinomycin on day 35. The data reported herein supports Schering-

Plough's "Quadrants of Performance" theory. Vaccination with Coccivac-B® gives the bird the means to develop immunity against coccidiosis caused by *E. acervulina, E. maxima,* and *E. tenella*. The vaccine causes initial immune challenge possibly retarding performance, but the vaccinated birds have the ability to rebound and improve performance before reaching market weight. The vaccinated birds were better equipped to deal with a coccidial challenge later in life.

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TABLE 1: Composition of diets used for broilers throughout experiment

	Age interval (days) and Treatments[1]				
	0 to 21		21 to 35		35 to 48
Ingredient, %	S	V and C	S	V and C	S, V, and C
Corn	58.3	58.3	64.529	64.529	64.529
Soybean meal (48 % CP)	34.56	34.56	28.21	28.21	28.21
Soybean oil	2.83	2.83	2.93	2.93	2.93
Dicalcium phosphate	1.87	1.87	1.98	1.98	1.98
Limestone	1.18	1.18	0.92	0.92	0.92
NaCl	0.35	0.35	0.29	0.29	0.29
Roche Vitamin Premix[2]	0.2	0.2	0.2	0.2	0.2
$NaHCO_3$	0.24	0.24	0.32	0.32	0.32
DL-Methionine	0.21	0.21	0.22	0.22	0.22
Huber trace mineral[3]	0.09	0.09	0.09	0.09	0.09
Lysine HCl	0.06	0.06	0.157	0.157	0.157
Selenium 600 premix	0.04	0.04	0.04	0.04	0.04
Threonine	0.02	0.02	0.05	0.05	0.05
Ethoxyquin	0.01	0.01	0.012	0.012	0.012
Choline Chloride	0.01	0.01	0	0	0
Copper Sulfate	0	0	0.002	0.002	0.002
L-Arginine	0.03	0.03	0.05	0.05	0.05
Sacox-60® (Salinomycin)	0.05	0.00	0.05	0.00	0.00
Stafac-20® (Virginiamycin)	0.05	0.05	0.05	0.05	0.05
Calculated Analysis					
Me_n (kcal/kg)	3053	3053	3,131	3,131	3,131
CP, %	22.1	22.1	19.8	19.8	19.8
Arg	1.38	1.38	1.30	1.30	1.30
Lys	1.12	1.12	1.14	1.14	1.14
Met	0.51	0.51	0.52	0.52	0.52
TSAA	0.83	0.83	0.88	0.88	0.88
Ca	0.90	0.90	0.80	0.80	0.80
P, available	0.44	0.44	0.40	0.40	0.40

[1]Treatments: C = Control; V = Vaccinated; S = Salinomycin.
[2]Supplied per kilogram of diet: vitamin A, 10,141 IU (retinyl acetate); cholecalciferol, 3,086 IU; vitamin E, 23.92 IU (dl-α-tocopheryl acetate); menadione, 2.87 mg; thiamine, 2.20 mg; riboflavin, 7.72 mg; niacin, 60.30 mg; d-pantothenic acid, 12.46 mg; pyridoxine, 3.75 mg; vitamin B_{12}, 0.017 mg; folic acid, 1.066 mg; d-biotin, 0.127 mg.
[3]Supplied per kilogram of diet: Ca,160 mg; Zn, 100 mg; Mn, 120 mg; Fe,75 mg; Cu, 10 mg; I, 2.5 mg.

TABLE 2: **Feed Consumption (FC), Body Weight (BW), Body Weight Gain (BW Gain), and Feed Efficiency (FE) of Male Broilers During Floor Pen Period**

Treatment	FC (g)	BW (g)	BW Gain (g)	FE (g/g)
Age 7 days				
C	160	190	145	0.91
S	158	187	142	0.90
V	166	195	150	0.91
Age 14 days				
C	415	492	299	0.72
S	395	485	296	0.75
V	410	501	305	0.75
Age 21 days				
C	606	936	442	0.73
S	607	923	440	0.72
V	584	914	411	0.70
Age 28 days				
C	993	1569	635	0.64
S	966	1529	603	0.63
V	930	1492	571	0.61
Age 35 days				
C	1188	2252	681	0.57
S	1189	2239	703	0.59
V	1135	2145	667	0.59
Age 42 days				
C	1370	3071	780	0.57
S	1368	3034	772	0.57
V	1332	2888	718	0.54

C = Control; S = Salinomycin; V = Vaccinated; g=gram; FC=Weekly feed consumption per bird; FE=BW Gain (g)/FC (g)

TABLE 3: Feed Consumption (FC), Body Weight (BW), Average Daily Gain (ADG), Body Weight Gain (BW Gain), and Feed Efficiency (FE) of Male Broilers During Challenge Periods

Treatment	Challenge	FC (g)	BW (g)	ADG (g/d)	BW Gain (g)	FE (g/g)
Age 20 days						
C	+	335^{a}	598^{a}	18^{a}	108^{a}	0.32^{a}
S	+	395^{b}	712^{b}	37^{b}	217^{b}	0.54^{b}
V	+	348^{a}	645^{c}	25^{c}	150^{c}	0.43^{c}
C	-	444^{c}	796^{d}	51^{d}	302^{d}	0.68^{d}
S	-	430^{cd}	777de	48de	282de	0.65^{d}
V	-	408^{bd}	752^{e}	44^{e}	258^{e}	0.63^{d}
	Trt	0.0004	0.0004	0.0003	0.0003	0.0003
P value	Chall	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Trt*Chall	0.0002	<0.0001	<0.0001	<0.0001	<0.0001
Age 27 days						
C	+	598^{a}	1093^{a}	29^{a}	169^{a}	0.28^{a}
S	+	710^{b}	1321^{b}	68^{b}	397^{b}	0.56^{bc}
V	+	677^{b}	1275^{c}	60^{c}	351^{c}	0.52^{bc}
C	-	714^{b}	1330^{b}	69^{b}	406^{b}	0.57^{b}
S	-	714^{b}	1313^{bc}	66^{bc}	389^{bc}	0.55^{bc}
V	-	712^{b}	1291^{bc}	63^{bc}	368^{bc}	0.52^{c}
	Trt	0.0042	<0.0001	<0.0001	<0.0001	<0.0001
P value	Chall	0.0002	<0.0001	<0.0001	<0.0001	<0.0001
	Trt*Chall	0.0024	<0.0001	<0.0001	<0.0001	<0.0001
Age 34 days						
C	+	720^{a}	1713^{a}	27^{a}	161^{a}	0.23^{a}
S	+	876^{b}	2059^{bc}	87^{bc}	507^{bc}	0.58^{b}
V	+	888^{bc}	2023^{b}	80^{c}	471^{bd}	0.53^{c}
C	-	919^{bc}	2088^{c}	92^{b}	536^{c}	0.58^{b}
S	-	924^{c}	2080^{c}	90^{b}	528^{c}	0.57^{b}
V	-	924^{bc}	2063^{bc}	87^{bc}	511^{cd}	0.55^{bc}
	Trt	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
P value	Chall	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Trt*Chall	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Age 41 days						
C	+	752^{a}	2261^{a}	8^{a}	45^{a}	0.06^{a}
S	+	841^{b}	2425^{b}	36^{b}	209^{b}	0.24^{b}
V	+	991^{c}	2673^{c}	78^{c}	457^{c}	0.45^{c}
C	-	1058^{cd}	2763^{cd}	93^{cd}	547^{cd}	0.51^{c}
S	-	1065^{cd}	2763^{cd}	93^{cd}	546^{cd}	0.51^{c}
V	-	1093^{d}	2771^{d}	95^{d}	555^{d}	0.51^{c}
	Trt	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
P value	Chall	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Trt*Chall	0.0016	<0.0001	<0.0001	<0.0001	<0.0001
Age 48 days						
C	+	801^{a}	2930^{a}	-16^{a}	-83^{a}	-0.11^{a}
S	+	840^{a}	3032^{a}	4^{a}	19^{a}	0.00^{a}
V	+	1144^{b}	3473^{b}	87^{b}	459^{b}	0.40^{b}
C	-	1079^{b}	3414^{b}	76^{b}	400^{b}	0.35^{b}
S	-	1046^{b}	3413^{b}	76^{b}	399^{b}	0.36^{b}
V	-	1095^{b}	3415^{b}	76^{b}	402^{b}	0.36^{b}
	Trt	<0.0001	0.0004	0.0004	0.0004	0.0007
P value	Chall	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	Trt*Chall	0.0002	<0.0001	<0.0001	<0.0001	0.0002

C = Control; S = Salinomycin; V = Vaccinated; + = Challenged; - = Unchallenged; g=gram; FE=BW Gain (g)/FC (g); Trt=Treatment (C, S, V); Chall=Challenge (+, -)

TABLE 4: Gross Lesion Scores for Male Broilers During Challenge Periods

Treatment	Challenge	Upper	Mid	Ceca
Age 20 days				
C	+	2.24[a]	1.64[a]	2.78[a]
S	+	0.42[b]	0.75[b]	2.05[b]
V	+	1.72[c]	1.14[b]	2.56[a]
C	-	-0.03[d]	-0.02[c]	0.02[c]
S	-	0.02[d]	0.01[c]	0.03[c]
V	-	1.05[e]	0.43[bc]	0.37[c]
	Trt	<0.0001	0.0115	0.0027
P value	Chall	<0.0001	<.0001	<0.0001
	Trt*Chall	<0.0001	0.0043	0.0151
Age 27 days				
C	+	0.41[a]	0.47[a]	2.34[a]
S	+	0.01[b]	-0.02[b]	0.62[b]
V	+	0.00[b]	-0.01[b]	1.27[c]
C	-	0.00[b]	0.01[b]	0.00[d]
S	-	0.00[b]	0.01[b]	0.00[d]
V	-	0.01[b]	-0.02[b]	0.00[d]
	Trt	0.0006	<0.0001	<0.0001
P value	Chall	0.0023	0.0005	<0.0001
	Trt*Chall	0.0002	<0.0001	<0.0001
Age 34 days				
C	+	0.55[a]	1.16[a]	2.79[a]
S	+	0.00[b]	0.12[b]	1.19[b]
V	+	0.00[b]	0.00[b]	1.32[b]
C	-	0.00[b]	-0.01[b]	0.01[c]
S	-	0.00[b]	0.00[b]	0.01[c]
V	-	0.00[b]	0.04[b]	-0.06[c]
	Trt	0.0005	<0.0001	<0.0001
P value	Chall	0.0023	<0.0001	<0.0001
	Trt*Chall	<0.0001	<0.0001	<0.0001
Age 41 days				
C	+	1.94[a]	2.13[a]	2.95[a]
S	+	1.96[a]	2.43[a]	2.75[a]
V	+	-0.01[b]	0.03[b]	0.87[b]
C	-	0.01[b]	0.00[b]	0.02[c]
S	-	0.01[b]	0.00[b]	0.03[c]
V	-	-0.01[b]	0.00[b]	-0.02[c]
	Trt	<0.0001	<0.0001	<0.0001
P value	Chall	<0.0001	<0.0001	<0.0001
	Trt*Chall	<0.0001	<0.0001	<0.0001
Age 48 days				
C	+	2.04[a]	2.43[a]	2.56[a]
S	+	1.76[b]	1.93[b]	1.28[b]
V	+	0.05[c]	0.03[c]	0.24[c]
C	-	-0.02[c]	-0.01[c]	0.00[c]
S	-	-0.02[c]	-0.02[c]	0.00[c]
V	-	0.03[c]	0.02[c]	-0.01[c]
	Trt	<0.0001	0.0004	0.0004
P value	Chall	<0.0001	<0.0001	<0.0001
	Trt*Chall	<0.0001	<0.0001	<0.0001

C = Control; S = Salinomycin; V = Vaccinated; + = Challenged; - = Unchallenged; Upper=Upper Small Intestine Lesion Score; Mid=Middle Small Intestine Lesion Score; Ceca=Cecal Lesion Score; Trt=Treatment (C, S, V); Chall=Challenge (+, -)

TABLE 5: Microscopic Lesion Scores for Male Broilers During Challenge Periods

Treatment	Challenge	*E. acervulina*	*E. maxima*	*E. tenella*
Age 20 days				
C	+	2.88[a]	2.51[a]	3.65[a]
S	+	1.43[b]	1.98[b]	2.83[b]
V	+	1.72[b]	1.24[c]	3.10[b]
C	-	0.06[c]	0.06[d]	0.13[c]
S	-	-0.01[c]	-0.01[d]	0.07[c]
V	-	0.92[d]	0.22[d]	0.50[c]
	Trt	<0.0001	0.0075	0.0229
P value	Chall	<0.0001	<0.0001	<0.0001
	Trt*Chall	<0.0001	0.0002	0.0138
Age 27 days				
C	+	0.38[a]	2.09[a]	3.67[a]
S	+	-0.01[b]	1.94[a]	1.00[b]
V	+	0.03[b]	0.11[b]	2.12[c]
C	-	0.00[b]	0.00[b]	0.00[d]
S	-	0.00[b]	0.00[b]	0.06[d]
V	-	0.04[b]	0.06[b]	0.31[d]
	Trt	0.0625	<0.0001	<0.0001
P value	Chall	0.0810	<0.0001	<0.0001
	Trt*Chall	0.0277	<0.0001	<0.0001
Age 34 days				
C	+	0.96[a]	2.67[a]	3.81[a]
S	+	0.05[b]	2.06[b]	1.40[b]
V	+	0.00[b]	0.00[c]	0.83[b]
C	-	0.00[b]	0.00[c]	0.00[c]
S	-	0.00[b]	0.00[c]	0.00[c]
V	-	-0.02[b]	0.00[c]	0.16[c]
	Trt	<0.0001	<0.0001	<0.0001
P value	Chall	<0.0001	<0.0001	<0.0001
	Trt*Chall	<0.0001	<0.0001	<0.0001
Age 41 days				
C	+	3.17[a]	2.95[a]	3.54[a]
S	+	3.43[a]	3.15[a]	3.80[a]
V	+	0.00[b]	0.00[b]	0.36[b]
C	-	0.00[b]	0.03[b]	-0.01[b]
S	-	0.00[b]	0.03[b]	0.04[b]
V	-	0.00[b]	-0.03[b]	0.31[b]
	Trt	<0.0001	<0.0001	<0.0001
P value	Chall	<0.0001	<0.0001	<0.0001
	Trt*Chall	<0.0001	<0.0001	<0.0001
Age 48 days				
C	+	2.39[a]	2.95[a]	3.30[a]
S	+	2.05[b]	2.40[b]	1.69[b]
V	+	0.16[c]	0.13[c]	0.04[c]
C	-	0.05[c]	0.06[c]	0.08[c]
S	-	0.00[c]	0.01[c]	0.03[c]
V	-	0.01[c]	-0.01[c]	0.01[c]
	Trt	<0.0001	<0.0001	0.0004
P value	Chall	<0.0001	<0.0001	<0.0001
	Trt*Chall	<0.0001	<0.0001	<0.0001

C = Control; S = Salinomycin; V = Vaccinated; + = Challenged; - = Unchallenged; *E. acervulina*= *Eimeria acervulina* lesion score; *E. maxima*= *Eimeria maxima* lesion score; *E. tenella*= *Eimeria tenella* lesion score; Trt=Treatment (C, S, V); Chall=Challenge (+, -)

TABLE 6: Heat Production Composition (HPc), Heat Production from Gas (HPg), Retained Energy (RE), and Retained Energy Efficiency (REE) for Male Broilers During Challenge Periods

Treatment	Challenge	HPc (kcal)	HPg (kcal)	RE (kcal)	REE
Age 20 days					
C	+	743[a]	619[ab]	289[a]	0.27[a]
S	+	658[bc]	694[ab]	542[b]	0.45[b]
V	+	694[ab]	590[b]	362[c]	0.34[c]
C	-	620[c]	714[a]	708[d]	0.52[d]
S	-	644[bc]	705[a]	664[d]	0.51[d]
V	-	634[c]	641[ab]	598[b]	0.48b[d]
	Trt	0.2610	0.5467	<0.0001	<0.0001
P value	Chall	<0.0001	0.0924	<0.0001	<0.0001
	Trt*Chall	0.0139	0.0869	<0.0001	<0.0001
Age 27 days					
1C	+	1448[a]	941[a]	406[a]	0.23[a]
3S	+	1272[bc]	1088[a]	946[b]	0.42[bc]
5V	+	1283[bc]	1045[a]	843[c]	0.40[b]
2C	-	1249[c]	1045[a]	1004[b]	0.45[c]
4S	-	1250[c]	1028[a]	984[b]	0.44[c]
6V	-	1365[ab]	1016[a]	879[bc]	0.39[b]
	Trt	0.0517	0.7501	<0.0001	<0.0001
P value	Chall	0.1259	0.9483	<0.0001	<0.0001
	Trt*Chall	0.0007	0.5813	<0.0001	<0.0001
Age 34 days					
C	+	1863[a]	1289[a]	410[a]	0.19[a]
S	+	1407[b]	1423[a]	1328[bc]	0.49[bc]
V	+	1529[b]	1403[a]	1272[c]	0.45[c]
C	-	1474[b]	1408[a]	1425[b]	0.50[b]
S	-	1422[b]	1367[a]	1445[b]	0.50[b]
V	-	1506[b]	1351[a]	1386[bc]	0.47[bc]
	Trt	<0.0001	0.9113	<0.0001	<0.0001
P value	Chall	0.0003	0.9697	<0.0001	<0.0001
	Trt*Chall	<0.0001	0.5836	<0.0001	<0.0001
Age 41 days					
C	+	2140[a]	1336[a]	145[a]	0.14[a]
S	+	1926[b]	1660[b]	654[b]	0.25[b]
V	+	1625[c]	1600[ab]	1364[c]	0.43[c]
C	-	1599[c]	1706[b]	1606[d]	0.51[d]
S	-	1667[c]	1572[ab]	1621[d]	0.50[cd]
V	-	1667[c]	1530[ab]	1675[d]	0.49[cd]
	Trt	0.0032	0.7065	<0.0001	0.0008
P value	Chall	<0.0001	0.4231	<0.0001	<0.0001
	Trt*Chall	0.0001	0.0764	<0.0001	<0.0001
Age 48 days					
C	+	2629[a]	1647[a]	-75[a]	0.29[ab]
S	+	2593[a]	1840[a]	-173[a]	0.24[a]
V	+	2041[b]	1862[a]	1437[b]	0.42[ab]
C	-	2143[b]	1781[a]	1219[b]	0.36[ab]
S	-	2125[b]	1686[a]	1082[b]	0.40[ab]
V	-	2115[b]	1726[a]	1344[b]	0.42[b]
	Trt	0.0134	0.8538	0.0012	0.1833
P value	Chall	0.1365	0.6458	<.0001	0.1299
	Trt*Chall	0.1222	0.4751	0.0087	0.3773

C = Control; S = Salinomycin; V = Vaccinated; + = Challenged; - = Unchallenged; HPc= Metobolizable energy (ME) consumed –RE; RE=Protein gain*5.65 + Fat gain*9.3; REE= RE/ME consumed; Trt=Treatment (C, S, V); Chall=Challenge (+, -) kcal=kilocalorie

TABLE 7: Protein, Fat, Water, and Ash Content of Male Broilers After Challenge Periods

Treatment	Challenge	Protein (g)	Fat (g)	Water (g)	Ash (g)
Age 20 days					
C	+	101[a]	60[a]	420[a]	15[a]
S	+	119[b]	74[b]	489[b]	17[b]
V	+	107[c]	66[c]	445[c]	16[b]
C	-	134[d]	86[d]	543[d]	20[c]
S	-	129[e]	82[e]	524[e]	19[d]
V	-	126[e]	80[e]	514[e]	18[d]
	Trt	<0.0001	<0.0001	<0.0001	<0.0001
P value	Chall	<0.0001	<0.0001	<0.0001	<0.0001
	Trt*Chall	<0.0001	<0.0001	<0.0001	<0.0001
Age 27 days					
C	+	187[a]	129[a]	734[a]	28[a]
S	+	226[bc]	164[bc]	870[bc]	33[bc]
V	+	219[c]	158[c]	847[c]	32[c]
C	-	230[b]	167[b]	881[b]	34[b]
S	-	229[bd]	167[b]	880[b]	34[b]
V	-	222[cd]	160[bc]	855[c]	33[c]
	Trt	<0.0001	<0.0001	<0.0001	<0.0001
P value	Chall	<0.0001	<0.0001	<0.0001	<0.0001
	Trt*Chall	<0.0001	<0.0001	<0.0001	<0.0001
Age 34 days					
C	+	297[a]	234[a]	1110[a]	44[a]
S	+	358[bc]	301[bc]	1305[bc]	53[bc]
V	+	353[b]	295[b]	1291[b]	52[c]
C	-	361[bc]	304[bc]	1317[bc]	53[bc]
S	-	365[c]	309[c]	1329[c]	54[b]
V	-	360[bc]	303[bc]	1318b[c]	53[bc]
	Trt	<0.0001	<0.0001	<0.0001	<0.0001
P value	Chall	<0.0001	<0.0001	<0.0001	<0.0001
	Trt*Chall	<0.0001	<0.0001	<0.0001	<0.0001
Age 41 days					
C	+	396[a]	345[a]	1427[a]	58[a]
S	+	429[b]	386[b]	1534[b]	63[b]
V	+	467[c]	436[c]	1659[c]	69[c]
C	-	479[cd]	452[cd]	1693[cd]	71[cd]
S	-	483[d]	457[d]	1710[d]	72[d]
V	-	485[d]	458[d]	1711[d]	72[d]
	Trt	<0.0001	<0.0001	<0.0001	<0.0001
P value	Chall	<0.0001	<0.0001	<0.0001	<0.0001
	Trt*Chall	<0.0001	<0.0001	<0.0001	<0.0001
Age 48 days					
C	+	516[a]	503[a]	1812[a]	77[a]
S	+	511[a]	500[a]	1801[a]	76[a]
V	+	594[b]	620[b]	2075[b]	89[b]
C	-	581[b]	604[b]	2030[b]	87[b]
S	-	576[b]	595[b]	2010[b]	86[b]
V	-	590[b]	613[b]	2060[b]	88[b]
	Trt	0.0013	0.0014	0.0014	0.0014
P value	Chall	<0.0001	<0.0001	<0.0001	<0.0001
	Trt*Chall	0.0124	0.0075	0.0137	0.0107

C = Control; S = Salinomycin; V = Vaccinated; + = Challenged; - = Unchallenged; g=gram; Trt=Treatment (C, S, V); Chall=Challenge (+, -)

TABLE 8: Protein Gain (PG), Fat Gain (FG), Water Gain (WG), and Ash Gain (AG) for Male Broilers During Challenge Periods

Treatment	Challenge	PG (g)	FG (g)	WG (g)	AG (g)
Age 20 days					
C	+	22[a]	17[a]	67[a]	3.0[a]
S	+	42[b]	33[b]	141[b]	6.0[b]
V	+	28[c]	22[c]	95[c]	4.0[c]
C	-	56[d]	42[d]	190[d]	8.0[d]
S	-	52[de]	40[d]	176[de]	7.4[de]
V	-	47[e]	36[b]	164[e]	6.8[e]
	Trt	<0.0001	<0.0001	<0.0001	<0.0001
P value	Chall	<0.0001	<0.0001	<0.0001	<0.0001
	Trt*Chall	<0.0001	<0.0001	<0.0001	<0.0001
Age 27 days					
C	+	30[a]	25[a]	110[a]	4.6[a]
S	+	69[bc]	60[b]	242[bc]	10.2[bc]
V	+	62[d]	53[c]	219[c]	9.2[b]
C	-	73[b]	64[d]	256[b]	10.9[c]
S	-	71[b]	62[d]	252[bc]	10.7[c]
V	-	64[cd]	55[bc]	227[c]	9.6[b]
	Trt	<0.0001	<0.0001	<0.0001	<0.0001
P value	Chall	<0.0001	<0.0001	<0.0001	<0.0001
	Trt*Chall	<0.0001	<0.0001	<0.0001	<0.0001
Age 34 days					
C	+	28[a]	27[a]	101[a]	4.3[a]
S	+	85[bc]	91[bc]	285[bc]	12.7[bc]
V	+	82[c]	87[c]	275[c]	12.2[b]
C	-	91[b]	98[b]	308[b]	13.6[c]
S	-	93[b]	99[b]	309[b]	13.8[c]
V	-	89[bc]	95[bc]	301[bc]	13.3b[c]
	Trt	<0.0001	<0.0001	<0.0001	<0.0001
P value	Chall	<0.0001	<0.0001	<0.0001	<0.0001
	Trt*Chall	<0.0001	<0.0001	<0.0001	<0.0001
Age 41 days					
C	+	9[a]	10[a]	25[a]	1.3[a]
S	+	38[b]	47[b]	120[b]	5.7[b]
V	+	78[c]	99[c]	250[c]	11.7[c]
C	-	92[d]	117[d]	292[cd]	13.8[d]
S	-	92[d]	118[d]	296[d]	13.9[d]
V	-	96[d]	121[d]	301[d]	14.3[d]
	Trt	<0.0001	<0.0001	<0.0001	<0.0001
P value	Chall	<0.0001	<0.0001	<0.0001	<0.0001
	Trt*Chall	<0.0001	<0.0001	<0.0001	<0.0001
Age 48 days					
C	+	-4[a]	-5[a]	-23[a]	-0.8[a]
S	+	-12[a]	-12[a]	-39[a]	-1.7[a]
V	+	73[b]	110[b]	238[b]	11.5[b]
C	-	61[b]	94[b]	196[b]	9.6[b]
S	-	54[b]	83[b]	171[b]	8.47[b]
V	-	69[b]	102[b]	225[b]	10.8[b]
	Trt	0.0012	0.0012	0.0012	0.0012
P value	Chall	<0.0001	<0.0001	<0.0001	<0.0001
	Trt*Chall	0.0124	0.0076	0.0142	0.0109

C = Control; S = Salinomycin; V = Vaccinated; + = Challenged; - = Unchallenged; g=gram; Trt=Treatment (C, S, V); Chall=Challenge (+, -)

TABLE 9: Protein, Fat, Water, and Ash Percent Content of Male Broilers After Challenge Periods

Treatment	Challenge	Protein (%)	Fat (%)	Water (%)	Ash (%)
Age 20 days					
C	+	16.7[a]	10.0[a]	69.6[ac]	2.41[a]
S	+	16.8[ab]	10.4[b]	69.0[ab]	2.45[bc]
V	+	16.8[ab]	10.3[c]	69.6[c]	2.44[b]
C	-	16.9[b]	10.8[d]	68.4[d]	2.47[c]
S	-	16.8[ab]	10.6[d]	68.6[bd]	2.46[bc]
V	-	16.9[b]	10.6[d]	68.8[bd]	2.46[bc]
	Trt	0.5080	0.1093	0.1309	0.3562
P value	Chall	0.0393	<0.0001	<0.0001	0.0001
	Trt*Chall	0.3131	<0.0001	0.2200	0.0297
Age 27 days					
C	+	17.0[a]	11.7[a]	66.7[ab]	2.51[a]
S	+	17.2[b]	12.5[b]	66.2[a]	2.54[b]
V	+	17.3[bc]	12.4[b]	66.6[ab]	2.54[b]
C	-	17.2[b]	12.5[bc]	66.1[a]	2.54[b]
S	-	17.4[c]	12.7[c]	67.0[b]	2.57[c]
V	-	17.3[bc]	12.5[bc]	66.7[ab]	2.55[bc]
	Trt	0.0025	<0.0001	0.6245	0.0027
P value	Chall	0.0020	<0.0001	0.6547	0.0019
	Trt*Chall	0.2993	<0.0001	0.0096	0.2364
Age 34 days					
C	+	17.3[a]	13.7[a]	64.7[a]	2.56[ab]
S	+	17.4[ab]	14.6[bc]	63.5[bc]	2.56[ab]
V	+	17.5[ab]	14.6[b]	64.0[ab]	2.57[ab]
C	-	17.3[a]	14.6[b]	63.2[c]	2.55[a]
S	-	17.6[b]	14.8[c]	63.9[abc]	2.59[b]
V	-	17.5[ab]	14.7[bc]	64.0[ab]	2.58[ab]
	Trt	0.0966	<0.0001	0.4844	0.1096
P value	Chall	0.4549	<0.0001	0.0896	0.4060
	Trt*Chall	0.3433	<0.0001	0.0009	0.3815
Age 41 days					
C	+	17.5[a]	15.3[a]	63.2[a]	2.59[a]
S	+	17.6[a]	15.8[b]	63.0[a]	2.60[a]
V	+	17.4[a]	16.2[c]	62.0[b]	2.58[a]
C	-	17.5[a]	16.5[cd]	62.0[b]	2.59[a]
S	-	17.5[a]	16.6[d]	62.1[b]	2.60[a]
V	-	17.5[a]	16.5[d]	61.7[b]	2.59[a]
	Trt	0.5048	<0.0001	0.0117	0.4852
P value	Chall	0.9232	<0.0001	0.0002	0.5914
	Trt*Chall	0.7063	0.0001	0.1660	0.8944
Age 48 days					
C	+	17.4[ab]	17.0[a]	61.4[a]	2.59[ab]
S	+	17.6[b]	17.0[a]	62.1[a]	2.61[b]
V	+	17.3[ac]	18.0[b]	60.4[b]	2.59[ab]
C	-	17.2[c]	17.7[b]	60.1[b]	2.57[a]
S	-	17.2[ac]	17.7[b]	60.2[b]	2.57[a]
V	-	17.2[ac]	17.9[b]	60.1[b]	2.58[ab]
	Trt	0.3312	0.0136	0.0333	0.6046
P value	Chall	0.0010	0.0139	<0.0001	0.0193
	Trt*Chall	0.3115	0.0883	0.0410	0.6339

C = Control; S = Salinomycin; V = Vaccinated; + = Challenged; - = Unchallenged; Protein (%) = Protein (g)/Body Weight (g) X 100; Fat (%) = Fat (g)/Body Weight (g) X 100; Water (%) = Water (g)/Body Weight (g) X 100; Ash (%) = Ash (g)/Body Weight (g) X 100; Trt=Treatment (C, S, V); Chall=Challenge (+, -)

TABLE 10: Heat Production (HP) per Metabolic Body Weight (HPMBW), Metabolizable Energy Consumption (MEc), and Heat Production per Kcal Metabolizable Energy (ME) Consumed (HPMEc) of Male Broilers During Challenge Periods

Treatment	Challenge	HPMB (kcal/kg$^{0.75}$)	MEc (kcal)	HPMEc
Age 20 days				
C	+	1120^{a}	1025^{a}	1.09^{a}
S	+	871^{b}	1198^{b}	0.73^{b}
V	+	968^{c}	1061^{a}	0.92^{c}
C	-	750^{bd}	1320^{c}	0.57^{d}
S	-	782^{d}	1320^{c}	0.60^{d}
V	-	795^{bd}	1231^{b}	0.65^{bd}
	Trt	0.0048	<0.0001	<0.0001
P value	Chall	<0.0001	<0.0001	<0.0001
	Trt*Chall	0.0001	<0.0001	<0.0001
Age 27 days				
C	+	1341^{a}	1869^{a}	0.72^{a}
S	+	1057^{bc}	2207^{bc}	0.48^{bcd}
V	+	1075^{bc}	2128^{b}	0.51^{bd}
C	-	1008^{b}	2253^{c}	0.45^{c}
S	-	1023^{b}	2241^{c}	0.46^{bc}
V	-	1148^{c}	2236^{c}	0.51^{d}
	Trt	0.0048	<0.0001	<0.0001
P value	Chall	0.0034	<0.0001	<0.0001
	Trt*Chall	<0.0001	<0.0001	<0.0001
Age 34 days				
C	+	1204^{a}	2318^{a}	0.52^{a}
S	+	824^{b}	2729^{b}	0.30^{bc}
V	+	916^{b}	2768^{bc}	0.33^{b}
C	-	853^{b}	2876^{d}	0.30^{bc}
S	-	832^{b}	2863^{cd}	0.29^{c}
V	-	911^{b}	2832^{cd}	0.32^{bc}
	Trt	<0.0001	<0.0001	<0.0001
P value	Chall	<0.0001	<0.0001	<0.0001
	Trt*Chall	<0.0001	<0.0001	<0.0001
Age 41 days				
C	+	1157^{a}	2301^{a}	0.51^{a}
S	+	993^{b}	2608^{b}	0.39^{b}
V	+	801^{c}	2980^{c}	0.27^{c}
C	-	747^{c}	3231^{d}	0.23^{c}
S	-	769^{c}	3316^{d}	0.23^{c}
V	-	794^{c}	3310^{d}	0.24^{c}
	Trt	0.0006	<0.0001	<0.0001
P value	Chall	<0.0001	<0.0001	<0.0001
	Trt*Chall	<0.0001	<0.0001	<0.0001
Age 48 days				
C	+	1167^{a}	2593^{a}	0.45^{a}
S	+	1151^{a}	2615^{a}	0.45^{a}
V	+	871^{b}	3361^{b}	0.26^{b}
C	-	828^{b}	3417^{b}	0.25^{b}
S	-	866^{b}	3240^{b}	0.28^{b}
V	-	880^{b}	3303^{b}	0.28^{b}
	Trt	0.2877	0.0079	0.0455
P value	Chall	0.0015	<0.0001	<0.0001
	Trt*Chall	0.0769	0.0012	0.0063

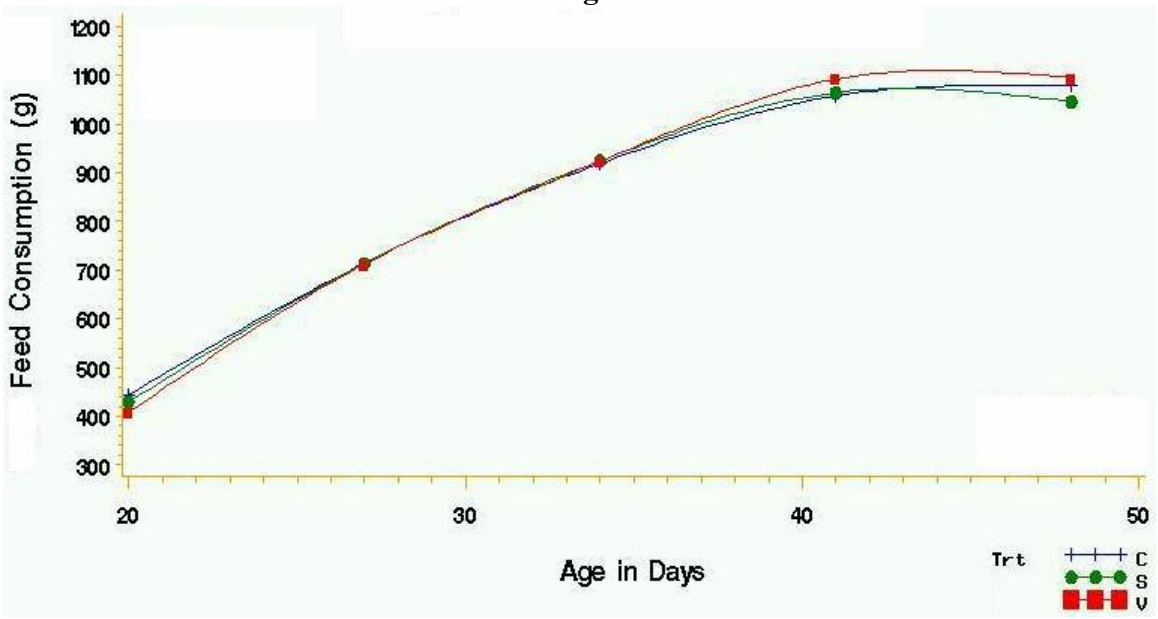
C = Control; S = Salinomycin; V = Vaccinated; + = Challenged; - = Unchallenged; HPMB= HP composition (HPc) /weight (kilograms)$^{0.75}$; MEc=ME consumed; HPMEc=HPc/MEc; Trt=Treatment (C, S, V); Chall=Challenge (+, -); kcal=kilocalorie

TABLE 11: Metabolizable Energy Consumption Reduction Due to Challenge (MEcr), Maintenance Energy Increase Due to Challenge (MC), and Total Caloric Cost of Challenge (TCC) of Male Broilers During Challenge Periods

Treatment		MEcr (kcal)	MC (kcal)	TCC (kcal)
Age 20 days				
C		337±300[a]	280±308[a]	617±466[a]
S		121±300[a]	73±308[a]	194±466[a]
V		181±300[a]	158±308[a]	339±466[a]
Age 27 days				
C		335±300[a]	445±308[a]	780±466[a]
S		-15±300[b]	80±308[b]	65±466[b]
V		109±300[ab]	-76±308[b]	33±466[b]
Age 34 days				
C		560±300[a]	71±3082[a]	1272±466[a]
S		132±300[b]	-33±308[b]	99±466[b]
V		22±300[b]	11±308[b]	33±466[b]
Age 41 days				
C		916±300[a]	1080±308[a]	1996±466[a]
S		786±300[a]	613±308[b]	1399±466[b]
V		222±300[b]	-72±308[c]	150±466[c]
Age 48 days				
C		780±300[a]	460±308[ab]	1240±466[a]
S		511±300[a]	711±308[a]	1222±466[a]
V		113±300[b]	269±308[b]	382±466[b]
P value	Trt	<0.0001	<0.0001	<0.0001
	Age	<0.0001	<0.0001	<0.0001
	Trt*Age	0.0215	<0.0001	<0.0001

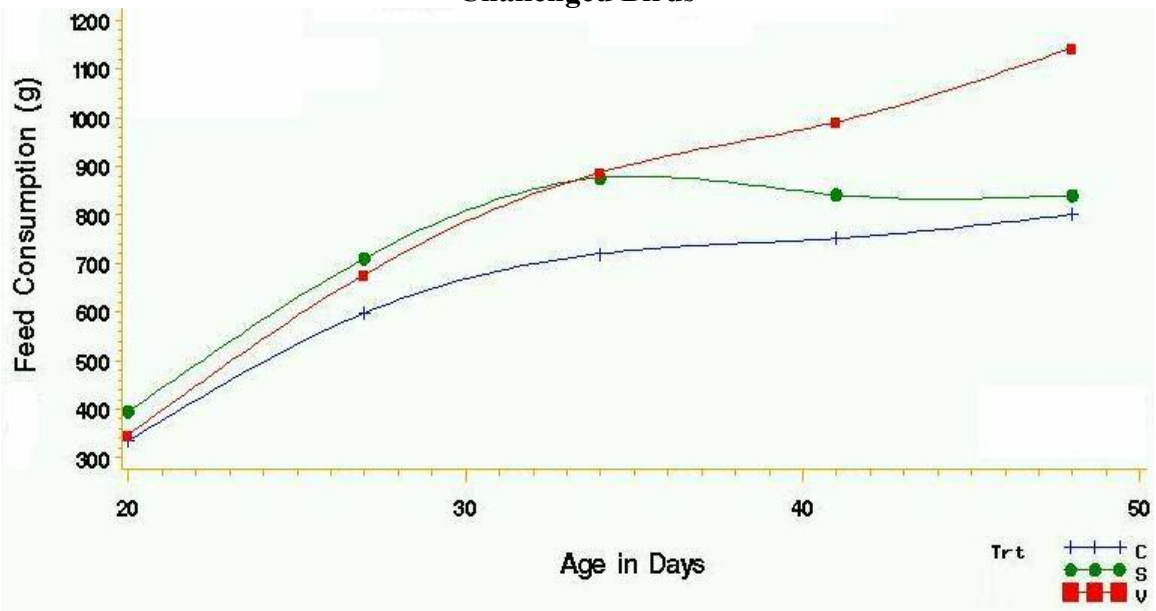
C = Control; S = Salinomycin; V = Vaccinated; MEcr=Metabolizable energy consumed by unchallenged bird – Metabolizable energy consumed by challenged Bird; MC=(Metabolizable energy consumed - (protein gain*5.65)/0.67 + (fat gain*9.3)/0.87)) – body weight$^{0.75}$ X 110 X 6) of challenged bird - (Metabolizable energy consumed - (protein gain*5.65)/0.67 + (fat gain*9.3)/0.87)) – body weight$^{0.75}$ X 110 X 6) of unchallenged bird; TCC=MEcr + MC; Trt= Treatment (C, S, V)

FIGURE 1: Treatment Effects on Challenge Period Feed Consumption Per Bird of Unchallenged Birds



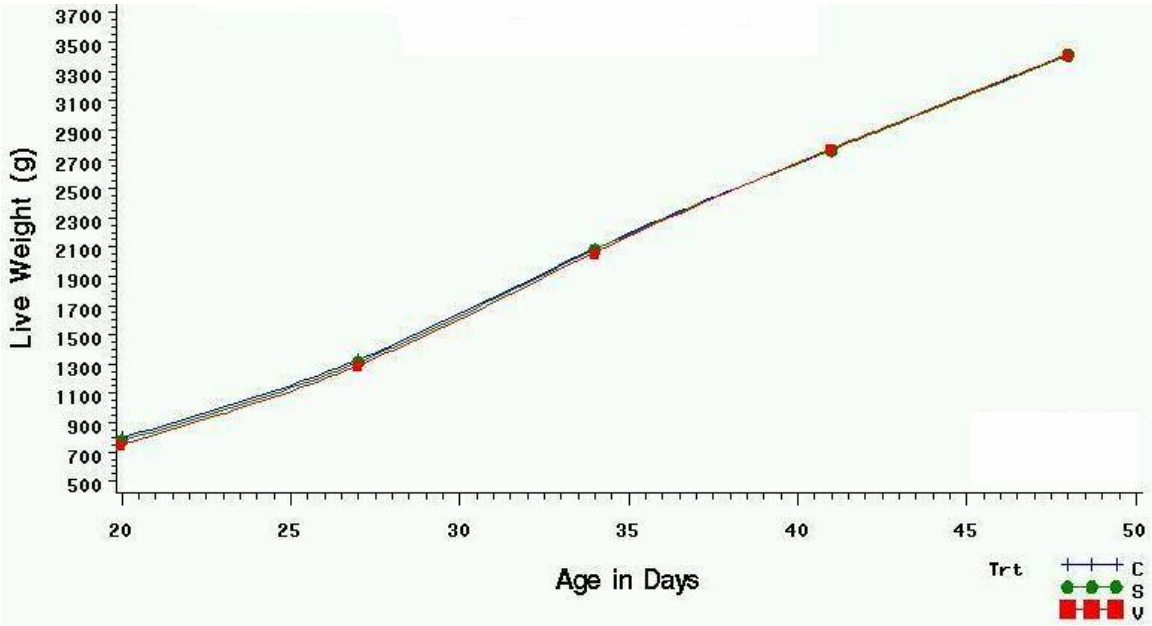
Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; g=grams

FIGURE 2: Treatment Effects on Challenge Period Feed Consumption Per Bird of Challenged Birds



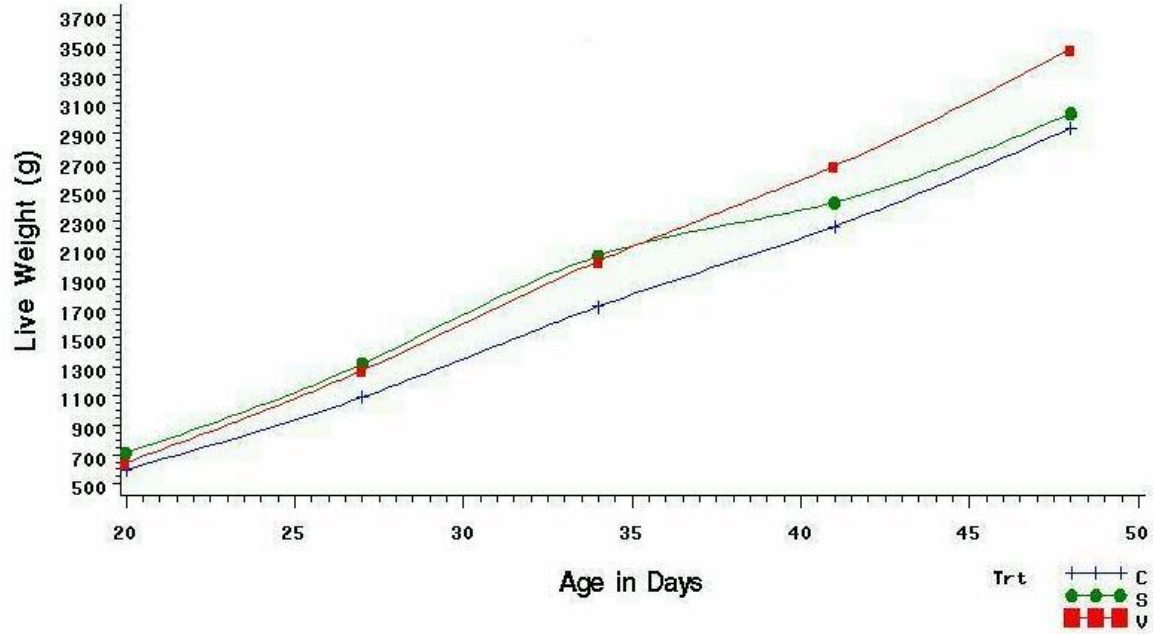
Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; g=grams

FIGURE 3: Treatment Effects on Challenge Period Live Weight of Unchallenged Birds



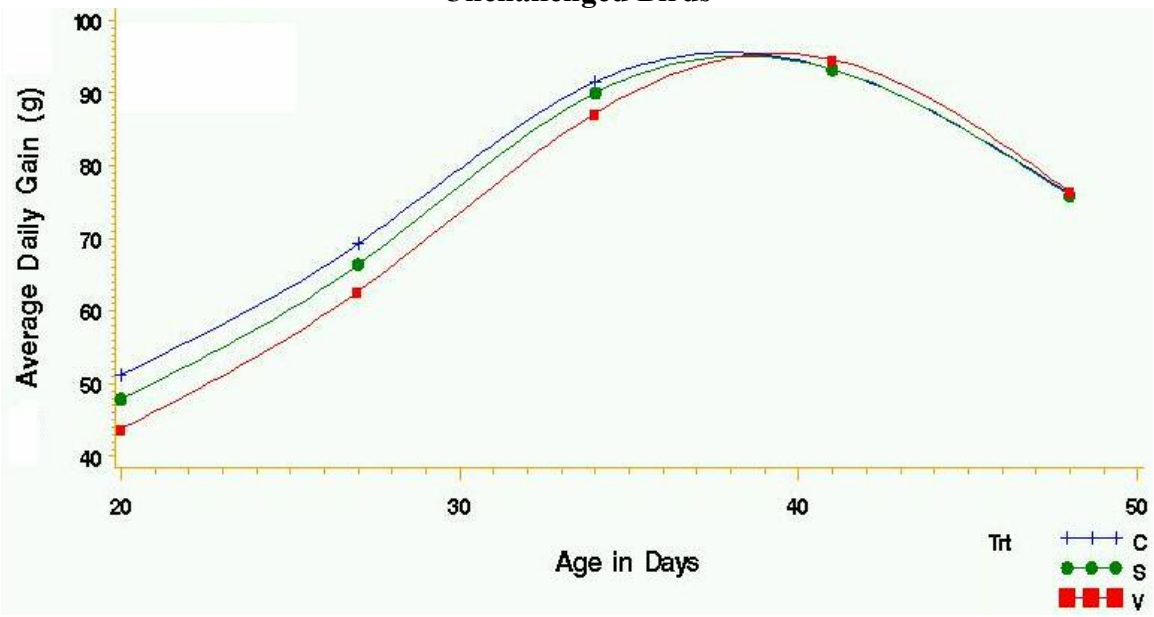
Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; g=grams

FIGURE 4: Treatment Effects on Challenge Period Live Weight of Challenged Birds



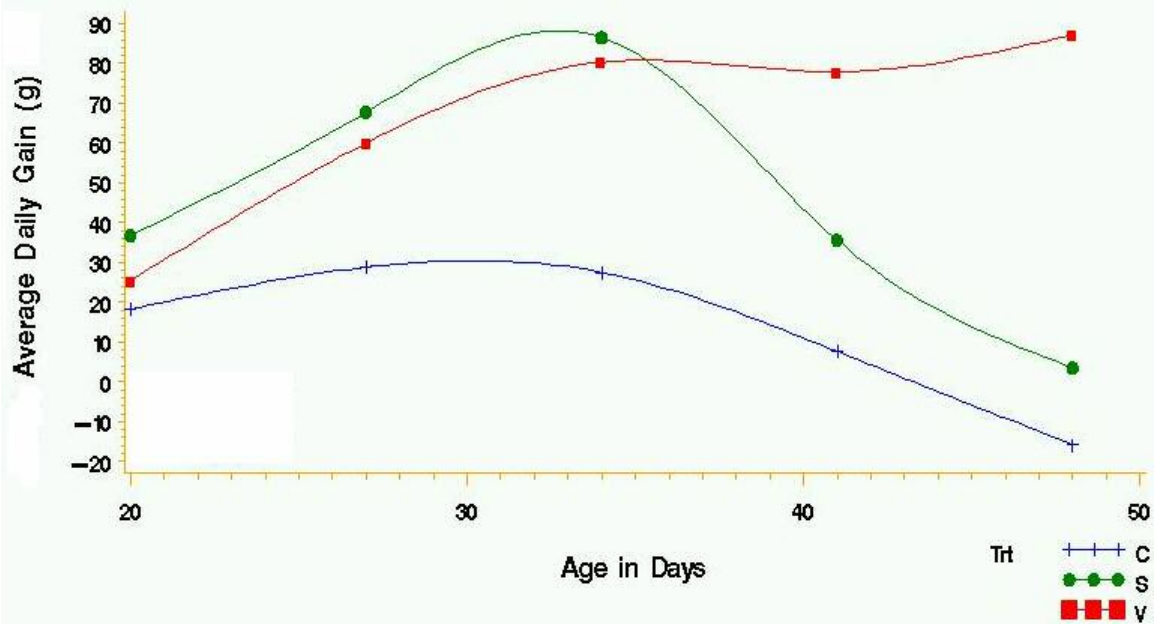
Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; g=grams

FIGURE 5: Treatment Effects on Challenge Period Average Daily Gain of Unchallenged Birds



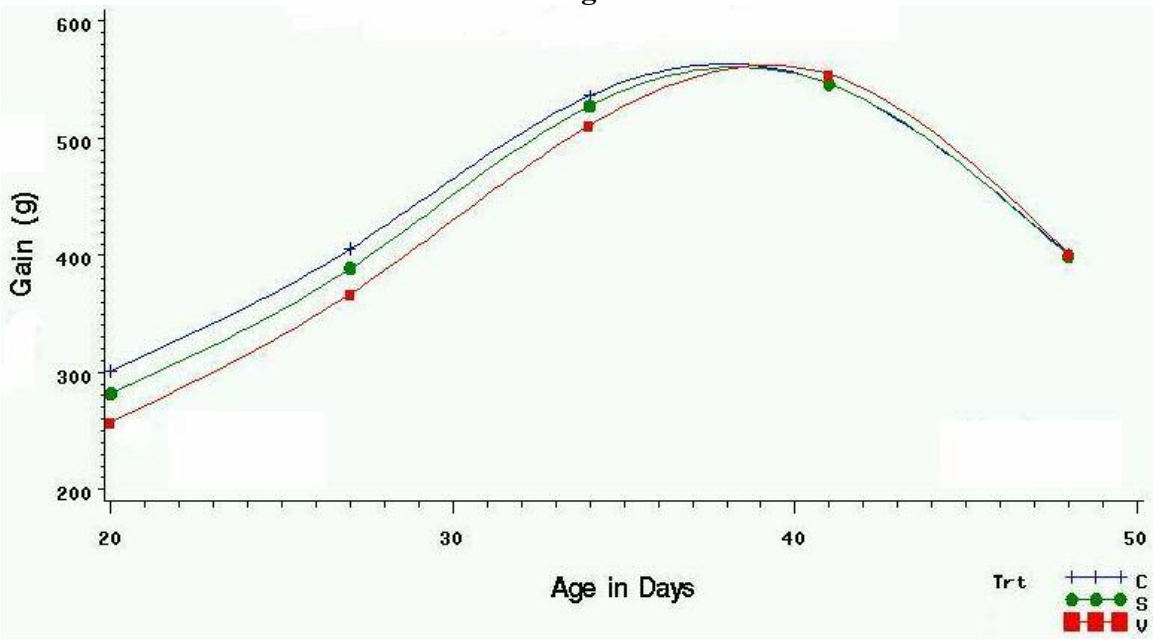
Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; g=grams

FIGURE 6: Treatment Effects on Challenge Period Average Daily Gain of Challenged Birds



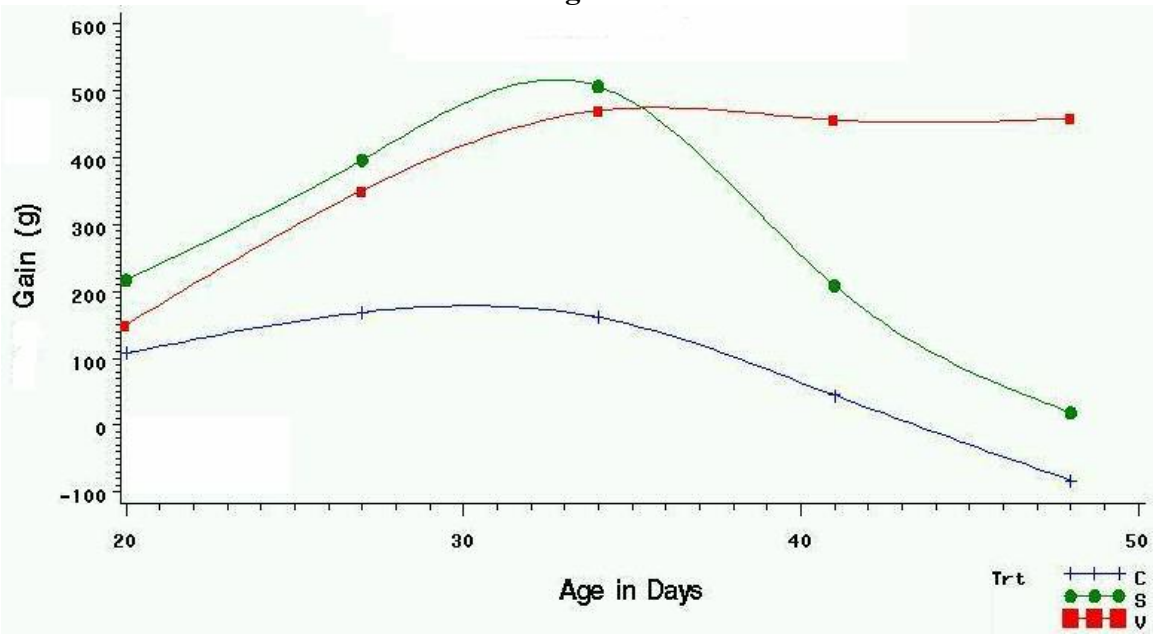
Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; g=grams

FIGURE 7: Treatment Effects on Challenge Period Body Weight Gain of Unchallenged Birds



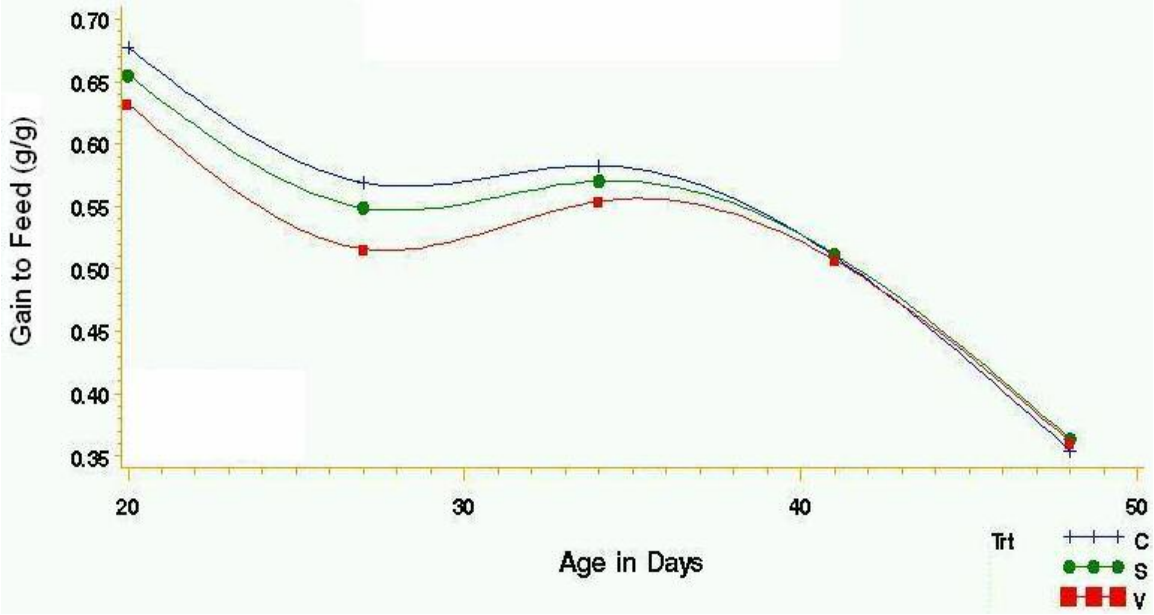
Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; g=grams

FIGURE 8: Treatment Effects on Challenge Period Body Weight Gain of Challenged Birds



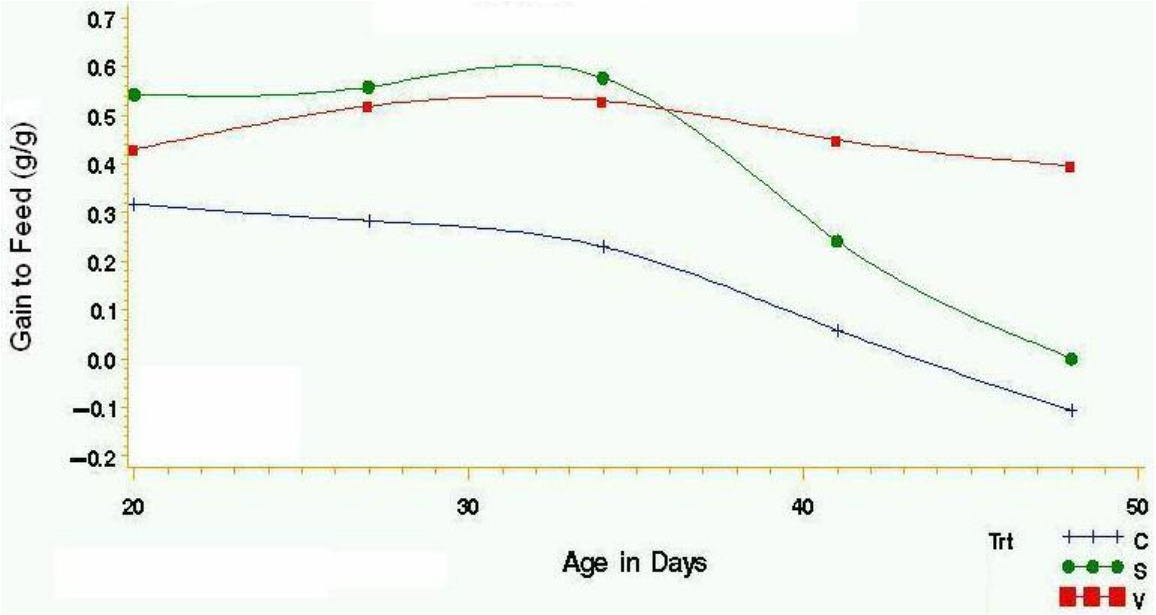
Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; g=grams

FIGURE 9: Treatment Effects on Challenge Period Feed Efficiency of Unchallenged Birds



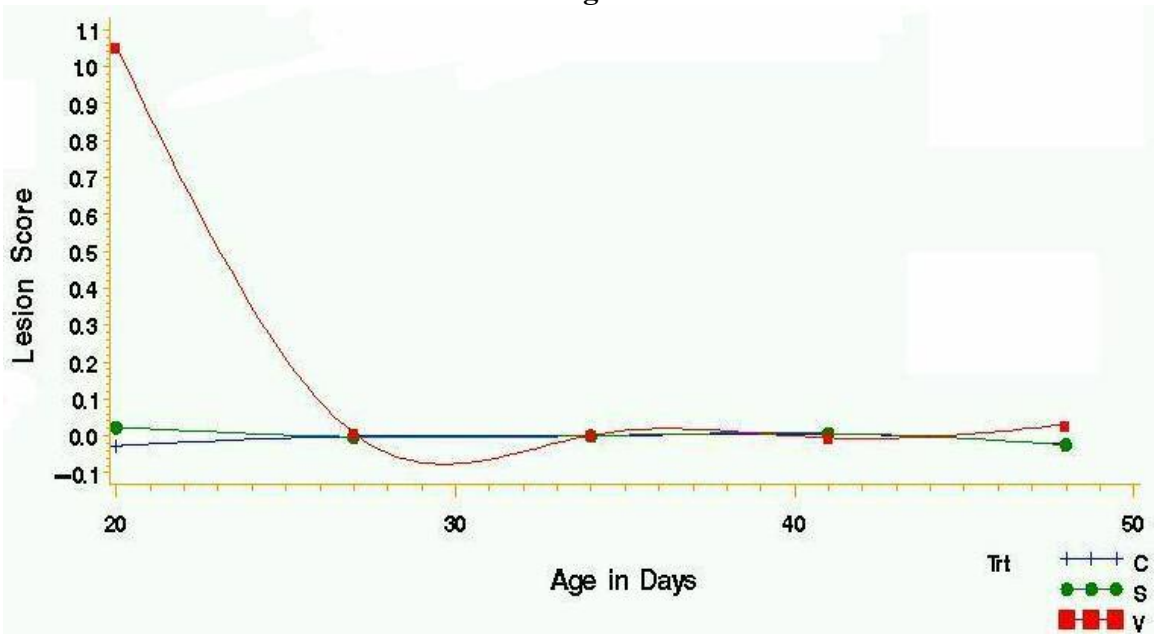
Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; Gain to Feed (g/g)=Body weight gain in grams/Feed consumption in grams; g=grams

FIGURE 10: Treatment Effects on Challenge Period Feed Efficiency of Challenged Birds



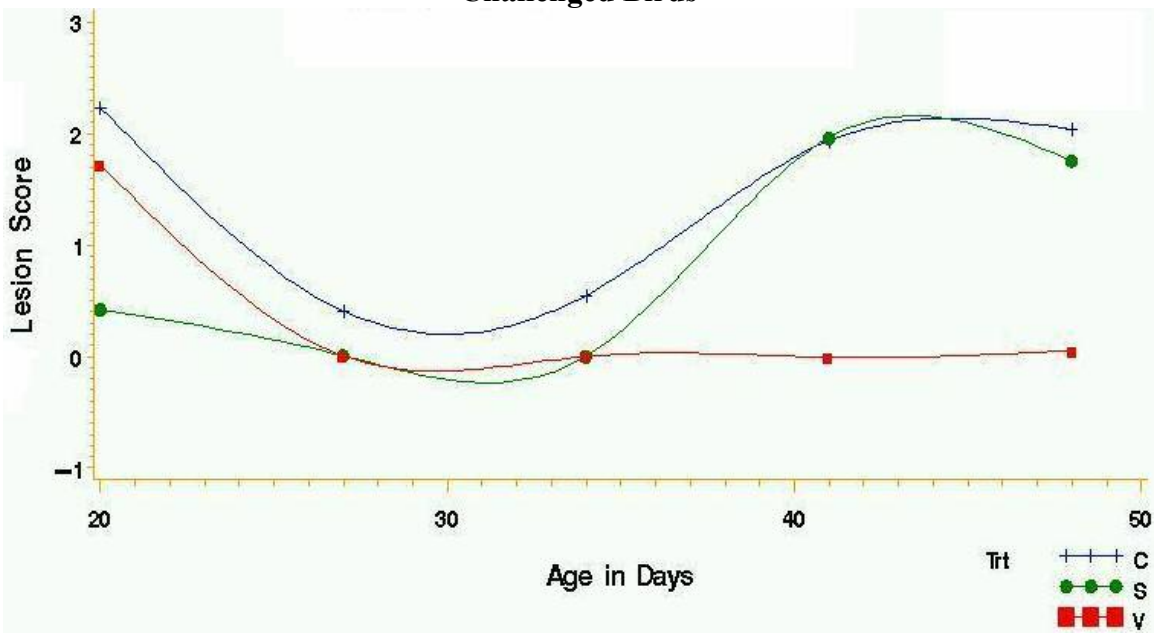
Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; Gain to Feed (g/g)=Body weight gain in grams/Feed consumption in grams; g=grams

FIGURE 11: Treatment Effects on Upper Small Intestine Lesion Score of Unchallenged Birds



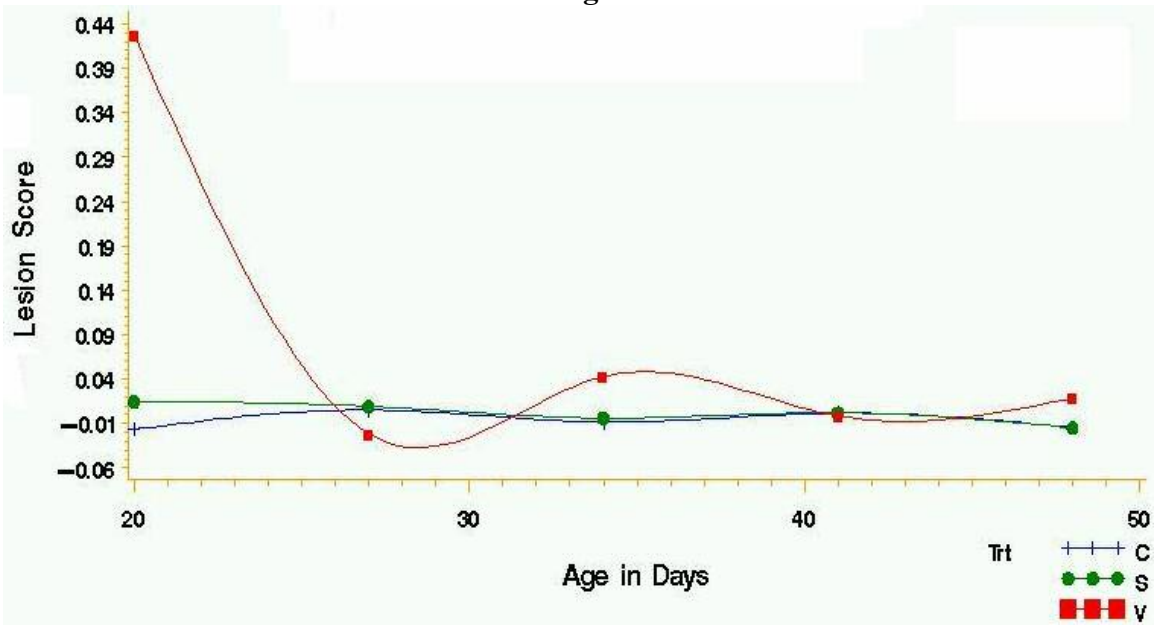
Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; Lesion scores range from 0 (no lesions) to 4 (many lesions)

FIGURE 12: Treatment Effects on Upper Small Intestine Lesion Score of Challenged Birds



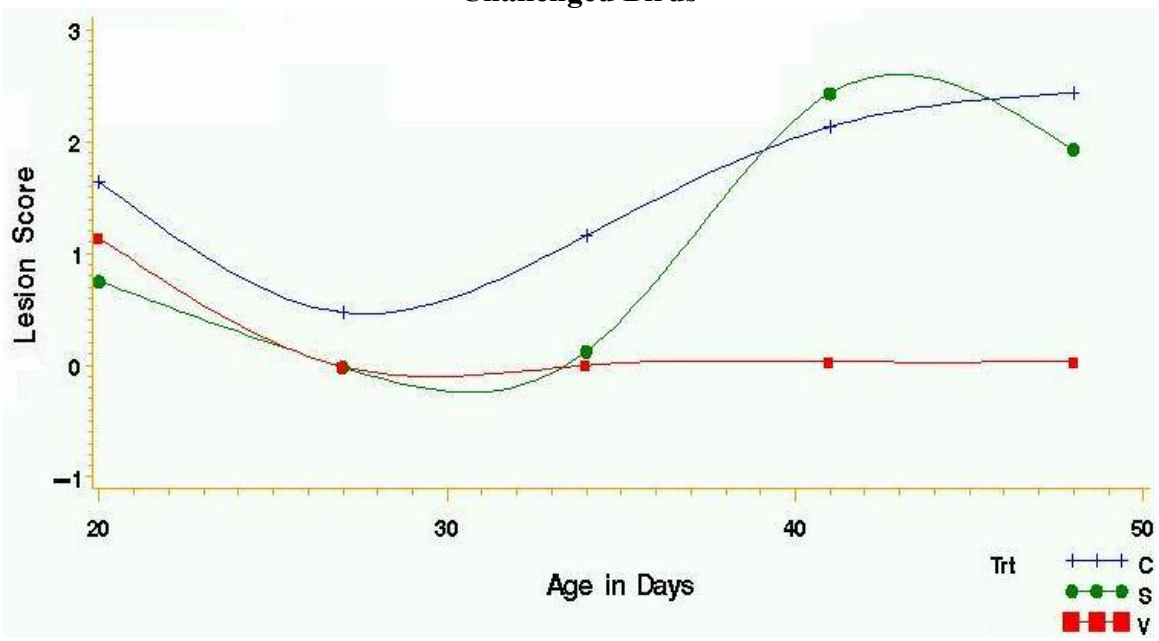
Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; Lesion scores range from 0 (no lesions) to 4 (many lesions)

FIGURE 13: Treatment Effects on Middle Small Intestine Lesion Score of Unchallenged Birds



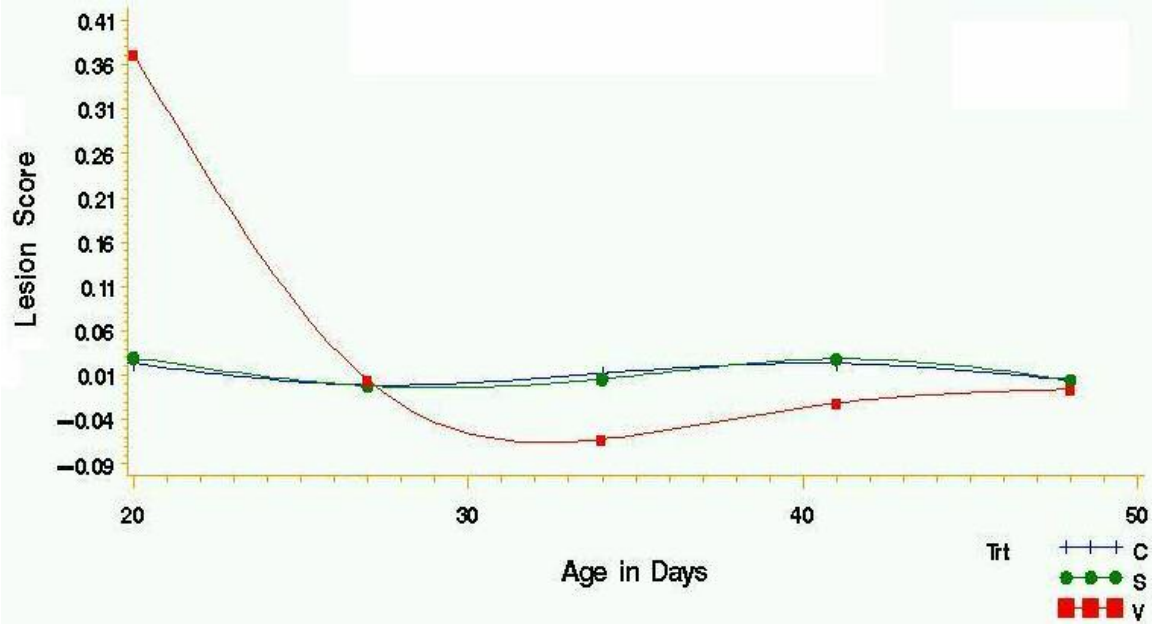
Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; Lesion scores range from 0 (no lesions) to 4 (many lesions)

FIGURE 14: Treatment Effects on Middle Small Intestine Lesion Score of Challenged Birds



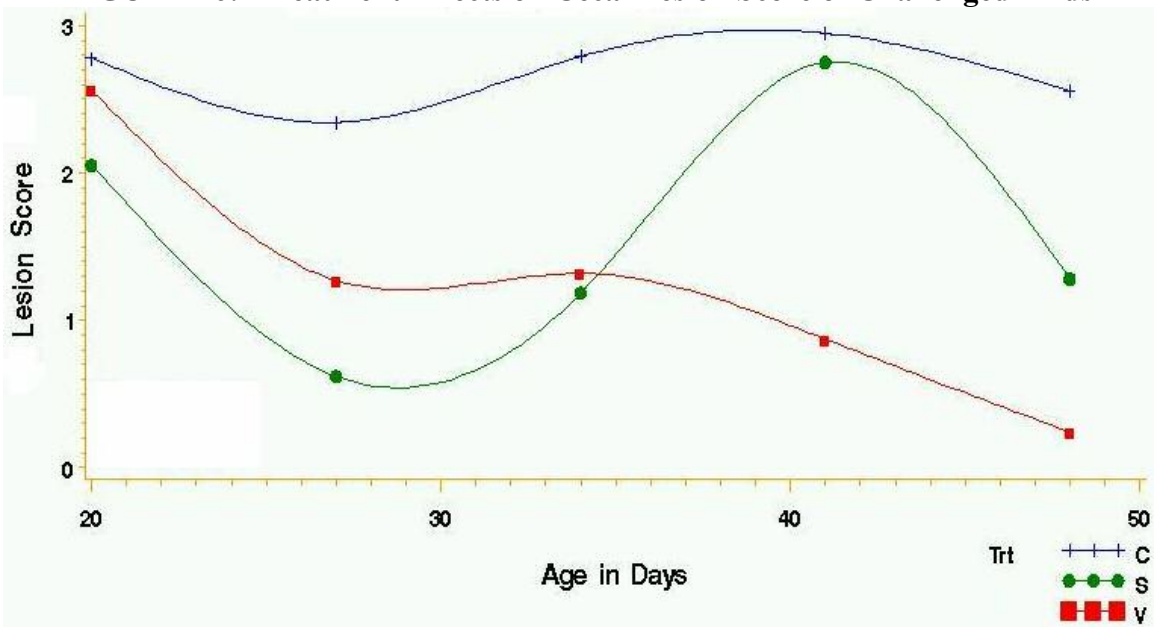
Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; Lesion scores range from 0 (no lesions) to 4 (many lesions)

FIGURE 15: Treatment Effects on Cecal Lesion Score of Unchallenged Birds



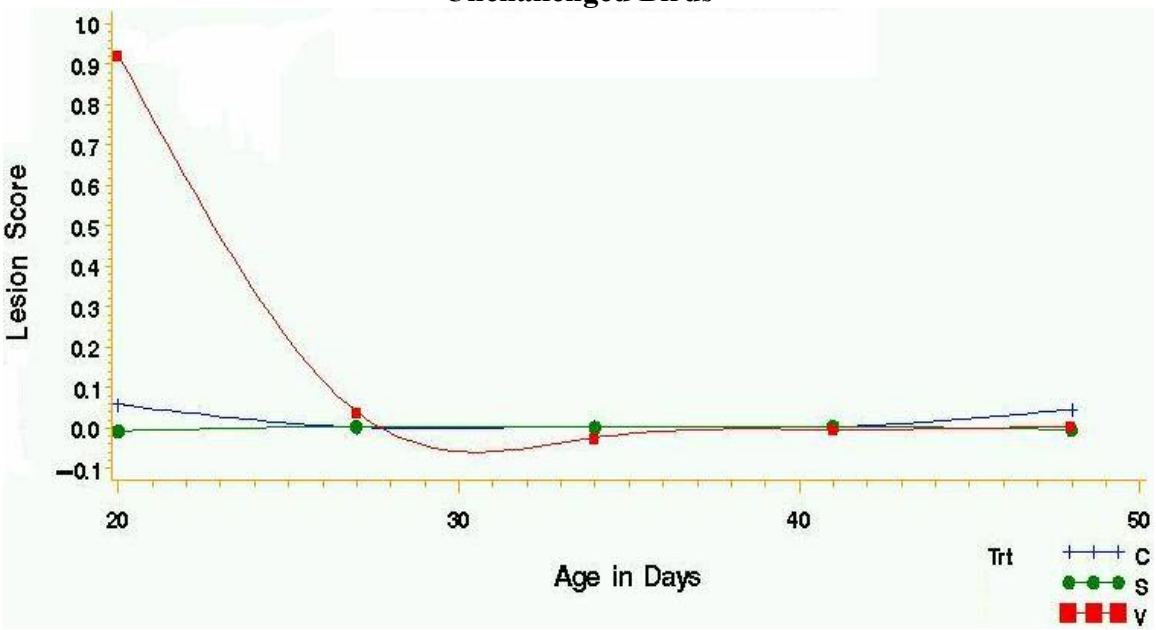
Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; Lesion scores range from 0 (no lesions) to 4 (many lesions)

FIGURE 16: Treatment Effects on Cecal Lesion Score of Challenged Birds



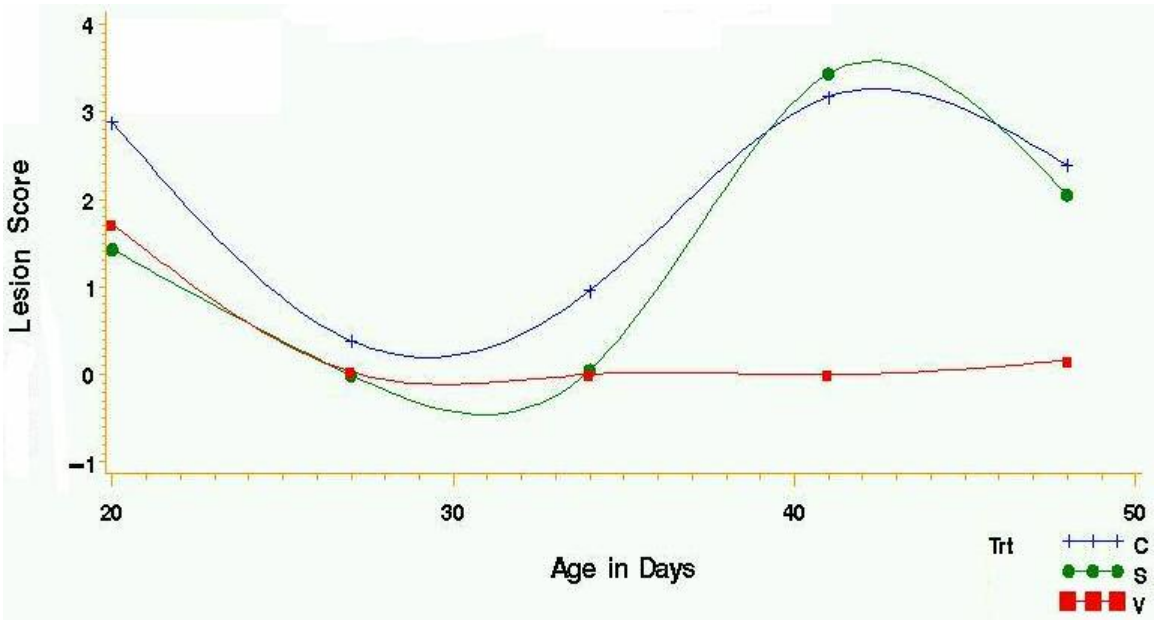
Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; Lesion scores range from 0 (no lesions) to 4 (many lesions)

FIGURE 17: Treatment Effects on *Eimeria Acervulina* Lesion Score of Unchallenged Birds



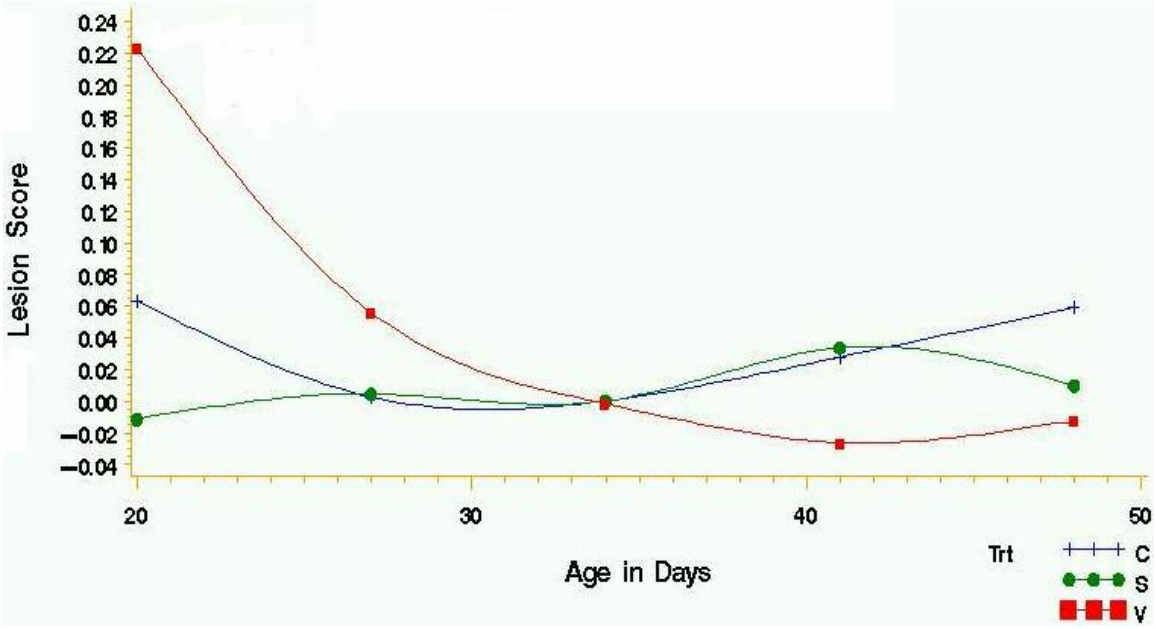
Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; Lesion scores range from 0 (no lesions) to 4 (many lesions)

FIGURE 18: Treatment Effects on *Eimeria Acervulina* Lesion Score of Challenged Birds



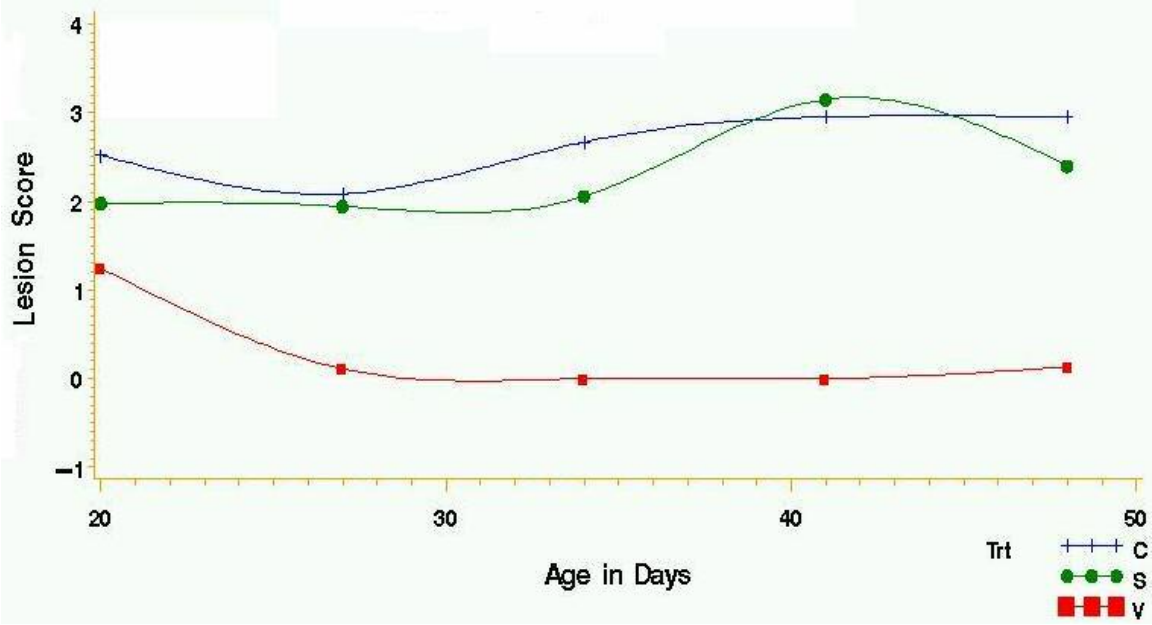
Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; Lesion scores range from 0 (no lesions) to 4 (many lesions)

FIGURE 19: Treatment Effects on *Eimeria Maxima* Lesion Score of Unchallenged Birds



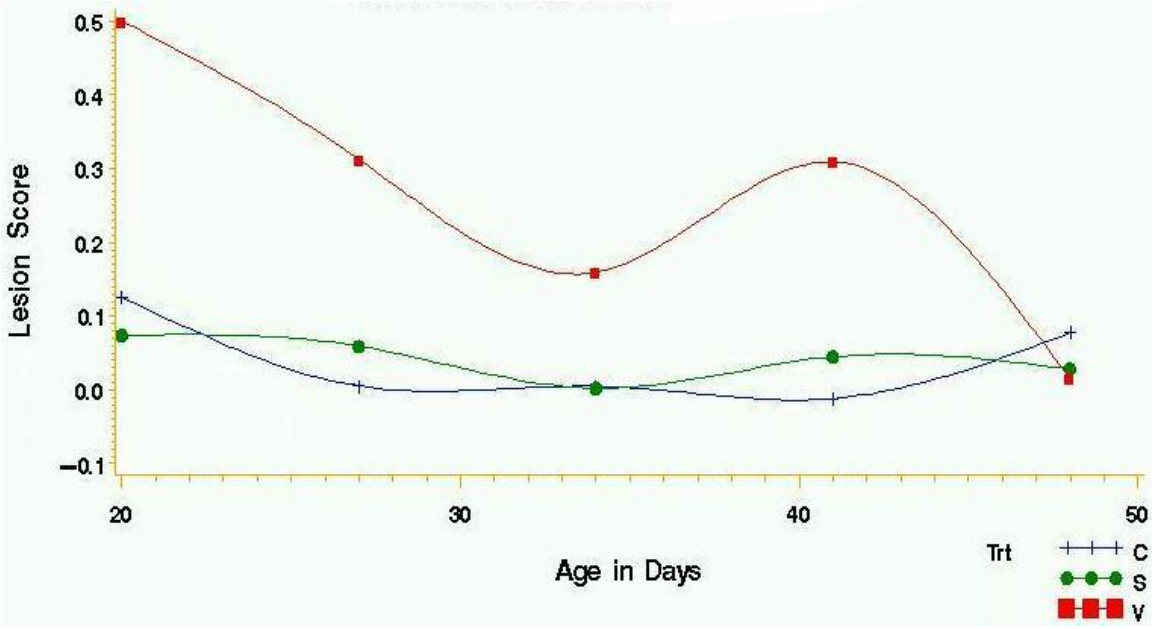
Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; Lesion scores range from 0 (no lesions) to 4 (many lesions)

FIGURE 20: Treatment Effects on *Eimeria Maxima* Lesion Score of Challenged Birds



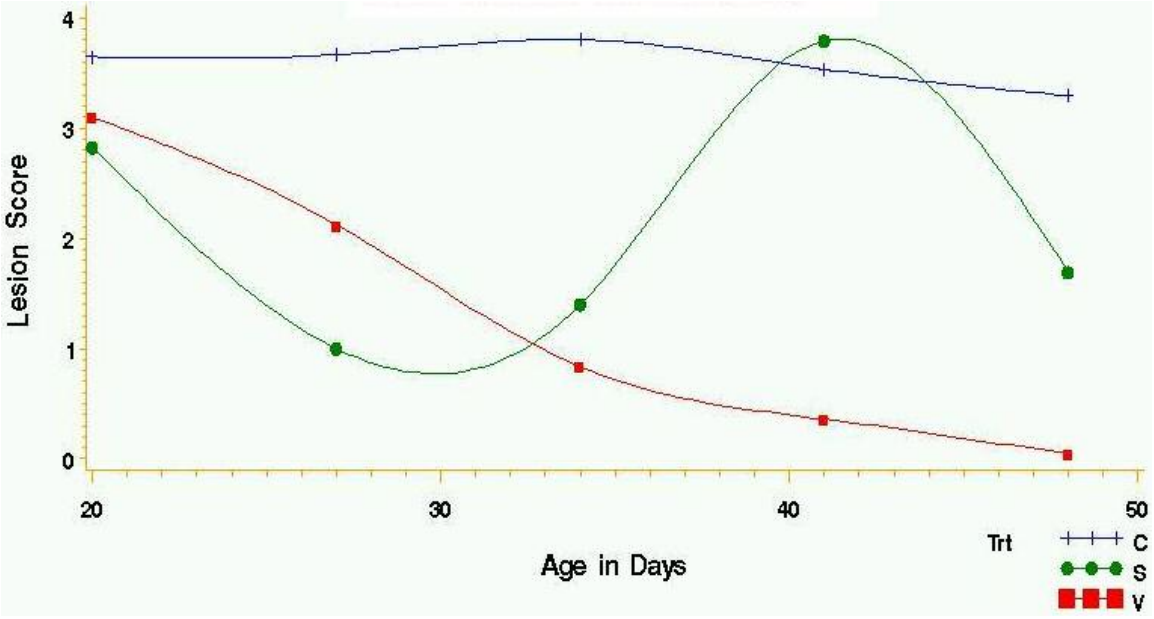
Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; Lesion scores range from 0 (no lesions) to 4 (many lesions)

FIGURE 21: Treatment Effects on *Eimeria Tenella* Lesion Score of Unchallenged Birds



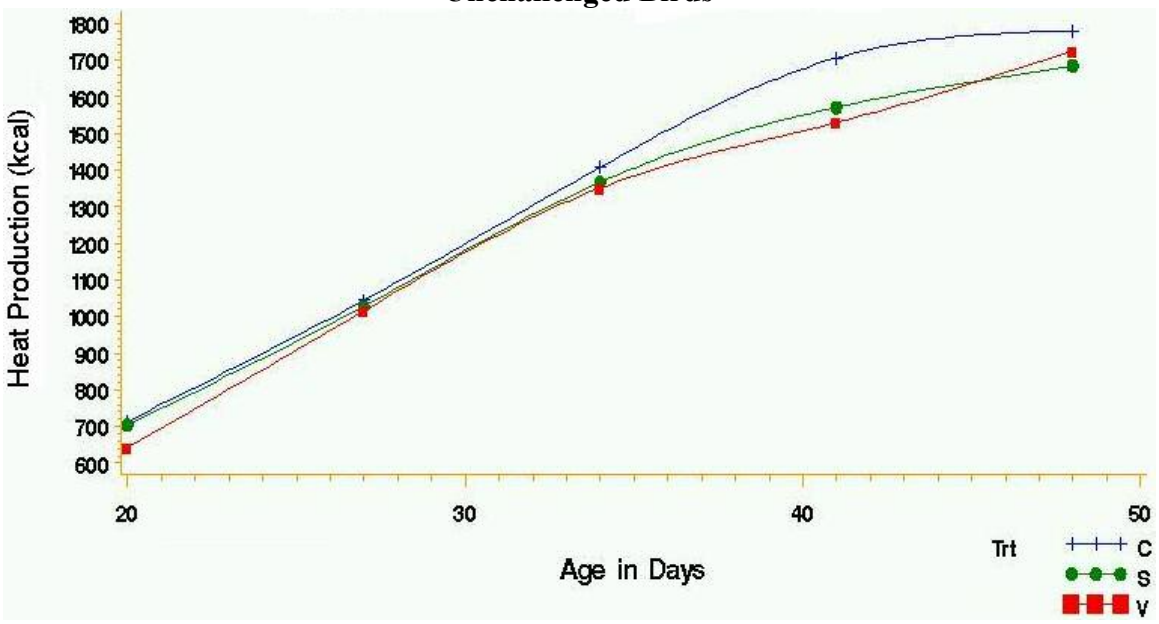
Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; Lesion scores range from 0 (no lesions) to 4 (many lesions)

FIGURE 22: Treatment Effects on *Eimeria Tenella* Lesion Score of Challenged Birds



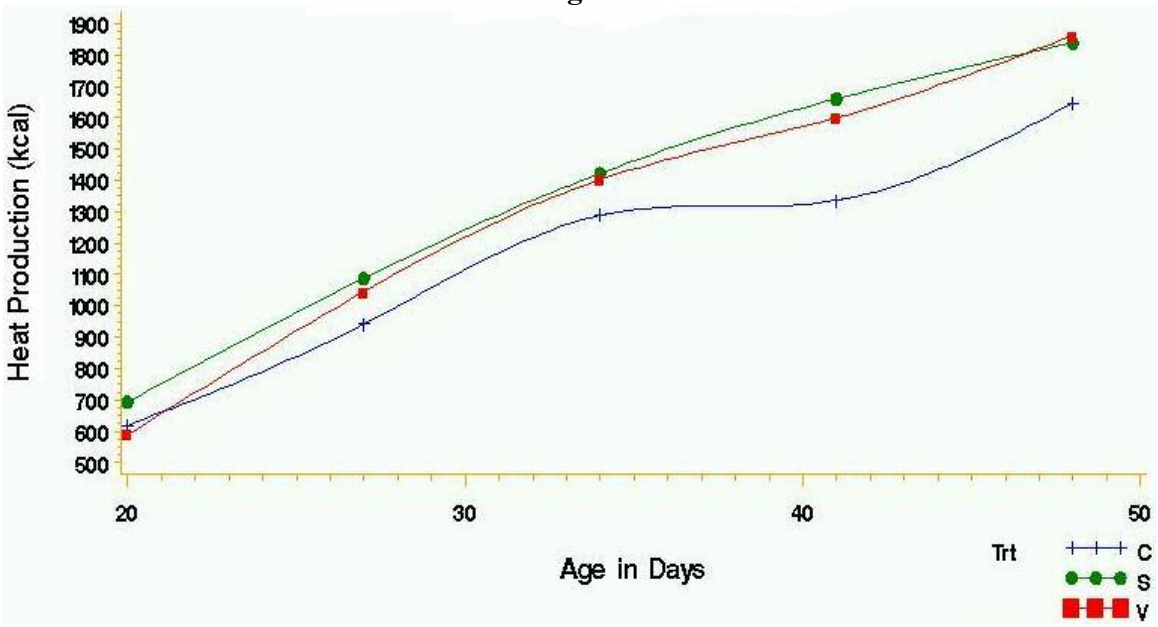
Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; Lesion scores range from 0 (no lesions) to 4 (many lesions)

FIGURE 23: Treatment Effects on Total Challenge Period Heat Production of Unchallenged Birds



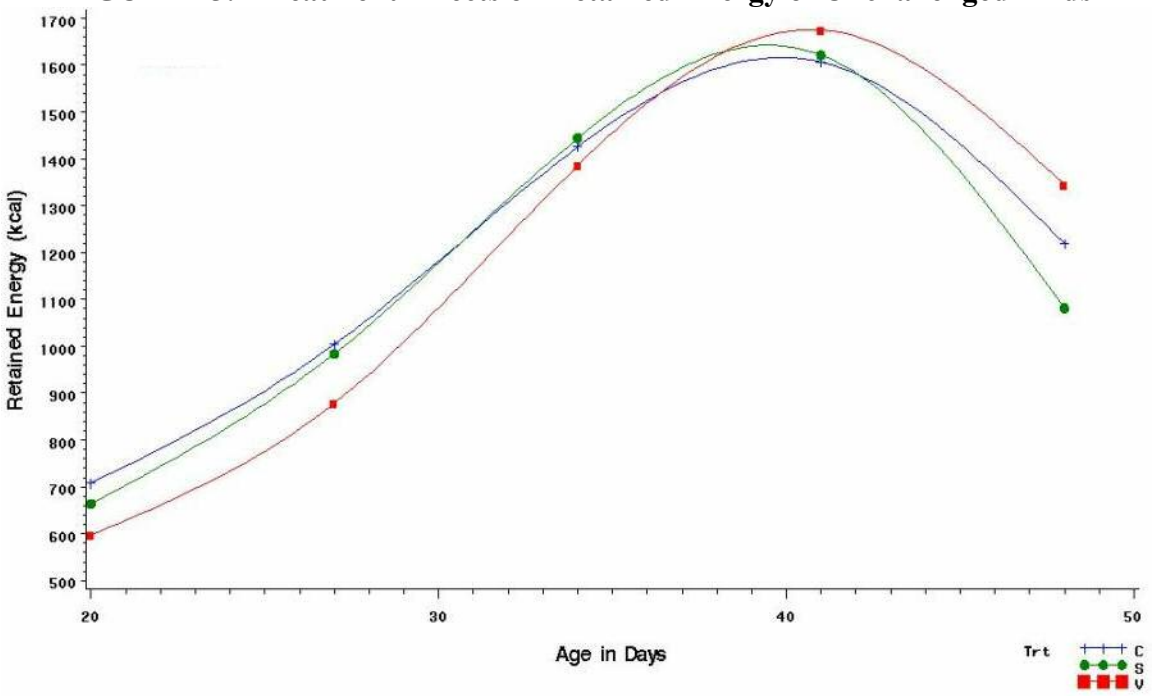
Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; kcal=kilocalories

FIGURE 24: Treatment Effects on Total Challenge Period Heat Production of Challenged Birds



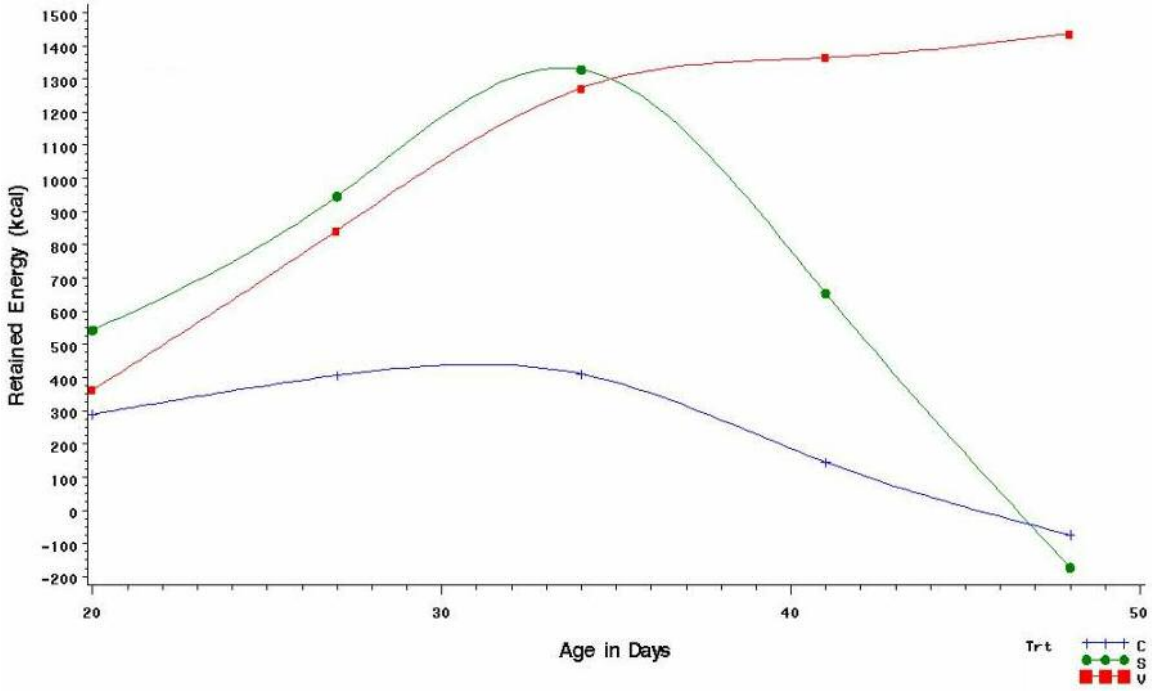
Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; kcal=kilocalories

FIGURE 25: Treatment Effects on Retained Energy of Unchallenged Birds



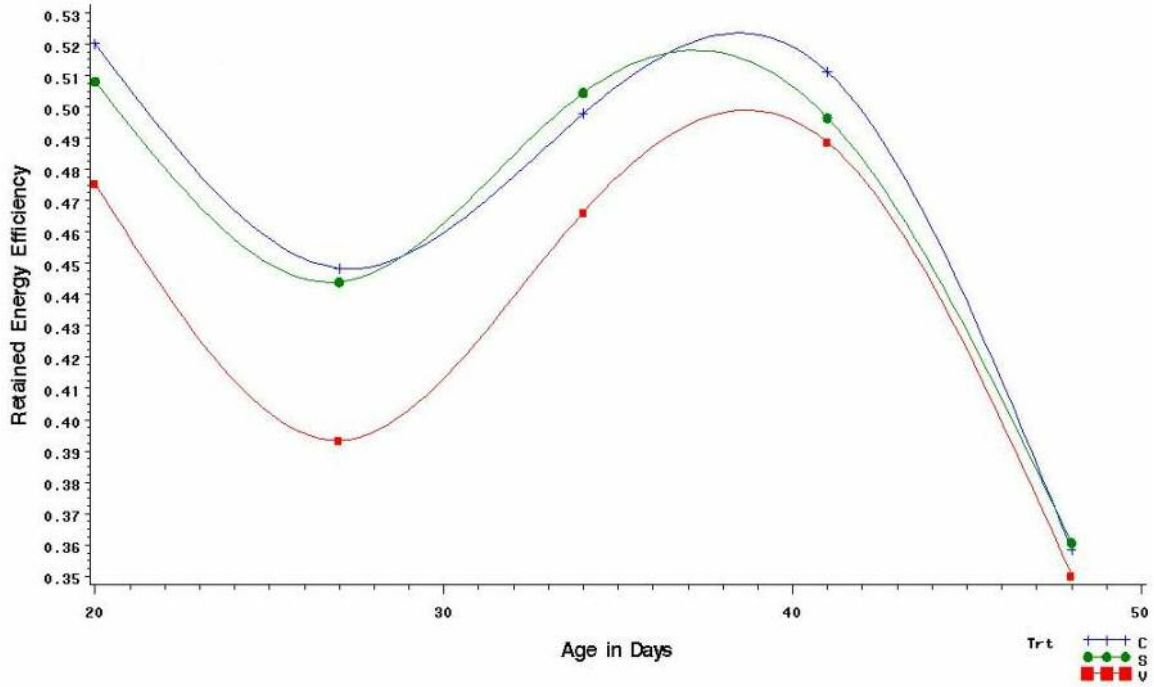
Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; kcal=kilocalories

FIGURE 26: Treatment Effects on Retained Energy of Challenged Birds



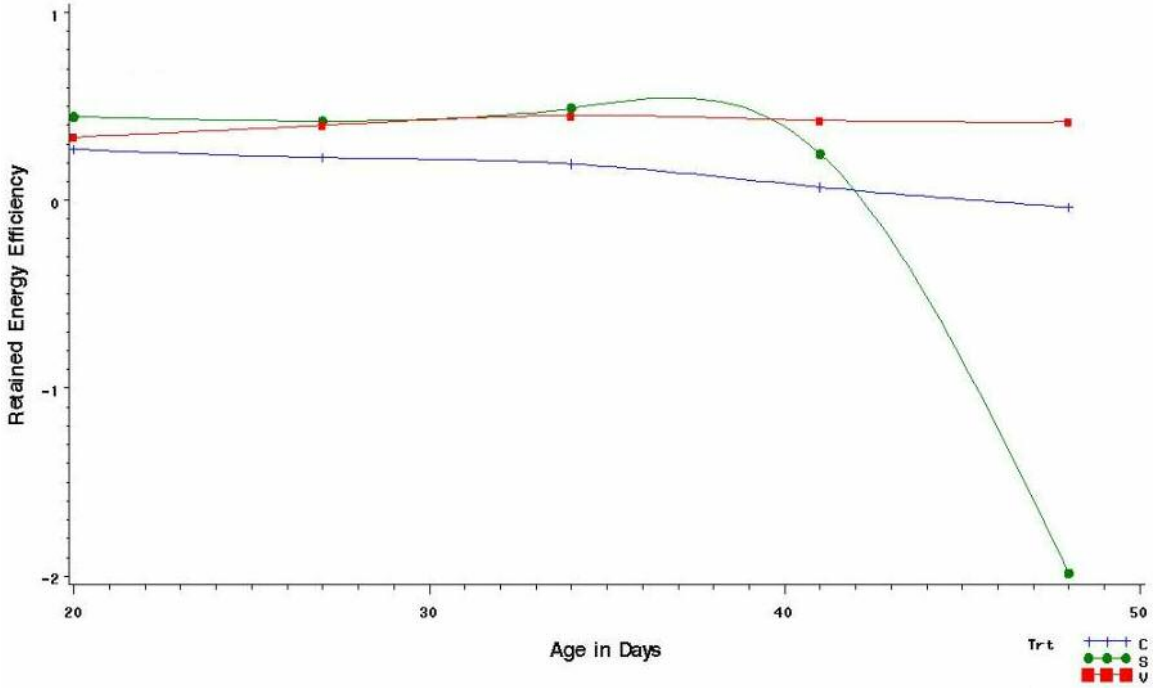
Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; kcal=kilocalories

FIGURE 27: Treatment Effects on Retained Energy Efficiency of Unchallenged Birds



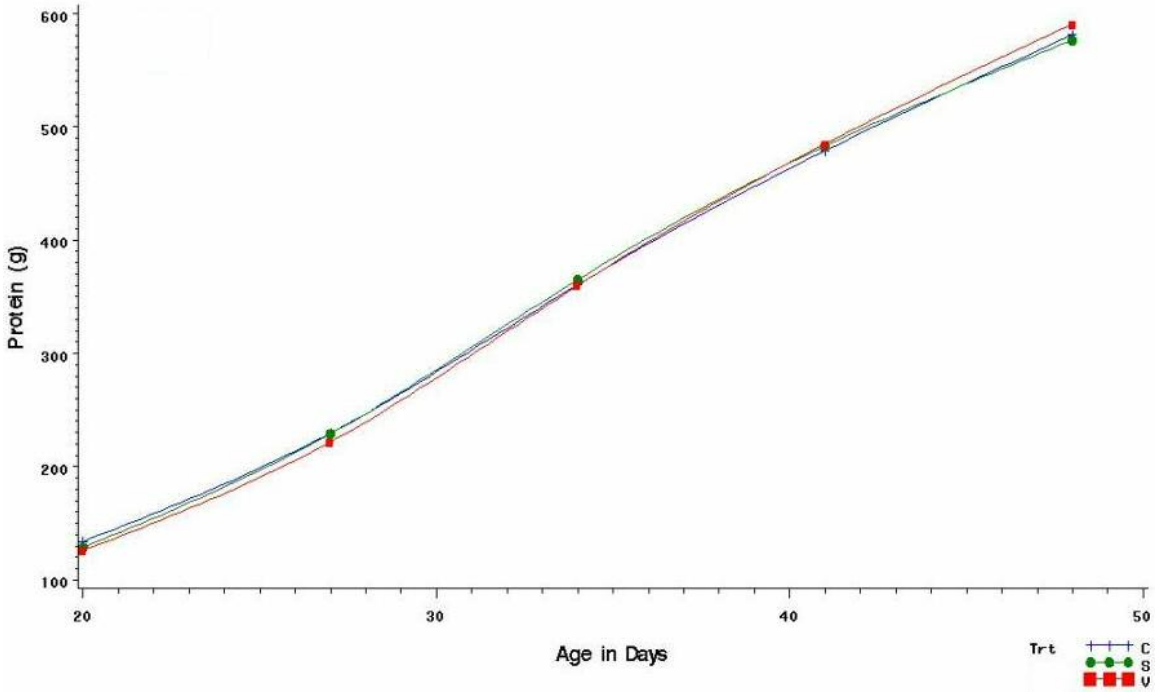
Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; Retained Energy Efficiency=Retained Energy/Metabolizable Energy consumed

FIGURE 28: Treatment Effects on Retained Energy Efficiency of Challenged Birds



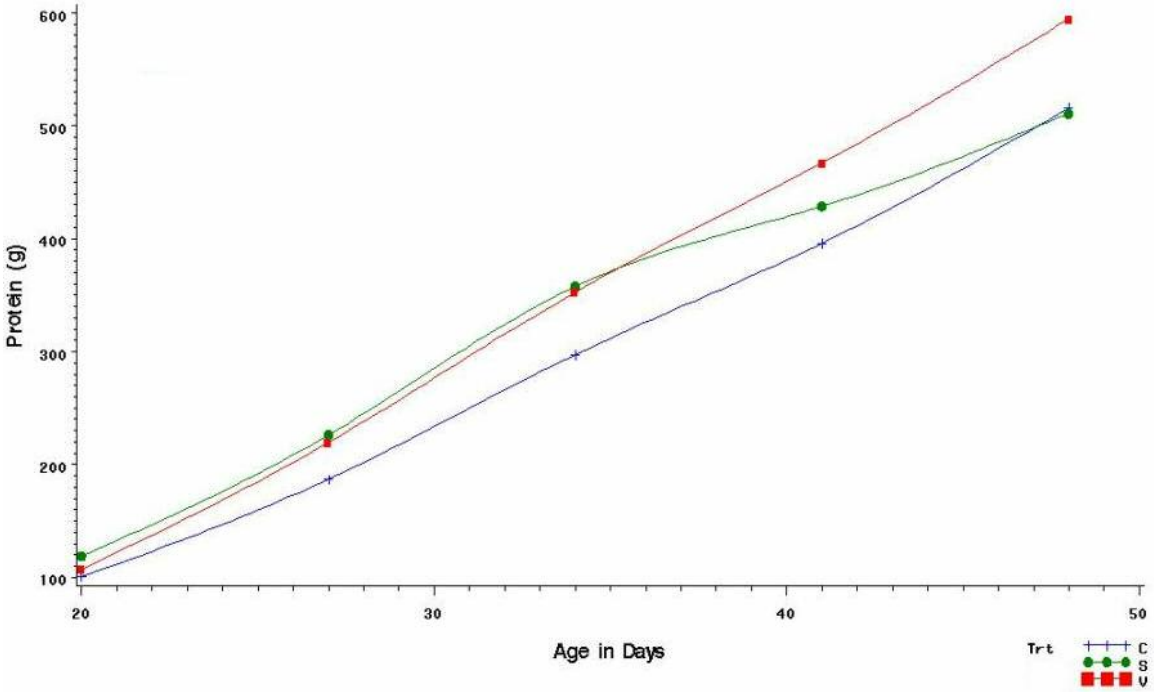
Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; Retained Energy Efficiency=Retained Energy/Metabolizable Energy consumed

FIGURE 29: Treatment Effects on Body Protein of Unchallenged Birds



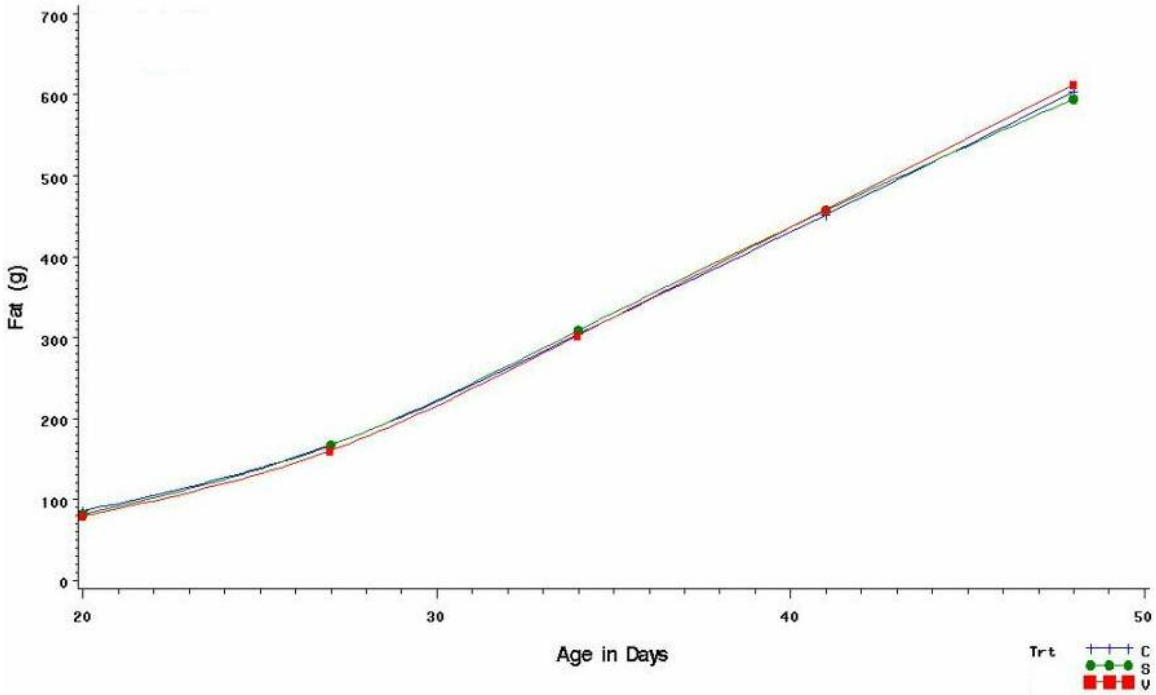
Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; g=grams

FIGURE 30: Treatment Effects on Body Protein of Challenged Birds



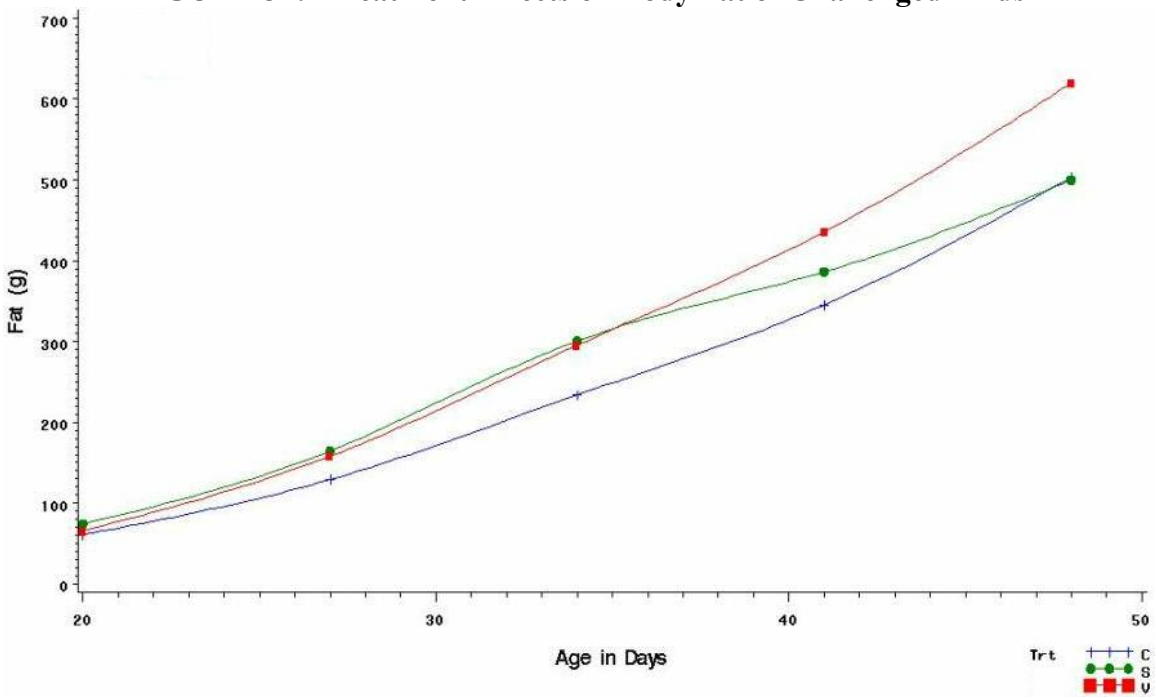
Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; g=grams

FIGURE 31: Treatment Effects on Body Fat of Unchallenged Birds



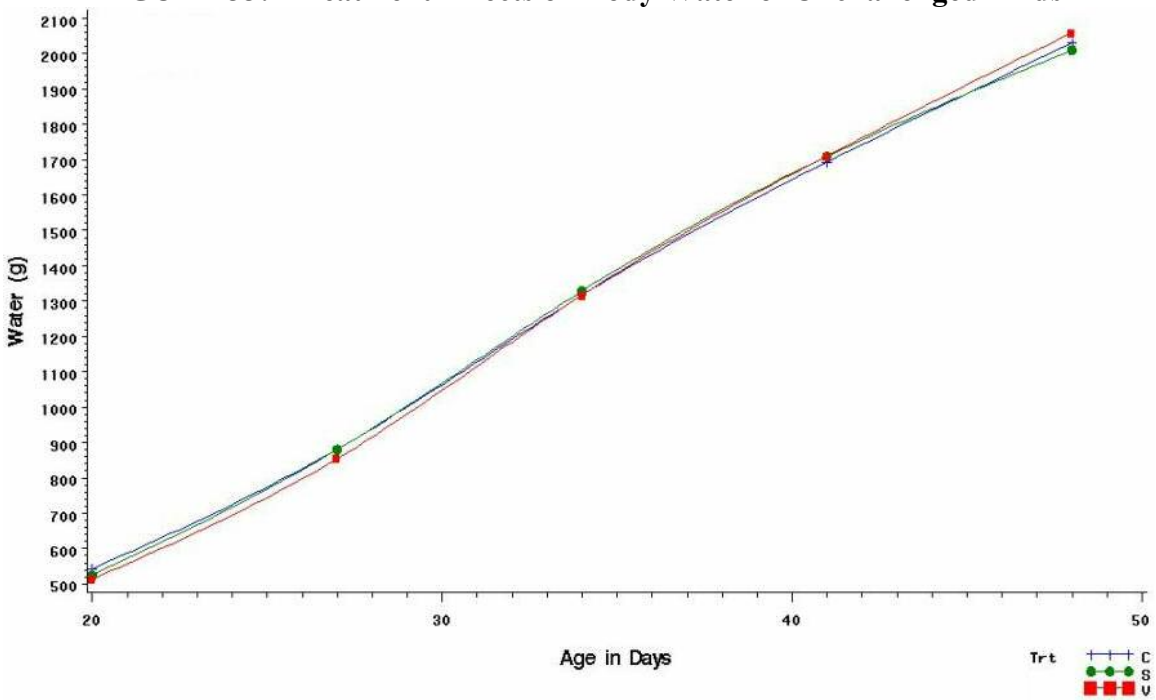
Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; g=grams

FIGURE 32: Treatment Effects on Body Fat of Challenged Birds



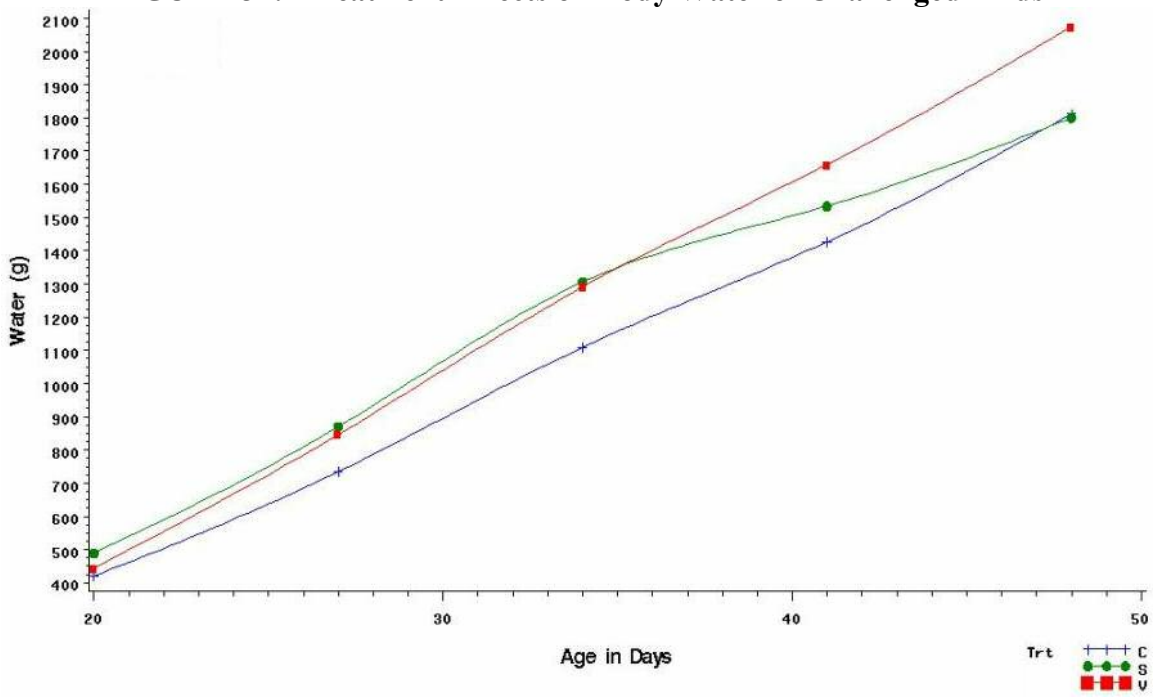
Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; g=grams

FIGURE 33: Treatment Effects on Body Water of Unchallenged Birds



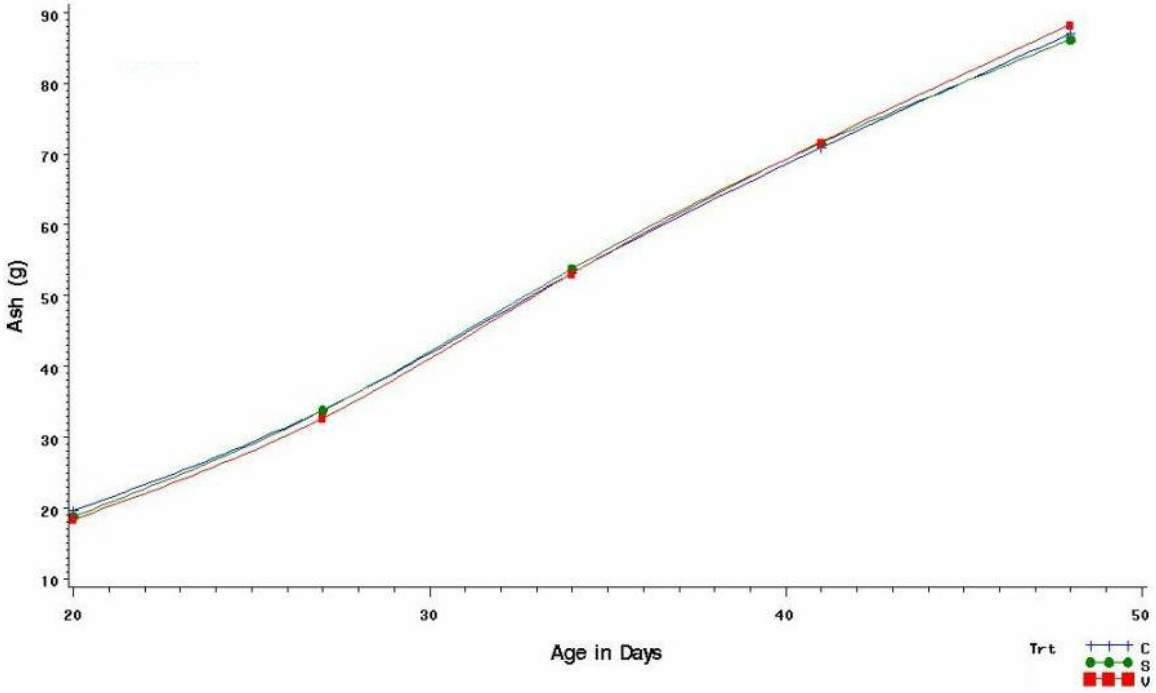
Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; g=grams

FIGURE 34: Treatment Effects on Body Water of Challenged Birds



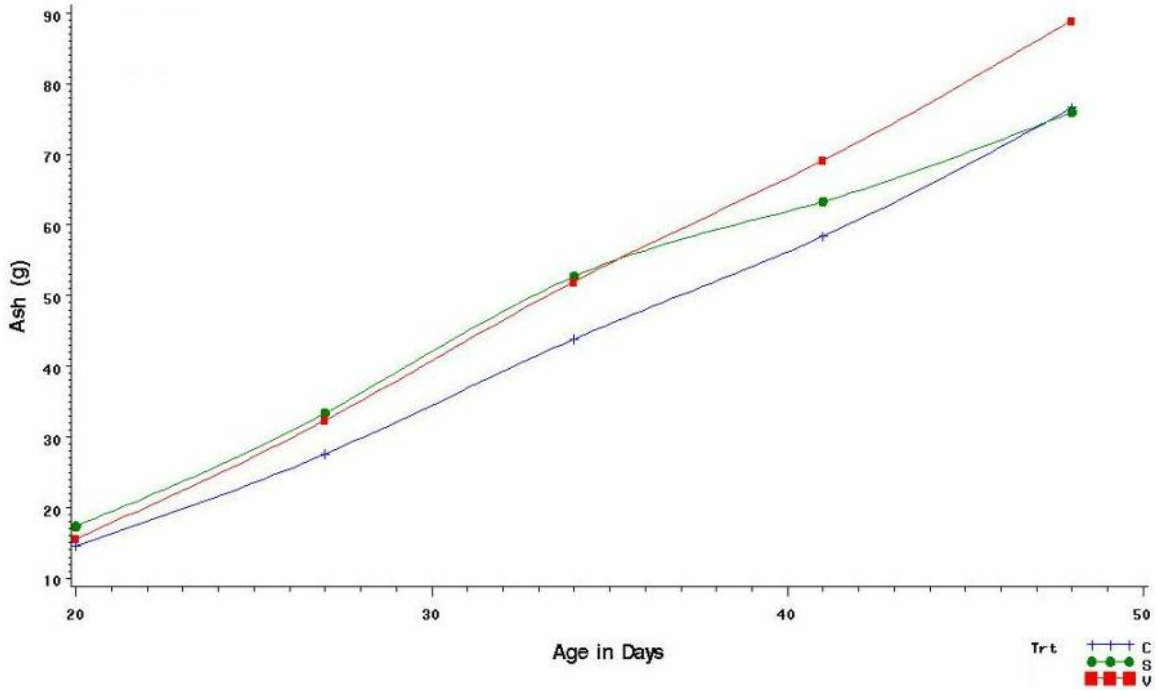
Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; g=grams

FIGURE 35: Treatment Effects on Body Ash of Unchallenged Birds



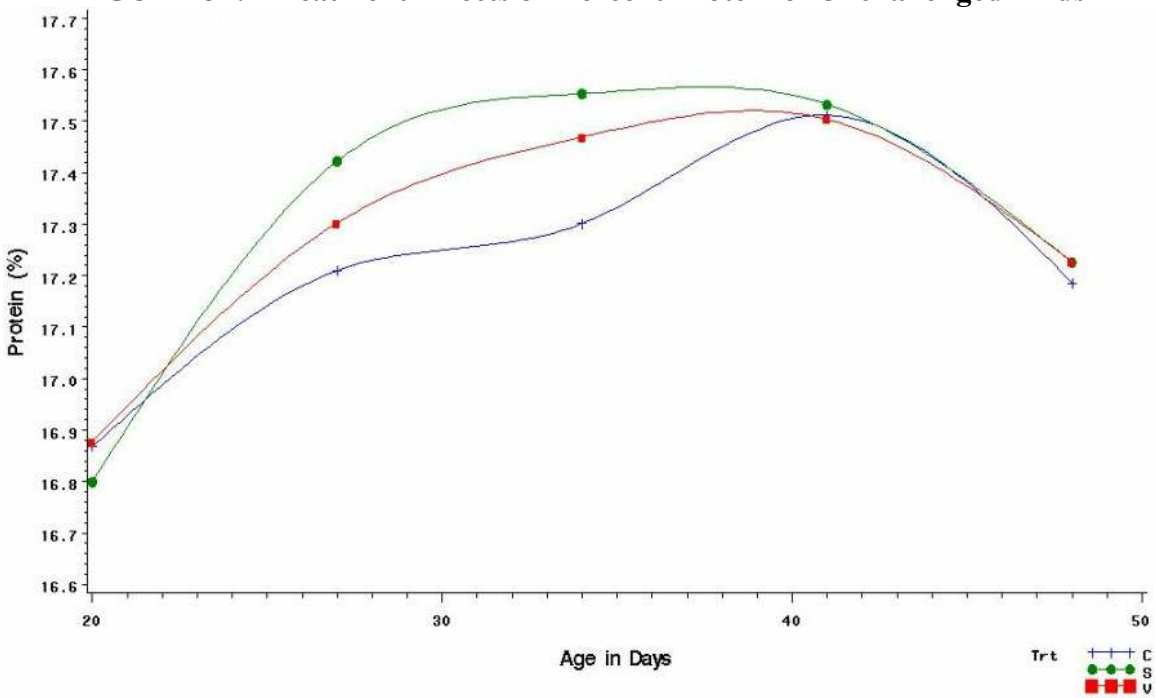
Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; g=grams

FIGURE 36: Treatment Effects on Body Ash of Challenged Birds



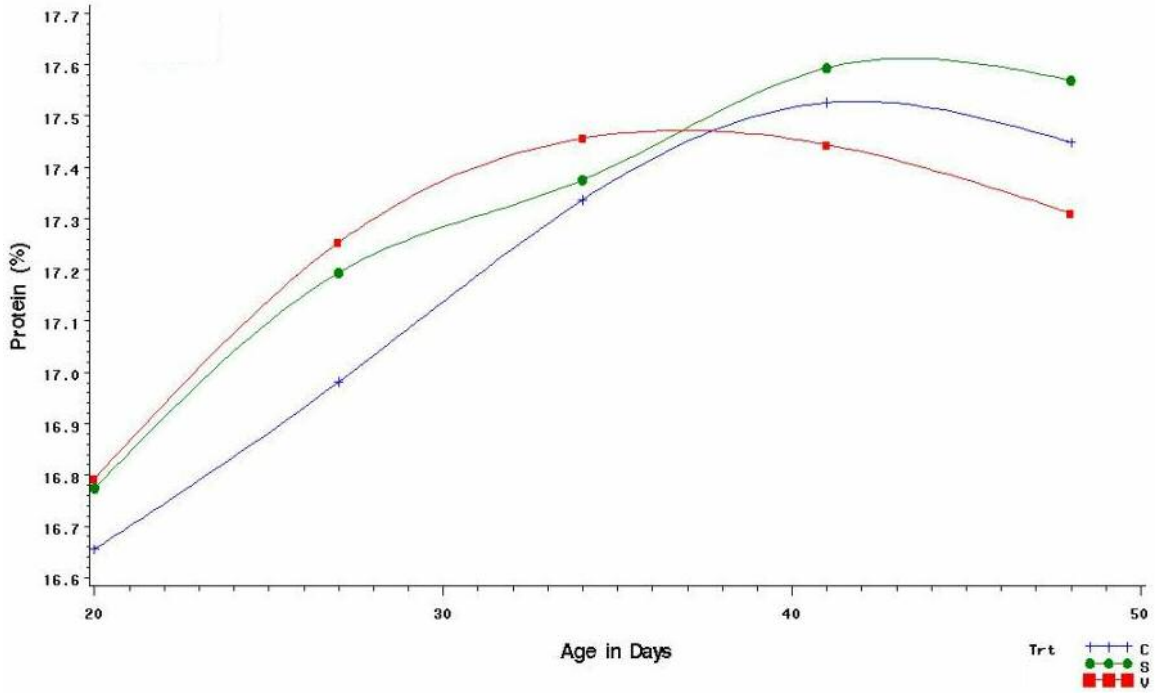
Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; g=grams

FIGURE 37: Treatment Effects on Percent Protein of Unchallenged Birds



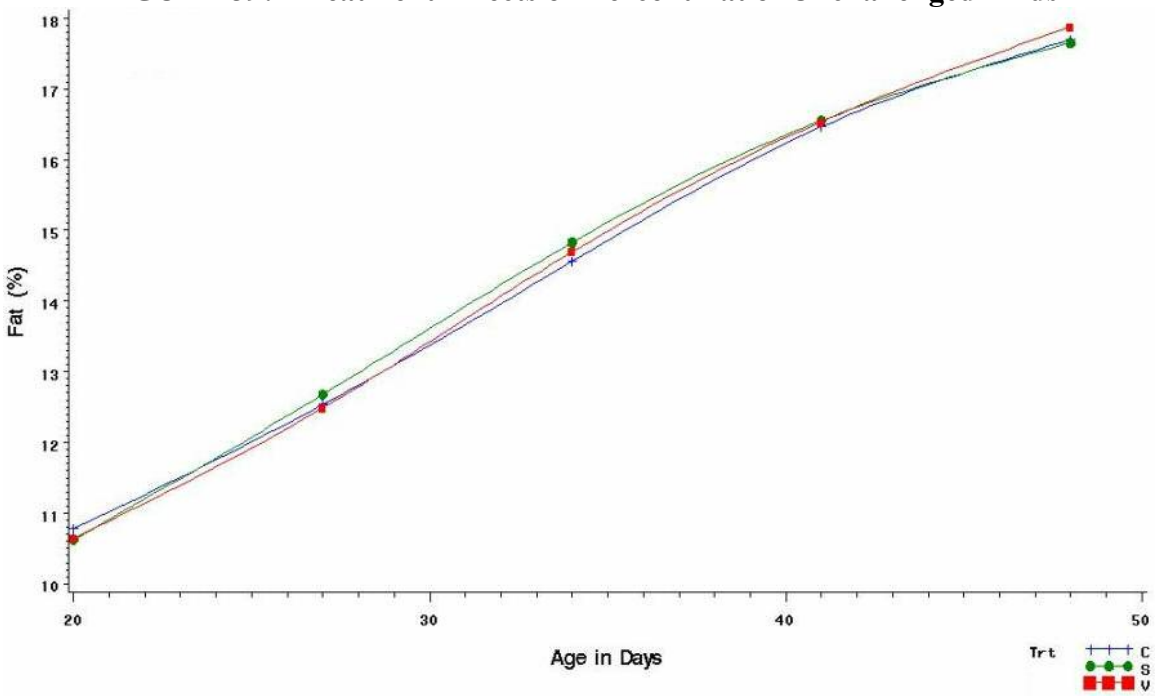
Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; Protein (%)=Protein (grams)/Live Weight (grams)

FIGURE 38: Treatment Effects on Percent Protein of Challenged Birds



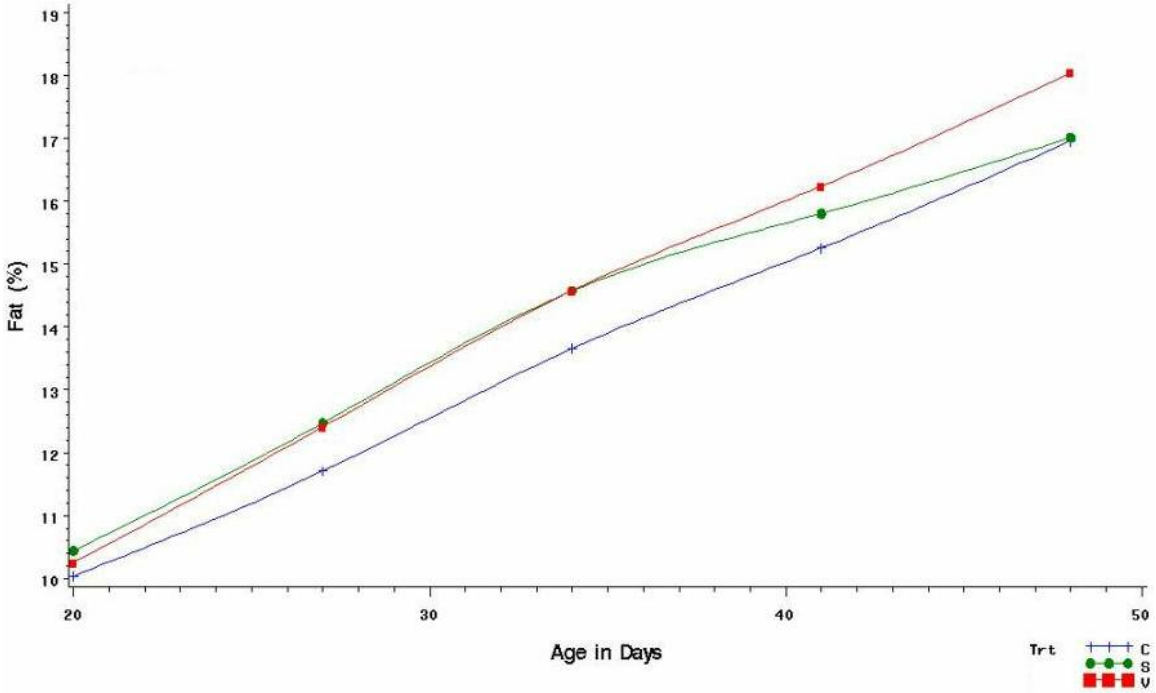
Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; Protein (%)=Protein (grams)/Live Weight (grams)

FIGURE 39: Treatment Effects on Percent Fat of Unchallenged Birds



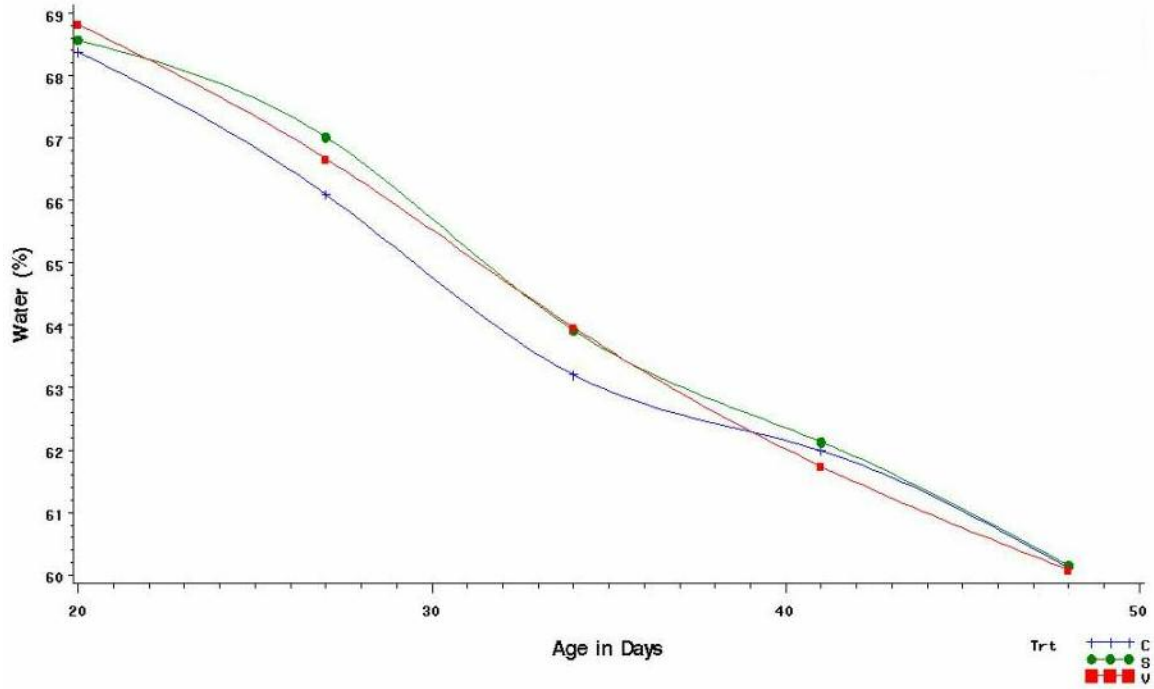
Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; Fat (%)=Fat (grams)/Live Weight (grams)

FIGURE 40: Treatment Effects on Percent Fat of Challenged Birds



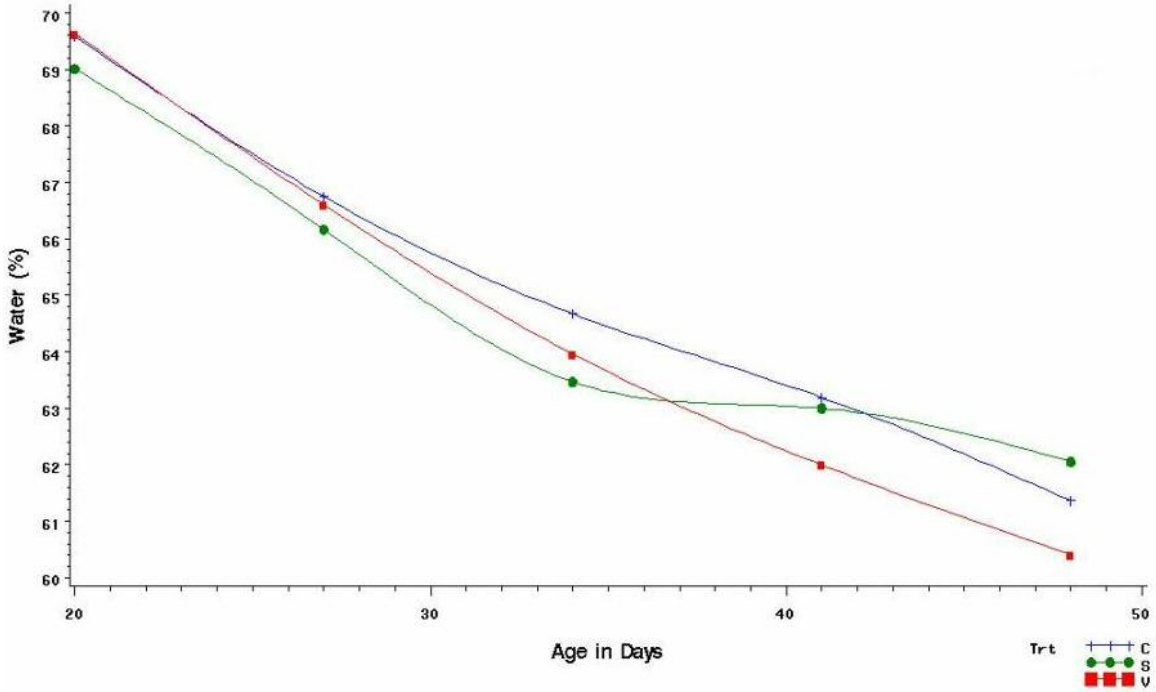
Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; Fat (%)=Fat (grams)/Live Weight (grams)

FIGURE 41: Treatment Effects on Percent Water of Unchallenged Birds



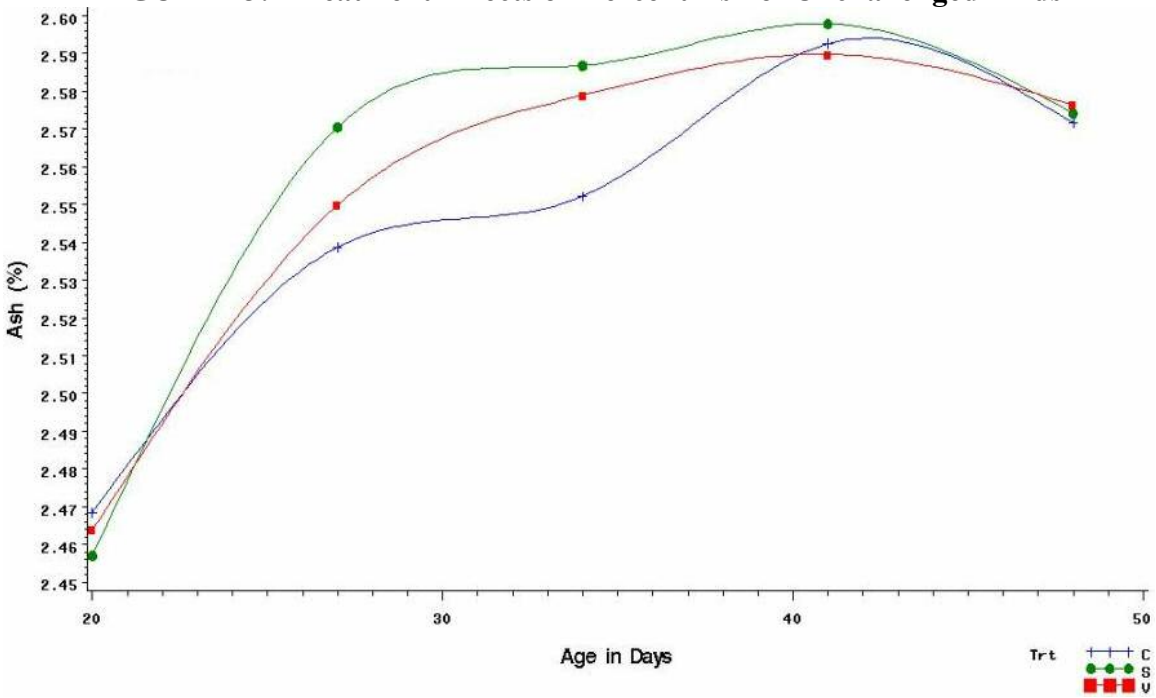
Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; Water (%)=Water (grams)/Live Weight (grams)

FIGURE 42: Treatment Effects on Percent Water of Challenged Birds



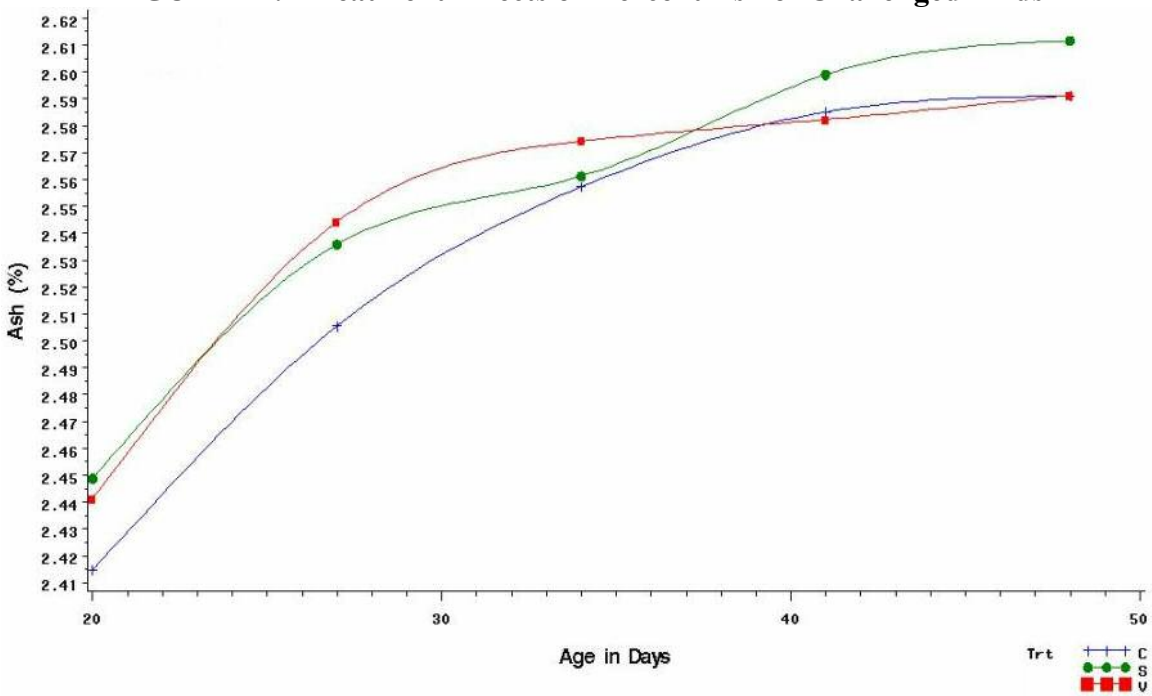
Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; Water (%)=Water (grams)/Live Weight (grams)

FIGURE 43: Treatment Effects on Percent Ash of Unchallenged Birds



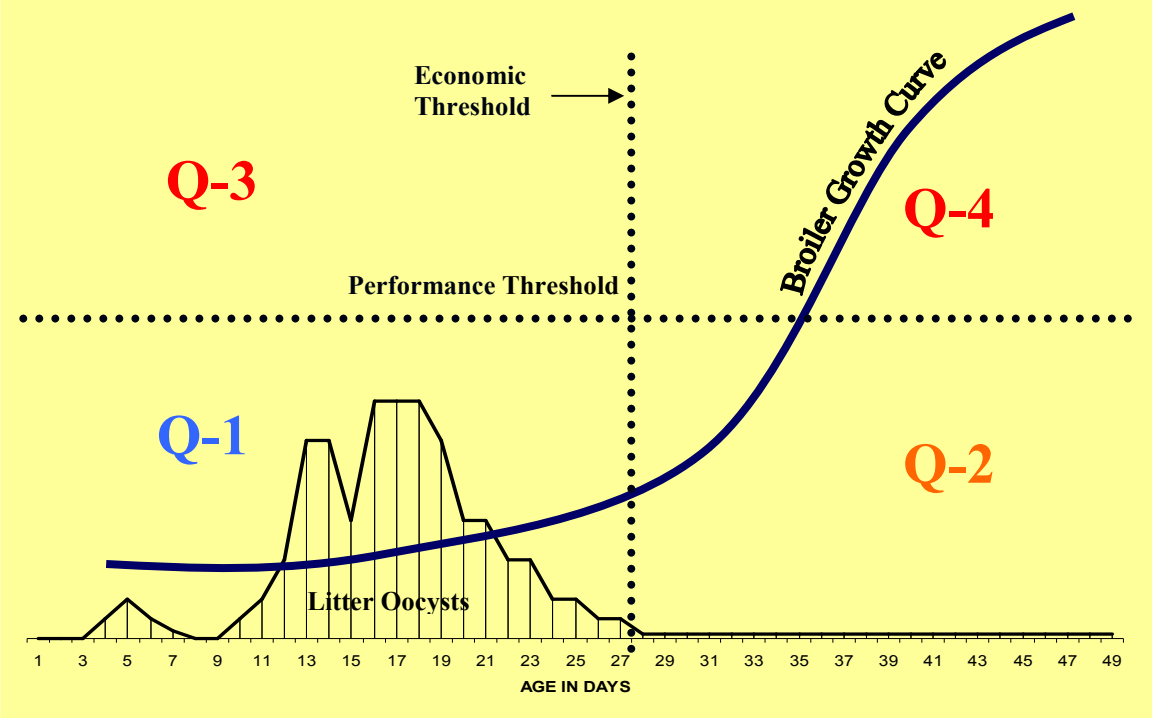
Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; Ash (%)=Ash (grams)/Live Weight (grams)

FIGURE 44: Treatment Effects on Percent Ash of Challenged Birds



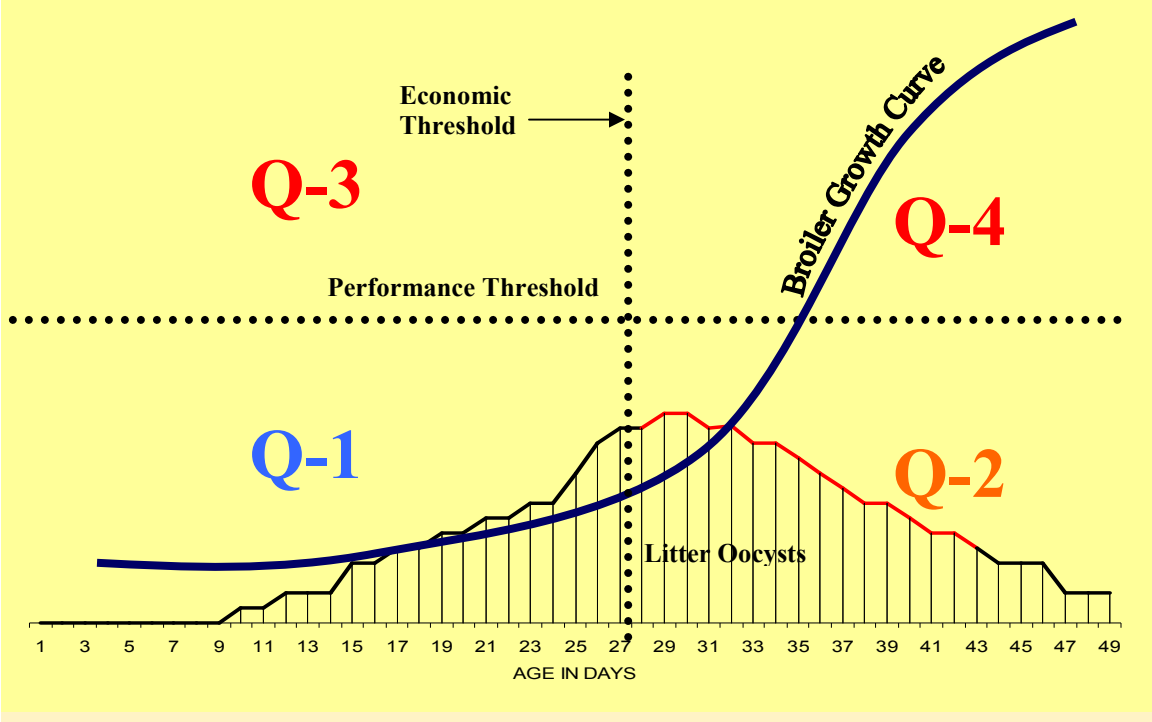
Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; Ash (%)=Ash (grams)/Live Weight (grams)

FIRGURE 45: Schering-Plough Quadrants of Performance of Broilers on a Vaccination Program Experiencing an Immune Challenge



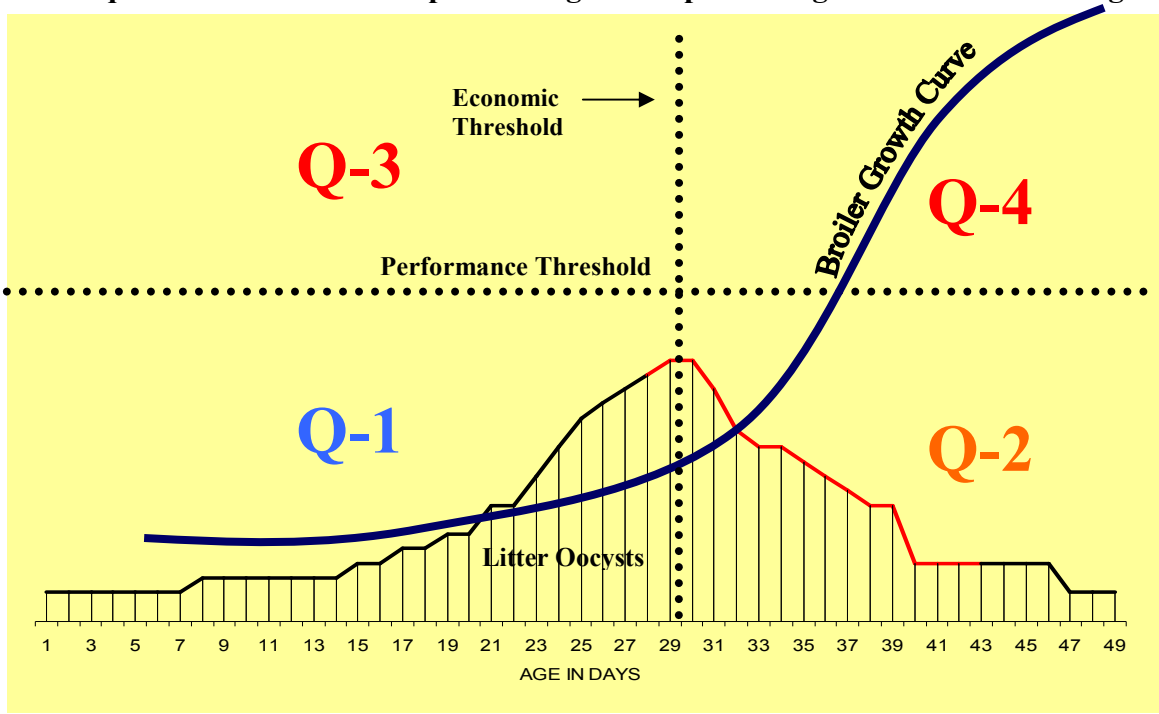
Q-1=Quadrant 1; Q-2=Quadrant 2; Q-3= Quadrant 3; Q-4=Quadrant 4

FIRGURE 46: Schering-Plough Quadrants of Performance of Broilers on an Ionophore Program Experiencing an Immune Challenge



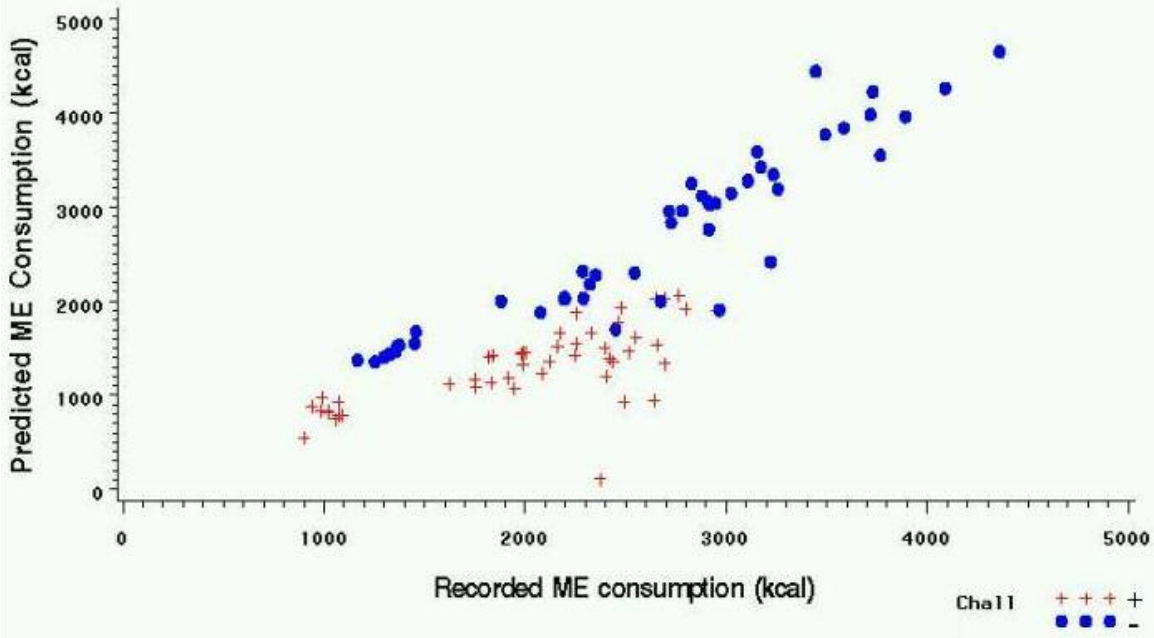
Q-1=Quadrant 1; Q-2=Quadrant 2; Q-3= Quadrant 3; Q-4=Quadrant 4

FIRGURE 47: Schering-Plough Quadrants of Performance of Broilers of Subsequent Flocks on an Ionophore Program Experiencing an Immune Challenge



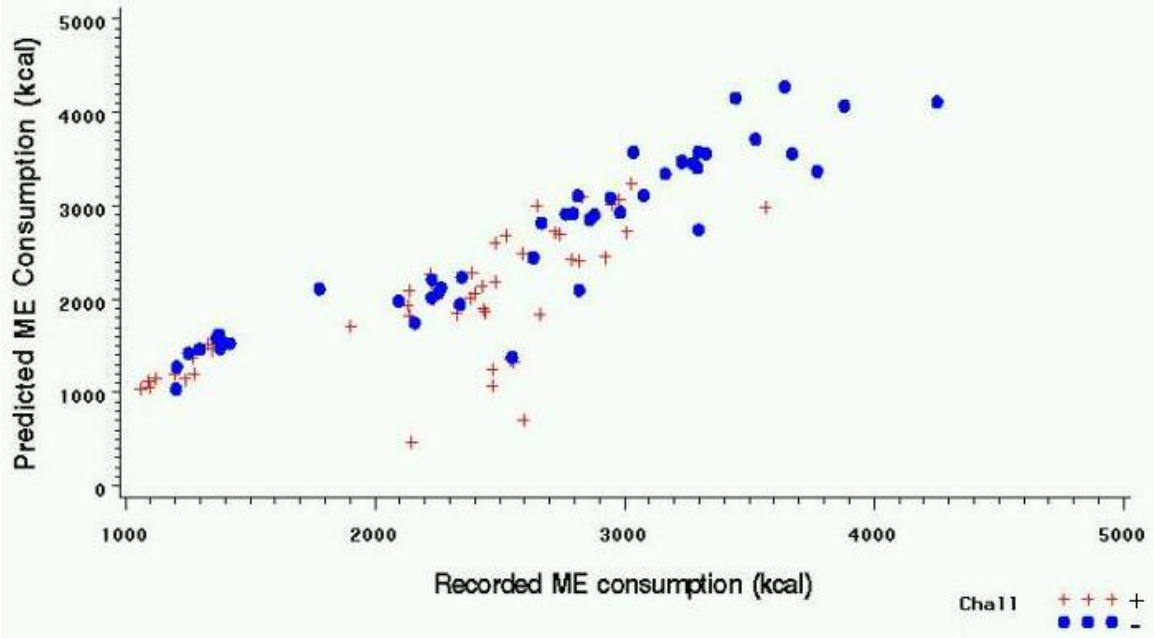
Q-1=Quadrant 1; Q-2=Quadrant 2; Q-3= Quadrant 3; Q-4=Quadrant 4

FIGURE 48: Recorded Metabolizable Energy Consumption vs. Predicted Metabolizable Energy Consumption for Control Birds



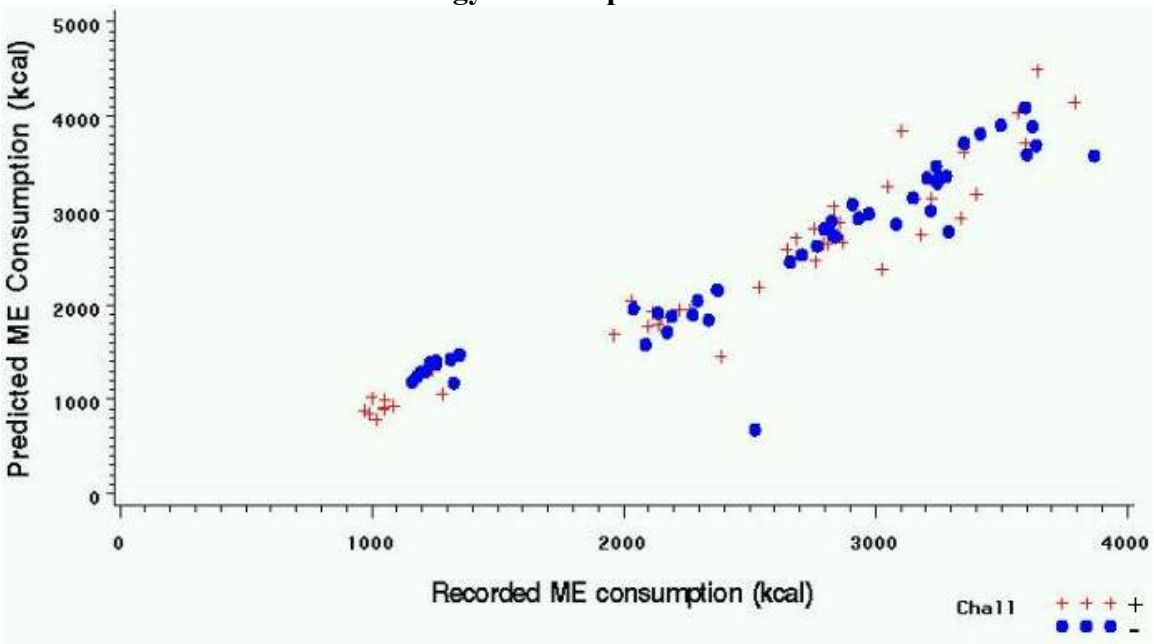
Kcal=kilocalorie; Chall=challenge group; +=challenged; - = unchallenged; ME=metabolizable energy; predicted ME consumption= 110 x body weight$^{0.75}$ x 6 + (protein gain x 5.65)/Kp + (fat gain x 9.3)/Kf; Kp=protein accretion efficiency constant (0.67); Kf=fat accretion efficiency constant (0.87)

FIGURE 49: Recorded Metabolizable Energy Consumption vs. Predicted Metabolizable Energy Consumption for Salinomycin Birds



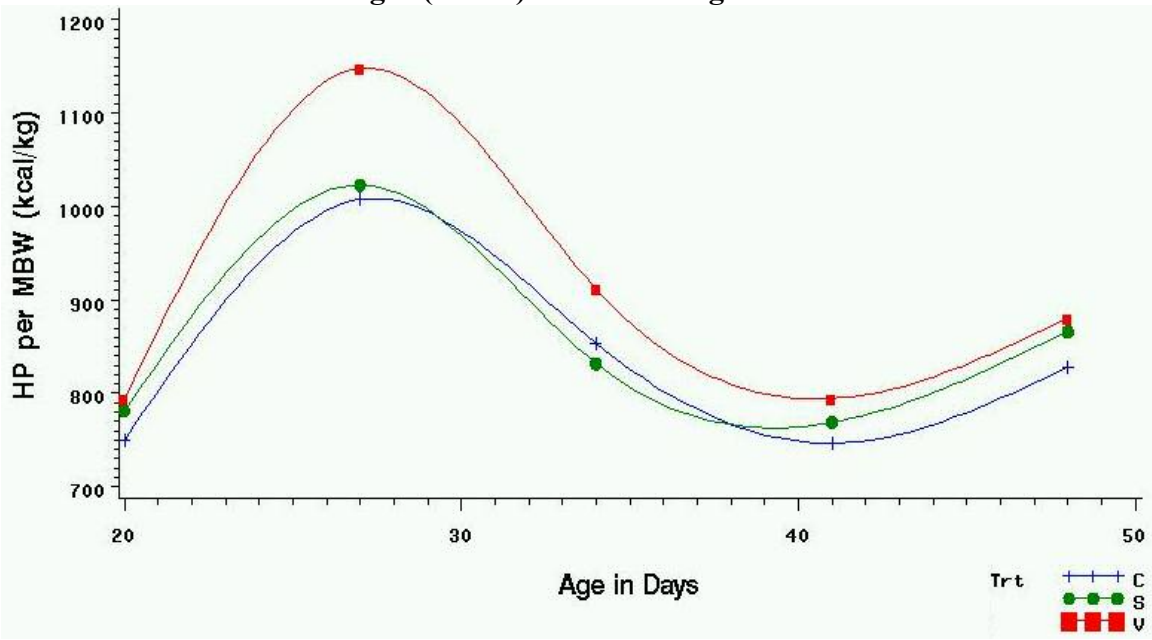
Kcal=kilocalorie; Chall=challenge group; +=challenged; - = unchallenged; ME=metabolizable energy; predicted ME consumption= 110 x body weight$^{0.75}$ x 6 + (protein gain x 5.65)/Kp + (fat gain x 9.3)/Kf; Kp=protein accretion efficiency constant (0.67); Kf=fat accretion efficiency constant (0.87)

FIGURE 50: Recorded Metabolizable Energy Consumption vs. Predicted Metabolizable Energy Consumption for Vaccinated Birds



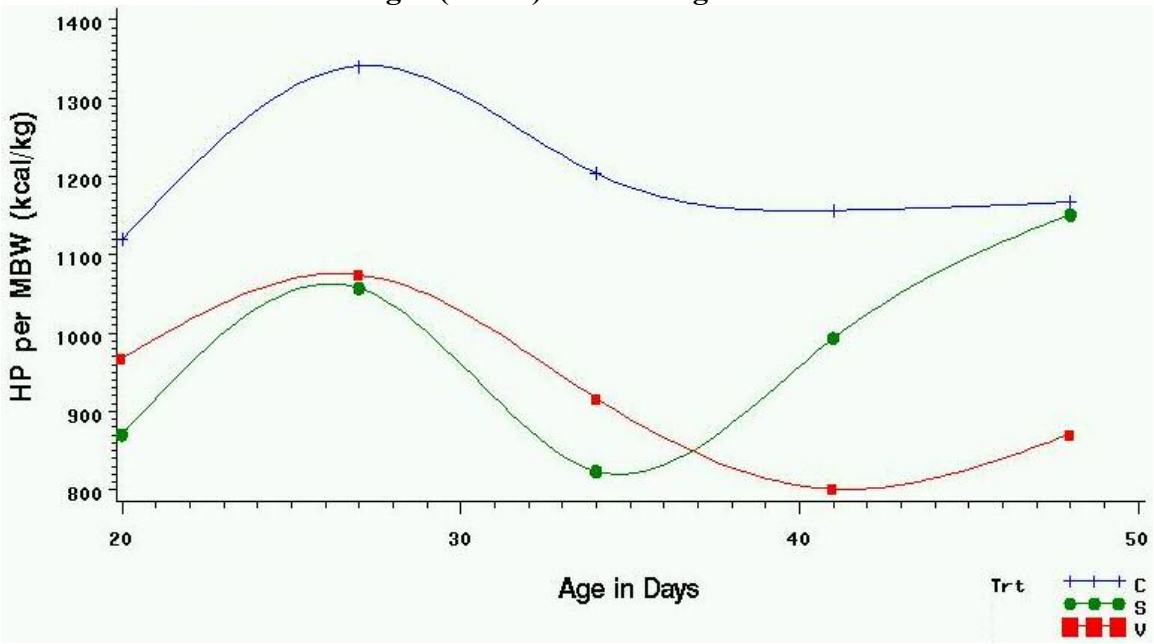
Kcal=kilocalorie; Chall=challenge group; +=challenged; - = unchallenged; ME=metabolizable energy; predicted ME consumption= 110 x body weight$^{0.75}$ x 6 + (protein gain x 5.65)/Kp + (fat gain x 9.3)/Kf; Kp=protein accretion efficiency constant (0.67); Kf=fat accretion efficiency constant (0.87)

FIGURE 51: Treatment Effects on Heat Production (HP) per Metabolic Body Weight (MBW) of Unchallenged Birds



Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; kcal=kilocalorie; kg=kilogram; MBW=Body weight$^{0.75}$

FIGURE 52: Treatment Effects on Heat Production (HP) per Metabolic Body Weight (MBW) of Challenged Birds



Trt=Treatment; C=Control; S=Salinomycin; V=Vaccinated; kcal=kilocalorie; kg=kilogram; MBW=Body weight$^{0.75}$

VITA

Chad Ernest Brown

Candidate for the Degree of

Master of Science

Thesis: EVALUATION OF COCCIVAC-B® AND SACOX 60® (SALINOMYCIN) FOR CONTROL OF 3 STRAINS OF EIMERIA IN BROILERS

Major Field: Animal Science

Biographical:

Education: Graduated from Conway High School in Conway, Arkansas in May 2001; received Bachelor of Science degree in Animal Science from the University of Arkansas, Fayetteville, Arkansas in May 2005; Completed the requirements for the Master of Science degree with a major in Animal Nutrition at Oklahoma State University in July 2007.

Experience: Employed at Chestnut Small Animal Clinic in Conway, Arkansas from May 1999 until August 2001; employed at Lunsford Veterinary Care Center in Tontitown, Arkansas from May 2003 until January 2005; employed as a part-time undergraduate worker in Poultry Science Department at the University of Arkansas in Fayetteville, Arkansas from January 2005 until May 2005; employed as graduate research assistant in Animal Science Department at Oklahoma State University from August 2005 to present.

Name: Chad Brown Date of Degree: July, 2007

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: EVALUATION OF COCCIVAC-B® AND SACOX 60® (SALINOMYCIN) FOR CONTROL OF 3 STRAINS OF EIMERIA IN BROILERS

Pages in Study: 139 Candidate for the Degree of Master of Science

Major Field: Animal Science

Scope and Method of Study: The purpose of the research experiment was to contrast Coccivac-B® vaccine with feed applied salinomycin (Sacox 60®) throughout the broiler's growth curve. The Schering-Plough theory of Quadrant Performance assumes that vaccinated birds will experience a minor coccidial challenge early in life, allowing them to develop solid immunity leading to their rebound in terms of performance. The experiment was conducted utilizing 936 Cobb x Cobb males in 6 treatments: control-unchallenged (C-); control-challenged (C+); Sacox 60®-unchallenged (60g/ton; 0-35 days; S-); Sacox 60®-challenged (S+); vaccinated-unchallenged (Coccivac-B® at hatch; V-); and vaccinated-challenged (V+). Challenge consisted of an oral dose of sterile saline (-) or a mixture of 3 *Eimeria* species as oocysts (+) at 14, 21, 28, 35, and 42 days. Variables examined included live weight, FE, gross lesion scores (upper small intestine, mid small intestine, and ceca), microscopic lesion scores (*E. maxima*, *E. tenella*, and *E. acervulina*), body composition, O_2 consumption, and CO_2 production.

Findings and Conclusions: C- birds exhibited superior performance than C+ birds at all ages ($P<0.01$) while results for other treatments are age dependent. In general, the performance criteria were similar for the – birds throughout the trial with the V- birds experiencing some depression on day 20. V+ and S+ birds were superior to C+ birds ($P<0.05$) post 20d. The V+ group exhibited better performance than the S+ group after day 34 in most areas. S+ birds were superior for most performance criteria to the C+ birds until day 41 when they became similar and remained so for the rest of the trial. Lesion scores for all intestinal tract areas and species of *Eimeria* correlate with performance data. The V- birds experienced slightly increased lesion scores on day 20 due to vaccine. Energy data also follows the general trends seen in performance. The data presented supports the Schering-Plough Animal Health theory for Quadrant Performance.

ADVISER'S APPROVAL: Dr. Robert Teeter