THE EFFICACY OF DL-METHIONINE METHYLSULFONIUM CHLORIDE ON

PERFORMANCE CHARACTERISTICS AND INTESTINAL TRACT

INTEGRITY IN BROILERS

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THE EFFICACY OF DL-METHIONINE METHYLSULFONIUM CHLORIDE ON PERFORMANCE CHARACTERISTICS AND INTESTINAL TRACT INTEGRITY IN BROILERS

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VITA

Ashley L. Shaw, daughter of Frank and Allison Hendricks, was born September 28, 1983 in Newport News, Virginia. She graduated from Kecoughtan High School in 2001. She attended Virginia Polytechnic Institute and State University in Blacksburg, Virginia and graduated with a Bachelor of Science in Animal and Poultry Sciences in May 2005. She then entered Graduate School at Auburn University in August 2005 in the Department of Poultry Science under the direction of John P. Blake, Ph.D.

THESIS ABSTRACT

THE EFFICACY OF DL-METHIONINE METHYLSULFONIUM CHLORIDE ON PERFORMANCE CHARACTERISTICS AND INTESTINAL TRACT INTEGRITY IN BROILERS

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DL-methionine methylsulfonium chloride (MMSC) is a methionine derivative previously shown to modulate the immune system and to protect intestinal membrane cells in humans and swine. It has also been shown to improve body weight gain and feed efficiency in cattle and hogs. Four 42-day trails were conducted to evaluate the effects of MMSC on growth performance, feed efficiency, and gut integrity in broilers.

The first two experiments utilized 384 day-old mixed-sex broilers that were randomly allotted to one of six dietary treatments. Experiment 1 employed a corn-soy basal diet with additions of MMSC at 0, 200, 400, 600, 800, or 1000 ppm. Experiment 2 utilized MMSC as a substitute for synthetic DL-methionine on a molecular weight equivalency at 0, 20, 40, 60, 80, or 100%.

Results from both experiments indicated no significant effects ($P > 0.05$) on final body weight gain, feed consumption, and feed efficiency due to additions of MMSC. The additions of MMSC in Experiment 1 failed to improve villi length, villi width, crypt depth, and mucosal depth of the small intestine. Beneficial effects to villi characteristics of the duodenum, jejunum, and ileum were seen in Experiment 2 as the amount of MMSC increased in the diet, with the greatest effects found in the duodenal measures.

The final two experiments utilized 384 (Experiment 3) or 480 (Experiment 4) day-old straight-run broilers that were randomly allotted to one of six dietary treatments. Both experiments employed a corn-soy basal diet with additions of MMSC at 0, 200, 400, 600, 800, or 1000 ppm. Experiment 3 birds were provided 1 ml of a cocci cocktail containing *E. acervulina* (125,000 oocytes/ml), *E. maxima* (25,000 oocytes/ml), and *E. tenella* (15,000 oocytes/ml) via oral gavage on day 10. Fecal scores were determined from day 4 to 10 post inoculation. Birds utilized in Experiment 4 were administered 0.1 ml of *Salmonella kentucky* (10^8 cfu/ml) on day of placement and re-dosed with 1ml (10^6 cfu/ml) on day 14. Çecal samples were collected weekly (4 birds/trt) from days 7 to 28 to determine presence of Salmonella.

Birds in neither experiment showed differences ($P > 0.05$) in body weight gain, feed consumption, or feed efficiency. In cocci-challenged birds, MMSC had little effect on the villi measures of length and width, but did provide varying results in the crypt depth measures as levels increased in the diet. MMSC was able to provided some positive effects in the intestinal tract of birds challenged with *S. Kentucky*, especially within the duodenum at the 800 and 1000 ppm addition levels. No differences of salmonella persistence were detected among the six treatments.

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I. GENERAL INTRODUCTION

Poultry is currently the leading meat product consumed in the United States. This is in part due to the competitive pricing compared with other meat products. In order to keep the prices of poultry fairly low, the industry works toward obtaining the greatest amount of weight gain for the lowest amount of production cost. Since the cost of feed amounts to approximately 70% of the production costs, there is a need for lowering feed consumption without relinquishing body weight gain.

DL-methionine is the primary limiting amino acid in the diet of poultry. Cysteine, another essential amino acid, has the ability to be synthesized from methionine. DL-methionine can be synthetically prepared, however the procedure to do so is quite costly. While the amount of methionine added to a typical broiler diet does not exceed more than $0.20 per 45 kg of feed, the cost can quickly add up with companies that produce multiple tonnes of feed daily.

Coccidiosis and salmonellosis are two pathogenic microorganisms that the poultry industry is faced with. Birds challenged with either of these microorganisms can show a decrease in growth performance, especially during the first few weeks of life, which can cause a decrease in overall weight gain compared with healthy birds. In addition, both salmonella and coccidia can cause irritation to the intestinal tract due to colonization. This in turn can lead to a decrease in absorptive function, leading to a decrease in feed efficiency.

DL-methionine methylsulfonium chloride (MMSC) has been shown to improve performance characteristics when added to the diet of several different species. As a methionine derivative, this compound may have the ability to meet methionine requirements of a typical broiler diet. Furthermore, evidence has shown that this compound protects against intestinal ulcerations in monogastrics. Due to these positive influences MMSC has shown within other species, it is possible that similar effects could be duplicated in poultry, a species that has not been a major subject of research with this compound.

II. REVIEW OF LITERATURE

DL-Methionine methylsulfonium chloride

DL-Methionine methylsulfonium chloride (MMSC) is a compound that naturally occurs in products such as milk, potatoes, corn, soybeans, asparagus, cabbage, various teas, and most flowering plants. (Hussain, 1983; Ohtsuki et al., 1984; Bourgis et al., 1999). MMSC has several other names such as methylmethioninesulfonium chloride and S-Methyl-L-methionine. Commonly, it is often referred to as either cabagin, since it was originally derived from cabbage juice, or Vitamin U, for its aid in healing and preventing ulcers. MMSC is not truly a vitamin though, as a vitamin is an essential nutrient that must be provided within the feed to maintain adequate health. MMSC is a methionine-derivative with the chemical formula $C_6H_{14}ClNO_2S$ and structure as shown in Figure 1. In the plant it is biosynthesized from S-adenosylmethionine and methionine by the enzyme S-methyltransferase (Greene and Davis, 1960). It is an active component of the methionine cycle in plants, serving as a substrate for methyl transferases (Augspurger et al., 2003; Mudd et al., 1966).

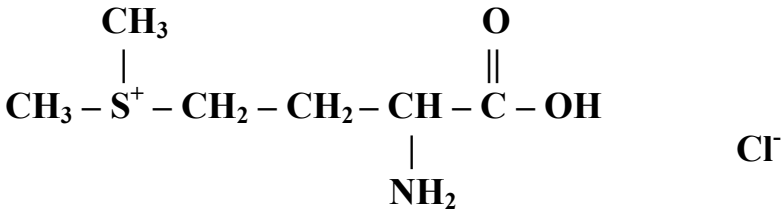

Figure 1. Structure of DL-Methionine methylsulfonium chloride (MMSC)

MMSC has been widely used in the past as either a cure or preventative against gastric and duodenal ulcers (Nakamura et al., 1981). Watanabe et al. (1996) reported that pre-treating an animal with a sulfhydryl-blocking compound prevented the protective effects of MMSC on ethanol-induced gastric damage, which suggested MMSC to be a latent sulfhydryl compound. Sulfhydryl compounds are known to function as cytoprotective agents in the stomach (Szabo et al., 1981; Rogers et al., 1988). These compounds provide gastroprotection via several different mechanisms including free radical scavenging (Hirashi et al., 1994), suppression of gastric motility (Takeuchi et al., 1989), vasoprotection (Szabo et al., 1984), and release of mucin (Lamont et al., 1983).

Many of the scientific studies on MMSC in relation to curing and preventing gastric ulcers have been performed with rats, swine, and humans. The original studies involving MMSC were conducted by Cheney (1950; 1952) during the early 1950's to determine possible therapeutic effects of the compound may have on peptic ulcers. During the mid-1970's Urazaeva (1976) showed that MMSC produced an antiphlogistic effect, while also providing protection against mucosal lesions. MMSC was shown to stimulate the healing of gastric erosions and decrease hemorrhaging often caused by this erosion in both swine and humans (Elbers et al., 1995; Salim, 1993). Administration of MMSC in rats was also found to increase the amount of mucin in the surface mucosa,

while depleting the amount of mucin in the deep mucosa (Watanabe et al., 2000). MMSC is able to convey protection of the gastric mucosa by sustaining the physiochemical properties of the mucosal barrier. In addition, it is able to bind cytotoxic oxyradicals, which are often the cause of mucosal damage (Lamont et al., 1983; Salim, 1989).

While MMSC has been shown to aid in the protection against ulcerations of the gastrointestinal tract, the extent of protection is often dose- and species-dependent. Salim (1992) showed that an oral dosage of 1 ml of distilled water with a 2% concentration of MMSC administered daily was required to significantly reduce injury in rats. MMSC decreased the severity of esophagogastric erosions and/or ulcers when added in the feed of fattening pigs at a level of 400 mg/kg (Elbers et al., 1995). Likewise, in humans a daily dosage of 2000 mg of MMSC was required to stimulate healing of erosive gastritis and protection against bleeding (Salim, 1993).

Little of the research involving MMSC deviates from the interest in its therapeutic effects on gastric ulcers within various species, however a handful of studies have been run to determine its effect on hypolipidemia, performance characteristics, and pregnancy. A reduction in both serum total cholesterol and phospholipid levels were demonstrated in rats, rabbits, and man (Seri et al., 1980; Nakamura et al., 1981). This hypolipidemic effect of MMSC is caused by acceleration of cholesterol catabolism and excretion of cholesterol into the feces. Administration of MMSC was also shown to increase urinary volume and decrease urinary protein excretion in patients undergoing nephritic complications (Seri et al., 1979).

When included in a hog-fattening ration at 25 mg/kg of feed, MMSC aided in an overall increased weight gain by 3.6% and decreased feed efficiency by 2.0%. This weight increase was mainly found in gilts, with little difference among boars (Solntsev and Filipovich, 1981). The growth-stimulating effect of this compound manifested primarily during the initial fattening period, but had virtually no effect during the second period (Solntsev and Filipovich, 1978). Tamas et al. (1987a; 1987b) provided several studies that showed this growth increase was able to decrease the entire fattening phase by 9 to 13 days. An addition of MMSC to the diet at a dose of 1 mg/kg of live weight was shown in fattening bull calves to strengthen the hooves and increase growth energy by providing an average increase of 22 kg compared with control animals (Kalinkhin, 1986). Contrary to the hog study, this research showed the most beneficial effects of MMSC during the second fattening period, from days 115 to 385 of age.

In 1980, the U.S. Department of Agriculture wanted to determine any toxic effects MMSC may have on fetuses of pregnant rats. Results indicated that the compound was not teratogenic to the fetuses when injected into gestating females (Nishe and Daxenbichler, 1980). Additionally, MMSC did not cause a growth depression in the female during gestation, nor did it have a significant increase in weight gain on the fetuses at birth.

Very little research exists on the uses and effects of MMSC in poultry. During the 1980's a few studies were conducted to determine the effects of MMSC in conjunction with different B-vitamins. This research was of some interest since some of the B-vitamins are associated with the regulation of methylation and MMSC could provide the methyl group required for this process to occur. Kovaleva (1986) sought the

effects of a diet containing a MMSC-folic acid mixture fed to broilers reared through 56 days of age. The results indicated an 11% increase in body weight gain and a decreased feed intake of 4.8% compared with the control group when the diet contained 3.5mg/kg MMSC. During a follow up study Kovaleva et al. (1987) sought the effects of the MMSC-folic acid combination on methylation in the liver. Results from this study indicated an increased methylating activity in the liver when provided at the previous dosage rate of 3.5 mg/kg. MMSC was able to decrease S-adenosyl homocysteine concentrations, hindering its actions in the methylation process, and enhancing liver metabolism.

In 1988, the interaction between MMSC and cyanocobalamin (Vitamin B_{12}) was studied to establish any physiological or growth changes in broilers fed a diet devoid of B_{12} (Ionauskene and Kanopkaite, 1988). Either 5 or 10 μg of MMSC was injected subcutaneously every 3 days and proved to increase concentrations of both individual and total B_{12} levels in the blood serum and liver, especially at 20 days of age. Injection of the compound was also found to increase body weight gain and to slightly increase blood glutathione levels.

Most recently research was conducted at the University of Illinois to determine the bioavailability of MMSC as either a choline or methionine source for broilers (Augspurger et al., 2005). In one experiment, the efficacy of MMSC on promoting weight gain in chicks deficient in methionine was sought. MMSC was added to the diet at a level of 1.3 g/kg both in the absence and presence of 0.8g/kg L-methionine. While MMSC was able to increase the body weight gain of chicks fed both diets in comparison

with the control that contained no MMSC or methionine, a 27 g increase in weight gain was observed in birds supplemented with both L-methionine and MMSC.

In a second experiment, Augspurger et al. (2005) sought to determine the methionine-sparing activity of MMSC when added to a diet deficient in methionine, but adequate in choline. For this diet, 1.1 g/kg of MMSC was supplemented, and did not positively impact growth performance in comparison with the control. Another experiment was conducted to determine the choline sparing activity of MMSC. In this third experiment, MMSC was included at 948 mg/kg into a diet that also contained 1 g/kg L-methionine. The compound was found to increase weight gain and gain:food ratio for all birds compared with those fed the control diet.

A final experiment was carried out to confirm the efficacy of MMSC for promoting growth of chicks fed diets deficient in choline or methionine and choline (Augspurger et al., 2005). MMSC was included at a rate of 1.4 g/kg for both diets. An increase in growth performance was observed for both MMSC containing diets in comparison with the control.

These experiments are among a small number of those conducted with poultry. For this reason, more research is needed to better determine the effects of MMSC in poultry. It is possible that MMSC, as a methionine-derivative, could provide methionine activity when added to the diet of birds in quantities higher than that used at the University of Illinois. MMSC has been shown to aid in the protection against ulcerations of the gastrointestinal tract, however it's effects have not been investigated with regards to other intestinal irritations. For these reasons, more research is needed in order to better assess the possibilities of MMSC within poultry.

DL-Methionine

Methionine is an indispensable amino acid required for normal growth and development of humans (Di Buono et al., 2003), other mammals, and avian species (NRC, 1994). In addition to being a substrate for protein synthesis, it is an intermediate in transmethylation reactions, serving as the major methyl group donor in vivo (Stipanuk, 1986; Grifith, 1987), including the methyl groups for DNA and RNA intermediates. It is also a precursor for several polyamines, which are essential in cell proliferation and development (Bardocz, 1995). Methionine is also of great interest metabolically for its trans-sulfuration to cysteine.

Given that it is not synthesized by the body and is required for a plethora of mechanisms, methionine is regarded as the first-limiting amino acid in poultry diets. This is primarily because poultry diets around the world are based on soybean meal, a protein source that is deficient in sulfur amino acids. Methionine has been accepted as the metabolic precursor for cysteine (Du Vigneaud et al.,1944) and is 100% efficient as such on a molar basis (Graber and Baker, 1971). For this reason, methionine is often added to the diet in order to meet total sulfur amino acid requirements, rather than just satisfying the need for methionine alone. This total sulfur amino acid requirement is determined as the dietary methionine intake in the absence of cysteine that satisfies the requirements for the animal.

Methionine is the only amino acid that can have both of its isomers utilized within the body, its structure is shown in Figure 2. The L-isomer can be 100% utilized by all mammal and avian species. The D-isomer of methionine is utilized well as an L-methionine precursor and is 100% bioavailable to animals such as poultry, swine and

dogs (Sunde, 1972; Cho et al., 1980). This same isomer has only about a 30% bioavailability for humans and apes (Kies et al., 1975; Stegink et al.,1980). The mixture of DL-methionine is only about 65% bioavailable to the aforementioned (Lewis and Bayley, 1995).

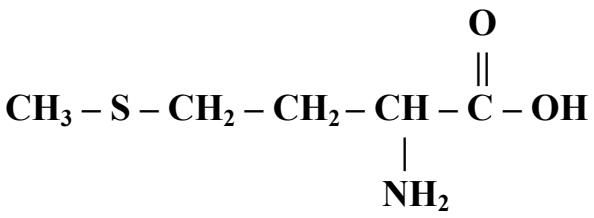

Figure 2. Structure of DL-Methionine.

The need for methionine in the diet falls within a specific range, and feeding outside of this range can lead to health problems. The feeding of diets deficient in methionine and methyl donor compounds leads to the production of fatty liver and an increase in hepatic gamma-glutamyltranspeptidase (Speisky et al., 1990). Methionine is the most toxic of the amino acids and causes severe growth depression when fed in excess amounts (Sauberlich, 1961; Harper et al., 1970). It has been found to oxidize much faster than other amino acids, perhaps allowing the toxic effects to appear sooner. One of the most consistent features of methionine toxicity is splenic hemosideroses caused by hemolytic anemia (Halter and Baker, 1978). Supplemental glycine has an ameliorative effect on methionine toxicity via its ability to enhance methionine oxidation (Benevenga and Harper, 1970). It is unclear as to whether this enhanced oxidation is due to the role of glycine in detoxifying the methyl group or for its role as a precursor of serine (Baker, 2006).

The only work that has been conducted to indicate that MMSC may be substituted for DL-methionine was performed by Augspurger et al. (2005). These results showed that MMSC was not capable of providing methionine-sparing activity at a dietary addition rate of 1.1 g/kg. Perhaps, when used at higher substitution levels, MMSC could have an effect on broiler performance.

Coccidiosis

Coccidiosis is one of the most common diseases to affect poultry. It is caused by protozoan parasites of the genus Eimeria, which multiply in the intestinal lumen and cause tissue damage. There are nine identified species of coccidia known to infect the chicken, eight of which are known to be pathogenic (Edgar and Siebold, 1964). *E. tenella* inhabits the ceca and causes bloody lesions and cecal cores. *E. acervulina* is most frequently encountered in North and South America (McDougald et al., 1997). This species is often found in the upper half of the small intestine, especially in the duodenal loop, and produces round white lesions. *E. maxima* infects the mid-small intestine from below the duodenal loop to beyond the yolk stalk, and is found as a large yellowish oocyst, often accompanied by yellow-orange mucus. *E. mitis* affects the lower small intestine from the yolk stalk to the cecal junction. The lesions of this species are easily overlooked since the oocysts do not colonize, but will cause the area it inhabits to appear pale and flaccid (McDougald, 2003). *E. praecox* is confined to the duodenal loop and may cause watery intestinal contents and small pinpoint hemorrhages on days 4-5 of infection. *E. necatrix* causes large white or red lesions and ballooning in the small intestine below the duodenal loop. *E. brunetti* is found in the lower small intestine from the yolk stalk to the cecal junction and can cause a caseous eroded surface over the entire

mucosa (McDougald, 2003). *E. mivati* affects the small intestine from the duodenal loop to the cloaca. This species causes lesions that resemble those of *E. acervulina*, though they are more circular in shape (Edgar, 1958).

Although there is variation in the number of asexual generations and time required for each developmental stage, the life cycle of all the Eimeria species are quite similar. The entire process takes 4-6 days, depending on the species, and is as follows: 1) In the presence of warmth, moisture and air, four sporocytes will develop within each oocyst. Each of the sporocytes contains two sporozoites. 2) The bird ingests the sporulated oocysts, allowing the first phase of infection to occur. Mechanical action and enzymes within the digestive tract remove the protective covering of the oocysts and sporocytes, thus releasing the sporozoites into the digestive tract for colonization. 3) Each of the newly released sporozoites will penetrate the wall of an epithelial cell and develops into a round body, this is known as a schizont. 4) The nucleus divides repeatedly. These newly replicated nuclei develop into merozites. The merozites will break free, invade uninfected epithelial cells, and create a second generation. 5) After two or three asexual generations, some merozites will enter new epithelial cells and develop into either microgametocytes (male cells) or macrogametocytes (female cells) so that the sexual phase can begin. 6) The microgametocytes are eventually released into the lumen and unite with a macrogametocyte to form a zygote. This newly formed zygote will then finish constructing its cell wall. Upon completion, the host cell is ruptured and the zygote is released. Once released, the zygote is considered an oocyte and is excreted from the bird passed through the bird in the feces to complete the coccidial lifecycle.

In 1936, sulfur was discovered to have an anticoccidial effect (Herrick and Holmes). A few years after this discovery, the "sulfa" drugs were developed and proved to be the first practical anticoccidial drugs (Collins, 1949). Today, it is common practice to include anticoccidials into the feed when raising poultry on litter floors. Selection of specific anticoccidials is based on its ability to improve weight gain and feed conversion, and to suppress development of coccidial lesions (Reid, 1975). Cost also plays an important role in choosing an anticoccidial treatment. There are many FDA approved anticoccidials listed in Table 1, some of which are readily available for use in the prevention or elimination of coccidiosis in a flock.

Coccidiosis is one of many challenges that the poultry industry is faced with. Birds undergoing coccidiosis challenge show a loss in performance such as decreased weight gain and an increased feed efficiency. In addition to this, the different species of coccidia can have various impacts along the small intestine. Since MMSC has been shown to aid in the protection of the gastrointestinal tract, it is possible that it could have positive effects on intestinal integrity. If MMSC is able to improve integrity, absorption could also be positively affected, which in turn could positively influence performance.

Salmonella

Salmonella is an important pathogenic microorganism in both humans and animals (Khakhria et al., 1997). It is one of 24 genera of the family Enterobacteriaceae, the largest, most heterogeneous collection of medically important gram-negative bacilli (Murray et al., 1994). The genus has grown to include over 2400 serotypes since Daniel E. Salmon, a USDA veterinary bacteriologist, discovered the first strain in 1885 (Gast, 2003). Infection of poultry with salmonellae can be grouped into two categories:

nonmotile and motile serotypes. The nonmotile serotypes are *S. pullorum*, which causes pullorum disease in chicks and poults, and *S. gallinarum*, which causes fowl typhoid in mature birds. Both species are host-specific for avian species and have previously caused drastic economic losses in the poultry industry. Motile serotypes are referred to collectively as paratyphoid salmonellae. These are not specific to any species and are often the cause of food-borne illness in humans (Gast, 2003). Paratyphoid *Salmonella* infections of poultry often colonize the intestinal tract, especially the cecal pouches, and often cause no outward signs of infection in the bird. They are often able to persist through slaughter, providing it the ability to contaminate the finished carcass.

Although there are more than 2400 known Salmonella serotypes, only about 10% of them have been isolated in poultry (Gast, 2003). Of the 10%, less than that account for the majority of Salmonella isolates often recovered. Distribution of these serotypes tends to vary geographically and alters over time. The U.S. Department of Agriculture stated that the most commonly identified paratyphoid serotypes in chickens were *S. heidelberg*, *S. kentucky*, *S. senftenberg*, *S. enteritidis*, and *S. thompson* (Ferris et al., 1999).

Due to salmonella being facultatively anaerobic, these bacteria are able to survive in both aerobic and anaerobic conditions. They grow best in an environment with a pH of 7 and a temperature of 37 C, though they have the ability to survive in a pH range of 4 to 9 and a temperature range of 5 to 45 C. The bacteria have little nutritional requirements, allowing most any environment or media with a source of carbon and nitrogen to support its growth (Gast, 2003).

Paratyphoid serotypes can be introduced to poultry flocks by many different sources. Contaminated feeds, especially those containing animal proteins, are often

likely sources of salmonella (Davies et al., 1997; Rose et al., 1999). Unpelleted feeds such as meal and mash are more likely to be contaminated, since the temperature provided during the pelleting process often destroys any *Salmonella* that is present (Himathongkham et al., 1996; Rose et al., 1999; Veldman et al., 1995). Contact with biological vectors such as insects, mice, wild or domestic birds, animal droppings, or humans can result in infection (Schlosser et al., 1999; Daoust et al., 2000; Kinde et al., 1997). Breeding birds can pass salmonella to their offspring through the egg. Eggs maintained for hatching, whether contaminated inside the shell or on the outside, have the ability to spread to other chicks during the piping and hatching periods (Cason et al., 1994). Contaminated poultry house environments are often the leading cause of *salmonella* infection (Kumar et al., 1971). This can be the result of horizontal transmission of the bacteria via bird-to-bird contact or ingestion of contaminated feces, litter, feed, or water.

Vaccination with either killed or live vaccines have been associated with significant protection against salmonellae, though neither type has provided a constant impenetrable barrier against infection. Live salmonella vaccines have often been associated with longer-lasting protective responses in poultry. This could be due to the live vaccine providing relevant antigens to the immune system more persistently or because of the adverse effects on protective antigens during the preparation of killed vaccines (Barrow et al., 1990). Deprivations of feed or water, along with environmental stresses such as heat, have the ability to compromise the effectiveness of vaccination as well (Nakamura et al., 1994).

Live attenuated vaccines need to persist in tissues long enough to induce a protective immune response but are often avirulent and are eventually removed from vaccinated birds (Gast, 1997). Oral or intramuscular administration of various *Salmonella* mutants has provided a reduction in fecal shedding, horizontal transmission, and egg contamination after oral challenge (Cooper et al., 1992; 1993). This protection was found to persist for up to 23 weeks post vaccination. However, similar live vaccine strains have provided inconsistent evidence for cross-protection against other epidemiologically important *Salmonella* serotypes (Cooper et al, 1992; Hassan and Curtiss, 1997).

The efficacy and utilization of antibiotics to prevent or treat Salmonella infections in poultry are topics of considerable debate. Injectable antibiotics such as gentamicin and spectinomycin in the hatchery have aided in the control of *S. arizonae* (Shivaprasad et al., 1997). Likewise, polymyxin B sulfate combined with trimethoprim prevented and cleared experimental infections (Goodnough and Johnson, 1991) and administration of flavophospholipol or salinomycin sodium reduced fecal shedding (Bolder et al., 1999). Current control practices for salmonellosis in poultry no longer regularly rely on antibiotics because of their poor history in salmonella elimination and the ability to jeopardize their medical usefulness by promoting microbial resistance.

Salmonella is an important pathogenic microorganism that the poultry industry must deal with. While it often negatively affects bird performance at a young age, the impact can lead to a decreased performance overall. If MMSC is able to provide cytoprotection to the intestinal tract, it could aid in decreasing the negative impact on growth and feed performances caused by this pathogenic microorganism. It may also aid

in ameliorating the salmonella infection, either partially or fully, and making it possible for utilization as another feed additive for birds presented with a salmonellosis infection.

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Table 1. Preventative anticoccidials approved by FDA for use in feed formulation[1]

Product Name	Trade Name	FDA approval Year
Sulfaquinoxaline	SQ, Sulquin	1948
Nitrofurazone	Nfz, Amifur	1949
Arsanilic acid (sodium arsnilate)	Pro-Gen	1949
Butynorate	Tinostat	1954
Nicarbazin	Nicarb	1955
Furazolidone	nf-180	1957
Nitromide + sulfanitran + roxarasone	Unistat-3	1958
Oxytetracycline	Terramycin	1959
Amprolium	Amprol	1960
Zoalene	Zoamix	1960
Buquinolate	Bonaid	1967
Clopidol (meticlopindol)	Coyden	1968
Decoquinate	Deccox	1970
Sulfadimethoxine + ormeoprim	Rofenaid	1970
Monensin	Coban	1971
Robendine	Robenz, Cycostat	1972
Lasalocid	Avatec	1976
Salinomycin	Bio-Cox	1983
Halofuginone	Stenorol	1987
Narasin	Monteban	1988
Madurimicin	Cygro	1989
Narasin + nicarbazin	Maxiban	1989
Semduramycin	Aviax	1995

Source: FDA, U.S. Food and Drug Administration, 1999.
[1]Historical, not all products available.

III. EFFECTS OF MMSC AS A FEED SUPPLEMENT IN BROILERS

INTRODUCTION

MMSC has been shown to improve performance characteristics when added to the diet of several different species. Furthermore, this compound has been shown to protect against intestinal ulcerations in monogastrics. Due to the positive influences MMSC has shown within the other species, it is likely that similar effects could be duplicated in poultry, a species that has not been largely utilized in research with this compound.

In order to further explore the use of MMSC, two separate nutritional studies were set forth. The first experiment was aimed at evaluating the affect of MMSC on performance characteristics and gut integrity of broilers through six weeks of age. This was used as a preliminary study to determine how the chosen levels of MMSC might affect birds not undergoing an infection.

As a methionine derivative, MMSC may have the ability to aid in meeting the methionine requirements of a growing broiler. In order to investigate this, a second experiment was proposed to evaluate the potential of MMSC as a substitute for synthetic DL-methionine, while monitoring effects on bird performance and intestinal integrity.

MATERIALS AND METHODS

Animals

Two experiments were conducted utilizing broiler chickens of mixed sex. Day-old chicks were commercially hatched from eggs that came from breeder flocks of varying and unknown age. Once obtained, 384 chicks were randomly allotted to one of six treatment groups with eight birds assigned to 48 pens. All were placed in Petersime[1] batteries within a temperature controlled room having continuous lighting. Experimental feed (mash) and water were provided *ad libitum* through the 42-day experimental period. Body weights and feed consumption were recorded bi-weekly, and the calculated feed conversion was corrected for mortality on a "bird day" basis. Animal handling procedures during experimentation were in accordance with guidelines of the Auburn University's Institutional Animal Care and Use Committee.

Intestinal samples were collected from four randomly selected birds per treatment to determine any effects of MMSC on gut integrity. These birds were sacrificed on day 42 and the entire intestinal tract was excised. Tissue samples were then collected from the descending loop of the duodenum, the jejunum, just above the yolk stalk, and the ileum at the cecal junction. Samples were then placed in formalin phosphate until submitted to the Thompson Bishop Sparks State Diagnostic Laboratory[2] for slide preparation and hematoxylin and eosin (H and E) staining. Histological sections were digitally photographed and measurements were taken using Image J[3] software. For each

[1] Petersime Incubator Co., Gettysburg, Ohio
[2] Thompson Bishop Sparks State Diagnostic Laboratory, Auburn, Alabama
[3] ImageJ, U. S. National Institutes of Health, Bethesda, Maryland

tissue section, replicate measures were collected for villi length, villi width, and crypt depth.

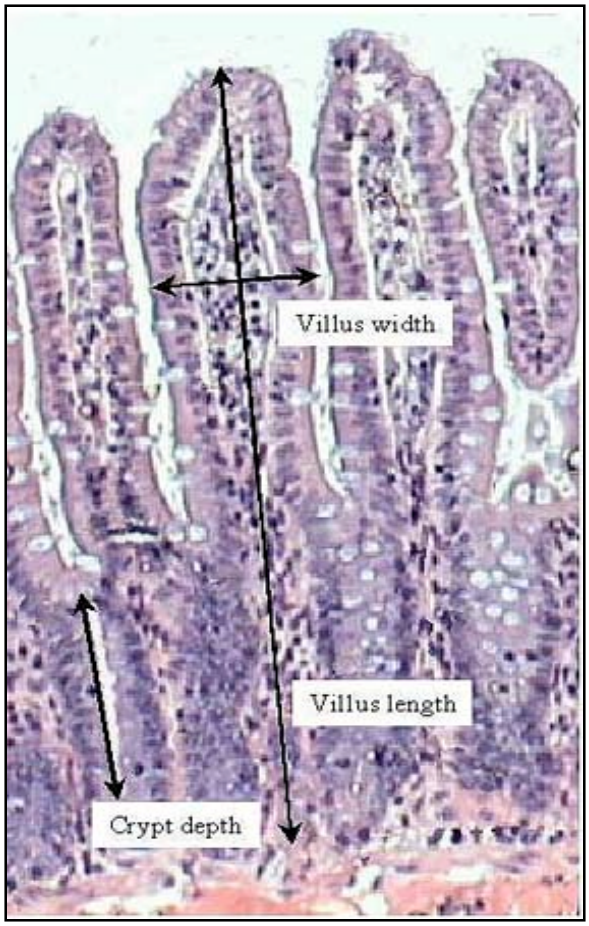

Figure 1. Villi measurements collected from each intestinal tissue sample for evaluation of intestinal integrity.

Statistical Analysis

Statistical analyses were performed using the general linear model procedure of the SAS® software (SAS Institute, 1990). All data were subjected to a one-way analysis of variance and the means were separated by Tukey's Honestly Significant Difference (HSD) procedure at the probability level of 0.05. Analyses of percentages involving mortality were performed after transformation with the arcsine of their square root.

Experiment 1. MMSC as a feed additive

All birds were fed a nutritionally complete corn-soybean meal basal diet, presented as a mash, through the duration of the experiment (Table 1). The basal starter diet, fed through the first four weeks, was calculated to contain 21.5% CP and 3142 kcal ME/kg. The basal grower diet was fed for the final two weeks of experimentation and was calculated to contain 19.5% CP and 3153 kcal ME/kg. Adding 0, 200, 400, 600, 800, or 1000 ppm of MMSC[4] to the basal diets generated the experimental treatments. Each dietary level was fed to birds, each of which was represented by eight replicate cages.

Intestinal tissues samples of four birds per treatment were collected on day 42 and ten replicate measurements were taken for the aforementioned measurements, as well as the measure of mucosal depth.

Experiment 2. Substitution of MMSC for DL-Methionine

The corn-soybean meal basal diets (Table 2) used for this experiment were deficient in methionine, but adequate in all other nutrients. The basal starter diet was formulated to contain 21.5% CP and 3142 kcal ME/kg, and the basal grower diet contained 19.5% CP and 3153 kcal ME/kg (Table 2). Experimental treatments were created by progressively adding MMSC and removing DL-methionine to the diet in 20% increments (Table 3). MMSC was calculated and added based on an equimolar equivalent of DL-methionine. For measurements of the intestinal tissue samples, six replicate measures were collected for each section of four birds per treatment on day 42 of age.

[4] Fluka Biochemika, Buchs, Switzerland

RESULTS

For both experiments, parameters of body weight, feed consumption, feed efficiency, and intestinal integrity were investigated. The basal diet fed to all birds of experiment 1 was formulated to satisfy NRC (1994) recommendations for broilers from 0 to 6 weeks of age. The basal diet utilized in experiment 2 complied with all recommendations, with the exception of methionine, which was later added to the basal diet in the form of DL-methionine, MMSC, or a combination of both.

Experiment 1

This experiment was used as a preliminary study to determine what effects MMSC may have on performance characteristics such as growth, feed, consumption, and feed efficiency in the modern broiler. While no advantage in body weight was found throughout the experiment, there was a positive impact on both feed consumption and feed efficiency during the 14 to 28 day period among diets containing MMSC, which differed ($P< 0.05$) from the control diet (Tables 4 and 5). This difference among the six diets was not seen prior to this period, nor did it persist into the 28 to 42 day feeding period.

The addition of MMSC, in comparison with the control diet, caused a decrease in duodenal villi length and width when included in the diet at any level ($P< 0.05$). Mucosal depth measures for the duodenum also showed a decreased depth ($P< 0.05$). The crypt depth, however, showed little to no change among the six different diets (Table 6).

Measures determined for both the jejunum and the ileum showed differences of significance ($P< 0.05$). No specific trend can be noted for the villi length of either

section, though the 800 ppm addition level of MMSC was similar in length when compared to the control. The crypt depth did not provide a definite trend either, but for both intestinal sections the 400 ppm level proved to be significantly shorter than the same measure found in all other diets. Similar to the previous intestinal section, the jejunal and ileal mucosal depths showed a decrease with the addition of MMSC at any level, except the 1000 ppm addition in the jejunum.

Experiment 2

This experiment was set forth to investigate the ability that MMSC may have in partially or fully replacing synthetic additions of DL-methionine in the diet of broilers. Body weights and feed consumption were also monitored throughout the duration of this experiment. The addition of MMSC at a level of 60% or lower, as well as at 100%, provided a weight gain advantage ($P< 0.05$) during the 0 to 14 day growth period when compared with the 80% addition rate (Table 7). This advantage did not continue in the following weeks. Neither feed consumption, nor feed efficiency were different among diets throughout the experiment (Table 8).

Villi length in the duodenum, while providing no definite trend, showed the longest length in those birds consuming an 80% substituted diet (Table 9). Increasing amounts of MMSC in the diet yielded wider villi ($P< 0.05$) and when provided in the diet at any level produced a deeper crypt compared with control birds. Moving down the intestinal tract to the jejunum, MMSC shows less of an effect on intestinal measures, however the 60% substitution rate provided longer villi and deeper crypts overall. By the time the ileum has been reached, diets containing MMSC have no effect on villi length,

though there is still some positive effects on villi width and crypt depth ($P < 0.05$) compared with the diet containing no MMSC.

DISCUSSION

Throughout these two nutritional studies the effects of MMSC in the diet were assessed. When MMSC was utilized as a feed additive in experiment 1, both feed efficiency and feed consumption were reduced by 11.2-11.7% and 10-16.1% respectively, during the 14 to 28 day feeding period, though this effect was not present before nor did it continue through other feeding periods. This was probably due to the bird's ability to better utilize the MMSC consumed during this specific period. Similar effects have been observed previously in both swine and cattle fed MMSC over multiple growth periods (Solenstev and Filipovich, 1978; Kalinikhin, 1986). These experiments showed an improvement in feed performances during the first and second fattening periods, respectively.

On day 14 of experiment 2, the substitution of MMSC for DL-methionine at 80% caused a reduced growth of birds compared with those fed one of the other five diets. It is not clear as to why this occurred, however this impact did not continue into the other periods, nor did it have significant effects on overall body weight gain. As stated previously, this period in the bird's development may allow for a better utilization of methionine sources and this negative impact could be due to the bird's inability to fully utilize MMSC as the main methionine source during this growth period.

Augspurger et al. (2005) noted no difference in growth performance of broilers when comparing a diet containing 1.1 g/kg MMSC as the sole methionine source and a

diet containing no source of methionine. These two diets were compared with a diet containing 0.5 g/kg L-methionine, and a difference in final weights (17 days of age) of 57g was found between the MMSC and L-methionine diets. This, however, was not the case in the present substitution of MMSC for DL-methionine. While substitution of MMSC on a percent methionine basis did not increase the final weights, it did not cause a decrease in final weights either. Given these results, it would be possible to utilize MMSC in the diet as a substitute for synthetic DL-methionine additions without negatively affecting weight gain at time of processing.

Villi are present throughout the length of the small intestine, and these villi steadily decrease in height as they descend through the intestinal tract (Bertschinger and Pohlenz, 1983; Pluske et al., 1996). This decrease in size is associated with a decreased absorptive function. For this reason, the most important section of concern for nutrient utilization is the duodenum. The duodenal samples of birds fed MMSC as a feed additive displayed a decrease in villi length and width when MMSC was included in the diet at any level. Since the decline of villi length is associated with a decreased absorptive function (Yasar and Forbes, 1999) it may be that MMSC negatively influences absorption in the duodenum at tested levels. The same results were not found in those birds fed MMSC in the diet as a substitute for DL-methionine. In these birds, the villi were longer and wider with increases in the percentage of MMSC in the diet. This difference in the two experiments could very well be related to the amount of MMSC actually provided in the diet. The highest amount of MMSC added into the substitution trial (Experiment 2) was three times larger than the highest amount provided in the additive trial (Experiment 1). Based on this, a greater amount of MMSC, as a feed additive, may be required in the

diet in order to show positive results in duodenal villi characteristics.

Mucosal depth measures for the duodenum were also decreased in birds fed diets containing MMSC. These results are similar to those found by Watanabe et al. (1996), which showed a decreased amount of mucin in the deep mucosa of rats. Based on the results concerning villi length of the jejunum for both studies, it could be concluded that the higher levels of MMSC may have more of an influence on the absorptive function lower in the intestinal tract. While this may be true, the absorptive functions of the lower gut have a decreased impact and MMSC was therefore unable to improve the bird's overall performance.

MMSC is well documented for its ability to heal and protect the intestinal tract from erosion due to ulcers (Elbers et al., 1995; Salim, 1993). It has also been shown to positively influence body weight gain in poultry that lacked certain B vitamins (Kovaleva, 1986; Ionauskene and Kanopkaite, 1988). While the addition of MMSC portrayed some ability to protect the intestinal tract, it was not enough to allow for better absorption, which could have lead to improvement in feed utilization and weight gain.

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Table 1. Composition of basal diets used in experiment 1 and offered as a starter from 0-4 weeks and grower from 4-6 weeks, % "as fed"

Ingredients	Starter	Grower
Ground Yellow Corn	55.85	63.07
Soybean meal (48% CP)	35.09	29.70
Poultry fat	4.53	3.29
Dicalcium phosphate (21.5%P; 18.5%Ca)	1.73	1.60
Ground Limestone	1.23	1.09
Iodized Salt	0.45	0.45
Trace-mineral premix[1]	0.25	0.25
Vitamin premix[2]	0.50	0.25
L-lysine (98.5%)	0.10	0.07
DL-methionine (99.9%)	0.27	0.23
Total	100.00	100.00
Calculated Analysis (%)		
Metabolizable Energy (kcal/kg	1425.0	1430.0
Crude protein	21.5	19.5
Calcium	0.94	0.84
Non-Phytate Phosphorus	0.45	0.42
Sodium	0.20	0.20
Lysine	1.27	1.10
Threonine	0.86	0.78
Methionine	0.62	0.56
Cystine	0.33	0.30

[1] Rovimix Premix, DSM Nutritional Products, Inc., Parsippany, New Jersey
[2]Auburn Chicken Trace Mineral Premix, Auburn University, Auburn, Alabama

Table 2. Composition of basal diets used in experiment 2 and offered as a starter from 0-4 weeks and grower from 4-6 weeks, % "as fed"

Ingredients	Starter	Grower
Ground Yellow Corn	55.78	63.07
Soybean meal (48% CP)	35.09	29.70
Poultry fat	4.53	3.29
Dicalcium phosphate (21.5%P; 18.5%Ca)	1.73	1.60
Ground Limestone	1.23	1.09
Iodized Salt	0.45	0.45
Trace-mineral premix[1]	0.25	0.25
Vitamin premix[2]	0.50	0.25
L-lysine	0.10	0.07
Total	99.66	99.77
Calculated Analysis (%)		
Metabolizable Energy (kcal/kg)	1425.0	1430.0
Crude protein	21.5	19.5
Calcium	0.94	0.84
Non-Phytate Phosphorus	0.45	0.42
Sodium	0.20	0.20
Lysine	1.27	1.10
Threonine	0.86	0.78
Cystine	0.33	0.30

[1] Rovimix Premix, DSM Nutritional Products, Inc., Parsippany, New Jersey
[2]Auburn Chicken Trace Mineral Premix, Auburn University, Auburn, Alabama

Table 3. Amount of DL-Methionine or MMSC added to the diet to create each experimental treatment, % "as fed" (Experiment 2)[1]

Dietary Treatment	Starter		Grower	
	Met	MMSC	Met	MMSC
100% Met, 0% MMSC	0.270	----	0.231	----
80% Met, 20% MMSC	0.216	0.072	0.186	0.062
60% Met, 40% MMSC	0.162	0.144	0.140	0.123
40% Met, 60% MMSC	0.108	0.217	0.091	0.186
20% Met, 80% MMSC	0.054	0.289	0.046	0.249
0% Met, 100% MMSC	----	0.361	----	0.311

[1]Molecular weights ascribed to each product for calculation of equimolar equivalencies were 199.70 g/M for MMSC and 149.22 g/M for DL-methionine.

Table 4. Growth performance of broilers fed varying levels of MMSC in the diet (Experiment 1)[1]

MMSC added	Body Weight, g				
	Initial	14 Day	28 Day	42 Day	Total Gain
0 ppm	44.92	424.13	1407.52	2391.30	2346.38
200 ppm	44.86	412.42	1357.59	2362.63	2317.77
400 ppm	44.68	423.40	1401.95	2330.50	2285.82
600 ppm	44.33	407.30	1349.22	2293.92	2249.59
800 ppm	44.89	410.89	1372.63	2279.22	2234.33
1000 ppm	44.96	404.56	1356.94	2276.28	2231.32
SEM[2]	0.63	7.60	26.54	35.31	35.20
P	NS	NS	NS	NS	NS

[1] Values are grand means involving 8 pens each with 8 chicks at placement
[2] SEM: Pooled standard error of the mean
NS: non-significant (P>0.05)

Table 5. Feed performance of broilers fed diets varying in levels of MMSC (Experiment 1)[1]

MMSC added	Day 0-14		Day 14-28		Day 28-42		Day 0-42	
	FC[2], g	FE[3]	FC, g	FE	FC, g	FE	FC, g	FE
0 ppm	528.73	1.248	1271.03[a]	1.293[a]	1958.94	1.959	3758.69	1.600
200 ppm	508.67	1.235	1109.66[b]	1.142[b]	2157.81	2.176	3776.15	1.606
400 ppm	528.77	1.249	1180.88[ab]	1.210[ab]	2033.46	2.015	3743.11	1.620
600 ppm	502.72	1.234	1125.03[b]	1.177[ab]	1985.16	2.087	3612.91	1.579
800 ppm	510.91	1.245	1143.94[b]	1.191[ab]	1989.53	2.075	3644.39	1.600
1000 ppm	502.90	1.243	1066.48[b]	1.148[b]	2038.25	2.163	3607.63	1.597
SEM[4]	8.74	0.02	27.72	0.03	56.32	0.07	66.69	0.03
P	NS	NS	***	*	NS	NS	NS	NS

[1] Values are grand means involving 8 pens each with 8 chicks at placement.
[2] Feed consumption is considered the amount of feed consumed per bird.
[3] Feed efficiency is the gain:feed and was corrected for mortality.
[4] SEM: Pooled standard error of the mean
NS: Non-significant (P>0.05)
*, P<0.05; ***, P<.001

Table 6. Measurements of intestinal tract integrity for broilers fed varying levels of MMSC in the diet (Experiment 1)[1]

Measurement, microns	Level of MMSC Addition						SEM[2]	P
	0 ppm	200 ppm	400 ppm	600 ppm	800 ppm	1000 ppm		
Duodenum								
Villi length	1860.73^{a}	1407.16^{bc}	1396.39^{bc}	1345.53^{c}	1483.92^{b}	1188.40^{d}	27.12	***
Villi width	181.35^{a}	126.46^{b}	122.63^{b}	126.09^{b}	120.35^{b}	122.27^{b}	4.72	***
Crypt depth	229.60^{aB}	251.04^{a}	201.90^{b}	258.72^{a}	195.77^{b}	260.35^{a}	9.21	***
Mucus depth	44.87^{a}	36.41^{bc}	33.94^{bc}	31.91^{c}	38.54^{b}	34.01^{bc}	1.33	***
Jejunum								
Villi length	1121.48^{ab}	977.03^{c}	1067.72^{b}	809.67^{d}	1196.51^{a}	1094.46^{b}	20.81	***
Villi width	125.67^{a}	108.17^{bc}	111.44^{bc}	100.37^{c}	114.39^{ab}	113.28^{abc}	3.27	***
Crypt depth	175.60^{a}	179.40^{a}	129.82^{b}	165.76^{a}	190.85^{a}	178.66^{a}	7.14	***
Mucus depth	35.14^{a}	28.90^{b}	30.40^{b}	27.63^{b}	29.45^{b}	35.13^{a}	0.96	***
Ileum								
Villi length	612.34^{ab}	529.30^{bc}	497.96^{c}	487.08^{c}	634.46^{a}	499.47^{c}	20.50	***
Villi width	143.93	133.55	138.09	130.01	137.66	141.62	4.22	NS
Crypt depth	126.54^{a}	129.97^{a}	102.38^{b}	131.02^{a}	134.45^{a}	123.90^{a}	5.22	***
Mucus depth	37.83^{a}	33.37^{b}	30.78^{b}	31.89^{b}	30.23^{b}	30.30^{b}	0.87	***

[1] Values are grand means involving 8 pens each with 8 chicks at placement.
[2] SEM: Pooled standard error of the mean
NS: Non-significant (P>0.05); ***, P<.001

Table 7. Growth performance of broilers fed diets containing MMSC as a substitute for DL-Methionine (Experiment 2)[1]

MMSC added	Body Weight, g				
	Initial	14 Day	28 Day	42 Day	Total Gain
0 %	36.72	379.45[a]	1247.44	2315.94	2279.22
20 %	36.09	377.73[ab]	1238.01	2286.70	2250.60
40 %	36.35	370.56[ab]	1250.97	2289.79	2253.44
60 %	36.09	376.56[ab]	1235.13	2264.79	2228.70
80 %	38.05	355.80[b]	1187.66	2175.94	2137.90
100 %	36.99	363.76[ab]	1243.44	2211.56	2174.58
SEM[2]	0.68	5.36	19.40	42.41	42.22
P	NS	*	NS	NS	NS

[1] Values are grand means involving 8 pens each with 8 chicks at placement
[2] SEM: Pooled standard error of the mean
NS: Non-significant (P>0.05)
*, P<0.05

Table 8. Feed performance of broilers fed diets containing MMSC as a substitute for DL-Methionine (Experiment 2)[1]

MMSC added	Day 0-14		Day 14-28		Day 28-42		Day 0-42	
	FC[2], g	FE[3]	FC, g	FE	FC, g	FE	FC, g	FE
0 ppm	469.69	469.69	1371.45	1.584	2037.75	1.958	3878.89	1.690
200 ppm	469.53	469.53	1358.95	1.576	2010.94	1.900	3839.42	1.668
400 ppm	452.24	452.24	1346.45	1.529	1999.31	1.946	3798.01	1.666
600 ppm	469.77	469.77	1333.38	1.562	2038.67	2.093	3841.82	1.712
800 ppm	454.55	454.55	1305.31	1.583	1937.45	1.963	3697.31	1.704
1000 ppm	461.39	461.39	1344.19	1.550	1998.53	2.112	3804.11	1.736
SEM[4]	10.09	10.09	20.44	0.02	48.66	0.08	64.93	0.02
P	NS	NS	NS	NS	NS	NS	NS	NS

[1] Values are grand means involving 8 pens each with 8 chicks at placement.
[2] Feed consumption is considered the amount of feed consumed per bird.
[3] Feed efficiency is the gain:feed and was corrected for mortality.
[4] SEM: Pooled standard error of the mean
NS: Non-significant (P>0.05)
*, P<0.05; ***, P<.001

Table 9. Measurements of intestinal tract integrity for broilers with MMSC substituted for DL-Methionine in the diet (Experiment 2)[1]

Measurement, microns	Level of MMSC Addition						SEM[2]	P
	0 %	20 %	40 %	60 %	80 %	100 %		
Duodenum								
Villi length	1455.81^{b}	2155.83^{a}	1657.14^{b}	1736.57^{b}	2377.01^{a}	1980.37^{b}	50.25	***
Villi width	59.38^{c}	113.40^{b}	100.78^{a}	124.85^{ab}	112.90^{b}	143.02^{a}	5.75	***
Crypt depth	69.23^{c}	176.66^{a}	114.81^{b}	127.37^{b}	117.80^{b}	112.56^{b}	9.07	***
Jejunum								
Villi length	774.07^{c}	1261.48^{ab}	997.08^{b}	1284.41^{a}	973.24^{b}	1058.56^{abc}	40.29	***
Villi width	75.89	97.34	79.75	97.39	91.16	88.37	6.01	NS
Crypt depth	74.57^{b}	88.76^{ab}	109.43^{ab}	124.05^{a}	105.65^{ab}	84.50^{bc}	8.44	***
Ileum								
Villi length	687.98^{a}	582.91^{ab}	461.13^{b}	598.73^{ab}	537.99^{ab}	645.49^{ab}	5.80	***
Villi width	54.88^{b}	92.59^{a}	77.29^{ab}	89.19^{a}	75.33^{ab}	84.20^{a}	4.32	***
Crypt depth	56.41^{c}	110.92^{ab}	99.45^{ab}	127.73^{a}	97.99^{b}	89.46^{b}	5.79	***

[1] Values are grand means involving 8 pens each with 8 chicks at placement.
[2] SEM: Pooled standard error of the mean
NS: Non-significant (P>0.05)
***, P<.001

IV. EFFECTS OF MMSC IN AMERLIORATING COCCIDIAL AND SALMONELLOSIS CHALLENGES IN BROILERS

INTRODUCTION

MMSC has been shown to protect against intestinal irritation while managing to improve performance in some animals. Little of this research has been conducted in poultry, despite the many intestinal challenges that poultry flocks may be faced with. Coccidiosis and salmonellosis are two major nuisances that the poultry industry is confronted with, both of which provide irritation to the intestinal tract and can result in a loss of performance in birds. Two separate experiments were conducted to evaluate the affect of MMSC, as a feed additive, on performance characteristics, health maintenance, and gut integrity while undergoing a challenge of either coccidiosis (Experiment 1) or Salmonellosis (Experiment 2).

MATERIALS AND METHODS

Animals

Day-old commercial broiler chicks were obtained and randomly allocated to one of six treatment groups for two separate experiments. Birds were placed in wire cages of Petersime[1] batteries. Lighting was continuous and the temperature was controlled within the room. Water and feed were continuously available. Birds were fed a starter diet through day 28 of the experiment and were then switched over to a grower diet through day 42. Body weights and feed consumption were recorded, and the calculated feed conversion was corrected for mortality on a "bird day" basis. Animal handling procedures during experimentation were in accordance with guidelines of Auburn University's Institutional Animal Care and Use Committee.

Intestinal samples were collected during each experiment to determine any effects of MMSC on gut integrity during infection. Four birds from each treatment had tissue samples collected from the descending loop of the duodenum, the jejunum, just above the yolk stalk, and the ileum at the cecal junction. These tissue samples were then placed in 10% buffered formalin phosphate and later sent to the diagnostic lab[2] for preparation and slide fixation. Histological sections were digitally photographed in order to collect measurements using Image J[3] software. Six replicate measures of villi length, villi width, and crypt depth were collected for each tissue sample.

[1] Petersime Incubator Co., Gettysburg, Ohio
[2] Thompson Bishop Sparks State Diagnostic Laboratory, Auburn, Alabama
[3] ImageJ, U. S. National Institutes of Health, Bethesda, Maryland

Experimental Diets

The corn-soy basal diets were formulated to meet or exceed all nutritional requirements set forth by NRC (1994). The basal starter diet, which was fed from 0-28 days of age, was formulated to contain 21.5% CP and 3142 kcal ME/kg (Table 1). The basal grower diet, fed from days 28-42, was calculated to contain 19.5% CP and 3153 kcal ME/kg.

Experimental treatments were generated by adding 0, 200, 400, 600, 800, or 1000 ppm of MMSC[4] to the basal diet. All dietary levels were fed to birds, each of which was represented by eight replicate cages.

Statistical Analysis

All data were statistically evaluated using the general linear model (GLM) procedure of SAS (1990). Data was subjected to an analysis of variance and means of all data were separated by Tukey's Honestly Significant Difference (HSD) procedure. Orthogonal polynomial contrasts were utilized to determine any trends present among treatments. Analyses of percentages involving mortality and cecal samples were performed after transformation to arcsine of their square root, whereas an estimate of the associated variance was expressed as the SEM of actual values.

Experiment 1. Coccidiosis study

Three hundred eighty-four chicks were allotted to one of six treatments with eight birds assigned to each of 48 pens. Birds and feed from all treatments were weighed on days 0, 10, 21, 28, and 42 for determining growth and feed performances.

[4] Fluka Biochemika, Buchs, Switzerland

Each of the coccidia species utilized were obtained from field isolates that had been maintained in potassium dichromate. These isolates required passage through chickens to obtain fresh, live oocysts.

In order to prepare these samples for passage, the excess potassium dichromate was removed. Distilled deionized water was then added to the resulting residue and was filtered by covering the beaker containing the sample with cheesecloth. The remaining solid residue was rinsed with distilled deioninzed water. The sample was then centrifuged at room temperature using the IECCR-6000 Centrifuge[7] at 2025 rpm (1000xg) for 10 minutes. After centrifugation, excess water was removed from the samples. Upon completing this process twice more, 1 ml of sample was diluted into 9 ml of water. This dilution was then placed on a hemocytometer for determining the number of sporulated oocysts.

Once counts were determined, several 10-day-old chicks were provided a 1 ml dosage of one of the three Eimeria species via oral gavage. From days four to seven post inoculation, outward signs of infection were sought and abnormal fecal droppings were collected. Abnormalities generally consisted of watery diarrhea (*E. acervulina*), bloody cecal droppings (*E. tenella*), and rust-like discolored feces (*E. maxima*). Potassium dichromate was added to fecal samples to maintain oocyst survivability through the duration of the collection. After the seventh day, all birds were euthanized via carbon dioxide inhalation and visible signs of infection within the intestine were sought. During this time, cecal cores were also collected and added to the fecal collection of those birds dosed with *E. tenella*. These cores are the result of cecal lining blood vessels that

[7] Damon/IEC, Needham Heights, Massachusetts

hemorrhage creating white pink masses in the ceca, which are formed from red blood cells, oocysts, pus and fecal material.

In order to prepare the samples for oral gavage, each species sample was blended in a mixer and strained using cheesecloth. After ensuring that unsporulated oocysts were present in each of the samples, more potassium dichromate was added and they were placed in a 30C water bath with steady agitation for at least 24 hours to ensure sporulation would occur.

MMSC treated birds were administered a 1 ml cocktail of coccidia via oral gavage at day 10 of age. The cocktail was composed of *Eimeria acervulina*, *E. maxima*, and *E. tenella* with concentrations of 125,000, 25,000, and 15,000 sporulated oocysts per milliliter, respectively.

Feces from each pen was visually assessed and scored daily from days 14-20, or 4-10 d post challenge. The number of abnormal fecal products seen determined the score, which ranged from 0-4 (Johnson and Reid, 1970). Papers were changed eight hours prior to scoring to ensure fresh fecal samples. Abnormalities mainly consisted of diarrhea, blood, and discoloration. On day 21 tissue samples were collected from the three sections of the small intestine and signs of infection were sought.

Experiment 2. Salmonella Study

Four hundred eighty chicks were allotted to one of six treatments, where ten birds were assigned to each of 48 pens. On days 7, 14, 21, 28, and 42 all birds and feed were weighed.

The *S. kentucky* strain utilized in the oral gavage of all birds was originally collected from a broiler house in Alabama. The original samples were maintained at 4C

until needed. Once removed from the freezer the isolate was grown on non-selective plate count agar (PCA)[5]. After 24 hours at 37C, colonies were selected and grown on xylose lysine tergitol-4 agar (XLT-4)[6], a media selective for salmonella, for 24 hours at 37C. Afterwards two black colonies were incubated in Brain Heart Infusion Broth (BHI)[7] for 24 hours at 37C in a shaker incubator[8] at 90 rpm. Following incubation, the bacteria were diluted using a tenfold dilution until a final dilution of 10^9 was obtained. A spiralplater[9] was used to verify the amount of salmonella used to inoculate the birds. Dilutions 10^{-7}, 10^{-8}, and 10^{-9} were plated onto PCA in duplicate using the spiralplater and incubated for 48 hours at 37C. After incubation, the number of salmonella colonies present was determined. Due to the limited number of suspect colonies, the spiralplater procedure was repeated, in duplicate, for 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} dilutions. Colonies from the final spiralplated plates were streaked onto XLT-4 agar for isolation. All plates were again incubated for 48 hours at 37C. From these plates, the amount of *S. kentucky* present was determined to be 10^8 cfu/ml.

On the day of placement, all chicks were provided a 0.1 ml dose of bacteria via oral gavage at a dosage rate of 10^8 cfu/ml. Ten birds, not allotted to treatments, were randomly selected and sacrificed to verify that the chicks from this particular hatch were salmonella negative. To determine if salmonella was present, the ceca were removed from the selected birds and placed in tubes containing 10 ml of Tetrathionate Broth Base,

[5] Difco, Becton Dickinson, Sparks, Maryland
[6] Difco, Becton Dickinson, Sparks, Maryland
[7] Difco, Becton Dickinson, Sparks, Maryland
[8] C24 Incubator Shaker, New Brunswick Scientific Co., Inc., Edison, New Jersey
[9] Whitley Automatic Spiral Plater, Don Whitley Scientific Ltd., Shipley, UK

Hajana (TTB-H)[10]. These samples were then incubated for 48 hours at 37C. On days 7 and 14 cecal samples were collected from 4 birds/trt to determine the presence and concentration of salmonella. The ceca and their contents were placed in pre-weighed bags and weights were determined. Saline solution (0.85%), which contained 8.5g of sodium chloride[11] per liter, was then added to the bag containing the ceca in a 10:1 ratio. All samples were mixed in a stomacher[12] for 90 seconds and then diluted out to 10^{-7}. Duplicate plates were streaked onto XLT-4 agar for the 10^{-5} and 10^{-7} dilutions and incubated at 37C for 48 hours. When no growth was noted, two plates each were plated on XLT-4 for the 10^{-2} and 10^{-4} dilutions and incubated. All original 10^{-1} samples from both collection days were enriched with 90 ml of TTB and incubated for 48 hours at 37C.

From the original counts it was determined that another bacterium was present, which may have suppressed the growth of *S. kentucky*. On day 14, all of the birds were re-inoculated with a 1 ml sample of *S. kentucky* via oral gavage at the dosage rate of 10^6 cfu/ml to ensure colonization of the species within the birds. Cecal samples were collected from 4 birds/trt on days 21, 28, and 42 to determine the presence salmonella. These cecal samples were placed in tubes containing 25 ml of TTB and incubated at 37C for 48 hours. Intestinal tissue samples were collected for histology on day 28 from the duodenum, jejunum, and ileum for evaluation.

[10] Difco, Becton Dickinson, Sparks, Maryland
[11] Fisher Scientific, Fairlawn, New Jersey
[12] Mix 1, AES Laboratoire, Combourg, France

RESULTS

Experiment 1

This experiment was performed to assess any effects MMSC may have on performance characteristics such as growth, feed, consumption, and feed efficiency in broilers infected with coccidiosis. Birds consuming diets containing MMSC showed no advantages ($P > 0.05$) in body weight gain (Table 2), feed consumption, or feed efficiency (Table3). Table 4 also presents results stating that MMSC, at the levels provided, was ineffective in lowering the fecal scores of treated diets when compared with the control.

Adding MMSC to the diet provided no difference in duodenal villi length or width (Table 5). There was an improvement in crypt depth ($P < 0.05$) found in birds fed a diet containing 400 ppm of MMSC in comparison with the control.

The measures collected for the jejunum showed a 22% difference ($P < 0.05$) in villi width between the 1000 ppm and 600 ppm levels. No specific trend could be noted for the villi length or crypt depth of the ileum, though differences of significance were discovered.

Experiment 2

This experiment was performed to assess any effects MMSC may have on performance characteristics such as growth, feed, consumption, and feed efficiency in broilers undergoing a salmonellosis infection. No advantages ($P > 0.05$) in body weight gain occurred in birds that consumed diets containing MMSC (Table 6). Neither feed consumption, nor feed efficiency proved to show any differences among diets throughout the experiment (Table 7). Table 8 provides data from the cecal enrichments, which showed no differences among treatments for any of the collection days.

In comparison with the control diet, adding MMSC to the diet at a level of 1000 ppm increased ($P< 0.05$) duodenal villi length (Table 9). This same additive level produced villi widths and crypt depths that were similar to those found in the control diet. The measures collected for jejunal tissue samples provided differences of significance ($P< 0.05$) for villi length and width. Though no specific trend can be noted for either measure of the villi, the 1000 ppm MMSC level increased size in comparison with the control. Villi length measures of the ileum showed a downward trend ($P< 0.05$) in length as the amount of MMSC decreased in the diet. However, the width of these villi did not differ in size among the six treatment levels. Crypt depth did not offer a definite trend, but the 1000 ppm level proved to be significantly greater than the same measure found in the control.

DISCUSSION

Both of these studies were carried out to assess the affects of MMSC on performance characteristics and gut integrity in the face of a pathogenic challenge. When MMSC was utilized as a feed additive in both challenge studies, neither feed efficiency nor feed consumption was reduced throughout the entire growth period. The original feed additive experiment showed that both of these characteristics decreased during the 14 to 28 day growth period when MMSC was included in the diet. One of the negative impacts associated with infections such as salmonellosis and coccidiosis is decreased weight gain and feed conversion (Gast, 2003; McDougald, 2003). It is likely that the birds were unable to show a decrease during the 14 to 28 day period of these experiments because of the body's inability to fully utilize the effects of MMSC during a time of infection. Since

no differences in body weight gain were noted among birds fed different diets during either study, it may be assumed that MMSC is unable to affect body weight gain during the presence of an infection when offered at the current levels.

Feeding a diet containing added MMSC to birds having been challenged with coccidia provided very little effect on villi characteristics for any of the three intestinal sections. Improvements were found in crypt depth of both the duodenum and ileum with increasing levels of MMSC. Both villous height and crypt depth are measures of integrity and damaging the intestine will cause these measures to decline (Yasar and Forbes, 1999). Because of the damage that coccidia inflicts upon the intestinal tract, it is possible that MMSC was unable to provide any positive enforcements for villi length and width, but was able to positively influence the lumen of the gut. Due to the lack of differences found among fecal scores, MMSC has no effects on alleviating the growth suppression associated with coccidiosis from birds, despite the fact that it is a sulfur-containing compound similar to the original sulfa-drugs originally used as anti-coccidials.

Villi length was significantly improved in all three intestinal tissue sections of salmonella-infected birds when fed a diet containing MMSC at a level of 800 to 1000 ppm. The small intestine is the main site for enzymatic nutrient digestion and absorption. Enlarging the villi allows for an increase in surface area of the mucus membrane (Wiese et al., 2003). Increasing the villi length in these birds may have allowed for an increase in digestibility and absorption of important nutrients. Despite the improvement in villi length, there was no improvement in growth or feed performance, which may be attributed to the salmonellosis infection. Had these levels been higher, similar to the levels provided in the methionine-substitution study, an improvement in feed efficiency

or growth rate may have been seen. It is possible that improvements could have also been noted in performance Eimeria challenged birds had the levels been increased.

The data collected for the duodenal villi width showed an asymptotic curve when compared utilizing orthogonal polynomial contrasts. Similar to the duodenal length, higher levels of MMSC increased villi width. The presence of salmonella may have also assisted in an increase of villi width and the lower levels of MMSC consumed were not high enough to overcome the colonization of Salmonella within the gastrointestinal tract. As the amounts of MMSC increased and began to have an effect on the salmonella present within the duodenum, the width was decreased until it reached a high enough level where the compound could properly aid in increased size.

Crypt depth of the duodenum showed little differences among the treatments, which would suggest that MMSC was unable to influence the lumen of these infected birds. While jejunal villi width and ileal crypt depth increased in size, especially at the 1000 ppm addition level, these improvements in villi characteristics most likely had a lesser impact on overall bird performance since the absorption of nutrients is decreased in these sections of the small intestine compared with that of the duodenum.

All cecal samples were enriched to determine the presence or absence of salmonella. Due to the small sample size collected from each treatment, no differences of significance were detected, though some treatments during the final collections showed differences ranging from 0 to 50% positive.

MMSC has been shown to positively influence body weight gain in poultry that lacked certain B vitamins (Kovaleva et al., 1987; Ionauskene and Kanopkaite, 1988). This compound is also well documented for its ability to heal and protect the intestinal

tract from erosion due to ulcers (Elbers et al., 1995; Salim, 1993). When added to the feed, MMSC was unable to provide performance advantages, or an increase in absorptive function for either study. While unable to produce a decrease in the colonization of salmonella within the intestinal tract, MMSC was able to provide some increase in surface area of the intestinal mucosa, indicated by an increase in villi length of all three intestinal sections as well as the crypt depth of the ileum.

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Table 1. Composition of basal diets used in experimentation and offered as a starter from 0-4 weeks and grower from 4-6 weeks, % "as fed"

Ingredients	Starter	Grower
Ground Yellow Corn	55.85	63.07
Soybean meal (48% CP)	35.09	29.70
Poultry fat	4.53	3.29
Dicalcium phosphate (21.5%P; 18.5%Ca)	1.73	1.60
Ground Limestone	1.23	1.09
Iodized Salt	0.45	0.45
Trace-mineral premix[1]	0.25	0.25
Vitamin premix[2]	0.50	0.25
L-lysine (98.5%)	0.10	0.07
DL-methionine (99.9%)	0.27	0.23
Total	100.00	100.00
Calculated Analysis (%)		
Metabolizable Energy (kcal/kg	1425.0	1430.0
Crude protein	21.5	19.5
Calcium	0.94	0.84
Non-Phytate Phosphorus	0.45	0.42
Sodium	0.20	0.20
Lysine	1.27	1.10
Threonine	0.86	0.78
Methionine	0.62	0.56
Cystine	0.33	0.30

[1] Rovimix Premix, DSM Nutritional Products, Inc., Parsippany, New Jersey
[2]Auburn Chicken Trace Mineral Premix, Auburn University, Auburn, Alabama

Table 2. Growth performance of cocci challenged broilers fed diets varying in levels of MMSC (Experiment 1)[1]

MMSC added	Body Weight, g					
	Initial	10 Day	21 Day	28 Day	42 Day	Total Gain
0 ppm	41.41	234.14	660.92	1103.68	2277.01	2235.60
200 ppm	42.66	243.20	670.00	1114.74	2253.77	2211.12
400 ppm	42.89	240.91	654.25	1110.51	2202.93	2160.04
600 ppm	41.56	236.42	645.13	1116.94	2261.43	2219.87
800 ppm	41.01	235.83	648.91	1100.35	2210.09	2169.07
1000 ppm	41.41	240.28	665.99	1135.75	2324.74	2283.33
SEM[2]	0.58	4.28	12.54	18.09	61.59	61.55
P	NS	NS	NS	NS	NS	NS

[1] Values are grand means involving 8 pens each with 8 chicks at placement
[2] SEM: Pooled standard error of the mean
NS: non-significant (P>0.05)

Table 3. Feed performance of cocci challenged broilers fed diets varying in levels of MMSC (Experiment 1)[1]

MMSC added	Day 0-10		Day 10-21		Day 21-28		Day 28-42		Day 0-42	
	FC[2], g	FE[3]	FC, g	FE	FC, g	FE	FC, g	FE	FC, g	FE
0 ppm	289.45	1.239	670.75	1.626	740.88	1.888	2246.74	2.237	3944.55	1.796
200 ppm	265.55	1.093	715.83	1.684	742.57	1.880	2228.54	2.184	3972.68	1.868
400 ppm	268.71	1.121	696.84	1.700	742.30	1.862	2204.66	2.071	3989.99	1.776
600 ppm	280.41	1.189	687.72	1.704	758.30	2.018	2218.12	1.826	3947.82	1.765
800 ppm	266.83	1.140	723.54	1.787	749.87	1.878	2232.43	1.876	3952.50	1.788
1000 ppm	285.40	1.186	712.11	1.748	765.76	1.875	2226.64	2.095	3912.51	1.828
SEM[4]	12.32	0.04	15.84	0.05	14.56	0.11	46.92	0.19	62.71	0.052
P	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[1] Values are grand means involving 8 pens each with 8 chicks at placement.
[2] Feed consumption is considered the amount of feed consumed per bird.
[3] Feed efficiency is the gain:feed and was corrected for mortality.
[4] SEM: Pooled standard error of the mean
NS: Non-significant (P>0.05)

Table 4. Fecal scores[1] of birds challenged with Coccidia (Experiment 1).

MMSC added	Days Post Innoculation						
	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
0 ppm	2.375	2.250	1.625	1.125	0.250	0.125	0.125
200 ppm	1.875	2.250	1.500	0.625	0.375	0.875	0.000
400 ppm	2.250	2.500	1.500	0.375	0.125	0.500	0.125
600 ppm	1.750	2.375	1.500	0.750	0.250	0.375	0.125
800 ppm	1.875	2.375	1.375	0.625	0.375	0.750	0.125
1000 ppm	2.000	2.250	1.625	0.500	0.250	0.375	0.375
SEM[2]	0.16	0.22	0.20	0.23	0.17	0.25	0.13
P	NS	NS	NS	NS	NS	NS	NS

[1]Scores: 0: No abnormal feces
1: 1-2 abnormalities
2: 3-6 abnormalities
3: 7-10 abnormalities
4: >10 abnormalities

[2] SEM: Pooled standard error of the mean

NS: Non-significant (P>0.05)

Table 5. Measurements of intestinal tract integrity for cocci challenged broilers fed diets varying in levels of MMSC (Experiment 1)[1]

Measurement, microns	Level of MMSC Addition						SEM[2]	P
	0 ppm	200 ppm	400 ppm	600 ppm	800 ppm	1000 ppm		
Duodenum								
Villi length	2309.09	1683.41	1840.64	1966.62	1617.52	1823.23	236.07	NS
Villi width	167.74	176.40	168.22	186.05	198.69	197.34	142.12	NS
Crypt depth	226.62^{b}	265.47^{ab}	333.62^{a}	311.39^{ab}	262.62^{ab}	232.63^{b}	20.01	***
Jejunum								
Villi length	759.01	731.92	744.05	756.80	691.04	674.45	32.97	NS
Villi width	159.71^{ab}	166.62^{ab}	145.08^{ab}	136.67^{b}	146.42^{ab}	175.29^{a}	8.98	*
Crypt depth	175.54	174.22	216.54	174.58	178.35	160.69	16.32	NS
Ileum								
Villi length	375.03^{b}	538.26^{a}	398.53^{a}	431.06^{b}	442.01^{a}	363.48^{b}	26.02	**
Villi width	123.53	106.11	81.47	76.42	105.09	104.49	12.43	NS
Crypt depth	91.22^{b}	133.22^{ab}	133.01^{ab}	124.95^{a}	117.17^{ab}	135.05^{a}	8.68	**

[1] Values are grand means involving 8 pens each with 8 chicks at placement.
[2] SEM: Pooled standard error of the mean
NS: Non-significant (P>0.05)
*, P<0.05; **, P<0.01; ***, P<.001

Table 6. Growth performance of broilers challenged with salmonellosis and fed diets varying in levels of MMSC (Experiment 2)[1]

MMSC added	Body Weight, g						
	Initial	7 Day	14 Day	21 Day	28 Day	42 Day	Total Gain
0 ppm	38.13	126.44	344.35	736.17	1233.65	2307.59	2269.47
200 ppm	38.26	128.10	356.43	726.19	1212.88	2316.17	2278.29
400 ppm	39.24	123.61	342.10	760.55	1310.39	2338.05	2261.27
600 ppm	39.30	132.14	349.45	746.72	1201.16	2284.57	2245.76
800 ppm	39.10	131.97	358.10	755.46	1242.03	2322.52	2283.72
1000 ppm	39.50	125.43	343.58	726.86	1241.31	2231.43	2192.38
SEM[2]	0.65	2.28	6.81	16.05	39.68	39.72	43.99
P	NS	NS	NS	NS	NS	NS	NS

[1] Values are grand means involving 8 pens each with 8 chicks at placement
[2] SEM: Pooled standard error of the mean
NS: Non-significant (P>0.05)

Table 7. Feed performance of broilers challenged with salmonellosis and fed diets varying in levels of MMSC (Experiment 2)[1]

MMSC added	Day 0-7		Day 7-14		Day 14-21		Day 21-28		Day 28-42		Day 0-42	
	FC[2], g	FE[3]	FC, g	FE	FC, g	FE	FC, g	FE	FC, g	FE	FC, g	FE
0 ppm	85.18	.674	304.46	1.214	537.63	1.464	776.19	1.722	2084.33	2.190	3787.81	1.743
200 ppm	81.57	.639	303.66	1.291	543.47	1.529	797.10	2.122	2113.12	2.132	3838.95	1.749
400 ppm	85.53	.698	301.09	1.268	542.52	1.524	805.85	1.629	2131.80	2.247	3805.12	1.764
600 ppm	87.71	.666	303.98	1.324	559.02	1.506	816.77	2.070	2037.62	2.099	3805.12	1.764
800 ppm	95.37	.720	328.25	1.329	562.63	1.497	815.23	2.002	2075.27	2.151	4244.27	1.768
1000 ppm	82.79	.660	291.34	1.189	537.41	1.505	784.96	1.716	2051.77	2.308	3748.28	1.770
SEM[4]	5.48	0.04	149.48	0.11	13.62	0.53	16.08	0.23	45.45	0.10	150.72	0.023
P	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[1] Values are grand means involving 8 pens each with 10 chicks at placement.
[2] Feed consumption is considered the amount of feed consumed per bird.
[3] Feed efficiency is the gain:feed and was corrected for mortality.
[4] SEM: Pooled standard error of the mean
NS: Non-significant (P>0.05)

Table 8. Presence of salmonella in cecal pouches of birds challenged with *Salmonella kentucky* (Experiment 2)

MMSC added	Enriched Cecal Samples				
	Day 7[1]	Day 14	Day 21	Day 28	Day 42
0 ppm	25	0	25	0	25
200 ppm	25	0	25	25	25
400 ppm	25	0	0	50	0
600 ppm	25	25	25	50	50
800 ppm	25	0	0	25	25
1000 ppm	25	0	50	0	25
SEM[2]	0.25	0.10	0.21	0.22	0.24
P	NS	NS	NS	NS	NS

[1] The value represents the percentage of positive samples
[2] SEM: Pooled standard error of the mean
NS: Non-significant (P>0.05)

Table 9. Measurements of intestinal tract integrity for broilers challenged with salmonellosis and fed diets varying in levels of MMSC (Experiment 2)[1]

Measurement, microns	Level of MMSC Addition						SEM[2]	P
	0 ppm	200 ppm	400 ppm	600 ppm	800 ppm	1000 ppm		
Duodenum								
Villi length	2070.02^{bc}	2098.52^{b}	1733.27^{c}	2069.42^{bc}	2134.55^{ab}	2476.39^{a}	77.36	***
Villi width	169.56^{a}	152.25^{ab}	129.64^{b}	137.27^{b}	149.04^{ab}	177.52^{a}	7.27	***
Crypt depth	235.80^{a}	231.64^{a}	157.14^{b}	210.68^{ab}	198.04^{ab}	217.44^{ab}	14.61	**
Jejunum								
Villi length	1017.85^{bc}	1174.01^{ab}	839.24^{c}	1270.10^{a}	1384.55^{a}	1256.95^{a}	55.14	***
Villi width	121.99^{bc}	129.94^{ab}	132.36^{ab}	100.57^{c}	122.17^{abc}	148.30^{a}	6.22	***
Crypt depth	142.49	162.40	149.25	155.67	141.90	169.03	10.12	NS
Ileum								
Villi length	460.98^{c}	500.45^{c}	570.18^{bc}	566.23^{bc}	708.68^{ab}	846.52^{a}	37.06	***
Villi width	103.27	98.75	95.81	105.66	107.70	112.39	6.01	NS
Crypt depth	103.66^{b}	109.71^{ab}	106.06^{b}	121.69^{ab}	111.51^{ab}	147.84^{a}	8.78	*

[1] Values are grand means involving 8 pens each with 10 chicks at placement.
[2] SEM: Pooled standard error of the mean
NS: Non-significant (P>0.05)
*, P<0.05; ***, P<.001

V. CONCLUSIONS

Improving performance characteristics in poultry continues to be of great importance within the poultry industry. Extensive research has been conducted to show that MMSC is capable of preventing and curing ulcers in mammals. A few studies have even shown that it can have positive effects on growth performance, hypolipidemia, and liver metabolism. Little of this research has been conducted in poultry, thereby requiring us to extrapolate the information found in mammals to avian species. The goal of the experiments described in the preceding chapters was to determine the effects of MMSC in the diet on growth performance and intestinal integrity under different situations.

All four experiments indicated that MMSC lacks the ability to increase growth performance or improve feed efficiency. The first experiment provided information suggesting that MMSC had no effect on the intestinal tract of healthy birds. This lack of effect was also shown in birds challenged with coccidia. Furthermore, when MMSC was included as an additive to birds undergoing a coccidiosis infection, there was no evidence to demonstrate its ability to decrease the numbers of coccidia oocysts shed in the fecal material. This was somewhat perplexing, considering some of the effective coccidiostats are also sulfur-containing compounds.

The substitution of MMSC in the diet for DL-methionine proved to work just as well in birds fed the 100% substitution rate those birds fed only DL-methionine to meet their methionine requirements. Furthermore, when MMSC was added to the diet at any

level, it allowed for an improvement in villi characteristics. Based on this information, it is possible to use MMSC as a synthetic dietary methionine source, either wholly or in part. It would be able to improve intestinal villi characteristics, thereby allowing for a better absorption and utilization of nutrients.

While MMSC was unable to decrease salmonella colonization of the cecal pouches when compared with the control, it did show signs of alleviating the negative consequences of the bacterium on intestinal villi characteristics. Villi enlargement, similar to what was seen in this experiment, aids in the increase in the surface area of the mucus membrane. This increase may have allowed for an increase in digestibility and absorption of important nutrients. Either the lack of improved growth and feed performance was a result of the salmonellosis infection, or the infection caused no decrease in bird performance and so there were no improvements to be needed. Without the presence of a negative control, this cannot be fully determined.

Due to the increased amount of MMSC utilized in the substitution trial compared with the additive trials (3000 ppm vs. 1000ppm, at highest levels), it is possible that the addition of MMSC at higher levels would provide more of an effect on colonization of either salmonella or coccidia in the intestinal tract. Furthermore, it may provide increased beneficial effects towards the protection of the intestinal tract. This could in turn lead to an increased absorption and possibly allow for an improvement of performance characteristics. Additional research could determine whether these possibilities could become a reality.