FEED ADDITIVES: ASSESSING THE NEXT STEP TOWARDS AN ANTIBIOTIC-FREE COMMERCIAL POULTRY INDUSTRY

by

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(Under the Direction of Adam Davis)

ABSTRACT

As the population on earth continues to grow, consumers are growing more and more cognizant of the animal protein that they consume. In their decision to eat meat from food animals, people want to know the animals were humanely reared. In addition to animal welfare concerns, these consumers do not want the animals they ingest to have been reared using antibiotics. Due to these concerns, the poultry industry is attempting to reduce antibiotic usage while continuing to produce more chickens. In order to achieve these goals, "antibiotic alternatives" are being used in the diets. Many of these alternatives are broadly classified as feed additives and include things such as aluminosilicates, essential oils, organic acids, prebiotics, and probiotics. The rapid transition away from antibiotic usage has outpaced scientific research, and new products are currently being used in the commercial poultry industry in various capacities throughout the vertically integrated system. The goal of the current research was to study a variety of these antibiotic alternatives in broiler production. Although some of the products tested in the current research improved bird growth and/or feed efficiency during some phases of the broiler grow-out, other products were detrimental to bird growth and feed utilization. The aluminosilicate- based product tested improved calcium and phosphorus digestibility in broilers

and reduced inflammatory processes in the birds, as evidenced by a reduction in an acute phase protein. The current research suggests that not all of these antibiotic alternatives are suited for all scenarios of broiler production. Though many of these products are intended for use throughout the broiler lifecycle, some are not beneficial and are actually detrimental under controlled conditions. Some of these products may be suited to aide in growth in the face of significant disease challenges, but using these products with more mild stressors is not recommended. Further studies will lead to better understanding of how each feed additive can be best used to maximize health and welfare of a flock. In doing so, the poultry industry may be able to meet the demands to produce antibiotic-free chickens.

INDEX WORDS: Antibiotic alternatives, broiler, intestinal health, aluminosilicate, essential oil, prebiotic, probiotic, acidifier, chemical

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CHAPTER 1

LITERATURE REVIEW

Introduction

According to the US Census Bureau, the earth's population of over 7.46 billion people is currently growing at 1.18% annually. This, in combination with people living longer, is estimated to result in a global population of almost 10 billion people by the year 2050 (The Economist, 2017). In addition to the increase in the human population, more people in developing countries are gaining the economic resources to support consumption of a higher animal protein diet. Agricultural science has worked hard to keep pace with the challenges of production in order to maintain relatively low prices on food animal products. Despite these agricultural achievements, land, soil, and water are becoming limiting resources as we attempt to grow enough production animals for an ever increasing demand.

In addition to a growing demand for meat products, there has been a shift in consumer ideology regarding the food consumed over the past several years. Many consumers are concerned about compounds that help promote growth given to food animals, as well as the welfare of the animals in these food production systems. There are also people in today's culture that have a desire for agriculture to revert some modern agricultural practices to its more traditional roots. The phrases "free range chickens" and "grass-fed cattle" are growing in popularity. "Cage free" and "No antibiotic" labels at the meat counter of grocers serve as a reminder of how industry is marketing towards these consumers. Many food producers and consumers are interested in the sustainability of agricultural systems in addition to welfare

practices in industrial agriculture. This is evidenced by a surge of press articles and books on this particular issue.

Many restaurant chains have increased the awareness and promotion of this consumer ideology as they created advertisements to target existing consumers willing to purchase and pay more for products produced using methods that fit their ideology. Panera Bread, Chipotle, McDonalds, KFC, Taco Bell, and Starbucks are just a few that have taken steps to promote less antibiotic use by requiring different levels of antibiotic exclusion. Some companies tout "antibiotic-free" chicken while others explain that their chicken is raised without "medically important antibiotics." Whole Foods Market is a large, specialty grocer that is specifically geared towards the previously described consumer. They state that the chicken they sell has to meet specific criteria, including no antibiotics ever, no animal byproducts in the feed, no physical alterations, and even appropriate litter for freedom to express natural behaviors (Whole Foods Market, 2018).

While these industrial giants certainly put a lot of pressure on the poultry industry, the government has also set policies in motion that will expedite this trend, as evidenced by the recent changes in food animal drug use imposed by the Veterinary Feed Directive (VFD). This law requires drugs incorporated into the diets of food animals to have a prescription for use. It also requires that a veterinarian visit the facility where the animals are housed, as well as coordinate with the feed mill, which cannot make the diet until they have veterinary approval. These steps will not completely exclude the use of preventatives being used in feeds for animal production, as the law specifically states it applies only for medically important drugs in human medicine. Many drugs, like ionophores, are not used in human medicine. Although this rule will

not exclude antibiotic use in the poultry industry, it definitely makes the use of those antibiotics used in human medicine less likely.

Consumers are becoming increasingly interested in minimizing antibiotic use while maintaining the current level of animal welfare. From the perspective of the poultry industry, this may be perceived as counterintuitive because when the antibiotics are provided in order to help mitigate disease, their use is improving animal welfare. Even so, consumer and regulatory pressures are driving the poultry industry to try to reduce antibiotic usage, and the industry is attempting to do this through the use of feed additives. For this dissertation, a feed additive will be defined as a nonessential component of a diet that is included to bring further benefit to the health and well-being of the animal. These benefits can come in a variety of flavors including increased digestibility of the diet, positive influences on the intestinal microbiome, and increased immune system activity. Because this definition is very inclusive, there is a wide range of ingredients that are considered additives. Some of these include probiotics, prebiotics, essential oils, acidifiers, phytogenic compounds, enzymes, and aluminosilicate-based (clay) products.

Aluminosilicate-based Products

Aluminosilicate-based substances contain a majority of aluminum and silicon in their matrices, typically in the form of silicon dioxide (SiO_2) and aluminum oxide (Al_2O_3). Aluminosilicates regularly have between 40% and 70% Al_2O_3 and 10% to 20% SiO_2 (Gilani, 2016). Aluminosilicate-based clays, including bentonite, zeolite, and kaolin, are included in diets to promote performance in food animal production systems. It is proposed that these clay products work via a variety of mechanisms including binding toxins, scavenging ammonia, creating an extra-caloric effect by slowing down transit time of consumed food, and influencing

the intestinal morphology and immunity (Adaszynska-Skwirzynska, 2017; Jarosz et al., 2017; Quisenberry, 1968; Wlazlo et al., 2016).

It is theorized that vertebrates in the Amazon Basin were consuming material in Collpas, or natural clay licks, to bind dietary toxins and promote intestinal health (Gilardi et al., 1999). Biologists believed this to be the case because they did not detect differences between this clay and other local sources. For several decades, other researchers have suggested that the sodium (or magnesium) content of a given clay entices various species to exhibit geophagia (Emmons and Stark, 1979; Powell et al., 2009). Their research suggests that the Peruvian deposits, which several species of animals (including parrots) chose to eat, were similar in clay content to other nearby clay banks, but the sodium and magnesium content of the soils where drastically different. Though debate remains as to why animals exhibit pica, there may be truth to both notions.

Scientists have used clay products in diets with varying degrees of success in their attempts to mimic nature. Toxin binding is a promising area of research with aluminosilicates. Kubena et al., (1998) investigated a hydrated aluminosilicate product and its ability to mitigate the effects of mycotoxicosis in broiler chicks. Chicks were reared to 21 days of age with 5 mg/kg of aflatoxin added to the diet. In this study, the researchers reported that when challenged with aflatoxin, 0.250% and 0.325% inclusions of hydrated aluminosilicate in the diet significantly reduced the feed conversion rate and increased the growth of the chicks. However, Kubena et al., also reported that tricocethene (T-2) toxicity was not assuaged by the hydrated aluminosilicate product they investigated, indicating that not all toxins were bound by the hydrated aluminosilicate. Chen et al., (2014), conducted a study in broiler chicks inoculated with 0.5 to 2 mg/kg of aflatoxin B1, which supports the previous findings. This group reported that a hydrated

sodium calcium aluminosilicate (HSCAS) product incorporated into the diet at 0.5% helped recover performance in inoculated groups on week three of the experiment. They also reported that the inclusion of HSCAS reduced the negative effects of the aflatoxin in the liver and increased expression of hepatic catalase and superoxide dismutase. Taken together, these studies support the use of aluminosilicate products in mitigating the costly influence of dietary aflatoxins.

In addition to binding toxins in the diet, clays have been used as a means to manage litter quality via decreasing NH_3 emissions (Luna et al., 2015; Wlazlo et al., 2016). Ammonia gas in excess of 25 ppm is considered harmful for a broiler's health, in addition to being a human safety concern. Decreasing the amount of aerosolized ammonia decreases secondary insults to the respiratory tract, thereby promoting poultry performance. Wlazlo et al., (2016) investigated bentonite and zeolite in their ability to reduce ammonia. They reported doses of one to 2% of either zeolite or bentonite added to laying hen manure reduced ammonia by almost 30% compared to the control. Prasai et al., (2017) also reported linear decreases in environmental ammonia as the inclusion of biochar, bentonite, or zeolite (each at 1%, 2%, and 4% inclusions) increased in broiler diets over a 46-day period. In addition, broilers in all of the treated groups performed better than the untreated control broilers in terms of body weight gain and feed conversion, but these improvements do not match the linear improvements in ammonia reduction.

Although scavenging ammonia is important for the health of the broiler flock, this might not be the driver, or at least not the only factor, for the improvement of broiler growth and feed utilization when aluminosilicate-based products are added to broiler diets. In the 1960s, J. Quisenberry at Texas A&M University conducted a series of experiments to observe the effects

of clay products in poultry (Quisenberry, 1968). In laying hens, he observed increased hen weights, increased egg weights, decreased mortality, and increased hen-day production in diets supplemented with bentonite at 2.5% and 5% inclusion. The control diet had an energy level of 945 kcal/pound, whereas the bentonite inclusion resulted in decreased dietary energy levels of 932 and 918 kcal/pound for the 2.5% and 5% diets, respectively. Though these diets had lower energy, the productivity of the hens remained similar (or better) than the control. From this, Quisenberry concluded that there are caloric-sparing effects of adding clay to the diet. He believed the increased feed utilization was due to a slower passage rate of the aluminosilicate diets, which allowed for more nutrients to be absorbed.

In addition to binding toxins and potentially slowing the intestinal passage rate of digesta, more recent publications have observed morphological and immunological changes with dietary inclusions of aluminosilicate products. A study with zeolite, an aluminosilicate of volcanic origin, was completed on Ross 308 broilers from one to 40 days of age fed diets supplemented with 2% and 3% zeolite (Adaszynska-Skwirzynska, 2017). These researchers reported that the zeolite increased villi surface area in the distal half of the small intestine. Jarosz et al., (2017) reported that broilers fed 2% and 3% zeolite had stimulated lymphocyte proliferation, specifically CD4 T cells, as well as pro-inflammatory cytokines such as interleukin 10 (IL10), compared to broilers fed a control diet. The researchers warned that dietary inclusions greater than or equal to 3% may cause intestinal inflammation and other negative consequences, however, both the 2% and 3% zeolite inclusions led to statistically increased body weights.

Alternatively, some researchers have reported no differences when experimenting with different inclusions of aluminosilicate products. Schneider et al., (2016) reported no differences in broilers fed 5 g/kg (0.5%) zeolites over a 42-day period compared to control diet-fed broilers.

In the same study, broilers raised on litter conditioned with 100 g/kg (10%) zeolite did not have improved body weight gain or feed conversion rates, but there was reduced litter moisture in this group compared to the control and the dietary zeolite inclusion groups.

The inconsistencies in the animal research results with aluminosilicate products may be expected given that parameters such as dietary toxin levels and ammonia production capacity are going to be highly variable from experiment to experiment. Overall, given the biological principles involved in toxin binding and decreased ammonia production as well as the positive research results, aluminosilicate products can have a role in improving broiler production. In addition to the research-defined mechanisms by which aluminosilicate products could positively affect broiler production efficiency, there also may be an unidentified nutrient(s) in some of these clay products that promote broiler growth by fulfilling unestablished nutrient requirements.

Rare Earth Elements

Rare Earth Elements (REE) have come to light recently in Western agricultural practices, but they have been incorporated in Asian agricultural production programs for decades. REEs have been described as 17 elements, which include: cerium (Ce), lanthanum (La), europium (Eu), gadolinium (Gd), holmium (Ho), erbium (Er). thulium (Tm), ytterbium (Yb), lutetium (Lu), promethium (Pm), neodymium (Nd), praseodymium (Pr), samarium (Sm), terbium (Tb), yttrium (Y), and scandium (Sc). The content of these elements within Earth's crust varies depending on the type of soil and geographical region (Hu et al., 2006). These elements can be separated into two groups based on physical attributes: light rare earth elements and heavy rare earth elements. Light REE's include lanthanum, cerium, praseodymium, neodymium, promethium, samarium, and europium. The rest of the elements in the lanthanide group from atomic number 64 (gadolinium) to 71(lutetium) are considered heavy REEs. Some also consider scandium (Sc) and

yttrium (Y) to be REE even though they do not belong to the lanthanide series. Lack of sensitive tests to detect these elements, as well as the diversity of samples being tested, has limited understanding of the distribution of these elements. Based on over 400 samples from around the world, China's soil appears to be one of the world's richest in REE with an average of 176.67 mg/kg of soil based on 279 samples tested. Based on testing 9 samples, Australia has an average of 104.83 mg REE/kg of soil, the USA has 57 mg/kg of soil based on 30 samples, and Germany has a lower average rate of 15.48 mg REE/kg of soil. The high REE content of China's soil results mainly from light REEs (over 90%), with lanthanum and cerium being the biggest contributors (Hu et al., 2006).

Rare Earth Elements have been used as a feed additive in a variety of food animal species, including aquatic species and poultry. A relatively recent study on gibel carp reported that the addition of higher amounts of REEs does not lead to better performance. In the study, carp fed diets supplemented with 0.08% (0.8 g/kg) REE gained more weight than carp fed the control diet or this diet supplemented with 0.4 (4 g/kg) and 0.8% (8 g/kg) REE (Zhou, Q., et al., 2016). In 1997, Shang and Liu conducted experiments with a product containing REEs in laying hens, broiler breeders and broilers, and reported positive trends in performance (Shang and Lui, 1997). In these experiments, a REE was classified as one belonging to the lanthanide series, as well as ytterium and scandium. In laying hens, they reported increased egg production and feed efficiency with dietary inclusions from 0.003 to 1% REE. Broilers given 0.002% to 0.04% REE experienced improvements from 4-14% in body weight gain, as well as improved feed conversion rates and eviscerated yield. Hatchability of eggs was also improved by the addition of the REEs in their research. Their broiler results agree with the research of He et al., (2009) who reported improvements in body weight gain of Ross broilers being fed REE supplements from

day of hatch to 35 days of age in two experiments. They also measured blood serum parameters including aspartate aminotransferase (AST), creatine kinase (CK), glucose, total protein, albumin, globulin, phosphorus, calcium, potassium, and sodium, and reported no differences. Improvements in body weight gain and feed conversion have also been reported in Japanese quail (*Coturnix coturnix*) fed diets supplemented with REE from zero to four weeks of age (Eleraky and Rambeck, 2011). A REE/yeast product utilized in older laying hen (52 weeks old) diets improved egg production in the supplemented hens after four weeks of consuming the amended diet (Cai et al., 2015). Although the yeast may have influenced the results beyond the REE inclusion, other research with specific REE, to be discussed subsequently, suggest that REE can have an influence on laying hen productivity.

Despite the positive results from the previously mentioned studies, not all REE studies have shown promising results for promoting poultry productivity. Schuller et al., (2002) added REE to diets in order to quantify performance alterations in swine and poultry production systems. They included a REE salt into their basal diet at 0.0075% and 0.03% inclusion rates. In swine, they reported higher daily weight gain and better feed conversion. However, there were no changes in the performance of the laying hens being fed REE.

Cerium and lanthanum have been explored individually in animal production systems. These experiments with individual elements in REE deposits help give insight into potential mechanism of action of these major components of REE products. In Lohman Brown laying hens from 22 to 32 weeks of age, cerium oxide supplemented at levels from 100 mg/kg to 400 mg/kg indicated that cerium may be one of REE that contributes to observed changes in performance (Bolukbasi et al., 2015). The hens supplemented with cerium had improved feed conversion rates and increased egg production compared to hens fed diets containing 0 mg/kg of

cerium oxide. Albumen weight, yolk weight, shell weight, specific gravity, Haugh units, and shell thickness were the same between treatments. The shell breaking strength was greater in the 300 mg/kg and 400 mg/kg cerium supplemented groups relative to the control group. Calcium and phosphorus levels were increased in the blood of hens fed diets supplemented with 100 mg/kg cerium oxide relative to control hens. Hens receiving diets with 200 mg/kg, 300 mg/kg, and 400 mg/kg cerium also had lower malondialdehyde and superoxide dismutase in their serum than hens fed the control and 110 mg/kg inclusion level diets. These experiments suggest that cerium may be used in poultry production without negatively impacting performance, and it may reduce oxidative stress while improving production.

Researchers from this same group also studied changes in egg production with the same inclusion rates of lanthanum oxide in the diet (Durmus and Bolukbasi, 2015). They reported that the highest dietary inclusion (400 mg/kg) rate resulted in greater egg production and lower feed conversion rates over the 10-week experimental period. Serum Ca and P concentrations were unchanged among treatments. Malondialdehyde was reduced in the group of hens fed 300 mg/kg lanthanum oxide and lower malondialdehyde was reported in the yolks of eggs produced by the hens in the 200 mg/kg, 300 mg/kg, and 400 mg/kg lanthanum oxide treatment groups.

In broilers, dietary lanthanum supplementation has resulted in inconsistent bird performance results. Agbede et al., (2011) reported that lanthanum oxide at 85.3, 171, and 256 ppm in the diet increased the total weight gain in Arbor Acres broilers in a 56-day experiment. A similar study conducted the next year did not detect performance differences between broilers fed the control diets or those fed diets containing 100, 200, 300, and 400 mg/kg of lanthanum oxide and lanthanum chloride (Igbasan and Adebayo, 2012). Furthermore, these researchers did not report differences in the organ weights, carcass yields, or biochemical and haemotological

parameters in the broilers from the different treatment groups. Though there is a possibility that lanthanum does not consistently alter broiler performance, it is important to note that the sample size (30 birds per treatment and 20 birds per treatment, respectively) utilized by the researchers was very small. It appears further research is needed.

Azomite

AZOMITE® is a hydrated sodium calcium aluminosilicate product that contains trace minerals and elements. It is mined near Nephi, Utah, which is south of Salt Lake City, Utah. The word "AZOMITE" is an acronym which stands for "A to Z of Minerals Including Trace Elements." Throughout the rest of this dissertation, this product will be referred to and written as "Azomite." Hydrated sodium calcium aluminosilicate has been classified as generally recognized as safe (GRAS) by the U.S. Food and Drug Administration (FDA). Similar to the Ceolpas in the Amazon Basin, Azomite may be acting via multiple mechanisms including toxin binding, providing nutrients, or providing some other benefit due to its distinct composition of hydrated sodium calcium aluminosilicate, rare earth elements, and other trace elements.

Though Azomite contains a high portion of aluminosilicate and is legally defined as a hydrated calcium aluminosilicate, its composition differs from other clays. One of the differences between clay products is the elements that compose them. Both calcium bentonite clay and Azomite consist of an aluminosilicate base. But, calcium bentonite clay only has a total of 15 additional elements, which is in stark contrast to the 74 elements regularly retained in Azomite. Azomite consists of 529.7 ppm total REEs or 529.7 mg REE/kg of soil (Azomite International-COA). It contains all of the elements that are considered REEs except for promethium (Pm) which is not typically found in the earth's crust (Hu et al., 2006). In Azomite, 492 ppm of the

total REE are light REE, giving the light REEs a total of 92.9% of the total REE content in Azomite.

Geologists have described Azomite as a volcanic [or rhyolitic] tuff breccia. In petrology (the study of rock), Azomite does not fit perfectly into any of the three major categories: igneous, sedimentary, or metamorphic rock (Ehlers and Blatt, 1982). Its volcanic origin puts it squarely into the category of igneous rock, but there is also some influence from sedimentary rock. Tuff and breccia are descriptors which both mean that a rock is composed of many different fragments and sediments that are bonded together with volcanic ash. Subtle differences distinguish them. A tuff is consolidated volcanic ash and dust that contains sediment (less than half). This ash sediment packs down to form the rock, and in doing so, forms small spaces within the rock. Breccia is similar, but it describes this compacted ash containing angular mineral fragments in excess of two millimeters within its compacted matrix (Ehlers and Blatt, 1982). Azomite is typically angular in appearance, though it can be rounded. The rock forming the Azomite deposit is light pink or coral in color with black, gray, red, and yellow streaks and dots, which are various mineral deposits.

Although Azomite has been used in agriculture for over 70 years, there are few published scientific studies examining the use of the product in animals. Initially, the product was used as a soil amendment for plant growth, yielding results in numerous horticultural species. Though peer reviewed university trials remain limited at this time, there are reports of Azomite being used successfully for cultivation of peaches, citrus, figs, tomatoes, wheat, and grapes (Azomite International, Studies and Tests).

More recently, research with Azomite has gravitated away from its use as a soil amendment to its potential use in agricultural animal production systems. Research in aquatic

species, mainly tilapia and shrimp, has suggested that Azomite is enhancing the immune systems of these organisms, thereby promoting growth (Liu et al., 2011; Tan et al., 2014). Azomite research has also been conducted in carp species, which are the most common fish produced in aquaculture worldwide. In a study on grass carp (*Ctenopharyngodon idella*), researchers reported increased growth and lower feed conversion rates in carp fed a diet supplemented with 0.2% Azomite for 8 weeks relative to carp fed a control diet without Azomite (Liu et al., 2011). However, dietary inclusion rates of 0.3% and 0.4% Azomite did not alter feed conversion rates. Liu et al., reported increased intestinal protease, lipase, and amylase levels and higher superoxide dismutase levels in the serum of the carp fed diets containing Azomite. Liu et al., (2011) concluded that Azomite was heightening the nonspecific innate immune system in addition to increasing intestinal enzyme activity. They also suggested that 0.2% may be the most ideal inclusion rate moving forward given the results of their study. Other researchers have investigated similar inclusion rates of Azomite in the diets of koi (*Cyprinus carpio*) fingerlings, an ornamental carp species. Jaleel et al., (2015) used dietary Azomite inclusion rates of 0.0, 0.2, 0.4, and 0.6 percent in their study. They reported growth rates and immune parameters were the most improved in the fish fed 0.4% Azomite in the eight-week study.

Tilapia (*Oreochromis sp*.) are second to carp in terms of worldwide aquaculture finfish production. In 2009, Liu et al., studied how tilapia (*Oreochromis niloticus* x *Oreochromis aureus*) performance was altered by including Azomite at 0, 0.25, 0.50, and 0.75 percent in the diet. They had three replicates of 20 fish for each dietary treatment and they recorded their findings after 30 days. In addition to reporting performance, they also analyzed intestinal morphology and some serum parameters. They reported that all dietary treatments containing Azomite had increased weight gain and decreased feed conversion rates. Compared to the

control, 0.25% and 0.50% inclusions of Azomite resulted in significantly increased villi height and width. The tilapia from these two dietary treatments also had increased protease activity in their intestine and stomach, increased dry matter digestibility, and increased *Lactobacillus* numbers in the intestine. The tilapia fed the 0.25% inclusion rate also had higher level of superoxide dismutase and lysozyme than the control tilapia. Azam et al., (2016) replicated the previous study by Liu et al., by using dietary Azomite inclusions at 0, 0.25, 0.5, and 0.75 percent inclusion rates in male tilapia (*Oreochromis sp.*) over a 49 day experimental period. They found that the tilapia fed the higher Azomite inclusion rates of 0.5 and 0.75% weighed more than the control tilapia and had lower feed conversion rates. The tilapia fed the diet containing 0.75% Azomite had an average final weight of 33.49 grams compared to 20.75 grams for the control tilapia and a feed conversion ratio of 2.22 compared to a 4.33 ratio for the control tilapia. They did not see increases in lipase enzymes in the Azomite treatments.

Musthafa et al., (2015) looked further into the functionality of the immunomodulatory changes in tilapia by designing a challenge study with *Aeromonas hydrophila*. This bacterium is known to cause elevated mortality in aquatic species, including fish and amphibians. The researchers had a positive and negative (infected) control as well as three dietary inclusions of Azomite (2 g/kg, 4 g/kg, and 6 g/kg). Each treatment was represented by three replicates of 25 tilapia. Thirty days after being acclimated to the diets the fish were inoculated with 100 microliters of PBS with 3.1×10^7 cfu ml^{-1} of *Aeromonas hydrophila* in the peritoneal cavity. Blood was collected on weeks 1, 2, and 4 post-inoculation. On weeks 2 and 4 post-inoculation, the tilapia fed 4 and 6 g/kg levels of Azomite had increased lysozyme activity, respiratory burst (measured via reactive oxygen species), and lower mortality.

Studies using Azomite in shrimp have had similar results to those of the finfish studies. In one study in Pacific white shrimp (*Litopenaeus vannamei*), Tan et al., (2014) reported that shrimp fed diets containing 2.0 and 4.0 g/kg of Azomite relative to control diet-fed shrimp had significantly greater weight gain and feed conversion ratio improvements over the six-week experimental period. In addition to these findings, the researchers also reported resistance to stressors (artificially induced hypoxic conditions) and improved survivability when inoculated with *Vibrio alginolyticus* in the shrimp fed diets supplemented with Azomite. Significant increases in stomach protease, hepatopancreas lipase, serum lysozyme, and phenoloxidase were observed in the shrimp fed the 4.0 g/kg Azomite diet. Shrimp fed the lower inclusion (2.0 g/kg) of Azomite had similar enzyme level trends, but these shrimp also had significantly increased levels of superoxide dismutase. Interestingly, shrimp fed diets with higher inclusion rates of Azomite at 6.0 and 8.0 g/kg did not exhibit the performance improvements seen with the lower inclusion rates, but they did have increased serum lysozyme and phenoloxidase levels compared to the control shrimp. The increased digestive enzymes would best explain the improvements observed in body weight gain and feed conversion rates in the Azomite treated groups (2.0 mg/kg and 4.0 mg/kg). The primed innate immunity might also help with growth if there were unrecognized pathogenic insults in the environment. With more robust immune systems, fighting non-clinical infections would be faster and there would be less malaise resulting in decreased feed consumption.

In an experiment with Black Tiger Prawn (*Panaeus monodon*), Azomite was added to three ponds prior to adding the larval shrimp (Azomite International, Black Tiger Shrimp). Three ponds without added Azomite served as the control. Comparisons indicate the Azomite-treated ponds exhibited an increase in the phytoplankton (algae) and zooplankton populations of the

aquaria. They also reported improved growth (grams per prawn) over a 120-day period and an improved survivability in all treated ponds. Although Azomite was not used in the diet, the influences in the ecosystem resulted in better performance in the treated group.

Azomite has also been reported to promote positive performance attributes in broilers. In a meta-analysis from 13 contract research farms and 10 integrator trials, Emerson and Hooge (2008) reported an increase in breast meat yield of 0.70 and 0.38%, respectively. Controls were represented by broilers grown without dietary Azomite and the treated group had the volcanic ash included in the diet. Unfortunately, the abstract from this 2008 meeting does not provide detail regarding the number of poultry included in the study, the inclusion rate of Azomite, nor details of the environment and experimental design.

For a laying hen trial, 96 W36 Hy-Line hens were split into two groups (Malheiros et al., 2018). The first group received a standard laying hen diet and the second group received the same diet supplemented with 0.25% Azomite. The experimental period was from 67 and 85 weeks of age and included a non-fasting molting period. Body weight, feed intake, feed conversion ratio, and the egg quality parameters (shell color, egg weight, Haugh unit, yolk color, and shell thickness) were not different between the groups. At 85-weeks, in the post molt period, the hens receiving Azomite had improved percent hen housed egg production compared to the control. Because there was no mortality, the percent hen day egg production was the same as the percent hen housed. The hens fed Azomite also had lighter tibia bone weight. The lighter tibia bones were likely a consequence of increased egg production.

While there are many studies that support the use of Azomite in different agricultural production systems, there is not a wealth of research exploring the potential benefits of Azomite's use in the poultry industry. Even less is known about potential mechanisms by which

Azomite might be improving the performance of poultry. Further research is needed to determine if Azomite could assist in the transition to antibiotic free poultry production.

Probiotics

Probiotics have been defined many ways. This term is continually being revised as these products are being used more frequently in conventional animal agriculture. Some of these definitions have focused on specific organisms within the microscopic ecosystem, while others included bacterial metabolites (Lilly and Stillwell, 1965; Parker, 1974). These metabolites are not living organisms, which contradicts the traditional definition of probiotics. Fuller (1989) describes the term probiotic as a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance. Though this definition may continue to be refined, it incorporates very important verbiage. As defined by Fuller, probiotic describes a live culture, as opposed to compounds that alter the flora. Therefore, metabolites like enzymes and antibiotic compounds are excluded. It also means that the product is consumed orally in order to benefit the host's microbial populations. The use of the term microbial is strategic as well. Though microbial might suggest bacteria, it does not exclude other microorganisms, including protozoa and fungi, which should be included in the definition.

Probiotics are added to human and animal diets in order to provide benefit to the host's microbiota. The intestinal microbiota of poultry is a dynamic community which changes throughout the life of the birds (Oakley et al., 2014). This ecosystem was influenced for decades with antibiotics in the diets, but as the commercial poultry industry steps away from antibiotic usage, the intestinal microbiota may also be influenced by incorporating prebiotics and probiotics. These products are being incorporated in order to mollify the transition away from antibiotic usage. They help competitively exclude pathogenic bacteria through a variety of

mechanisms. Probiotics are suggested to be most valuable at early life stages or after stressful events. Just as probiotics prime the intestine, they can be used to increase the microbial diversity of fresh litter (Maurer et al., 2013). As the litter matures, regardless of how the chickens are supplemented, microbial diversity expands.

Recycled litter has served as one of the most important probiotics in the poultry industry for decades, but has recently attained more attention. Torok et al., (2009) reported that cecal microbial populations were significantly altered in broilers at two and four weeks of age when using reused litter as opposed to fresh litter. No differences in microbiota were observed when comparing six different fresh bedding materials.

There may be a tendency to evaluate the litter as a whole, but the litter microbiota throughout the house differs slightly depending on location within the house. Locatelli et al., (2017) reported that in a commercial broiler house, the litter under and near drinker lines had significantly different bacterial populations than other areas of the house (near the cooling pad, near the fans, and "bulk litter locations"). The authors advised that sampling location in a poultry house would be an important consideration when interpreting bacterial populations in litter across houses. This information may also be useful for mindful brooding as the host's early microbiome becomes more of a focus.

Although there may be concerns about pathogen proliferation in systems that encourage the use of reused litter, this concern may be unfounded. Chinivasagam et al., (2016) reported that in a multi-year study observing a new litter, partially replaced litter, and recycled litter, there were no differences in *Escherichia coli* or *Campylobacter jejuni* between groups. The research was conducted in Queensland, Australia, where they practice thinning, or an initial harvesting of the poultry in the house, to make room for others in the poultry house. For *Campylobacter* in

particular, the level of bacteria in the house at thin-out was often non-detectible, but they reported several logs of the bacteria a few weeks later when rest of the chickens were harvested. Though this may be a coincidence, it might also suggest that fomite (human boots and catching equipment) introduction is of greater concern for *Campylobacter* in a poultry house than the practice of reused litter. Further research will be needed to determine the potential benefits of utilizing recycled litter rather than fresh litter.

Many different bacteria are used as probiotics to inoculate the gastrointestinal tract with live organisms to benefit the host. Currently, some of the more common probiotics used in poultry production include *Bacillus sp.*, *Lactobacillus sp.*, *Bifidobacter sp*., and *Pediococcus sp*. (Ajuwon, 2016; Noohi et al., 2016). These are often obtained from healthy chickens or healthy environments and subjected to various tests, such as tolerance to acidic conditions and bile acids, susceptibility to antibiotics, secretion of enzymes, antibacterial properties, and biofilm characteristics (Noohi et al., 2016). Rigorous testing helps ensure the bacteria will make it through feed manufacturing and the physiology of the upper gastrointestinal tract. It also gives the researchers insight into how the bacteria may benefit the animal. Lastly, in vitro testing shows whether the bacteria can be controlled with antibiotics and whether or not it can be used in tandem with other drugs. Broadly, probiotics help inoculate the gastrointestinal tract. More specific mechanisms of action have been reported. Generally speaking, probiotics are thought to work by competitively excluding pathogenic and less beneficial bacteria, by enhancing the immune system, and by altering host metabolism and digestion (Ajuwon, 2016; Fuller, 1989). The gastrointestinal flora can be an effective weapon in battling pathogenic insults. Keerqin et al., (2017) transfaunated ileal and cecal flora from a flock previously challenged with necrotic enteritis to broiler chicks. They subsequently recreated a necrotic enteritis challenge. The broilers

that were cloacally inoculated with the flora maintained lower feed conversion rates under challenged conditions. The chicks administered cecal flora also had a lower small intestinal lesion score analogous to the unchallenged control. Intestinal microflora may be effective in promoting growth and efficiency, but more research needs to be performed to identify effective bacterial strains and modes of action.

Bacillus are a genus of spore-forming, gram-positive bacteria endogenous to the environment. Probiotics in the genus *Bacillus* are currently popular for use in poultry. Their ability to form spores makes them a very promising candidate because the dried spores can handle a broad range of environmental conditions. Many strains of *Bacillus subtilis* have been used in the poultry industry to promote broiler performance (Balamuralikrishnan et al., 2017; Fritts et al., 2000; Wang et al., 2016). Jayaraman et al., (2017) suggested that one strain (PB6) could be used in place of antibiotic growth-promoting drugs. They reported increased villi height and villus/crypt ratios in the duodenum and jejunum of broilers receiving the probiotic, as compared to the control and antibiotic groups. The researchers also reported improved weight gain and feed conversion ratio with the *Bacillus* product, as compared to the non-medicated control. Their data suggests that the probiotic may be encouraging intestinal health, thus improving broiler performance. However, in this experiment there was no pathogenic challenge introduced.

Improved growth and feed efficiency data has been reported in broilers fed various *Bacillus subtilis* strains and combinations (Fritts et al., 2000; Wang et al., 2016). Fritts et al., (2000) reported that in addition to an improvement in growth, broilers fed a *Bacillus* product also had lower levels of *Coliforms, Campylobacter,* and *Salmonella* on the carcass pre-chill than the control birds. Balamuralikrishanan et al., (2017) reported similar improvements in broiler

performance with a combination of *Bacillus* sp. included in the diet. They also reported increase in *Lactobacillus* bacteria in the excrement of the broilers fed the probiotic. However, this is a contradiction to the reports by Wang et al., (2016), who did not report any changes in *Lactobacillus* concentrations due to inclusion of *Bacillus* species in the diet. Broadly speaking, these experiments suggest that *Bacillus* probiotics are altering the microbial community to favor less pathogenic bacterial strains, which may also be contributing to better growth and performance.

Reis et al., (2017) also observed improvements in growth and feed conversion at multiple stages of their experiment when adding a *Bacillus subtilis* probiotic to the diet, although there were no significant differences in final body weight and overall feed conversion at the conclusion of the six week experiment. These researchers also measured pH of various portions of the intestine and reported increased pH in the distal gastrointestinal tract (jejunum and ileum) as a result of feeding the *Bacillus subtilis* probiotic. This report is contrary to others, who have suggested that *Bacillus* sp. may increase the lactic acid producing bacteria and thus lactic acid levels (Balamuralikrishnan et al., 2017). Reis et al., also reported differences in total tract digestibility between the diets, with the broilers fed the probiotic having increased dry matter digestibility as well as apparent metabolizable energy (AME_N). They suggested that increased nutrient utilization is likely the reason for the reported decrease in feed conversion rate in the probiotic-supplemented group. Because no changes were observed in the ileal digestibility, it is likely the observed overall improvements may have resulted from increased cecal digestion. If the probiotic bacteria were resulting in improved digestion through traditional means, like producing exogenous proteases or xylanases, the increased digestibility should have been observed by the time the digesta reached the ileum. While enzyme secretion may not be the

mechanism by which this strain of *Bacillus subtilis* worked, other *Bacillus subtilis* strains are very effective at secreting protease into the environment (Uddin et al., 2017). The previously described studies suggest probiotics help improve performance without pathogenic stress, but others have reported that these introduced bacteria also help stave off infectious agents. Hayashi et al., (2018) challenged broilers with *Salmonella enterica* serovar Heidelberg. Broilers supplemented with *Bacillus* sp. in the diet had reduced colony-forming units compared to the unsupplemented control broilers in both the liver and ceca.

Bacillus licheniformis is another probiotic being used in the poultry industry. The efficacy and diverse functions are similar to *Bacillus subtilis* strains. Liu et al., (2012) reported broilers consuming *Bacillus licheniformis* (No. 1265) exhibit increased body weight gain and decreased feed conversion rates when no challenges were introduced. They also reported broilers fed *Bacillus licheniformes* had improved meat quality compared to broilers fed a control diet. This species of *Bacillus* has also been reported to be effective in helping broilers cope with necrotic enteritis challenge (Knap et al., 2010; Zhou, M. et al., 2016). Knap et al., (2010) reported, based on a series of three experiments, that feeding *Bacillus licheniformis* (DSM 17236) increased body weight gain, decreased feed conversion rate, decreased lesion scores, and decreased mortality when broilers were subjected to a necrotic enteritis challenge. They suggested this bacterium could have had the reported effects through a variety of mechanisms, including immune modulation, bacterocin production, and enzyme production, but concluded the exact mechanism(s) was unclear. Zhou, M. et al., (2016) reported similar findings, but they also reported that feeding a *Bacillus licheniformis* probiotic decreased oxidative stress during a necrotic enteritis challenge.

Several *Bacillus* species have been investigated for enzyme production. While many of these have been studied as a means to degrade industrial waste, they have also been used to help digest products that are high in keratin, like feathers and feather meal, which could have implications for some commercial poultry diets (Daroit and Brandelli, 2014). While both *Bacillus subtilis* species and *Bacillus licheniformis* have been investigated, mixed results exist due to differences in each bacterial strain. For example, Marzotto et al., (2011) reported that a strain of *B. subtilis* outcompeted another *B. subtilis* and *B. licheniformis* strains in terms of keratinase production and feather degradation. On the other hand, Hmidet et al., (2010) reported that the *Bacillus licheniformis* they tested had higher production of a-amylase and protease than other *Bacillus* species tested. As suggested by Knap et al., (2010), the enzymes produced may also help to degrade toxic compounds like the alpha toxin and the NetB toxin released by *Clostridium perfringens* species during some necrotic enteritis infections. Thus, these exogenous enzymes produced by these probiotic species may serve to increase available sugars and amino acids from ingested carbohydrates and protein in some diets, as well as degrade toxic compounds produced in the intestine.

Bacillus species are also efficacious under introduced toxic conditions. Ma et al., (2012) introduced mold from corn at different levels and reported the *Bacillus subtilis* evaluated (ANSB060) reduced gross anatomical lesions in the liver. They also reported the probiotic helped reduce oxidative stress by maintaining superoxide dismutase and glutathione peroxidase levels and reducing malondialdehyde concentrations in toxemic birds.

Probiotics and the intestinal microflora have been suggested to help mitigate stress. Acute, maintained stressors, such as heat stress and feed deprivation, have been documented to alter intestinal morphology and intestinal microflora in six week old broiler cockerels with heat

stress causing a decreased crypt depth in the distal ileum (Burkholder et al., 2008). Ashraf et al., (2013) reported that inclusion of a *Lactobacillus*-based probiotic in the diet during cyclic heat stress insults helped the intestine microarchitecture cope with the insult. The villi height, crypt depth, and surface area of the duodenum were decreased in the heat stressed control birds relative to the heat stressed birds fed the diet containing the probiotic. Improvements in villi height and surface area were also reported in the ileum and the jejunum of the heat stressed birds fed the probiotic. Deng et al., (2012) reported similar findings in laying hens supplemented with *Bacillus licheniformis* under heat-stressed conditions. In addition to maintaining longer villi in the ileum and ceca, the hens supplemented with *Bacillus* also had lower corticosterone levels and acute phase protein (interleukin 1) compared to the heat-stressed control hens. Under ideal, non heat stress, conditions, laying hens fed a *Bacillus licheniformis* probiotic did not have a decrease in corticosterone, but these hens did have a decrease in adrenal cortical hormone (Lei et al., 2013). Broilers fed a probiotic mixture (*Bacillus subtilis, Bacillus licheniformis,* and *Lactobacillus plantarum*) had improved jejunal intestinal morphology, altered microbiota, and decreased feed-to-gain ratios compared to controls, regardless if they were heat stressed or not (Song et al., 2014). Taken together, these studies suggest probiotic treatments might be able to preserve intestinal morphology, while also decreasing stress hormone release, and thus maintain feed-to-gain ratios and body weight gains during a heat stress event.

Research on *Bacillus* species used in poultry has yielded a wide range of results. As a whole, these products are thought to aid the host by outcompeting pathogenic organisms, by modulating the immune system, and by releasing enzymes to aide in nutrient utilization. The diversity of the proposed mechanisms and results may be due to the wide variety of strains of

Bacillus bacteria available. Each may be interacting with the host differently to achieve similar, yet distinctive results.

Prebiotics

As the commercial poultry industry moves away from antibiotic usage in order to satisfy consumer sentiment, it has looked extensively for solutions. Many of these solutions attempt to modulate the intestinal microbial population in order to benefit the host. One previously discussed option is the use of direct-fed microbial products in order to inoculate the intestine with beneficial bacterial that outcompete the more harmful bacterial species. An alternative method is to selectively nourish the intestinal microbiota, such that the beneficial bacteria survive, multiply, and flourish.

The addition of fiber and other less digestible substances to the diet for growth promotion in poultry production is not a novel concept, with studies dating back to the 1930s (Tasaki and Kibe, 1959). These original prebiotics were frequently wood-based products. Prebiotics have been described as nondigestible oligosaccharides that stimulate the growth of endogenous bacteria (Gibson and Roberfroid, 1995). This selective encouragment of the endogenous bacteria is theorized to enrich the intestine and competitively exclude pathogenic bacteria, but there are also prebiotics that are thought to bind bacteria and to enhance the immune system (Patterson and Burkholder, 2003). The prebiotic compounds used in poultry include, but are not limited to, fructo-oligosaccharides, galacto-oligosaccharides, gluco-oligosaccharides, malto-oligosaccharides, mannan-oligosaccharides, and other disaccharides. Oligosaccharides are short chains of monosaccharides that vary in their sugars and linkages (Campbell and Farrell, 2008). Disaccharides are composed of two monosaccharides with glycosidic linkages. When animals consume disaccharides, like sucrose, they hydrolyze the glycosidic linkages to make the

monosaccharide components (glucose and fructose) available, but they do not produce the enzymes necessary to break down all glycosidic linkages, such as beta 1,4 linkages. Thus, yeasts and bacteria that can hydrolyze this bond are needed in order to utilize these carbohydrates, and these organisms can proliferate in the gastrointestinal tract when these carbohydrates are provided.

In a series of experiments utilizing oligosaccharides in broiler chick diets, Biggs et al., (2007) compared feeding a basal corn/soy bean diet to this diet supplemented with inulin, oligofructose, mannanoligosaccharide, short-chain fructooligosaccharide, or transgalactooligosaccharide. They reported that at 4 g/kg (0.4%) inclusions, these products were not deleterious to dietary metabolizable energy or digestible amino acid values, but at higher inclusions (8 mg/kg) these prebiotics tended to decrease dietary metabolizable energy and digestible amino acid values. These experiments were conducted in un-cleaned battery brooder cages in order to help inoculate the intestine. However, this might have resulted in inconsistency in microbial inhabitation of the birds. These results highlight the delicate balance needed in determining the correct dietary inclusion level of prebiotics to prevent negative effects on animal performance.

Many organisms contain indigestible prebiotic components, but currently coproducts from yeast, algae, and horticultural products are most commonly used in the commercial poultry industry. Due to differences between product type and the method of culturing, these products are not simple to compare side by side. Diamond V prebiotic supplements are some of the more investigated yeast culture products that are currently used in the poultry industry. Multiple studies have reported that these products increase growth in broiler chickens (Gao et al., 2008; Roto et al., 2017). Gao et al., (2008) reported that the use of this yeast product in broilers from 0

to 42 days of age improved growth and enhanced both innate and humoral immunity. Corn and soybean meal-based diets were mixed with 0, 0.25, 0.50, and 0.75 percent Diamond V XP yeast culture. They concluded that the lower dose, 2.5 g/kg (0.25%), was the best inclusion rate based on their research in a controlled, low stress environment. Feeding the Diamond V product also improved calcium and phosphorus digestibility. The researchers suggested this may be partially due to the presence of phytase within the yeast culture. The authors suggested the improved immunity measures could be a function of the yeast cells acting as an adjuvant or some other unidentified property of the oligosaccharides in the product. It is important to note the birds in this research were reared in cages off of the floor and thus had limited contact with their excrement, which may have lessened the potential disease challenge. The researchers also suggested that the more intense immune response generated from the higher inclusions could have been at the expense of growth and lower fed conversion values.

Roto et al., (2017), reported similar findings with this product when they fed broilers from 0 to 42 days of age a diet supplemented with Diamond V XPC at 0.125% in the starter and grower periods and at 0.0625% in the finisher period. The birds were also challenged with a coccidiosis vaccine and by the use of previously used litter. Relative to broilers fed the control diet, broilers fed the diet supplemented with Diamond V XPC had increased body weight gain in the starter and grower periods. The researchers also reported decreased detection of *Salmonella* isolates in the XPC-treated broilers relative to the control broilers. However, the broilers were not inoculated with *Salmonella,* which might have influenced its initial prevalence. Feye et al., (2016), also reported that Diamond V XPC can be effective against *Salmonella*. These researchers inoculated the broilers with antibiotic-resistant *Salmonella typhimurium* and reported decreased fecal shedding, virulence, and antibiotic resistance genes in broilers fed 0.125%

Diamond V XPC from 0 to 42 days of age. They also reported increased weight gain in the Diamond V XPC fed broilers at the conclusion of the 49-day experiment, relative to broilers fed a control diet without the Diamond V product. The increased weight gain occurred primarily from 21 and 49 days of age which was the opposite of the report by Roto et al., (2017). The discrepancy in the reports may be due to the Diamond V product inclusion rate differences in the finisher/withdraw diets between the two studies.

These previous studies report specific examples of Diamond V products influencing the relative abundance of specific organisms. Changes in the microbial population of the intestine would be expected when feeding a prebiotic. However, other researchers, like Park et al., (2017) reported the cecal microbiome in broilers was influenced more by temporal aspects of the study than by treating with dietary salinomycin or Diamond V XPC. This result contradicts the work of Wang et al., (2016), who reported an increased *Lactobacillus* concentration in the ileum as a result of feeding a mannan-oligosaccharide and beta glucan prebiotic to broilers from 0 to 42 days of age. The difference in the findings between the two studies might have arisen from several places. While Park et al., (2017) used Diamond V in their study, Wang et al used a mannan oligosaccharide and beta-glucan product. In addition to using different prebiotics, one sampled the ceca while the other sampled from the ileum. Lastly, each group used different 16s RNA primers when quantifying the relative abundance of different bacterial genera.

Beyond the influences Diamond V XPC has on gastrointestinal bacteria in broilers, researchers have also reported feeding this product decreased lesion severity in laying hens challenged with subclinical *Eimeria maxima* (Lensing et al., 2012). This 37-day study was fairly short in duration to detect differences in egg production, especially given that the 18-week-old Brown Nick laying hens would have just began their laying cycle. Thus, it was not surprising

that no differences in egg production were detected between the control hens and those fed the Diamond V supplement. The age of the hens also might have altered the findings in this experiment, as they would have had mature immune systems at that age and may have been exposed to *Eimeria* species prior to the experiment.

In addition to helping poultry cope with pathogenic insults, a recent study suggested that Diamond V XPC influences the stress response associated with heat in broilers (Price et al., 2018). At 42 days of age, after two weeks of heat stress, broilers fed a diet containing Diamond V XPC had decreased plasma corticosterone levels, as well as a decreased heterophil:lymphocyte ratio, compared to control broilers. Houshmand et al., (2012) did not observe improvements in circulating corticosterone nor heterophil:lymphocyte ratios when sampling broilers at 42 days of age that were not heat stressed.

The list of products investigated for use as a prebiotic is expansive. In addition to yeast and yeast derivatives, some of the plants investigated include chickory, coconut, lupin (albus), potato starch, rice hulls, and stevia leaves (Atteh et al., 2008; Geigerova et al., 2017; Huff et al., 2015; Sundu et al., 2012; Yun et al., 2017). One complication with using prebiotic products from plants and yeast is that they contain several carbohydrates and compounds that make it more difficult to assess which components are most important. Some have reported performance differences using very specific disaccharides, including lactulose. This non-digestible disaccharide has been reported to decrease the feed-to-gain ratio, increase *Lactobacillus* populations, and decrease ammonia and hydrogen sulfide in excreta in broilers fed for 28 days lactulose at inclusions of 0, 1, or 2 g/kg diet (Cho and Kim, 2014). Zhao et al., (2016) also reported increased body weight gain and decreased feed conversion rate in broilers fed a diet containing 1.5 g/kg lactulose from 0 to 35 days of age compared to broilers fed a diet not

supplemented with lactulose. The significant differences in body weight gain and feed conversion were only detected in the last two weeks of the trial and for the overall experimental period and not detected in the starter (0-21 days of age) period.

Plant products are currently being used in the poultry industry to promote growth and to modulate the immune system. These botanical or phytogenic products may also fit under the prebiotic umbrella. Of these products, yucca (*Yucca schidigera*) is one of the more widely used horticultural species. Yucca containing products have been reported to deodorize manures by reducing volatile odorous compounds, including ammonia, dimethylamine, and hydrogen sulfide (Matusiak et al., 2016). This research report focused on these changes from a human and animal welfare point of view. The research involved tests on laying hen manure outside of the production facility, and thus did not report egg performance differences between groups of laying hens. Chepete et al., (2012) also reported that ammonia was reduced during a 12 week experiment when Hy-Line W-36 laying hens were fed a diet supplemented with yucca at 100 ppm and that the addition of the yucca did not impact egg production in the supplemented hens relative to hens fed a control diet containing no yucca. Spray application of a yucca solution on various litter substrates did not reduce ammonia production (Onbasilar et al., 2014). Even though there are mixed reports on efficacy, some yucca products are marketed specifically for their deodorizing effects.

Broiler experiments suggest that dietary additions of *Yucca schidigera* might be beneficial. In the research by Sun et al., (2018), Arbor Acres broilers were reared under standard conditions until 14 days of age. From 15 to 42 days of age the broilers were fed diets supplemented with 0, 100, 200, or 300 mg/kg of *Yucca schidigera* extract. Broilers fed the 100 mg/kg inclusion level gained more weight than the broilers fed the other dietary treatments and

had higher antibody titers to Newcastle's disease virus and increased interleukin 6 levels. The researchers paired these results to suggest the observed increased growth may be due to enhanced immunity. Crevens et al., (2015) challenged broilers by giving them a coccidiosis vaccine and rearing them on litter that was previously inoculated with *Clostridium perfringens*. Under these conditions broilers fed a diet supplemented with yucca had better body weight gain than those that received no additive in their diet. Galli et al. (2018), observed a decrease in *Eimeria* oocyst per gram of feces in broilers fed a yucca extract product compared to those fed a control diet. The birds fed the yucca extract also exhibited increased body weight gain compared to the control-fed broilers.

Another potential aspect to consider when a diet is supplemented with probiotics, is the amino acids and other nutrients contained within the bacteria-laden cecal excretions of the broilers. In caged systems, these feces would fall below and become a waste product, but in the litter the substrate becomes a food source for bacteria and chickens alike. Although this is not typically considered as a benefit of prebiotics, the birds do exhibit coprophagic behavior, similar to a rabbit, and may attain those nutrients. In this case, the prebiotics would be serving to grow the bacteria that would later be consumed as protein-rich substrate.

Essential oils

Feeding the beneficial organisms in the intestine with prebiotics and modulating the host's immune system can indirectly lead to better growth and development in animal production systems. Other compounds, such as essential oils, may have a more direct impact on the host and on any pathogens present. Essential oils are extracts acquired from a variety of plants including oregano, sage, thyme, basil, cinnamon, peppermint, eucalyptus, garlic, turmeric, and several other phytogenic products (Adaszynska-Skwirzynska, 2017). Essential oils have also been

described as volatile oils which may be a more accurate description of these terpenoid and aromatic compounds. Some of the compounds in these extracts are reported to act as immune-stimulatory agents while others are reported to have antifungal, antimicrobial, and/or parasitacidal characteristics.

Some essential oils have been suggested to increase the immune response in broilers when foreign material is introduced (Farhadi et al., 2017; Habibi et al., 2015). Farhadi et al., (2017) studied the immunoglobulin response to intravenous injection of sheep red blood cells to broiler chicks that were fed a diet containing either eucalyptus (*Eucalyptus globulus* L.) essential oil or not. While the added essential oil had no significant effect on body weight gain in the broilers, primary antibody levels were increased (most likely due to increased IgM levels according to the authors) in response to the sheep red blood cell infusion in the broilers fed the eucalyptus extract. Habibi et al., (2015) completed a study measuring the immunoglobulin response to intramuscular injection of sheep red blood cells in broilers fed a control diet or this diet supplemented with either cumin (*Cuminum cyminum*) or wormwood (*Artemisia absinthium*). Neither essential oil had a significant effect on body weight gain in the broilers, but broilers fed either cumin or wormwood essential oil both had a significant increase in immunoglobulins, as shown by increased hemagglutination titers to the sheep red blood cell injections. These studies suggest some essential oils may be effective in promoting adaptive immune responses in chickens.

Another potential use for essential oils is as an antimicrobial agent. Yin et al., (2017), completed a study in which they fed a diet that contained a blend of thymol and carvacrol to broilers that were then challenged with *Clostridium perfringens*. Thymol, or 2-isopropyl-5-methylphenol, is a compound found in relative abundance in common spices, including thyme

(*Thymus vulgaris*) and oregano (*Origanum vulgare sp.*). Carvacrol, or 2-Methyl-5-(propan-2-yl) phenol, is also found in these two spices. Broilers fed diets containing these essential oils had decreased mortality and decreased intestinal lesions compared to those fed a control diet. Yin et al., (2017) also reported that the host microbiome changed in response to the added blend of essential oils as there was increased numbers of *Lactobacillus crispatus* and *Lactobacillus agilis* in the ileum, and the authors hypothesized that the decreased gut lesions and mortality were at least partially due to this change in microbiome.

Amerah et al., (2012) reported that supplementing the diet with a blend of cinnamaldehyde and thymol reduced horizontal transmission and environmental detection of *Salmonella enterica* serovar Heidelberg. In this study, the *Salmonella* Heidelberg challenge did not negatively alter the growth and performance of the inoculated groups, which was attributed to the strain and/or dose used. Nonetheless, they reported reduced *Salmonella* Heidelberg present in the ceca of non-inoculated pen mates and in environmental drag-swabs in the broilers given the dietary essential oils.

Some essential oils (especially those containing oregano extracts) have been reported to help control coccidiosis. Alp et al., (2012) reported dietary inclusion of 100 mg/kg (5% essential oil) of a powdered oregano product decreased fecal *Eimeria* oocyst counts in broilers at 20 days of age and 40 days of age, as compared to counts in broilers fed a control diet containing no additives. A third dietary treatment group consisted of broilers fed an anticoccidial drug had the lowest level of fecal shedding of oocysts. In this study, there was no difference in body weight gain among the dietary treatments, but both the oregano and drug supplemented groups had improved feed-to-gain ratios. This improved feed conversion due to the dietary presence of oregano essential oil was also reported by Mohiti-Asli et al., (2015) in an experiment where the

broilers were challenged with coccidiosis. In this experiment, although it was less effective than the anticoccidial drug diclazuril, the dietary administration of oregano oil at 500 ppm significantly decreased gross intestinal lesions and fecal oocyst counts (Mohiti-Asli et al., 2015).

Another protozoal disease that challenges the poultry industry is blackhead disease, which is caused by *Histomonas meleagridis*. When turkey poults fed a control diet were challenged with *Histomonas meleagridis* they experienced a 50% mortality rate, but poults fed the control diet supplemented with an essential oil mixture only experienced a 20% mortality rate (Hafez and Hauck, 2006). Although essential oil products are not as effective as antiprotozoal drugs, they show promise in helping mitigate the effects of the two most common protozoal diseases effecting poultry.

Essential oils have also been investigated as alternative acaracidal treatments. *Dermanyssus gallinae*, commonly referred to as the poultry red mite, are nocturnal, blood-eating ectoparasites that feed on poultry at night and hide in the cracks and crevices around the house during the day (Swayne et al., 2013). These parasites are an issue in laying hens and breeding operations in North America and Europe. As the industry shifts to include more alternative rearing techniques, such as free-range laying hens and cage-free laying hen facilities, these mites will likely become more prevalent. One research group in the United Kingdom, where these mites are currently a larger concern, has found that various essential oil products are effective in killing the poultry red mite. They observed these mites, which can live in dormancy for several weeks between meals, were more vulnerable to essential oils three weeks post-feeding than mites having consumed blood more recently (3-13 days post feeding)(George et al, 2008). They also found mites could be killed by fumigation using certain essential oils rather than direct contact

(George et al., 2009). Of the essential oils tested, the essential oil from thyme was found the most effective in killing mites (George et al., 2010).

Chemicals

Although feed additives appear as though they will serve a vital role in the poultry industry moving forward, they are not currently used in such a way as to replace antibiotics completely. In an antibiotic-free poultry industry, there are other ways to help reduce pathogens that cause disease in animals (as well as in humans). *Salmonella* and other foodborne pathogens contaminate various feed ingredients which are combined in feed mills to make a formulated diet. *Salmonella* are known to have prolonged survival times on many of these organic substrates (Jones, 2011). Cognizant ingredient sourcing, thermal processing, and chemical preservatives have been used to reduce feed contamination. Briefly heat-conditioning and pelleting the diet successfully reduces bacterial loads, but thermotolerant bacterial strains have increased contamination concerns and encouraged concurrent chemical sterilization protocols (Boroojeni et al., 2014).

Exposure of animal diets to formaldehyde and other chemicals used in the commercial poultry industry can be very effective in drastically reducing viral, bacterial, and fungal pathogens (Ruano et al., 2001). Formaldehyde is an effective antimicrobial chemical which can eliminate pathogens, like *Salmonella*, at low inclusion rates (Wales et al., 2010). Although formaldehyde products have been used successfully in combination with heat treatment in eliminating bacterial pathogens in feed, there remains concern about how this treatment may effect nutrient availability (Yakhkeshi et al., 2014).

Summary

As the use of antibiotics decreases in poultry production due to consumer demand and regulatory mandates, a niche has opened up for the use of feed additives that did not previously exist. A holistic approach in which improvements in management, nutrition, medicine, and welfare are all contributing to lessen the need for the use of antibiotics during poultry production, will be necessary to help fill this niche. Rather than eradicating a handful of pathogens with drugs, the industry is currently moving to nourish the beneficial bacteria to outcompete pathogens through the use of prebiotics and probiotics, to mitigate or neutralize pathogens with clay based and essential oil dietary additives, and to use chemical and heat sterilization to lessen exposure to pathogens in the diets consumed by poultry.

CHAPTER 2

STATEMENT OF PURPOSE

Animal protein is a valuable source of amino acids and other vital nutrients for humans. As the world's population grows and life expectancy rises, efficient animal production systems will become increasingly vital. Poultry are among the most efficient food animals and commercial poultry production has the most developed vertical integration, which translates to effective operation management and cost-efficient production. Although cost is a big motivating factor for consumers, there are growing numbers of consumers that also desire to know more about the food they are consuming and the welfare of the animals being grown for consumption. Concerns by these consumers include, but are not limited to, use of antibiotics and other drugs, providing adequate space for animals, enriching the animals' environment, and providing cage-free production systems. Consumer desire has led many fast food restaurants such as Chipotle, Panera Bread, Subway, McDonalds, Chick-fil-a, and Wendy's and grocery chains like Whole Foods to advertise they are only selling poultry meat or eggs produced under conditions they have dictated.

In addition to consumer-driven corporate pressure from their purchasers, the poultry industry is having to respond to new revised governmental regulations. Recently, the Veterinary Feed Directive was implemented, which requires a veterinary signature for the use of antibiotics in poultry diets. Because this prescription requires the drugs to be used as labeled, it greatly reduces the likelihood of using these drugs as growth-promoting compounds. Frequently, in opposition to the consumer- and regulatory-driven push to reduce or eliminate antibiotic usage in

poultry production, are regulations on the food safety side which continue to decrease the allowable tolerances of food borne pathogens while increasing the rigor of the detection of these pathogens in fresh poultry products.

In order to simultaneously satisfy these demands, the poultry industry is taking steps to meet these diverse needs. One step is the incorporation of a variety of feed additives in order to promote intestinal health and reduce the need for antibiotic usage. These feed additives include probiotics, prebiotics, essential oils, aluminosilicate-based products, and acidifiers. Another step is the use of chemical and heat protocols in feed manufacturing to reduce pathogen-contamination of the diets fed to poultry. Regretfully, the pace of the changes dictating these new feeding protocols outpaces the scientific research to determine the effectiveness and best administration of these feeding protocols. This is especially true given these products that reduce the need for antibiotics are being added to diets at the same time as new enzyme products are being added to poultry diets to enhance the digestion and utilization of the nutrients in the diets. Therefore, the goal of this research was 1) to determine the efficacy of 5 different *Bacillus* probiotics, of 4 different prebiotics (cellobiose, yeast extract, and 2 yucca products), of a product containing a blend of essential oils, and of an Rare Earth Element product (Azomite) during broiler production, and 2) to determine if the application of a formaldehyde treatment to the diet combined with an extended heat treatment decreased the true digestible energy and/or digestible amino acid content of a broiler diet.

CHAPTER 3

MATERIALS AND METHODS

Animals

Male chicks for experiments 1-5 and 7-8 were unvaccinated, male by-product broiler chicks (Cobb 500) from a female parent stock obtained from the Cobb hatchery in Cleveland, GA. Male chicks for experiment 6 were Cobb 700 and were obtained from the Tyson Hatchery in Ogelthorpe, GA. Cobb 500 mixed sex chicks for experiment 9 were obtained from the Pilgrims Hatchery in Athens. GA. The Bovans White Single Comb White Leghorn roosters used for experiment 10 were obtained from Centurion Poultry Inc. (Lexington, GA). All animal procedures were in accordance with and approved by the University of Georgia Animal Care and Use Committee (IACUC), Athens, GA.

Experiment 1

The first experiment evaluated the performance of broilers from 0 to 21 days of age fed diets supplemented with Azomite at 0, 0.125, 0.250, and 0.500% of the diet. The composition of the basal diet is presented in **Table 3.1**. The diets were fed in crumble form. The dietary treatments were equally distributed and randomized among pens in three Petersime battery brooders each equipped with 24 pens, to create 18 replicate pens for each dietary treatment. Each replicate pen consisted of 5 chicks. Individual pens measured 98 cm long by 35 cm wide by 23 cm high. Prior to placement, the chicks were sorted by weight and re-assimilated to keep the starting weights of each pen similar, thus minimizing initial variation. Any chicks with extreme weights or physical abnormalities, including open navels and splayed legs, were excluded from the study. The chicks were given ad libitum access to food and water.

A computerized controller for the room housing the batteries regulated a gas-fired furnace, an exterior evaporative cooling system for intake air, a 46-cm ceiling circulation fan, and a 53-cm exhaust fan at the end of the room for heating, cooling, and ventilation. Ambient temperature was set to 34 °C on day 0 and decreased by 0.28 °C each day. For the duration of the study, light intensity was 20 lux for 24 hours a day.

Body weight and total feed consumption on a pen basis were determined every 7 days. In addition, to determine apparent calcium and phosphorus digestibility, the exact amount of feed consumed during the last 48 hours of the experiment was determined and total feces was collected for each pen during this time. To ensure clearance of the digestive tract of food, the feeding troughs were removed 12 hours prior to the start of this feeding period and 12 hours before the end of the study when the feces were collected.

On the last day of the experiment, after weighing the birds and feed, blood samples were collected from the control and the 0.500% Azomite- supplemented group. After collection, each blood sample was transitioned into borosilicate glass tubes for serum collection. Collected serum was transferred to Eppendorf tubes and frozen at -80°C. These serum samples were subsequently analyzed with the ABCAM Chicken Alpha-1-acid Glycoprotein Sandwich ELISA (Cambridge, MA, USA). The samples were processed and analyzed as indicated by the manufacturer's protocol.

Experiment 2

This experiment mirrored Experiment 1 except the dietary inclusion rates of Azomite were 0, 0.0625, 0.125, and 0.250 %. Instead of collecting blood samples at 21 days of age

Table 3.1. Composition of the diet for Experiment 1 and 2.

Ingredient	Diet[1]
	Positive control
	%
Corn	57.625
Soybean meal	32.320
Corn DDGS	3.000
Soybean oil	2.593
Calcium Carbonate	0.646
Defluorinated Phosphate	1.237
Salt	0.353
L-Lysine, HCl 78.8%	0.236
DL- Methionine 99%	0.298
L-Threonine, 98%	0.077
Choline Chloride 60%	0.045
Sand	0.490
Vitamin mix[2]	0.386
Mineral mix[3]	0.075
Azomite/Solka-Floc[4]	0.500
Quantum Blue phytase (5,000 FTU/g)	0.060
Econase XT25	0.010
Coban	0.050
Calculated analysis	
AME (kcal/kg)	2964
Crude protein (%)	20.090
Calcium (%)	0.950
Available phosphorus (%)	0.480
Digestible total sulfur (%)	0.857
Digestible lysine (%)	1.127
Digestible threonine (%)	0.733

[1]Starter diet was fed from day 1 to 21 days of age.
[2]Vitamin mix provided the following per 100 g of diet: vitamin A, 551 IU; vitamin D_3, 110 IU; vitamin E, 1.1 IU; vitamin B_{12}, 0.001mg; riboflavin, 0.44 mg; niacin, 4.41 mg; d-pantothenic acid, 1.12 mg; choline, 19.13 mg; menadione sodium bisulfate, 0.33 mg; folic acid, 0.55 mg; pyridoxine HCl, 0.47 mg; thiamin, 0.22 mg; d-biotin, 0.011 mg; and ethoxyquin, 12.5 mg.
[3]Mineral mix provided the following in mg per 100 g of diet: Mn, 6.0; Zn, 5.0; Fe, 3.0; I, 1.5; and Se, 0.5.
[4]Azomite was added at the expense of Solka Floc.

intestinal samples were collected. Samples for histological measurements were taken from the tip of the duodenal loop as well as four centimeters distal to Meckel's diverticulum. In order to ensure the villi were properly preserved, the content was flushed with 10% formalin (3.7% formaldehyde) prior to submerging each sample in 10% formalin. Feces samples were not collected in this experiment.

Experiment 3

This floor pen experiment was conducted to determine the performance of broilers fed Azomite throughout the entire rearing period (0-49 days) until processing. This experiment was conducted in a facility with 2 identical, but separate rooms. Each room was equipped with 48 (1.52 m by 1.22 m) floor pens. All pens were equipped with 7 nipple drinkers originating from a common water line and 1 pan feeder (0.09 m^2). Prior to chick placement, litter from 5 previous flocks was top dressed with 2 cm of new pine shavings for each pen. A standard industry lighting program was implemented with a light intensity of 20 lux for 24 hours (0 to 4 days), 20 lux for 20 hours (5 to 7days), 10 lux for 18 hours (8 to 14 days), and 2 lux for 18 hours (15 to 49 days). Light intensity was verified by placing a Light ProbeMeter™ (model 403125, Extech Instruments Corp. Waltham, MA) into the pens.

For each room, a computerized controller regulated 2 gas-fired furnaces, an exterior evaporative cooling system present on both sides of the room for intake air, six 45.7 cm ceiling circulation fans, and two 91.4 cm exhaust fans and one 61cm exhaust fan. Ambient temperature was set to 34 °C on day 1 and decreased by 0.28 °C until 24 °C was reached and then maintained. No significant differences in temperature and humidity were noted throughout the studies between the 2 rooms.

The experiment consisted of six dietary treatments, three made from a positive control basal diet and three made from a negative control basal diet. The positive control basal diet was formulated to mimic poultry industry standards in the United States. The negative control basal diet had a 2% reduction in both apparent metabolizable energy and essential digestible amino acids levels and was used to provide a slight dietary stress on the birds. To create the six dietary treatments, Azomite was added at a rate of 0, 0.125, or 0.250% to both the positive and negative control basal diets (**Table 3.2)**. The starter diets were fed from days 1 to 14 of age, the grower diets were fed from days 14-28 of age, the finisher diets were fed from 28 to 42 days of age and the withdrawal diet was fed from 42 to 49 days of age. The starter diets were in crumble form while the grower, finisher, and withdrawal diets were in pellet form.

Prior to placing chicks, each of the 96 pens were assigned to one of the 6 dietary treatments in a random block design [16 replicates per treatment (8 replicates in each room)]. The chicks were sorted and those with extreme weights or with visual physical abnormalities were discarded before the remaining birds were assigned to the 96 pens (22 birds per pen). Feed and water were provided ad libitum throughout the duration of the experiment. Diets were formulated on a digestible amino acid basis.

For each room, humidity, temperature, water consumption, and pen mortality were recorded daily. Birds and feed were weighed on a pen basis on days 0, 14, 28, 42, and 49 to determine body weight, feed intake, body weight gain, and feed conversion. On day 49, the mean bird weight for each pen was determined and then ten broilers within 300 grams of the average pen weight were selected from each of the pens from the negative control treatments (negative control diet supplemented with 0%, 0.125%, and 0.250% Azomite) and placed in a coop feed withdrawal procedures. Individual weights for the selected birds were recorded and each bird

Table 3.2. Composition of the diets for Experiment 3.

Ingredient	Diets[1]							
	Starter		Grower		Finisher		Withdraw	
	Positive Control	Negative control	Positive control	Negative control	Positive control	Negative control	Positive control	Negative control
	%							
Corn	56.107	58.642	61.083	63.549	66.138	68.522	71.367	73.666
Soybean meal	33.106	31.826	29.238	28.038	25.178	24.064	20.912	19.886
Soybean oil	2.440	1.173	2.418	1.139	2.374	1.092	2.278	0.993
Corn DDGS	4.500	4.500	3.716	3.716	3.059	3.059	2.533	2.533
Limestone	0.663	0.670	0.640	0.647	0.620	0.626	0.603	0.608
Defluorinated P	1.174	1.182	0.944	0.951	0.714	0.719	0.481	0.486
Salt	0.351	0.350	0.357	0.356	0.363	0.362	0.368	0.367
L-Lysine, HCl 78.8%	0.284	0.289	0.265	0.271	0.252	0.258	0.244	0.250
DL- Methionine 99%	0.318	0.308	0.283	0.274	0.247	0.238	0.210	0.202
L-Threonine, 98%	0.090	0.091	0.090	0.091	0.090	0.090	0.089	0.090
Choline Cl 60%	0.000	0.004	0.000	0.004	0.000	0.003	0.000	0.003
Vitamin mix[2]	0.567	0.567	0.567	0.567	0.567	0.567	0.567	0.567
Mineral mix[3]	0.079	0.079	0.079	0.079	0.079	0.079	0.079	0.079
Sand[4]	0.250	0.250	0.250	0.250	0.250	0.250	0.250	0.250
Coban	0.050	0.050	0.050	0.050	0.050	0.050	0.000	0.000
Phytase 5,000 FTU/g	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
Econase XT25	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010
Calculated analysis								
AME (kcal/kg)	3025	2964	3075	3013	3125	3062	3175	3111
Crude protein (%)	21.620	21.210	19.920	19.540	18.180	17.830	16.390	16.080
Calcium (%)	0.950	0.950	0.850	0.850	0.750	0.750	0.650	0.650
Available P (%)	0.480	0.480	0.430	0.430	0.380	0.380	0.330	0.330
Digestible Met (%)	0.618	0.604	0.564	0.551	0.508	0.496	0.451	0.440
Digestible TSAA (%)	0.900	0.882	0.828	0.812	0.755	0.740	0.679	0.665
Digestible Lys (%)	1.200	1.176	1.090	1.068	0.980	0.960	0.870	0.853
Digestible Thr (%)	0.780	0.764	0.719	0.705	0.657	0.643	0.592	0.580
Digestible Ile (%)	0.847	0.827	0.773	0.754	0.696	0.678	0.616	0.600

Digestible Val (%)	0.900	0.882	0.828	0.812	0.755	0.740	0.679	0.665
Digestible Trp (%)	0.243	0.236	0.221	0.215	0.198	0.192	0.174	0.169

[1]The starter diet was fed from day 0 to 14 days of age. The grower diet was fed from day 15 to 28 days of age, the finisher was fed from day 29 to 42 days of age and the withdraw diet was fed from day 43-49 of age.
[2]Vitamin mix provided the following per 100 g of diet: vitamin A, 551 IU; vitamin D_3, 110 IU; vitamin E, 1.1 IU; vitamin B_{12}, 0.001mg; riboflavin, 0.44 mg; niacin, 4.41 mg; d-pantothenic acid, 1.12 mg; choline, 19.13 mg; menadione sodium bisulfate, 0.33 mg; folic acid, 0.55 mg; pyridoxine HCl, 0.47 mg; thiamin, 0.22 mg; d-biotin, 0.011 mg; and ethoxyquin, 12.5 mg.
[3]Mineral mix provided the following in mg per 100 g of diet: Mn, 6.0; Zn, 5.0; Fe, 3.0; Cu, 0.5; I, 0.15; and Se, 0.05.
[4]Azomite was added at the expense of sand.

was leg banded prior to placement in a coop for an overnight feed withdrawal before processing. On day 50, birds were weighed again and then processed at the University of Georgia's Pilot Processing Plant as previously described (Hidalgo et al., 2004). Subsequently, eviscerated hot carcass weights were recorded for each bird prior to static chilling in an ice bath. After a four-hour chill, carcasses were drained prior to cut up and deboning. Weights were recorded for: drained chilled carcass, *pectoralis major*, *pectoralis minor*, wings, and leg quarters of each bird. As the *pectoralis majors* were weighed and handled, one person observed the muscle and noted white striping and woody breast. Woody breast was judged based on the presence or absence of the condition using the scoring system described by Tijare et al., (2016). White striping was scored using a scoring system adapted from Kuttappan et al., (2012). Percent yield calculations were based on the fasted, live weight of the bird.

To determine nitrogen corrected true metabolizable energy value and the calcium and phosphorus digestibility values, on day 49, 20 broilers were selected from the negative control treatment supplemented with 0% Azomite and from the negative control treatment containing 0.125% Azomite. These broilers were moved to individual, suspended cages equipped with a single drinker for ad libitum access to water. The birds were fasted for 20 hours to allow existing digesta to be expelled. After fasting, each of the 12 birds from each treatment were allowed access to a known quantity of food for 12 hours. At the conclusion of the 12-hour period, remaining food was weighed so the feed intake for each bird could be calculated. The remaining eight birds in each group were not given access to food and served as the endogenous controls. During the 12-hour feeding period and for the subsequent 36 hours, excreta were collected from each individual bird. Collected feces was dried, weighed, and analyzed for gross energy, calcium and phosphorus content.

Experiment 4

Experiment 4 was performed in order to evaluate efficacy of three different Bacillus probiotic products supplemented to standard United States broiler production diets which were fed to the broilers from 0 to 42 days of age. This experiment was conducted in the same facility as experiment 3 and followed the same management guidelines except that de-caked, used litter from 7 previous flocks was utilized and not top dressed with new pine shavings.

For this experiment, there were 8 dietary treatments consisting of a control diet (**Table 3.3)**, or this diet supplemented with *Bacillus licheniformis* probiotic product 1 at 0.2%, with *Bacillus subtilis* probiotic 1 at 0.1, 0.2, and 0.4% or with *Bacillus licheniformis* probiotic product 2 at 0.1, 0.2, and 0.4%. Each dietary treatment consisted of 12 replicate pens (6 replicates per room) with each pen having 23 chicks.

Birds and feed were weighed on a pen basis on days 0, 14, 28, and 42 to determine body weight, feed intake, body weight gain, and feed conversion. On day 42, the foot pads of 15 randomly selected broilers form each pen evaluated and scored using the procedure of Bilgili et al., (2006). The same individual scored all of the foot pads to decrease variation.

Experiment 5

Experiment 5 evaluated the use of a strain of *Bacillus subtilis* in broilers from 0 to 42 days of age when 1) utilizing a diet more similar to what might be found in European countries 2) it was added to a diet that was marginally deficient relative to industry standard diets in energy and 3) utilized in this amino acid and energy deficient diet in combination with a commercial carbohydrase enzyme mixture (two carbohydrase complexes) that enhances feed

Table 3.3. Composition of the diets for Experiment 4.

Ingredient	Diets[1]		
	Starter	Grower	Finisher
		%	
Corn	59.790	63.839	67.539
Soybean meal	29.865	25.710	22.327
Soybean oil	1.253	1.321	1.548
Corn DDGS	2.500	3.000	3.594
MBM	3.500	3.285	2.233
Limestone	0.697	0.705	0.729
Defluorinated P	0.204	0.000	0.000
Salt	0.419	0.419	0.407
L-Lysine, HCl 78.8%	0.253	0.244	0.235
DL- Methionine 99%	0.317	0.281	0.246
L-Threonine, 98%	0.084	0.084	0.084
Choline Cl 60%	0.012	0.007	0.000
Vitamin mix[2]	0.567	0.567	0.567
Mineral mix[3]	0.079	0.079	0.079
Sand[4]	0.400	0.400	0.400
Diclazuril	0.050	0.050	0.000
Phytase 5,000 FTU/g	0.010	0.010	0.010
Calculated analysis			
AME (kcal/kg)	2964	3013	3062
Crude protein (%)	21.611	19.948	18.149
Calcium (%)	0.950	0.850	0.750
Available P (%)	0.475	0.425	0.375
Digestible Met (%)	0.604	0.551	0.497
Digestible TSAA (%)	0.882	0.812	0.740
Digestible Lys (%)	1.176	1.068	0.960
Digestible Thr (%)	0.764	0.705	0.643
Digestible Ile (%)	0.800	0.726	0.653
Digestible Val (%)	0.895	0.825	0.748
Digestible Trp (%)	0.233	0.210	0.189

[1]The starter diet was fed from day 0 to 14 days of age. The grower diet was fed from day 15 to 28 days of age and the finisher was fed from day 29 to 42 days of age.
[2]Vitamin mix provided the following per 100 g of diet: vitamin A, 551 IU; vitamin D_3, 110 IU; vitamin E, 1.1 IU; vitamin B_{12}, 0.001mg; riboflavin, 0.44 mg; niacin, 4.41 mg; d-pantothenic acid, 1.12 mg; choline, 19.13 mg; menadione sodium bisulfate, 0.33 mg; folic acid, 0.55 mg; pyridoxine HCl, 0.47 mg; thiamin, 0.22 mg; d-biotin, 0.011 mg; and ethoxyquin, 12.5 mg.
[3]Mineral mix provided the following in mg per 100 g of diet: Mn, 6.0; Zn, 5.0; Fe, 3.0; Cu, 0.5; I, 0.15; and Se, 0.05.
[4]The test products were added at the expense of sand.

digestibility. This experiment was conducted in the same facility as experiment 3 and followed the same management guidelines except that de-caked, used litter from eight previous flocks top dressed with 2 cm of new pine shavings was utilized.

For this experiment, there were 6 dietary treatments (**Table 3.4**) consisting of a positive control diet, a negative control diet or this diet supplemented with *Bacillus subtilis* probiotic 2, carbohydrase complex, *Bacillus subtilis* probiotic 2 plus carbohydrase complex, or *Bacillus subtilis* probiotic 3. The dietary level of both probiotics was 0.05% of the diet. Carbohydrase complex was spray applied to crumbled or pelleted diets at a rate of 200 mL per 1000 kg. For the diets that did not contain carbohydrase complex, an equivalent amount of water was applied to the diet. Each dietary treatment consisted of 16 replicate pens (8 replicates per room) with each pen having 24 chicks.

Birds and feed were weighed on a pen basis on days 0, 14, 28, and 42 to determine body weight, feed intake, body weight gain, and feed conversion. On day 42, the foot pads of 10 randomly selected broilers from each pen were evaluated and scored as described for Experiment 4. Litter moisture and litter ammonia level were also assessed on day 42. For litter moisture, a plug from the center of each pen from the top of the litter to the concrete floor was collected. Each sample was homogenized, and from this sample, 100-grams was removed and dried at 82°C for 48 hours. The dried sample was then weighed again to determine moisture loss.

The ammonia release from the litter was analyzed with a Dräger CMS analyzer (Notting Hill, Victoria, Australia) equipped with 10-150 ppm sensor chips. The sensor was attached to a chamber placed on the top of the litter. The chamber was equipped with a 7.6 cm fan powered

with a nine-volt battery to circulate the air. The air was allowed to equilibrate for 120 seconds in the chamber before the ammonia level of the air was measured.

Experiment 6

The last probiotic study compared broiler performance from days 0 to 42 of age when diets were supplemented with the same *Bacillus subtilis* probiotic used in experiment 5 (*Bacillus subtilis* probiotic 2) or two different yucca products. This experiment was conducted in the same facility as experiment 3 and followed the same management guidelines except that de-caked, used litter from 9 previous flocks that was top dressed with 2 cm of new pine shavings was utilized.

For this experiment, there were 4 dietary treatments (**Table 3.5**) consisting of a standard industry control diet, or this diet supplemented with 0.05% Magni-Phi (Phibro Animal Health Corporation, Teaneck, NJ), 0.05% Micro-Aid (DPI Global, Porterville, CA), or 0.05% *Bacillus subtilis* probiotic 2. Each dietary treatment consisted of 22 replicate pens (11 replicates per room) with each pen having 25 chicks.

Birds and feed were weighed on a pen basis on days 0, 14, 28, and 42 to determine body weight, feed intake, body weight gain, and feed conversion. On day 42, the mean bird weight for each pen was determined and then 7 broilers within 300 grams of the average pen weight were selected for processing after an overnight fast as described previously for experiment 3.

Experiment 7

This experiment compared broiler performance from 0 to 42 days of age when diets were supplemented with a yeast-based prebiotic or a purified carbohydrate that is indigestible to poultry. This experiment was conducted in the same facility as experiment 3 and followed the

Table 3.4. Composition of the diets for Experiment 5.

Ingredient	Diets					
	Starter		Grower		Finisher	
	Positive control	Negative control	Positive control	Negative control	Positive control	Negative control
			%			
Corn	51.966	47.491	56.512	51.689	61.529	56.511
Soybean Meal	36.788	34.180	32.513	30.226	27.813	25.711
Soybean Oil	4.683	3.832	4.602	3.782	4.456	3.646
Wheat Middlings	1.000	5.000	1.000	5.000	1.000	5.000
Rye	1.000	5.000	1.000	5.000	1.000	5.000
Limestone	1.356	1.389	1.307	1.337	1.260	1.289
Monocalcium P	1.695	1.631	1.603	1.537	1.514	1.447
Salt	0.502	0.502	0.478	0.478	0.454	0.454
L-Lysine HCl 78%	0.170	0.170	0.160	0.160	0.163	0.163
DL-Methionine 99%	0.323	0.310	0.289	0.278	0.251	0.242
L-Threonine 98%	0.074	0.071	0.074	0.071	0.077	0.074
Choline-Cl, 60%	0.052	0.057	0.072	0.075	0.093	0.095
Vitamin Mix[1]	0.243	0.243	0.243	0.243	0.243	0.243
Mineral Mix[2]	0.075	0.075	0.075	0.075	0.075	0.075
Sand/probiotic[3]/enzyme[4]	0.073	0.050	0.073	0.050	0.073	0.050
Calculated analysis						
AME (kcal/kg)	3025	2934	3065	2973	3105	3012
Crude Protein	21.428	21.071	19.746	19.513	17.912	17.753
Dig Lysine	1.160	1.120	1.055	1.022	0.950	0.921
Dig Methionine	0.607	0.583	0.554	0.534	0.496	0.479

Dig Met+Cys	0.882	0.851	0.812	0.787	0.736	0.714
Dig Threonine	0.754	0.728	0.696	0.675	0.636	0.617
Dig Valine	0.882	0.860	0.812	0.796	0.736	0.723
Dig Tryptophan	0.253	0.247	0.231	0.227	0.207	0.203
Total Calcium	0.950	0.950	0.900	0.900	0.850	0.850
Available P	0.475	0.475	0.450	0.450	0.425	0.425

[1]Vitamin mix provided the following per 100 g of diet: vitamin A, 551 IU; vitamin D_3, 110 IU; vitamin E, 1.1 IU; vitamin B_{12}, 0.001mg; riboflavin, 0.44 mg; niacin, 4.41 mg; d-pantothenic acid, 1.12 mg; choline, 19.13 mg; menadione sodium bisulfate, 0.33 mg; folic acid, 0.55 mg; pyridoxine HCl, 0.47 mg; thiamin, 0.22 mg; d-biotin, 0.011 mg; and ethoxyquin, 12.5 mg.
[2]Mineral mix provided the following in mg per 100 g of diet: Mn, 6.0; Zn, 5.0; Fe, 3.0; Cu, 0.5; I, 0.15; and Se, 0.05.
[3]Probiotic was added at the expense of sand.
[4]The enzyme complex is a primarily composed of two carbohydrase complexes.

Table 3.5. Composition of the diets for Experiment 6.

Ingredient	Diets[1]		
	Starter	Grower	Finisher
		%	
Corn	60.495	63.091	67.627
Soybean Meal	27.961	23.877	19.659
DDGSs	5.000	5.000	5.000
Meat&Bone 50%	4.000	4.000	4.000
Limestone	0.668	1.983	2.012
Soybean Oil	0.404	0.668	0.603
Salt	0.364	0.366	0.367
DL-Methionine 99%	0.288	0.244	0.209
Dicalcium Phospahte	0.243	0.232	0.208
L-Lysine HCl 78%	0.212	0.210	0.081
Choline-Cl, 60 %	0.098	0.079	0.063
L-Threonine 98%	0.093	0.077	0.041
Mineral Mix[2]	0.075	0.075	0.032
Vitamin Mix[3]	0.025	0.025	0.025
Enzyme Blend (0.5 lb/ton)	0.025	0.025	0.025
Sand[4]	0.050	0.050	0.050
Calculated analysis			
AME (kcal/kg)	2987	3108	3164
Crude protein (%)	21.744	19.931	18.218
Calcium (%)	0.942	0.924	0.840
Available P (%)	0.449	0.440	0.400
Total Met (%)	0.622	0.557	0.501
Total TSAA (%)	0.972	0.884	0.808
Total Lys (%)	1.314	1.193	1.073
Total Thr (%)	0.873	0.790	0.729

[1]The starter diet was fed from day 0 to 14 days of age. The grower diet was fed from day 15 to 28 days of age and the finisher diet was fed from day 29 to 42 days of age.
[2]Mineral mix provided the following in mg per 100 g of diet: Mn, 6.0; Zn, 5.0; Fe, 3.0; Cu, 0.5; I, 0.15; and Se, 0.05.
[3]Vitamin mix provided the following per 100 g of diet: vitamin A, 551 IU; vitamin D_3, 110 IU; vitamin E, 1.1 IU; vitamin B_{12}, 0.001mg; riboflavin, 0.44 mg; niacin, 4.41 mg; d-pantothenic acid, 1.12 mg; choline, 19.13 mg; menadione sodium bisulfate, 0.33 mg; folic acid, 0.55 mg; pyridoxine HCl, 0.47 mg; thiamin, 0.22 mg; d-biotin, 0.011 mg; and ethoxyquin, 12.5 mg.
[4]The test products were added at the expense of sand.

same management guidelines except that de-caked, used litter from 4 previous flocks top dressed with 2 cm of new pine shavings was utilized.

For this experiment, there were 6 dietary treatments (**Table 3.6**) consisting of an industry standard control diet, or this diet supplemented with 0.05% bacitracin methylene disalicylate (BMD, from Nutra Blend, Neosho, MO), a yeast-based product, or 0.01, 0.025, and 0.05% cellobiose (Pfeifer & Langen, Cologne, Germany). Each dietary treatment consisted of 16 replicate pens (8 replicates per room) with each pen having 22 chicks. Birds and feed were weighed on a pen basis on days 0, 21, 35, and 42 to determine body weight, feed intake, body weight gain, and feed conversion.

Experiment 8

This experiment examined the efficacy of an acidified essential oil product on performance and intestinal health parameters of broilers from 0 to 15 days of age. This experiment was conducted in the same facility as experiment 3 and followed the same management guidelines except that only 1 room was utilized for a total of 48 pens and that de-caked, used litter from 2 previous flocks top dressed with 2 cm of new pine shavings was utilized.

For this experiment, there were 4 dietary treatments (**Table 3.7**) consisting of an industry standard control diet, or this diet supplemented with 0.0055% bacitracin methylene disalicylate (BMD, from Nutra Blend, Neosho, MO), or 0.015, 0.02, and 0.03% essential oil/acid product-1. Each dietary treatment consisted of 12 replicate pens with each pen having 22 chicks. Birds and feed were weighed on a pen basis on 0, 8, and 15 days of age to determine body weight, feed intake, body weight gain, and feed conversion. On day 15 of age, one chick from each pen with a

weight within 10% of the pen's overall mean body weight was selected for intestinal sampling (12 replicate birds for each treatment). A five-centimeter portion of the ascending duodenum and a five-centimeter segment of the jejunum containing Meckel's diverticulum in the center were collected for subsequent histological measurements as described in experiment 2.

Experiment 9

This experiment examined the efficacy of essential oil products in broilers from 0 to 42 days of age that were given a coccidiosis vaccine at day of hatch. This experiment was conducted in the same facility as experiment 3 and followed the same management guidelines except that only 1 room was utilized for a total of 48 pens and that de-caked, used litter from 2 previous flocks top dressed with 2 cm of new pine shavings was utilized

For this experiment, there were 6 treatments. The first treatment consisted of coccidiosis vaccinated chicks fed a standard industry control diet (**Table 3.8**). The next treatment consisted of unvaccinated chicks fed the control diet. The remaining treatments (3-6) all utilized coccidiosis vaccinated chicks fed the control diet. In treatment 3, the control diet was supplemented with 0.002% virginiamycin in the starter, 0.005% BMD and 0.005% salinomycin in the grower, and 0.006% salinomycin in the finisher. In treatment 4, essential oil mix 2 was added to the control diet at a rate 0.1% in the starter and grower diets and at a rate of 0.045% in the finisher diet. Treatment 5 contained essential oil mix 3 added to the control diet at a rate 0.636% in the starter and grower diets and at 0.045% in the finisher diet. Treatment 6 combined the essential oil mix 2 and 3 at the same rates applied for treatments 4 and 5. The vaccinated chicks received a spray coccidiosis vaccine at the hatchery. The unvaccinated control chicks

Table 3.6. Composition of the diets for Experiment 7.

Ingredient	Diet[1]		
	Starter	Grower	Finisher
		%	
Corn	55.417	60.751	64.017
Soybean Meal	38.950	34.003	30.852
Soybean Oil	2.216	2.121	2.309
Limestone (Calcium Carbonate)	0.573	0.566	0.554
Defluorinated phosphate	1.219	0.981	0.729
Salt	0.370	0.373	0.377
L-Lysine HCl 78%	0.172	0.162	0.151
DL-Methionine 99%	0.331	0.292	0.267
L-Threonine 98%	0.075	0.074	0.074
Choline-Cl, 60%	0.031	0.031	0.023
Vitamin Mix[2]	0.386	0.386	0.386
TM Mix[3]	0.075	0.075	0.075
Phytase (Quantum Blue 5000 FTU/g)	0.010	0.010	0.010
Coban 90	0.050	0.050	0.050
Sand/prebiotic[4]	0.125	0.125	0.125
Calculated analysis			
AME (kcal/kg)	2950	3000	3050
Crude Protein (%)	22.170	20.270	19.040
Dig Lysine (%)	1.200	1.080	1.000
Dig Methionine (%)	0.620	0.560	0.520
Dig Met+Cys (%)	0.900	0.820	0.770
Dig Threonine (%)	0.780	0.710	0.670
Dig Valine (%)	0.900	0.820	0.770
Dig Tryptophan (%)	0.250	0.230	0.210
Total Calcium (%)	0.950	0.850	0.750
Available P (%)	0.480	0.430	0.380

[1]Starter diet was fed from 0-21 days, grower diet was fed from 21-35 days, and the finisher diet was fed from 35-42 days.
[2]Vitamin mix provided the following per 100 g of diet: vitamin A, 551 IU; vitamin D_3, 110 IU; vitamin E, 1.1 IU; vitamin B_{12}, 0.001mg; riboflavin, 0.44 mg; niacin, 4.41 mg; d-pantothenic acid, 1.12 mg; choline, 19.13 mg; menadione sodium bisulfate, 0.33 mg; folic acid, 0.55 mg; pyridoxine HCl, 0.47 mg; thiamin, 0.22 mg; d-biotin, 0.011 mg; and ethoxyquin, 12.5 mg.
[3]Mineral mix provided the following in mg per 100 g of diet: Mn, 6.0; Zn, 5.0; Fe, 3.0; Cu, 0.5; I, 0.15; and Se, 0.05.
[4]Prebiotic will be added at the expense of sand.

Table 3.7. Composition of the diet for Experiment 8.

Ingredient	Diet[1]
	Starter
	%
Corn	48.599
Soybean meal	43.755
Soybean oil	3.796
Limestone	1.299
Dicalcium Phosphate	1.163
Salt	0.293
Sodium Carbonate	0.229
L-Lysine, HCl 78.8%	0.050
DL- Methionine 99%	0.346
L-Threonine, 98%	0.051
Choline Chloride 60%	0.020
Quantum Phytase XT 2,500	0.020
Vitamin mix[2]	0.227
Mineral mix[3]	0.075
Coban	0.046
SolkaFloc[4]	0.030
Calculated analysis	
AME (kcal/kg)	3031
Crude protein (%)	24.021
Calcium (%)	0.950
Available phosphorus (%)	0.475
Digestible total sulfur (%)	0.950
Digestible lysine (%)	1.250
Digestible threonine (%)	0.812

[1]Starter diet will be fed from day 1 to 15 days of age.
[2]Vitamin mix provided the following per 100 g of diet: vitamin A, 551 IU; vitamin D_3, 110 IU; vitamin E, 1.1 IU; vitamin B_{12}, 0.001mg; riboflavin, 0.44 mg; niacin, 4.41 mg; d-pantothenic acid, 1.12 mg; choline, 19.13 mg; menadione sodium bisulfate, 0.33 mg; folic acid, 0.55 mg; pyridoxine HCl, 0.47 mg; thiamin, 0.22 mg; d-biotin, 0.011 mg; and ethoxyquin, 12.5 mg.
[3]Mineral mix provided the following in mg per 100 g of diet: Mn, 6.0; Zn, 5.0; Fe, 3.0; Cu, 0.5; I, 0.15; and Se, 0.05.
[4]SolkaFloc was used as an inert filler and additions of BMD or Essential oil/acid product-1 were at its expense.

Table 3.8. Composition of the diets for Experiment 9.

Ingredient	Diets[1]		
	Starter	Grower	Finisher
		%	
Corn	57.730	61.825	63.925
Soybean meal	35.300	30.300	28.000
Soybean oil	2.500	3.500	4.000
Limestone	1.600	1.550	1.500
Dicalcium Phosphate	1.400	1.350	1.200
Salt	0.405	0.405	0.405
L-Lysine, HCl 78.8%	0.185	0.240	0.200
DL- Methionine 99%	0.300	0.275	0.250
L-Threonine, 98%	0.040	0.040	0.030
Choline Chloride 60%	0.100	0.075	0.050
Quantum phytase, 2,500 FTU	0.020	0.020	0.020
Vitamin mix[2]	0.225	0.225	0.225
Mineral mix[3]	0.075	0.075	0.075
SolkaFloc[4]	0.120	0.120	0.120
Calculated analysis			
AME (kcal/kg)	3060	3160	3200
Crude protein (%)	21.000	19.000	18.000
Calcium (%)	0.940	0.900	0.850
Available P (%)	0.440	0.420	0.380
Total Met (%)	0.630	0.570	0.530
Total TSAA (%)	0.980	0.900	0.850
Total Lys (%)	1.350	1.250	1.150
Total Thr (%)	0.880	0.800	0.750

[1]Starter diet was fed from day 1 to 14 days of age, the grower diet from 14 to 28 days of age, and the finisher diet from 28 to 42 days of age.
[2]Vitamin mix provided the following per 100 g of diet: vitamin A, 551 IU; vitamin D_3, 110 IU; vitamin E, 1.1 IU; vitamin B_{12}, 0.001mg; riboflavin, 0.44 mg; niacin, 4.41 mg; d-pantothenic acid, 1.12 mg; choline, 19.13 mg; menadione sodium bisulfate, 0.33 mg; folic acid, 0.55 mg; pyridoxine HCl, 0.47 mg; thiamin, 0.22 mg; d-biotin, 0.011 mg; and ethoxyquin, 12.5 mg.
[3]Mineral mix provided the following in mg per 100 g of diet: Mn, 6.0; Zn, 5.0; Fe, 3.0; Cu, 0.5; I, 0.15; and Se, 0.05.
[4]Test ingredients were added at the expense of SolkaFloc

were from the same hatchery and breeder flock, but were simply removed prior to application of the sprayed vaccine.

Each dietary treatment consisted of 8 replicate pens with each pen having 21 chicks. Birds and feed were weighed on a pen basis on days 0, 14, 28, and 42 days of age to determine body weight, feed intake, body weight gain, and feed conversion.

Experiment 10

One source for the introduction of pathogens in poultry production can be the feed. Extended heat conditioning of diets prior to pelleting combined with the addition of preservatives such as formaldehyde has proven effective in reducing the risk of *Salmonella* in poultry diets. However, there has been a concern that the addition of formaldehyde to diets subjected to extended heat treatment could decrease the metabolizable energy and amino acid availability of the diet. Thus, the purpose of this experiment was to determine if the application of a specific formaldehyde feed additive, Termin-8 (Anitox Co., Lawrenceville, GA), at a 0.3% inclusion rate in combination with extended heat treatment, reduced the TME_N and available amino acid content of a broiler starter diet.

A single one ton batch of broiler starter diet (**Table 3.9**) was mixed and equally divided into two bulk tote containers. The feed in one container was designated as the control diet and added back to the mixer for subsequent heat treatment and pelleting. The feed in the second tote was mixed in a Davis Horizontal Double Ribbon Mixer equipped with an air atomizing liquid application system for the application of Termin-8 at an inclusion rate of 0.3%. The Termin-8 treated diet was then heat treated and pelleted, exactly as the control diet.

Prior to pelleting, both the control and Termin-8 feeds were preconditioned at 82°C for 60 seconds using steam and subsequently subjected to extended heat treatment (4.5 minutes at 82°C). Each heat-treated diet was then immediately pelleted. When pellet temperature had achieved 82°C for 5 minutes, the flow of pellets was diverted to the crumbler. The crumbled diets were then used for the nitrogen corrected true metabolizable energy (TME_N) bioassay and the digestible amino acid bioassay.

TME_N Determination

TME_N was determined according to the method of Sibbald (1976) as modified by Dale and Fuller (1984). Forty Single Comb White Leghorn roosters (65 weeks of age) were fasted for 30 hours to empty the digestive tract. Roosters were then transferred to individual wire cages measuring 30.48 cm wide by 45.72 cm deep by 50.8 cm high. Each cage was equipped with a nipple drinker to provide free access to water and a stainless-steel excreta collection pan. Roosters (20 per treatment) were each precision-fed 35 grams of either the control diet or Termin-8 diet. The 20 roosters per treatment were subdivided into 5 replicate groups of 4 birds each. Excreta were collected for 48 hours post feeding. To estimate endogenous energy excretion, 10 roosters remained unfed for the 48-hour collection period.

Excreta were quantitatively collected from each individual pan, dried and weighed. Crude protein and moisture of the feces and diets were determined (AOAC 934.01, 2006; AOAC 990.03, 2006), with gross energy of feed and feces measured with a bomb calorimeter by the University of Georgia Agricultural and Environmental Laboratories (Athens, GA). The gross energy of each diet was obtained by averaging the values obtained from three samples of each diet.

Table 3.9. Composition of the experimental diet for experiment 10.

Ingredient	
	%
Corn	57.550
Soybean meal, 48% CP	34.146
Poultry fat	3.207
Dicalcium phosphate	1.719
Limestone	1.283
Sodium Chloride	0.452
DL – Methionine, 99%	0.313
L – Lysine, HCL 99%	0.130
Trace mineral mix[1]	0.100
Choline chloride, 50%	0.050
Vitamin mix[2]	0.050
Calculated analysis	
AME (Kcal/kg)	2900
Crude protein (%)	21
Calcium (%)	1.00
Available phosphorus (%)	0.45
Lysine (%)	1.15
Methionine	0.50

[1]Supplied per kilogram of diet: Cu, 8 mg; Zn, 75 mg; Fe, 80 mg; Mn, 100 mg; se 0.15 mg; I, 0.35 mg.

[2]Supplied per kilogram of diet: Vitamin A (retinyl acetate), 9500 IU; Vitamin D3, 2500 IU; Vitamin K, 2.65 mg; Vitamin B1, 2 mg; Vitamin B2, 6 mg; Vitamin B12, 0.025 mg; Vitamin E (alpha-tocopherol acetate), 30 IU; Biotin, 0.0325 mg; Folic acid, 1.25 mg; Pantothenic acid, 12 mg; Niacin, 50 mg.

Digestible Amino Acid Determination

The determination of the digestible amino coefficients of each diet followed the same procedures utilized for the TME_N determination except that 57 week old cecectomized roosters were utilized. The amino acid content of the diets and feces were determined (AOAC 982.30, 2006) by the University of Missouri Agricultural Experiment Station Chemical Laboratories (Colombia, MO) for calculation of the digestible amino acid coefficients for each diet. The amino acid composition of each diet was obtained by averaging the values obtained from 3 samples of each diet.

Statistics

Data from each experiment were subjected to ANOVA according to the General Linear Model (GLM) with dietary treatment, battery block (Experiments 1 and 2), room block (Experiments 3-7), and pen position block within a room (Experiments 3-9) as factors within the statistical model. Tukey's multiple-comparison procedure (Neter et al., 1990) was used to detect significant differences among individual dietary treatments. Differences were considered significant when $P < 0.05$. All statistical procedures were completed with the Minitab statistical software package (Minitab Release 16, State College, PA).

CHAPTER 4

RESULTS

Experiment 1

Broilers fed a control diet supplemented with 0.250 or 0.500 percent Azomite had lower feed to gain ratios than the broilers fed the control diet (**Tables 4.1**, **4.2** and **4.3**). Body weight gain for broilers fed the control diet supplemented with 0.125% Azomite was greater than those fed the control diet (**Table 4.3**).

Apparent calcium and phosphorus digestibilities were improved and serum levels of alpha-1-acid glycoprotein (AGP), an acute phase protein, were decreased at 21 days of age in the broilers fed the control diet supplemented with 0.5% Azomite (**Table 4.4**).

Experiment 2

Broilers fed a control diet supplemented with the lowest Azomite inclusion level (0.0625%) had greater body weight gains and improved feed to gain ratios relative broiler fed the control diet in the first 7 days of the experiment (**Table 4.5**), but these differences did not persist through 0 to 14 or 0 to 21 days of age (**Table 4.6** and **Table 4.7**). Although mortality was, numerically, very similar across the treatments, during the last week of the study four birds fed the 0.0625% Azomite diet developed splayed legs.

There were no significant differences in intestinal villi height, crypt depth, and villi/crypt ratio measured in the duodenum and the jejunum of broilers fed the control diet or the diets supplemented with 0.0625% and 0.250% Azomite (**Table 4.8** and **Table 4.9**).

Experiment 3

As expected, the broilers fed the negative control diet with less available energy and essential digestible amino acids than the positive control diet had decreased feed to gain ratios compared to the broilers fed the positive control diet in all phases of the experiment except the withdrawal phase (**Table 4.10 - Table 4.16)**. The addition of Azomite to the negative or positive control diets did not alter broiler performance (**Table 4.10 - Table 4.16)**. Although no differences were reported when assessing these groups individually, there were differences between main effect means in the negative control treatments with Azomite increasing body weight gain from 42 to 49 days (mean ± SEM, 718 ± 12g and 673 ± 32g for Azomite and control, respectively) and from 0 to 49 days (mean ± SEM, 4020 ± 20g and 3945 ± 33g for Azomite and control, respectively).

Calcium digestibility was increased, and phosphorus digestibility tended ($P = 0.07$) to improve in broilers fed the negative control diet supplemented with 0.125% Azomite (**Table 4.17**). All of the broilers from the negative control treatments were weighed individually and 10 birds per pen were processed. The variability in body weight was less in broilers fed the negative control diet supplemented with 0.125% Azomite relative to those fed the control diet (**Table 4.17)**. Processing yields and the incidence of woody breast were unaffected by supplementing the diet with Azomite except for an increase in the weight of leg quarters in broilers fed the negative control diet supplemented with 0.250% Azomite (**Table 4.18 – Table 4.21**).

Experiment 4

Although none of the broilers fed the control diet supplemented with any probiotic had weight gains or feed to gain ratios that were different from the control broilers throughout the experiment (**Table 4.22 – 4.26**), the broilers fed the control diet supplemented with any level of

Table 4.1. Body weight, body weight gain and feed efficiency of broilers fed diets containing 0%, 0.125%, 0.250% or 0.500% Azomite from 0 to 7 days of age[1] (Experiment 1).

Dietary treatments	Body weight	Body weight gain	Feed to gain	Feed Intake	Mortality
		g/bird			#(%)
Control	185 ± 2	142 ± 2	0.996 ± 0.008 [a]	139 ± 2	1 (1.11)
0.125% Azomite	191 ± 2	148 ± 2	0.965 ± 0.009[b]	144 ± 2	1 (1.11)
0.250% Azomite	187 ± 2	144 ± 2	0.966 ± 0.007[b]	139 ± 3	0 (0.00)
0.500% Azomite	190 ± 2	147 ± 2	0.958 ± 0.006[b]	142 ± 2	1 (1.11)

[1]The values are means ± SEM, n = 18 replicate pens for the dietary treatments. [a-b]Values with different superscripts for a given parameter differ, ($P < 0.05$).

Table 4.2. Body weight, body weight gain and feed efficiency of broilers fed diets containing 0%, 0.125%, 0.250% or 0.500% Azomite from 0 to 14 days of age[1] (Experiment 1).

Dietary treatments	Body weight	Body weight gain	Feed to gain	Feed Intake	Mortality
		g/bird			# (%)
Control	527 ± 4	485 ± 4	1.174 ± 0.008 [a]	562 ± 4	1 (1.11)
0.125% Azomite	542 ± 6	500 ± 6	1.151 ± 0.009[ab]	569 ± 6	3 (3.33)
0.250% Azomite	536 ± 4	493 ± 4	1.144 ± 0.005[b]	559 ± 6	2 (2.22)
0.500% Azomite	543 ± 4	500 ± 4	1.128 ± 0.005[b]	573 ± 6	2 (2.22)

[1]The values are means ± SEM, n = 18 replicate pens for the dietary treatments. [a-b]Values with different superscripts for a given parameter differ, ($P < 0.05$).

Table 4.3. Body weight, body weight gain and feed efficiency of broilers fed diets containing 0%, 0.125%, 0.250% or 0.500 % Azomite from 0 to 21 days of age[1] (Experiment 1).

Dietary treatments	Body weight	Body weight gain	Feed to gain	Feed Intake	Mortality
		g/bird			#(%)
Control	970 ± 6[b]	927 ± 6[b]	1.356 ± 0.008[a]	1241 ± 11	1 (1.11)
0.125% Azomite	1004 ± 10[a]	961 ± 10[a]	1.316 ± 0.007[b]	1253 ± 11	4 (4.44)
0.250% Azomite	991 ± 8[ab]	948 ± 8[ab]	1.313 ± 0.007[b]	1243 ± 10	3 (3.33)
0.500% Azomite	987 ± 6[ab]	944 ± 6[ab]	1.318 ± 0.007[b]	1254 ± 10	2 (2.22)

[1]The values are means ± SEM, n = 18 replicate pens for the dietary treatments. [a-b]Values with different superscripts for a given parameter differ, ($P < 0.05$).

Table 4.4. AME_N, apparent Ca digestibility and apparent P digestibility in broilers fed diets containing 0 % or 0.500 % Azomite from 0 to 21 days of age[1] (Experiment 1).

Dietary treatments	AME_N as is basis (Kcal/kg)	AME_N Dry basis (Kcal/kg)	Apparent P digestibility (%)	Apparent Ca digestibility (%)	AGP[2] ug/uL
Control	2939 ± 14	3372 ± 16	51.32 ± 1.15[b]	48.61 ± 1.44[b]	255 ± 12[a]
0.500% Azomite	2937 ± 12	3371 ± 13	54.80 ± 0.83[a]	56.48 ± 1.53[a]	224 ± 7[b]

[1]The values are means ± SEM, n = 18 replicate pens for the dietary treatments and 17 and 16 replicates for the control and 0.5% Azomite treatments, respectively. [a-b]Values with different superscripts for a given parameter differ, ($P < 0.05$).
[2]Alpha-1-acid Glycoprotein determined by a chicken ELISA kit (Abcam, Cambridge, United Kingdom).

Table 4.5. Body weight, body weight gain and feed efficiency of broilers fed diets containing 0%, 0.0625%, 0.125% or 0.250 % Azomite from 0 to 7 days of age[1] (Experiment 2).

Dietary treatments	Body weight	Body weight gain	Feed to gain	Feed Intake	Mortality
		g/bird			#(%)
Control	182 ± 2[b]	142 ± 2[b]	1.034 ± 0.007[a]	141 ± 3	2 (2.22)
0.0625% Azomite	190 ± 2[a]	150 ± 2[a]	0.989 ± 0.007[b]	142 ± 4	1 (1.11)
0.125% Azomite	185 ± 2[ab]	145 ± 2[ab]	1.013 ± 0.007[a]	151 ± 2	1 (1.11)
0.250% Azomite	182 ± 2[b]	142 ± 2[b]	1.029 ± 0.005[a]	146 ± 2	1 (1.11)

[1]The values are means ± SEM, n = 18 replicate pens for the dietary treatments. [a-b]Values with different superscripts for a given parameter differ, ($P < 0.05$).

Table 4.6. Body weight, body weight gain and feed efficiency of broilers fed diets containing 0%, 0.0625%, 0.125% or 0.250 % Azomite from 0 to 14 days of age[1] (Experiment 2).

Dietary treatments	Body weight	Body weight gain	Feed to gain	Feed Intake	Mortality
		g/bird			# (%)
Control	517 ± 6	477 ± 6	1.161 ± 0.010	538 ± 9	4 (4.44)
0.0625% Azomite	536 ± 7	496 ± 7	1.143 ± 0.009	548 ± 10	3 (3.33)
0.125% Azomite	527 ± 5	488 ± 5	1.144 ± 0.007	558 ± 10	3 (3.33)
0.250% Azomite	518 ± 6	478 ± 6	1.148 ± 0.008	546 ± 6	3 (3.33)

[1]The values are means ± SEM, n = 18 replicate pens for the dietary treatments.

Table 4.7. Body weight, body weight gain and feed efficiency of broilers fed diets containing 0%, 0.0625%, 0.125% or 0.250 % Azomite from 0 to 21 days of age[1] (Experiment 2).

Dietary treatments	Body weight	Body weight gain	Feed to gain	Feed Intake	Mortality
			g/bird		#(%)
Control	1005 ± 10	965 ± 9	1.258 ± 0.009	1193 ± 18	4 (4.44)
0.0625% Azomite	1034 ± 12	944 ± 12	1.244 ± 0.012	1198 ± 11	4 (4.44)
0.125% Azomite	1029 ± 8	989 ± 8	1.228 ± 0.005	1190 ± 14	3 (3.33)
0.250% Azomite	1008 ± 7	968 ± 7	1.246 ± 0.007	1187 ± 12	4 (4.44)

[1]The values are means ± SEM, n = 18 replicate pens for the dietary treatments.

Table 4.8. Duodenum histology measurements of broilers fed diets containing 0%, 0.0625%, 0.125% or 0.250 % Azomite from 0 to 21 days of age[1] (Experiment 2).

Dietary treatments	Total length[2]	Villi height	Crypt depth	Villi/crypt ratio
			mm	
Control	2.418 ± 0.051	2.156 ± 0.047	0.262 ± 0.008	8.608 ± 0.255
0.0625% Azomite	2.454 ± 0.041	2.188 ± 0.036	0.265 ± 0.010	8.704 ± 0.289
0.125% Azomite	Not collected and determined			
0.250% Azomite	2.523 ± 0.040	2.236 ± 0.031	0.288 ± 0.013	8.305 ± 0.298

[1]The values are means ± SEM, n = 18 replicate pens for the dietary treatments, intestinal measurements were made on 3 birds from each pen on day 21 of age.
[2]Total length represents the length from the base of the crypt to the tip of the villus.

Table 4.9. Ileum histology measurements of broilers fed diets containing 0%, 0.0625%, 0.125% or 0.250 % Azomite from 0 to 21 days of age[1] (Experiment 2).

Dietary treatments	Total length[2]	Villi height	Crypt depth	Villi/crypt ratio
			mm	
Control	1.714 ± 0.043	1.426 ± 0.037	0.288 ± 0.013	5.298 ± 0.229
0.0625% Azomite	1.698 ± 0.037	1.411 ± 0.037	0.286 ± 0.013	5.324 ± 0.285
0.125% Azomite	Not collected and determined			
0.250% Azomite	1.708 ± 0.044	1.426 ± 0.045	0.282 ± 0.009	5.381 ± 0.254

[1]The values are means ± SEM, n = 18 replicate pens for the dietary treatments, intestinal measurements were made on 3 birds from each pen on day 21 of age.
[2]Total length represents the length from the base of the crypt to the tip of the villus.

Table 4.10. Body weight, body weight gain and feed efficiency of broilers fed a basal diet or this diet supplemented with Azomite from 0 to 14 days of age[1] (Experiment 3).

Dietary treatments	Body weight	Body weight gain	Feed to gain	Feed Intake	Mortality
		g/bird			% (#)
Positive control (PC)	475 ± 3^{ab}	430 ± 3^{ab}	1.265 ± 0.006^{b}	543 ± 4	1.705 (6)
PC + 0.125% Azomite	475 ± 2^{ab}	431 ± 2^{ab}	1.256 ± 0.004^{b}	539 ± 2	0.568 (2)
PC + 0.250% Azomite	483 ± 3^{a}	439 ± 3^{a}	1.254 ± 0.004^{b}	545 ± 3	2.557 (9)
Negative control (NC)	469 ± 2^{b}	424 ± 2^{b}	1.290 ± 0.004^{a}	545 ± 2	1.705 (6)
NC + 0.125% Azomite	470 ± 3^{b}	426 ± 3^{b}	1.292 ± 0.004^{a}	549 ± 3	1.136 (4)
NC + 0.250% Azomite	467 ± 3^{b}	422 ± 3^{b}	1.304 ± 0.006^{a}	549 ± 3	1.420 (5)

[1]The values are means ± SEM, n = 16 replicate pens for the dietary treatments. $^{a-b}$Values with different superscripts for a given parameter differ, ($P < 0.05$).

Table 4.11. Body weight, body weight gain and feed efficiency of broilers fed a basal diet or this diet supplemented with Azomite from 14 to 28 days of age[1] (Experiment 3).

Dietary treatments	Body weight	Body weight gain	Feed to gain	Feed Intake	Mortality
		g/bird			% (#)
Positive control (PC)	1752 ± 8^{ab}	1278 ± 6^{ab}	1.462 ± 0.005^{b}	1859 ± 13	0.568 (2)
PC + 0.125% Azomite	1734 ± 10^{abc}	1261 ± 9^{ab}	1.472 ± 0.004^{b}	1846 ± 11	0.568 (2)
PC + 0.250% Azomite	1766 ± 9^{a}	1284 ± 6^{a}	1.470 ± 0.006^{b}	1887 ± 14	0.000 (0)
Negative control (NC)	1714 ± 9^{c}	1247 ± 7^{b}	1.509 ± 0.007^{a}	1880 ± 9	0.568 (2)
NC + 0.125% Azomite	1726 ± 12^{bc}	1256 ± 10^{ab}	1.507 ± 0.005^{a}	1884 ± 13	0.852 (3)
NC + 0.250% Azomite	1717 ± 10^{bc}	1251 ± 9^{b}	1.510 ± 0.006^{a}	1886 ± 11	0.568 (2)

[1]The values are means ± SEM, n = 16 replicate pens for the dietary treatments. $^{a-b}$Values with different superscripts for a given parameter differ, ($P < 0.05$).

Table 4.12. Body weight, body weight gain and feed efficiency of broilers fed a basal diet or this diet supplemented with Azomite from 0 to 28 days of age[1] (Experiment 3).

Dietary treatments	Body weight	Body weight gain	Feed to gain	Feed Intake	Mortality
		g/bird			% (#)
Positive control (PC)	1752 ± 8^{ab}	1707 ± 8^{ab}	1.411 ± 0.005^{b}	2376 ± 15	2.273 (8)
PC + 0.125% Azomite	1734 ± 10^{abc}	1690 ± 10^{abc}	1.416 ± 0.004^{b}	2364 ± 13	1.136 (4)
PC + 0.250% Azomite	1766 ± 9^{a}	1722 ± 9^{a}	1.413 ± 0.004^{b}	2402 ± 14	2.557 (9)
Negative control (NC)	1714 ± 9^{c}	1670 ± 9^{c}	1.452 ± 0.005^{a}	2398 ± 10	2.273 (8)
NC + 0.125% Azomite	1726 ± 12^{bc}	1682 ± 12^{bc}	1.450 ± 0.004^{a}	2410 ± 15	1.989 (7)
NC + 0.250% Azomite	1717 ± 10^{bc}	1673 ± 10^{bc}	1.457 ± 0.005^{a}	2412 ± 12	1.989 (7)

[1]The values are means ± SEM, n = 16 replicate pens for the dietary treatments. $^{a-b}$Values with different superscripts for a given parameter differ, ($P < 0.05$).

Table 4.13. Body weight, body weight gain and feed efficiency of broilers fed a basal diet or this diet supplemented with Azomite from 28 to 42 days of age[1] (Experiment 3).

Dietary treatments	Body weight	Body weight gain	Feed to gain	Feed Intake	Mortality
		g/bird			% (#)
Positive control (PC)	3374 ± 21	1625 ± 18	1.758 ± 0.011^{c}	2851 ± 27	0.284 (1)
PC + 0.125% Azomite	3367 ± 16	1641 ± 14	1.758 ± 0.014^{c}	2824 ± 19	0.568 (2)
PC + 0.250% Azomite	3384 ± 22	1625 ± 16	1.763 ± 0.011^{bc}	2822 ± 21	0.568 (2)
Negative control (NC)	3322 ± 15	1612 ± 15	1.798 ± 0.012^{a}	2868 ± 17	1.136 (4)
NC + 0.125% Azomite	3369 ± 22	1645 ± 18	1.790 ± 0.012^{ab}	2907 ± 18	0.852 (3)
NC + 0.250% Azomite	3346 ± 27	1635 ± 24	1.799 ± 0.016^{a}	2890 ± 24	1.136 (4)

[1]The values are means ± SEM, n = 16 replicate pens for the dietary treatments. $^{a-c}$Values with different superscripts for a given parameter differ, ($P < 0.05$).

Table 4.14. Body weight, body weight gain and feed efficiency of broilers fed a basal diet or this diet supplemented with Azomite from 0 to 42 days of age[1] (Experiment 3).

Dietary treatments	Body weight	Body weight gain	Feed to gain	Feed Intake	Mortality
		g/bird			% (#)
Positive control (PC)	3374 ± 21	3330 ± 21	1.576 ± 0.006[b]	5173 ± 38	2.557 (9)
PC + 0.125% Azomite	3367 ± 16	3323 ± 16	1.579 ± 0.007[b]	5140 ± 32	1.705 (6)
PC + 0.250% Azomite	3384 ± 22	3340 ± 22	1.578 ± 0.005[b]	5175 ± 33	3.125 (11)
Negative control (NC)	3322 ± 15	3277 ± 15	1.616 ± 0.006[a]	5211 ± 21	3.409 (12)
NC + 0.125% Azomite	3369 ± 22	3325 ± 22	1.613 ± 0.005[a]	5261 ± 30	2.841 (10)
NC + 0.250% Azomite	3346 ± 27	3302 ± 27	1.620 ± 0.007[a]	5248 ± 32	3.125 (11)

[1]The values are means ± SEM, n = 16 replicate pens for the dietary treatments. [a-b]Values with different superscripts for a given parameter differ, ($P < 0.05$).

Table 4.15. Body weight, body weight gain and feed efficiency of broilers fed a basal diet or this diet supplemented with Azomite from 42 to 49 days of age[1] (Experiment 3).

Dietary treatments	Body weight	Body weight gain	Feed to gain	Feed Intake	Mortality
		g/bird			% (#)
Positive control (PC)	4021 ± 39	652 ± 28	2.413 ± 0.068	1527 ± 27	2.557 (9)
PC + 0.125% Azomite	4031 ± 28	692 ± 23	2.387 ± 0.060	1522 ± 26	1.989 (7)
PC + 0.250% Azomite	4050 ± 24	678 ± 18	2.391 ± 0.047	1526 ± 16	1.420 (5)
Negative control (NC)	3989 ± 33	673 ± 32	2.383 ± 0.082	1516 ± 29	0.852 (3)
NC + 0.125% Azomite	4077 ± 26	715 ± 18	2.277 ± 0.027	1564 ± 15	0.568 (2)
NC + 0.250% Azomite	4051 ± 30	721 ± 17	2.319 ± 0.028	1549 ± 20	1.989 (7)

[1]The values are means ± SEM, n = 16 replicate pens for the dietary treatments.

Table 4.16. Body weight, body weight gain and feed efficiency of broilers fed a basal diet or this diet supplemented with Azomite from 0 to 49 days of age[1] (Experiment 3).

Dietary treatments	Body weight	Body weight gain	Feed to gain	Feed Intake	Mortality
		g/bird			% (#)
Positive control (PC)	4021 ± 39	3977 ± 39	1.698 ± 0.005[b]	6696 ± 60	5.114 (18)
PC + 0.125% Azomite	4031 ± 28	3987 ± 28	1.698 ± 0.004[b]	6656 ± 52	3.693 (13)
PC + 0.250% Azomite	4050 ± 24	4006 ± 24	1.700 ± 0.003[b]	6679 ± 46	4.545 (16)
Negative control (NC)	3989 ± 33	3945 ± 33	1.729 ± 0.006[a]	6703 ± 43	4.261 (15)
NC + 0.125% Azomite	4077 ± 26	4033 ± 26	1.720 ± 0.005[a]	6794 ± 40	3.409 (12)
NC + 0.250% Azomite	4051 ± 30	4007 ± 30	1.729 ± 0.006[a]	6771 ± 48	5.114 (18)

[1]The values are means ± SEM, n = 16 replicate pens for the dietary treatments. [a-b]Values with different superscripts for a given parameter differ, ($P < 0.05$).

Table 4.17. Coefficient of variation in body weight at 49 days of age in broilers fed a basal diet or this diet supplemented with Azomite as well as Ca/P digestibility[1] (Experiment 3).

Dietary treatments	Coefficient of variation in body weight[1]	P Digestibility[2]	Ca Digestibility[2]
		%	
Negative control (NC)	7.73 ± 0.42[a]	57.32 ± 4.58	48.95 ± 4.09[a]
NC + 0.125% Azomite	6.44 ± 0.36[b]	68.41 ± 3.16	67.82 ± 1.95[b]
NC + 0.250% Azomite	6.68 ± 0.31[ab]	Not determined	Not determined

[1]The values are means ± SEM, n = 16 replicate pens for the dietary treatments. [a-b]Values with different superscripts for a given parameter differ, ($P < 0.05$).
[2]Ca and P digestibility were determined at 42 days of age. [a-b]Values with different superscripts for a given parameter differ, ($P < 0.05$). The *P*-value for P digestibility equals 0.07.

Table 4.18. Processing yields from 49 day old broilers fed a basal diet or this diet supplemented with Azomite[1] (Experiment 3).

Dietary treatments	Live weight	Fasted weight (day 50 of age)	Loss of weight
	G	g	%[2]
Negative control (NC)	4000 ± 32	3782 ± 29	5.44 ± 0.09
NC + 0.125% Azomite	4071 ± 30	3853 ± 29	5.35 ± 0.10
NC + 0.250% Azomite	4059 ± 30	3845 ± 27	5.25 ± 0.13

[1]The values are means ± SEM, n = 16 replicate pens with 10 birds per pen selected.
[2]As a percent of live weight.

Table 4.19. Processing yields from 50 day old broilers fed a basal diet or this diet supplemented with Azomite[1] (Experiment 3).

Dietary treatments	Hot carcass		Chilled carcass		Frame	
	g	%[2]	g	%[2]	g	%[2]
Negative control (NC)	2798 ± 23	73.98 ± 0.12	2817 ± 22	74.48 ± 0.15	631 ± 5	16.69 ± 0.06
NC + 0.125% Azomite	2865 ± 25	74.35 ± 0.23	2877 ± 24	74.66 ± 0.17	636 ± 6	16.50 ± 0.10
NC + 0.250% Azomite	2848 ± 22	74.04 ± 0.13	2867 ± 24	74.54 ± 0.17	633 ± 6	16.46 ± 0.10

[1]The values are means ± SEM, n = 16 replicate pens with 10 birds per pen selected.
[2]As a percent of live fasted weight.

Table 4.20. Processing yields from 50 day old broilers fed a basal diet or this diet supplemented with Azomite[1] (Experiment 3).

Dietary treatments	Pectoralis major		Pectoralis minor		Total white meat[2]		Wings		Leg quarters	
	g	%[3]	g	%[3]	g	%[3]	g	%[3]	g	%[3]
Negative control (NC)	807 ± 12	21.3 ± 0.2	156 ± 2	4.12 ± 0.04	962 ± 13	25.41 ± 0.2	296 ± 2	7.84 ± 0.02	916 ± 5[b]	22.93 ± 0.15
NC + 0.125% Azomite	830 ± 10	21.5 ± 0.1	159 ± 2	4.13 ± 0.04	989 ± 11	25.68 ± 0.1	301 ± 2	7.82 ± 0.04	938 ± 7[ab]	23.06 ± 0.11
NC + 0.250% Azomite	821 ± 9	21.3 ± 0.1	160 ± 2	4.17 ± 0.04	981 ± 10	25.49 ± 0.1	299 ± 3	7.77 ± 0.04	941 ± 7[a]	23.19 ± 0.11

[1]The values are means ± SEM, n = 16 replicate pens with 10 birds per pen selected. [a-b]Values with different superscripts for a given parameter differ, ($P < 0.05$) among treatments.
[2]Pectoralis major plus pectoralis minor
[3]As a percent of live fasted weight.

Table 4.21. Incidence of woody breast/green muscle and white striping score at 50 days of age in broilers fed a basal diet or this diet supplemented with Azomite (Experiment 3).

Dietary treatments	Number of birds	Incidence	White Striping score
		%[1]	
Negative control (NC)	15	9.38	2.03 ± 0.08
NC + 0.125% Azomite	12	7.50	1.97 ± 0.07
NC + 0.250% Azomite	11	6.88	2.06 ± 0.05

[1]Percent of processed birds per treatment.

Table 4.22. Body weight, body weight gain and feed efficiency of broilers fed diets containing different types and levels of *Bacillus* probiotic products from 0 to 14 days of age[1] (Experiment 4).

Dietary treatments	Body weight	Body weight gain	Feed to gain	Feed Intake	Mortality and culls
		g/bird			% (#)
Control (C)	485 ± 4^{ab}	441 ± 4^{ab}	1.269 ± 0.006^{ab}	557 ± 4	1.81 (5)
C + 0.2% *B. licheniformis* 1	475 ± 4^{b}	431 ± 4^{b}	1.278 ± 0.006^{a}	547 ± 4	1.45 (4)
C + 0.1% *B. subtilis* 1	490 ± 3^{a}	447 ± 3^{a}	1.244 ± 0.006^{d}	556 ± 4	1.81 (5)
C + 0.2% *B. subtilis* 1	490 ± 4^{a}	446 ± 4^{a}	1.255 ± 0.005^{bcd}	552 ± 4	3.62 (10)
C + 0.4% *B. subtilis* 1	490 ± 3^{a}	447 ± 3^{a}	1.256 ± 0.006^{bcd}	556 ± 4	2.54 (7)
C + 0.1% *B. licheniformis* 2	485 ± 2^{ab}	441 ± 2^{ab}	1.264 ± 0.005^{abc}	552 ± 4	2.90 (8)
C + 0.2% *B. licheniformis* 2	486 ± 3^{ab}	442 ± 3^{ab}	1.258 ± 0.004^{bcd}	550 ± 3	1.81 (5)
C + 0.4% *B. licheniformis* 2	490 ± 4^{a}	446 ± 4^{a}	1.248 ± 0.003^{cd}	551 ± 3	2.17 (6)

[1]The values are means ± SEM, n = 12 replicate pens for the dietary treatments. $^{a-d}$Values with different superscripts for a given parameter differ, ($P < 0.05$).

Table 4.23. Body weight, body weight gain and feed efficiency of broilers fed diets containing different types and levels of *Bacillus* probiotic products from 14 to 28 days of age[1] (Experiment 4).

Dietary treatments	Body weight	Body weight gain	Feed to gain	Feed Intake	Mortality and culls
		g/bird			% (#)
Control (C)	1667 ± 13	1182 ± 10	1.542 ± 0.005^{abc}	1820 ± 16	0.36 (1)
C + 0.2% *B. licheniformis* 1	1624 ± 13	1150 ± 11	1.565 ± 0.006^{a}	1794 ± 15	0.72 (2)
C + 0.1% *B. subtilis* 1	1665 ± 20	1175 ± 18	1.528 ± 0.008^{bc}	1798 ± 24	1.09 (3)
C + 0.2% *B. subtilis* 1	1670 ± 16	1183 ± 13	1.523 ± 0.007^{c}	1795 ± 17	0.00 (0)
C + 0.4% *B. subtilis* 1	1679 ± 19	1191 ± 17	1.531 ± 0.008^{bc}	1809 ± 21	0.72 (2)
C + 0.1% *B. licheniformis* 2	1667 ± 10	1183 ± 11	1.551 ± 0.005^{ab}	1832 ± 19	0.00 (0)
C + 0.2% *B. licheniformis* 2	1670 ± 9	1186 ± 8	1.537 ± 0.006^{bc}	1817 ± 13	0.72 (2)
C + 0.4% *B. licheniformis* 2	1676 ± 15	1188 ± 13	1.547 ± 0.006^{ab}	1829 ± 16	1.45 (4)

[1]The values are means ± SEM, n = 12 replicate pens for the dietary treatments. [a-c]Values with different superscripts for a given parameter differ, ($P < 0.05$).

Table 4.24. Body weight, body weight gain and feed efficiency of broilers fed diets containing different types and levels of *Bacillus* probiotic products from 0 to 28 days of age[1] (Experiment 4).

Dietary treatments	Body weight	Body weight gain	Feed to gain	Feed Intake	Mortality and culls
		g/bird			% (#)
Control (C)	1667 ± 13	1623 ± 13	1.467 ± 0.006^{abc}	2320 ± 36	2.17 (6)
C + 0.2% *B. licheniformis* 1	1624 ± 13	1580 ± 13	1.486 ± 0.006^{a}	2289 ± 35	2.17 (6)
C + 0.1% *B. subtilis* 1	1665 ± 20	1621 ± 20	1.449 ± 0.006^{c}	2264 ± 30	2.90 (8)
C + 0.2% *B. subtilis* 1	1670 ± 16	1627 ± 16	1.449 ± 0.005^{c}	2261 ± 32	3.62 (10)
C + 0.4% *B. subtilis* 1	1679 ± 19	1635 ± 19	1.456 ± 0.006^{bc}	2281 ± 39	3.26 (9)
C + 0.1% *B. licheniformis* 2	1667 ± 10	1623 ± 10	1.472 ± 0.005^{ab}	2308 ± 40	2.90 (8)
C + 0.2% *B. licheniformis* 2	1670 ± 9	1626 ± 9	1.461 ± 0.004^{bc}	2301 ± 35	2.54 (7)
C + 0.4% *B. licheniformis* 2	1676 ± 15	1632 ± 15	1.464 ± 0.003^{bc}	2277 ± 24	3.62 (10)

[1]The values are means ± SEM, n = 12 replicate pens for the dietary treatments. [a-c]Values with different superscripts for a given parameter differ, ($P < 0.05$).

Table 4.25. Body weight, body weight gain and feed efficiency of broilers fed diets containing different types and levels of *Bacillus* probiotic products from 28 to 42 days of age[1] (Experiment 4).

Dietary treatments	Body weight	Body weight gain	Feed to gain	Feed Intake	Mortality and culls[2]
		g/bird			% (#)
Control (C)	3196 ± 43	1531 ± 37	1.779 ± 0.018	2708 ± 36	19.20 (53)
C + 0.2% *B. licheniformis* 1	3102 ± 34	1480 ± 28	1.790 ± 0.013	2651 ± 32	17.03 (47)
C + 0.1% *B. subtilis* 1	3148 ± 64	1484 ± 46	1.758 ± 0.019	2627 ± 71	19.56 (54)
C + 0.2% *B. subtilis* 1	3133 ± 43	1466 ± 33	1.773 ± 0.018	2580 ± 47	16.30 (45)
C + 0.4% *B. subtilis* 1	3187 ± 47	1514 ± 38	1.789 ± 0.018	2703 ± 64	20.29 (56)
C + 0.1% *B. licheniformis* 2	3220 ± 37	1554 ± 33	1.765 ± 0.016	2756 ± 66	20.29 (56)
C + 0.2% *B. licheniformis* 2	3219 ± 36	1549 ± 33	1.745 ± 0.015	2730 ± 44	19.20 (53)
C + 0.4% *B. licheniformis* 2	3186 ± 52	1510 ± 41	1.777 ± 0.021	2690 ± 54	21.74 (60)

[1]The values are means ± SEM, n = 12 replicate pens for the dietary treatments

[2]At the end of the day 33 of the experiment a thundershower caused a power failure at the University of Georgia Poultry Research Farm and the back-up generator was not powerful enough to run all of the circulation fans and with an outside temperature in excess of 26°C the rooms overheated, and birds died before full power was restored. If this event is excluded total mortality in this period is 1.449, 1.449, 1.812, 1.449, 1.449, 0.362, 1.087, and 1.812 percent for treatments 1 through 8, respectively.

Table 4.26. Body weight, body weight gain and feed efficiency of broilers fed diets containing different types and levels of *Bacillus* probiotic products from 0 to 42 days of age[1] (Experiment 4).

Dietary treatments	Body weight	Body weight gain	Feed to gain	Feed Intake	Foot pad lesion score[2]
		g/bird			
Control (C)	3196 ± 43	3152 ± 43	1.607 ± 0.008[ab]	4991 ± 55	0.49 ± 0.07
C + 0.2% *B. licheniformis* 1	3102 ± 34	3058 ± 34	1.624 ± 0.007[a]	4887 ± 44	0.28 ± 0.06
C + 0.1% *B. subtilis* 1	3148 ± 64	3104 ± 64	1.585 ± 0.008[b]	4884 ± 98	0.54 ± 0.05
C + 0.2% *B. subtilis* 1	3133 ± 43	3089 ± 43	1.596 ± 0.007[ab]	4872 ± 62	0.46 ± 0.07
C + 0.4% *B. subtilis* 1	3187 ± 47	3143 ± 47	1.605 ± 0.009[ab]	4973 ± 64	0.54 ± 0.10
C + 0.1% *B. licheniformis* 2	3220 ± 37	3176 ± 37	1.605 ± 0.006[ab]	5045 ± 68	0.38 ± 0.07
C + 0.2% *B. licheniformis* 2	3219 ± 36	3175 ± 36	1.590 ± 0.007[b]	5000 ± 48	0.36 ± 0.08
C + 0.4% *B. licheniformis* 2	3186 ± 52	3142 ± 52	1.602 ± 0.009[ab]	4972 ± 63	0.45 ± 0.07

[1]The values are means ± SEM, n = 12 replicate pens for the dietary treatments. [a-c]Values with different superscripts for a given parameter differ, ($P < 0.05$).
[2]Score range is from 0 to 2.

Bacillus subtilis product 2 had improved feed to gain ratios relative to the broilers fed the control diet supplemented with *Bacillus licheniformis* product 1. Similarly, the broilers fed the control diet supplemented with 0.2% *Bacillus licheniformis* product 2 had an improved feed to gain ratio in the 0-14, 14-28, and 0-28 day of age periods compared to the broilers fed an equivalent amount *Bacillus licheniformis* product 1.

Regretfully, during the finisher phase of this experiment at the end of the day 33 of the experiment, a thunderstorm caused a power failure at the University of Georgia Poultry Research Farm and the back-up generator failed to switch on to power the facility. With an outside temperature in excess of 26°C, the rooms overheated and there was a significant mortality event (**Table 4.27)**.

Experiment 5

As expected, the feed to gain ratio of the birds fed the negative control diet was significantly higher than the birds fed the positive control diet for every period (0-14, 14-28, 0-28, 28-42, and 0-42 days of age (**Table 4.28 – 4.32**). The enzyme mixture of carbohydrate digesting enzymes naturally lacking in poultry that was added to the diet did not increase broiler performance. During the starter period the dietary addition of both of *Bacillus subtilis* probiotic products to the negative control diet actually increased the feed to gain ratio of the broilers fed these diets relative to those fed the negative control diet. During the grower period this increase in the feed to gain ratio persisted for the broilers fed the diet supplemented with *Bacillus subtilis* probiotic product 3.

On 42 days of age all of the broilers in each pen were individually weighed. The coefficient of variation in body weight did not vary among the dietary treatments (**Table 4.33).** Although litter score, litter ammonia production levels and litter moisture did not differ among

the dietary treatments, the foot pad score, which is another indicator of litter quality, of the broilers fed the positive control diet and the negative control diet supplemented with *Bacillus subtilis* probiotic product 3 was decreased relative to the score in broilers fed the negative control diet or this diet supplemented with *Bacillus subtilis* probiotic product 2.

Because the diets contained no coccidiostat, the incidence of severe gross necrotic enteritis observed in mortalities and culls during the experiment was recorded (**Table 4.34**). While the overall incidence was low, the addition of *Bacillus subtilis* probiotic product 2 did not appear to provide any protection against the development of necrotic enteritis.

Experiment 6

The feed to gain ratio did not differ between the broilers fed the 4 dietary treatments during any of the periods of the experiment (**Table 4.35 - 4.39**). At 14 days of age the body weight of the broilers fed the control diet supplemented with Magni-Phi was greater than the body weight of the broilers fed the control diet or the control diet supplemented with Micro-Aid and the difference in body weight between the broilers fed Magni-Phi and Micro-Aid were also present at day 28 and 42 of age.

The broilers did not receive any coccidiostats or antibiotics in their diets and the overall mortality rate relative to the control treatment was not reduced by Magni-Phi or the probiotic product (**Table 4.39**). Mortality for the broilers fed the control diet supplemented with the probiotic product was actually over 30% less than the rate of mortality in the broilers fed the control diet at day 14 (Table 4.35), but by the end of the experiment the overall mortality rate was equal between the 2 treatments (Table 4.39). The equaling out of the mortality rate in these 2 treatments resulted in part from a higher rate of broilers dying from necrotic enteritis in the probiotic treatment relative to the control treatment as the experiment progressed (**Table 4.40)**.

Table 4.27. Overall experimental mortality of broilers fed diets containing different types and levels of *Bacillus* products from 0 to 42 days of age[1] (Experiment 4).

Dietary treatments	Mortality and culls **without** including power outage birds on day 34[2]	Mortality and culls **with** including power outage birds on day 34
	% (#)	
Control (C)	3.623 (10)	21.377 (59)
C + 0.2% *B. licheniformis* 1	3.623 (10)	19.203 (53)
C + 0.1% *B. subtilis* 1	4.710 (13)	22.464 (62)
C + 0.2% *B. subtilis* 1	5.072 (14)	19.928 (55)
C + 0.4% *B. subtilis* 1	4.710 (13)	23.551 (65)
C + 0.1% *B. licheniformis* 2	3.261 (9)	23.188 (64)
C + 0.2% *B. licheniformis* 2	3.623 (10)	21.739 (60)
C + 0.4% *B. licheniformis* 2	5.435 (15)	25.362 (70)

[1]The values are means ± SEM, n = 12 replicate pens for the dietary treatments.
[2]At the end of the day 33 of the experiment, a thundershower caused a power failure at the University of Georgia Poultry Research Farm and the back-up generator did not switch on to power the facility. With an outside temperature in excess of 26°C the rooms overheated and birds died before full power was restored.

Table 4.28. Body weight, body weight gain and feed to gain of broilers fed a positive control diet, a negative control diet with about 3% less energy and digestible amino acid levels than the positive control diet or the negative control diet supplemented with a carbohydrase enzyme mixture, *Bacillus subtilis* probiotic product 2, or *Bacillus subtilis* probiotic product 3 from 0 to 14 days of age[1] (Experiment 5).

Dietary treatments	Body weight	Body weight gain	Feed to gain	Feed Intake	Mortality /culls
		g/bird			% (#)
Positive control	445 ± 3[a]	405 ± 3[a]	1.255 ± 0.004[d]	508 ± 3	1.04 (4)
Negative control (NC)	437 ± 3[abc]	398 ± 3[abc]	1.293 ± 0.004[c]	509 ± 4	1.82 (7)
NC + *Bacillus subtilis* 2	435 ± 3[bc]	395 ± 3[bc]	1.315 ± 0.008[ab]	516 ± 3	1.82 (7)
NC + Enzyme	443 ± 3[ab]	403 ± 3[ab]	1.286 ± 0.003[c]	515 ± 4	2.34 (9)
NC + Enzyme & *B. subtilis* 2	444 ± 3[ab]	404 ± 3[ab]	1.300 ± 0.005[bc]	519 ± 5	1.56 (6)
NC + *B. subtilis* 3	433 ± 3[c]	393 ± 3[c]	1.322 ± 0.005[a]	518 ± 3	1.56 (6)

[1]The values are means ± SEM, n = 16 replicate pens for the dietary treatments. [a-d]Values with different superscripts for a given parameter differ, ($P < 0.05$).

Table 4.29. Body weight, body weight gain and feed to gain of broilers fed a positive control diet, a negative control diet with about 3% less energy and digestible amino acid levels than the positive control diet or the negative control diet supplemented with a carbohydrase enzyme mixture, *Bacillus subtilis* probiotic product 2, or *Bacillus subtilis* probiotic product 3 from 14 to 28 days of age[1] (Experiment 5).

Dietary treatments	Body weight	Body weight gain	Feed to gain	Feed Intake	Mortality /culls
		g/bird			% (#)
Positive control	1609 ± 9[a]	1167 ± 7	1.467 ± 0.004[c]	1708 ± 12	0.78 (3)
Negative control (NC)	1591 ± 9[abc]	1158 ± 7	1.501 ± 0.005[b]	1726 ± 10	1.04 (4)
NC + *Bacillus subtilis* 2	1574 ± 11[bc]	1142 ± 9	1.503 ± 0.004[b]	1714 ± 14	0.52 (2)
NC + Enzyme	1603 ± 8[abc]	1163 ± 6	1.494 ± 0.005[b]	1735 ± 11	0.52 (2)
NC + Enzyme & *B. subtilis* 2	1607 ± 9[ab]	1168 ± 7	1.492 ± 0.004[b]	1732 ± 13	0.78 (3)
NC + *B. subtilis* 3	1569 ± 8[c]	1140 ± 7	1.530 ± 0.007[a]	1730 ± 11	1.56 (6)

[1]The values are means ± SEM, n = 16 replicate pens for the dietary treatments. [a-c]Values with different superscripts for a given parameter differ, ($P < 0.05$).

Table 4.30. Body weight, body weight gain and feed to gain of broilers fed a positive control diet, a negative control diet with about 3% less energy and digestible amino acid levels than the positive control diet or the negative control diet supplemented with a carbohydrase enzyme mixture, *Bacillus subtilis* probiotic product 2, or *Bacillus subtilis* probiotic product 3 from 0 to 28 days of age[1] (Experiment 5).

Dietary treatments	Body weight	Body weight gain	Feed to gain	Feed Intake	Mortality /culls
		g/bird			% (#)
Positive control	1609 ± 9[a]	1569 ± 9[a]	1.409 ± 0.003[c]	2219 ± 15	1.82 (7)
Negative control (NC)	1591 ± 9[abc]	1551 ± 9[abc]	1.443 ± 0.004[b]	2243 ± 11	2.86 (11)
NC + *Bacillus subtilis* 2	1574 ± 11[bc]	1534 ± 11[bc]	1.451 ± 0.004[b]	2235 ± 15	2.34 (9)
NC + Enzyme	1603 ± 8[abc]	1563 ± 8[abc]	1.437 ± 0.004[b]	2257 ± 13	2.86 (11)
NC + Enzyme & *B. subtilis* 2	1607 ± 9[ab]	1567 ± 9[ab]	1.439 ± 0.003[b]	2258 ± 17	2.34 (9)
NC + *B. subtilis* 3	1569 ± 8[c]	1529 ± 8[c]	1.472 ± 0.005[a]	2250 ± 13	3.12 (12)

[1]The values are means ± SEM, n = 16 replicate pens for the dietary treatments. [a-c]Values with different superscripts for a given parameter differ, ($P < 0.05$).

Table 4.31. Body weight, body weight gain and feed to gain of broilers fed a positive control diet, a negative control diet with about 3% less energy and digestible amino acid levels than the positive control diet or the negative control diet supplemented with a carbohydrase enzyme mixture, *Bacillus subtilis* probiotic product 2, or *Bacillus subtilis* probiotic product 3 from 28 to 42 days of age[1] (Experiment 5).

Dietary treatments	Body weight	Body weight gain	Feed to gain	Feed Intake	Mortality /culls
		g/bird			% (#)
Positive control	3272 ± 35	1672 ± 29	1.672 ± 0.009[c]	2786 ± 41	0.78 (3)
Negative control (NC)	3226 ± 35	1649 ± 31	1.746 ± 0.018[ab]	2849 ± 38	2.34 (9)
NC + *Bacillus subtilis* 2	3215 ± 32	1648 ± 26	1.758 ± 0.017[a]	2887 ± 31	2.86 (11)
NC + Enzyme	3253 ± 35	1658 ± 33	1.739 ± 0.014[ab]	2852 ± 42	2.08 (8)
NC + Enzyme & *B. subtilis* 2	3261 ± 31	1665 ± 28	1.727 ± 0.018[ab]	2841 ± 26	2.08 (8)
NC + *B. subtilis* 3	3236 ± 28	1674 ± 27	1.712 ± 0.013[bc]	2864 ± 34	1.56 (6)

[1]The values are means ± SEM, n = 16 replicate pens for the dietary treatments. [a-c]Values with different superscripts for a given parameter differ, ($P < 0.05$).

Table 4.32. Body weight, body weight gain and feed to gain of broilers fed a positive control diet, a negative control diet with about 3% less energy and digestible amino acid levels than the positive control diet or the negative control diet supplemented with a carbohydrase enzyme mixture, *Bacillus subtilis* probiotic product 2, or *Bacillus subtilis* probiotic product 3 from 0 to 42 days of age[1] (Experiment 5).

Dietary treatments	Body weight	Body weight gain	Feed to gain	Feed intake	Mortality/ culls
		g/bird			% (#)
Positive control	3272 ± 35	3232 ± 35	1.535 ± 0.004[c]	5065 ± 52	2.60 (10)
Negative control (NC)	3226 ± 35	3186 ± 35	1.585 ± 0.007[ab]	5157 ± 36	5.21 (20)
NC + *Bacillus subtilis* 2	3215 ± 32	3175 ± 32	1.597 ± 0.007[a]	5175 ± 46	5.21 (20)
NC + Enzyme	3253 ± 35	3213 ± 35	1.579 ± 0.005[ab]	5160 ± 55	4.95 (19)
NC + Enzyme & *B. subtilis* 2	3261 ± 31	3221 ± 31	1.575 ± 0.007[b]	5175 ± 33	4.43 (17)
NC + *B. subtilis* 3	3236 ± 28	3196 ± 28	1.588 ± 0.006[ab]	5202 ± 37	4.69 (18)

[1]The values are means ± SEM, n = 16 replicate pens for the dietary treatments. [a-c]Values with different superscripts for a given parameter differ, ($P < 0.05$).

Table 4.33. Coefficient of body weight, litter score, foot pad score, litter moisture and litter ammonia levels at 42 days of age for broilers fed a positive control diet, a negative control diet with about 3% less energy and digestible amino acid levels than the positive control diet or the negative control diet supplemented with a carbohydrase enzyme mixture, *Bacillus subtilis* probiotic product 2, or *Bacillus subtilis* probiotic product 3 from 0 to 42 days of age[1] (Experiment 5).

Dietary treatments	Coefficient of variation in body weight (%)	Litter Score[2]	Foot pad score[3]	Litter moisture (%)	Litter ammonia level (ppm)
Positive control	6.50 ± 0.33	1.34 ± 0.26	0.31 ± 0.05[b]	26.57 ± 2.68	112 ± 9
Negative control (NC)	6.66 ± 0.44	1.86 ± 0.17	0.56 ± 0.05[a]	33.21 ± 3.10	123 ± 9
NC + *Bacillus subtilis* 2	6.54 ± 0.43	2.00 ± 0.29	0.56 ± 0.08[a]	32.80 ± 2.25	110 ± 12
NC + Enzyme	6.71 ± 0.35	1.84 ± 0.27	0.42 ± 0.06[ab]	29.46 ± 2.34	123 ± 9
NC + Enzyme & *B. subtilis* 2	6.91 ± 0.36	1.72 ± 0.18	0.57 ± 0.07[a]	26.66 ± 2.53	121 ± 12
NC + *B. subtilis* 3	6.13 ± 0.33	1.55 ± 0.23	0.28 ± 0.05[b]	27.51 ± 1.98	98 ± 12

[1]The values are means ± SEM, n = 16 replicate pens for each dietary treatment for coefficient of body weight, litter score and foot pad score and n = 8 for litter moisture and litter ammonia level. [a-b]Values with different superscripts for a given parameter differ, ($P < 0.05$).

[2]Litter score is based on a scale from 0 to 10, with a score of zero meaning no caked litter in the pen and a score of 10 meaning 100% of the litter in the pen is caked.

[3]Foot pad score is based on a scale from 0 to 2 with a score of 0 given to footpads with no scab or lesion present, a score of 1 is given for a mild lesion on both feet, and a score of 2 is given for severe lesions on both feet.

Table 4.34. Incidence of necrotic enteritis of broilers fed a positive control diet, a negative control diet with about 3% less energy and digestible amino acid levels than the positive control diet or the negative control diet supplemented with a carbohydrase enzyme mixture, *Bacillus subtilis* probiotic product 2, or *Bacillus subtilis* probiotic product 3 from 0 to 14 days of age[1] (Experiment 5).

Dietary treatment	Incidence
Positive control	1
Negative control (NC)	3
NC + *Bacillus subtilis* 2	9
NC + Enzyme	4
NC + Enzyme & *B. subtilis* 2	3
NC + *B. subtilis* 3	5

[1]The birds were assessed based on gross pathological findings. Severe diffuse intestinal mucosal necrosis was required in order to label the cause of death necrotic enteritis.

Table 4.35. Body weight, body weight gain and feed efficiency of broilers fed a control diet or this diet supplemented with yucca products Magni-Phi and Micro-Aid or *Bacillus subtilis*-probiotic product 2 from 0 to 14 days of age[1](Experiment 6).

Dietary treatments	Body weight	Body weight gain	Feed to gain	Feed Intake	Mortality/culls
		g/bird			% (#)
Control (C)	395 ± 4^{b}	358 ± 4^{b}	1.315 ± 0.005	463 ± 4	3.45 (19)
C + 0.05% Magni-Phi	409 ± 4^{a}	372 ± 4^{a}	1.303 ± 0.004	472 ± 4	5.09 (28)
C + 0.05% Micro-Aid	392 ± 4^{b}	355 ± 4^{b}	1.317 ± 0.007	462 ± 4	3.09 (17)
C+0.05% *Bacillus subtilis* 2	401 ± 3^{ab}	364 ± 3^{ab}	1.313 ± 0.006	474 ± 4	2.18 (12)

[1]The values are means ± SEM, n = 22 replicate pens for the dietary treatments. $^{a-b}$Values with different superscripts for a given parameter differ, ($P < 0.05$).

Table 4.36. Body weight, body weight gain and feed efficiency of broilers fed a control diet or this diet supplemented with yucca products Magni-Phi and Micro-Aid or *Bacillus subtilis*-probiotic product 2 from 14 to 28 days of age[1] (Experiment 6).

Dietary treatments	Body weight	Body weight gain	Feed to gain	Feed Intake	Mortality/culls
		g/bird			% (#)
Control (C)	1526 ± 10^{ab}	1132 ± 7^{ab}	1.525 ± 0.005	1707 ± 12^{b}	2.18 (12)
C + 0.05% Magni-Phi	1554 ± 10^{a}	1147 ± 7^{a}	1.531 ± 0.006	1747 ± 10^{a}	2.00 (11)
C + 0.05% Micro-Aid	1509 ± 10^{b}	1118 ± 7^{b}	1.543 ± 0.005	1721 ± 11^{ab}	1.82 (10)
C+0.05% *Bacillus subtilis* 2	1537 ± 8^{ab}	1137 ± 7^{ab}	1.537 ± 0.006	1717 ± 10^{ab}	3.27 (18)

[1]The values are means ± SEM, n = 22 replicate pens for the dietary treatments. [a-b]Values with different superscripts for a given parameter differ, ($P < 0.05$).

Table 4.37. Body weight, body weight gain and feed efficiency of broilers fed a control diet or this diet supplemented with yucca products Magni-Phi and Micro-Aid or *Bacillus subtilis*-probiotic product 2 from 0 to 28 days of age[1] (Experiment 6).

Dietary treatments	Body weight	Body weight gain	Feed to gain	Feed Intake	Mortality/culls
		g/bird			% (#)
Control (C)	1526 ± 10^{ab}	1489 ± 10^{ab}	1.474 ± 0.004	2158 ± 17	5.64 (31)
C + 0.05% Magni-Phi	1554 ± 10^{a}	1517 ± 10^{a}	1.473 ± 0.005	2199 ± 14	7.09 (39)
C + 0.05% Micro-Aid	1509 ± 10^{b}	1472 ± 10^{b}	1.488 ± 0.003	2176 ± 15	4.91 (27)
C+0.05% *Bacillus subtilis* 2	1537 ± 8^{ab}	1501 ± 8^{ab}	1.481 ± 0.005	2174 ± 16	5.45 (30)

[1]The values are means ± SEM, n = 22 replicate pens for the dietary treatments. [a-b]Values with different superscripts for a given parameter differ, ($P < 0.05$).

Table 4.38. Body weight, body weight gain and feed efficiency of broilers fed a control diet or this diet supplemented with yucca products Magni-Phi and Micro-Aid or *Bacillus subtilis*-probiotic product 2 from 28 to 42 days of age[1] (Experiment 6).

Dietary treatments	Body weight	Body weight gain	Feed to gain	Feed Intake	Mortality/culls
		g/bird			% (#)
Control (C)	3109 ± 19[ab]	1586 ± 14	1.781 ± 0.007	2785 ± 22	2.36 (13)
C + 0.05% Magni-Phi	3155 ± 19[a]	1601 ± 11	1.786 ± 0.009	2820 ± 24	2.18 (12)
C + 0.05% Micro-Aid	3096 ± 20[b]	1587 ± 15	1.790 ± 0.006	2803 ± 21	1.09 (6)
C+0.05% *Bacillus subtilis* 2	3107 ± 18[ab]	1572 ± 14	1.803 ± 0.007	2786 ± 19	2.54 (14)

[1]The values are means ± SEM, n = 22 replicate pens for the dietary treatments. [a-b]Values with different superscripts for a given parameter differ, ($P < 0.05$).

Table 4.39. Body weight, body weight gain and feed efficiency of broilers fed a control diet or this diet supplemented with yucca products Magni-Phi and Micro-Aid or *Bacillus subtilis*-probiotic product 2 from 0 to 42 days of age[1] (Experiment 6).

Dietary treatments	Body weight	Body weight gain	Feed to gain	Feed Intake	Mortality/culls
		g/bird			% (#)
Control (C)	3109 ± 19[ab]	3072 ± 19[ab]	1.629 ± 0.004	4900 ± 38	7.33 (44)
C + 0.05% Magni-Phi	3155 ± 19[a]	3118 ± 19[a]	1.630 ± 0.005	4991 ± 37	8.50 (51)
C + 0.05% Micro-Aid	3096 ± 20[b]	3059 ± 20[b]	1.642 ± 0.003	4943 ± 35	5.50 (33)
C+0.05% *Bacillus subtilis* 2	3107 ± 18[ab]	3070 ± 18[ab]	1.642 ± 0.004	4921 ± 30	7.33 (44)

[1]The values are means ± SEM, n = 22 replicate pens for the dietary treatments. [a-b]Values with different superscripts for a given parameter differ, ($P < 0.05$)

Table 4.40. Incidence of necrotic enteritis in broilers fed a control diet or this diet supplemented with yucca products Magni-Phi and Micro-Aid or *Bacillus subtilis*-probiotic product 2 from 0 to 42 days of age[1] (Experiment 6).

Dietary treatment	Incidence
Control (C)	7
C + 0.05% Magni-Phi	6
C + 0.05% Micro-Aid	3
C+0.05% *Bacillus subtilis* 2	12
Room	
J1	22
J4	6

[1]The birds were assessed based on gross pathological findings. Severe diffuse intestinal mucosal necrosis was required in order to label the cause of death necrotic enteritis.

Table 4.41. Mortality incidence by room for broilers fed a control diet or this diet supplemented with yucca products Magni-Phi and Micro-Aid or *Bacillus subtilis*-probiotic product 2 from 0 to 42 days of age[1] (Experiment 6).

Room	Starter period (0-14 days)	Grower period (14-28 days)	Finisher period (28-42 days)	Total (0-42 days)
J1	45	37	29	111
J4	31	14	16	61

Death from necrotic enteritis also played a significant role in the overall mortality rate being greater in one of the experimental rooms than the other (**Table 4.40 and Table 4.41**).

Table 4.42 reports the live weight, fasted weight, and loss of weight between dietary treatments. There were no differences in fasted weight or weight loss (**Table 4.43**). Magni-Phi had a higher live weight than the Micro-Aid group at the start of processing. There were no differences in hot carcass or chilled carcass weights or percentages between dietary treatments. The frame weight as a percentage of live fasted weight was lower in Magni-Phi compared to Micro-Aid.

The broilers selected for processing reflected the same statistical differences in body weight between the dietary treatments as the whole population (**Table 4.39 and Table 4.42**). Although the broilers fed the diet supplemented with Magni-Phi had live body weights greater than those fed the diet supplemented with Micro-Aid (**Table 4.42**), the percent of the live fasted body weight represented by the carcass frame was less (**Table 4.43**) and represented by total weight meat yield was greater for the birds fed Magni-Phi than Micro-Aid (**Table 4.44**).

Experiment 7

Although through 0 to 14 days of age body weight and body weight gain did not differ between the broilers fed the control diet and the broilers in any of the supplemented diets (**Table 4.45**), in the periods from 14 to 21 days of age and 0 to 21 days of age the broilers fed the control diet supplemented with the yeast-based product had gained less weight and had a decreased body weight than the broilers fed the control diet (**Tables 4.46 and 4.47**). However, after 21 days of age, there were no differences in body weight or weight gain between any of the dietary treatments (**Table 4.48 - Table 4.54**). The feed to gain ratio was also increased in the broilers fed the diet supplemented with the yeast product relative to those fed the control diet in the starter

period from 0 to 21 days of age. But this difference in the feed to gain ratio between these 2 dietary treatments did not persist in any of the measured time points after 21 days of age.

The overall mortality incidence through 42 days of age was 60% greater in the broilers fed the control diet supplemented with a yeast-based product and was decreased by 50% in the broilers fed the control diet supplemented with the lowest dose of cellobiose compared to the broilers fed the control diet (**Table 4.51**).

Experiment 8

No differences were detected in body weight, body weight gain, or feed-to-gain ratio between the broilers fed a BMD supplemented diet or those fed the different levels of the essential oil and acid mix (**Table 4.55** and **Table 4.56)**. The average villi height and crypt depth in the duodenum of the broilers fed a diet containing 0.03% of the essential oil and acid mix was greater than those in the broilers fed the diet containing BMD (**Table 4.57**).

Experiment 9

In the starter period (0 to 14 days of age), the feed-to-gain ratio for the vaccinated broilers fed the diet supplemented with a combination of essential oils was lower than vaccinated broilers fed the control diet (**Table 4.58**). For the remainder of the experiment there were no differences in body weight, body weight gain or the feed to gain ratio detected between the broilers fed the different dietary treatments (**Table 4.59 – 4.62**). The mortality rate was 4.17% for in the vaccinated broilers fed the control diet and was 0.59% for the unvaccinated broilers fed the control diet (**Table 4.62**).

Table 4.42. Processing yields from 43 day old broilers fed a control diet or this diet supplemented with yucca products Magni-Phi and Micro-Aid or *Bacillus subtilis*-probiotic product 2 from 0 to 42 days of age[1] (Experiment 6).

Dietary treatments	Live weight (day 42 of age)	Fasted weight (day 43 of age)	Loss of weight
	g	g	%[2]
Control (C)	3106 ± 19[ab]	2930 ± 19	5.66 ± 0.10
C + 0.05% Magni-Phi	3149 ± 22[a]	2968 ± 21	5.76 ± 0.13
C + 0.05% Micro-Aid	3092 ± 21[b]	2914 ± 21	5.75 ± 0.09
C+0.05% *Bacillus subtilis* 2	3099 ± 16[ab]	2923 ± 17	5.67 ± 0.11

[1]The values are means ± SEM, n = 22 replicate pens with 7 birds per pen selected for processing.
[a-b]Values with different superscripts for a given parameter differ, ($P < 0.05$) among treatments.
[2]As a percent of live weight.

Table 4.43. Processing yields from 43 day old broilers fed a control diet or this diet supplemented with yucca products Magni-Phi and Micro-Aid or *Bacillus subtilis*-probiotic product 2 from 0 to 42 days of age[1] (Experiment 6).

Dietary treatments	Hot carcass		Chilled carcass		Frame	
	g	%[2]	g	%[2]	g	%[2]
Control (C)	2191 ± 14	74.78 ± 0.06	2163 ± 12	73.83 ± 0.14	542 ± 4	18.47 ± 0.11[ab]
C + 0.05% Magni-Phi	2223 ± 17	74.87 ± 0.11	2195 ± 15	73.97 ± 0.14	546 ± 5	18.34 ± 0.14[b]
C + 0.05% Micro-Aid	2187 ± 17	75.01 ± 0.12	2159 ± 15	74.08 ± 0.20	543 ± 4	18.65 ± 0.12[a]
C+0.05% *Bacillus subtilis* 2	2189 ± 13	74.81 ± 0.11	2160 ± 12	73.83 ± 0.13	541 ± 3	18.47 ± 0.12[ab]

[1]The values are means ± SEM, n = 22 replicate pens with 7 birds per pen selected for processing, except for the frame values which n equals 19, 20, 20 and 19 for the control, Magni-Phi, Micro-Aid and *Bacillus subtilis* 2 treatments, respectively due to an error in that the breast skin weight was discarded and not included in the frame weight for the birds from initial replicate pens of each treatment.
[a-b]Values with different superscripts for a given parameter differ, ($P < 0.05$) among treatments.
[2]As a percent of live fasted weight.

Table 4.44. Processing yields from 43 day old broilers fed a control diet or this diet supplemented with yucca products Magni-Phi and Micro-Aid or *Bacillus subtilis*-probiotic product 2 0 to 42 days of age[1] (Experiment 6).

Dietary treatments	Pectoralis major		Pectoralis minor		Total white meat[2]		Wings		Leg quarters	
	g	%[3]	g	%[3]	g	%[3]	g	%[3]	g	%[3]
Control (C)	670 ± 5[ab]	22.8 ± 0.1[ab]	132 ± 1[ab]	4.49 ± 0.03	801 ± 6[ab]	27.33 ± 0.1[a]	214 ± 1	7.32 ± 0.04	682 ± 5	23.28 ± 0.07[b]
C + 0.05% Magni-Phi	679 ± 7[a]	22.9 ± 0.2[a]	134 ± 2[a]	4.52 ± 0.04	814 ± 8[a]	27.39 ± 0.2[a]	220 ± 2	7.41 ± 0.04	691 ± 5	23.30 ± 0.09[b]
C + 0.05% Micro-Aid	653 ± 7[b]	22.4 ± 0.1[b]	130 ± 1[b]	4.46 ± 0.04	782 ± 8[b]	26.81 ± 0.2[b]	215 ± 2	7.39 ± 0.04	691 ± 6	23.72 ± 0.10[a]
C+0.05% *Bacillus subtilis* 2	665 ± 5[ab]	22.7 ± 0.1[ab]	133 ± 1[ab]	4.54 ± 0.03	798 ± 6[ab]	27.26 ± 0.1[ab]	215 ± 2	7.37 ± 0.04	684 ± 4	23.37 ± 0.06[b]

[1]The values are means ± SEM, n = 22 replicate pens with 7 birds per pen selected for processing. [a-b]Values with different superscripts for a given parameter differ, ($P < 0.05$) among treatments.
[2]Pectoralis major plus pectoralis minor
[3]As a percent of live fasted weight.

Table 4.45. Body weight, body weight gain and feed efficiency of broilers fed a control diet or this diet supplemented with bacitracin methylene disalicylate (BMD) or prebiotics from 0 to 14 days of age[1] (Experiment 7).

Dietary treatments	Body weight	Body weight gain	Feed to gain	Feed Intake	Mortality
		g/bird			% (#)
Control (C)	477 ± 2^{ab}	434 ± 2^{ab}	1.214 ± 0.004^{b}	526 ± 3	1.42 (5)
C + 0.05% BMD	479 ± 2^{a}	436 ± 2^{a}	1.206 ± 0.004^{b}	525 ± 2	0.28 (1)
C + yeast product	469 ± 2^{b}	426 ± 2^{b}	1.234 ± 0.004^{a}	524 ± 2	0.85 (3)
C + 0.01% cellobiose	480 ± 2^{a}	437 ± 2^{a}	1.213 ± 0.003^{b}	528 ± 2	0.85 (3)
C + 0.025% cellobiose	475 ± 3^{ab}	432 ± 3^{ab}	1.215 ± 0.004^{b}	522 ± 3	0.85 (3)
C + 0.050% cellobiose	476 ± 2^{ab}	434 ± 2^{ab}	1.204 ± 0.003^{b}	521 ± 3	0.57 (2)

[1]The values are means ± SEM, n = 16 replicate pens for the dietary treatments. $^{a-b}$Values with different superscripts for a given parameter differ, ($P < 0.05$).

Table 4.46. Body weight, body weight gain and feed efficiency of broilers fed a control diet or this diet supplemented with bacitracin methylene disalicylate (BMD) or prebiotics from 14 to 21 days of age[1] (Experiment 7).

Dietary treatments	Body weight	Body weight gain	Feed to gain	Feed Intake	Mortality
		g/bird			% (#)
Control (C)	1001 ± 5^{a}	524 ± 3^{a}	1.356 ± 0.006^{ab}	710 ± 4^{a}	0.57 (2)
C + 0.05% BMD	993 ± 3^{a}	514 ± 2^{ab}	1.349 ± 0.005^{b}	694 ± 4^{ab}	0.57 (2)
C + yeast product	971 ± 6^{b}	502 ± 4^{b}	1.375 ± 0.005^{a}	691 ± 5^{b}	0.28 (1)
C + 0.01% cellobiose	992 ± 5^{a}	513 ± 4^{ab}	1.365 ± 0.003^{ab}	700 ± 5^{ab}	0.28 (1)
C + 0.025% cellobiose	983 ± 6^{ab}	508 ± 4^{b}	1.362 ± 0.005^{ab}	692 ± 4^{b}	0.85 (3)
C + 0.050% cellobiose	984 ± 4^{ab}	508 ± 3^{b}	1.364 ± 0.005^{ab}	691 ± 3^{b}	0.00 (0)

[1]The values are means ± SEM, n = 16 replicate pens for the dietary treatments. $^{a-b}$Values with different superscripts for a given parameter differ, ($P < 0.05$).

Table 4.47. Body weight, body weight gain and feed efficiency of broilers fed a control diet or this diet supplemented with bacitracin methylene disalicylate (BMD) or prebiotics from 0 to 21 days of age[1] (Experiment 7).

Dietary treatments	Body weight	Body weight gain	Feed to gain	Feed Intake	Mortality
		g/bird			% (#)
Control (C)	1001 ± 5[a]	959 ± 5[a]	1.291 ± 0.005[b]	1233 ± 7	1.99 (7)
C + 0.05% BMD	993 ± 3[a]	950 ± 3[a]	1.283 ± 0.004[b]	1217 ± 6	0.85 (3)
C + yeast product	971 ± 6[b]	928 ± 6[b]	1.310 ± 0.003[a]	1213 ± 7	1.14 (4)
C + 0.01% cellobiose	992 ± 5[a]	950 ± 5[a]	1.295 ± 0.003[b]	1224 ± 7	1.14 (4)
C + 0.025% cellobiose	983 ± 6[ab]	940 ± 6[ab]	1.294 ± 0.004[b]	1208 ± 7	1.70 (6)
C + 0.050% cellobiose	984 ± 4[ab]	941 ± 4[ab]	1.290 ± 0.003[b]	1212 ± 5	0.57 (2)

[1]The values are means ± SEM, n = 16 replicate pens for the dietary treatments. [a-b]Values with different superscripts for a given parameter differ, ($P < 0.05$).

Table 4.48. Body weight, body weight gain and feed efficiency of broilers fed a control diet or this diet supplemented with bacitracin methylene disalicylate (BMD) or with prebiotics from 14 to 35 days of age[1] (Experiment 7).

Dietary treatments	Body weight	Body weight gain	Feed to gain	Feed Intake	Mortality
		g/bird			% (#)
Control (C)	2471 ± 10	1994 ± 9	1.519 ± 0.005[ab]	3019 ± 16	0.57 (2)
C + 0.05% BMD	2473 ± 11	1995 ± 11	1.511 ± 0.005[b]	2985 ± 15	1.14 (4)
C + yeast product	2433 ± 12	1964 ± 11	1.528 ± 0.005[a]	2972 ± 16	1.42 (5)
C + 0.01% cellobiose	2452 ± 15	1972 ± 13	1.530 ± 0.005[a]	3015 ± 18	0.28 (1)
C + 0.025% cellobiose	2445 ± 12	1970 ± 10	1.519 ± 0.003[ab]	2968 ± 13	1.70 (6)
C + 0.050% cellobiose	2441 ± 11	1966 ± 10	1.534 ± 0.005[a]	2993 ± 16	0.85 (3)

[1]The values are means ± SEM, n = 16 replicate pens for the dietary treatments. [a-b]Values with different superscripts for a given parameter differ, ($P < 0.05$).

Table 4.49. Body weight, body weight gain and feed efficiency of broilers fed a control diet or this diet supplemented with bacitracin methylene disalicylate (BMD) or prebiotics from 21 to 35 days of age[1](Experiment 7).

Dietary treatments	Body weight	Body weight gain	Feed to gain	Feed Intake	Mortality
		g/bird			% (#)
Control (C)	2471 ± 10	1469 ± 8	1.578 ± 0.005[ab]	2312 ± 14	0.00 (0)
C + 0.05% BMD	2473 ± 11	1480 ± 9	1.568 ± 0.006[b]	2303 ± 12	0.57 (2)
C + yeast product	2433 ± 12	1462 ± 8	1.582 ± 0.006[ab]	2298 ± 13	1.14 (4)
C + 0.01% cellobiose	2452 ± 15	1460 ± 11	1.588 ± 0.006[ab]	2316 ± 14	0.00 (0)
C + 0.025% cellobiose	2445 ± 12	1462 ± 8	1.575 ± 0.005[ab]	2294 ± 10	0.85 (3)
C + 0.050% cellobiose	2441 ± 11	1459 ± 9	1.593 ± 0.006[a]	2317 ± 11	0.85 (3)

[1]The values are means ± SEM, n = 16 replicate pens for the dietary treatments. [a-b]Values with different superscripts for a given parameter differ, ($P < 0.05$).

Table 4.50. Body weight, body weight gain and feed efficiency of broilers fed a control diet or this diet supplemented with bacitracin methylene disalicylate (BMD) or prebiotics from 0 to 35 days of age[1] (Experiment 7).

Dietary treatments	Body weight	Body weight gain	Feed to gain	Feed Intake	Mortality
		g/bird			% (#)
Control (C)	2471 ± 10	2428 ± 10	1.464 ± 0.004[ab]	3539 ± 18	1.99 (7)
C + 0.05% BMD	2473 ± 11	2431 ± 11	1.455 ± 0.004[b]	3511 ± 16	1.42 (5)
C + yeast product	2433 ± 12	2390 ± 12	1.475 ± 0.004[a]	3498 ± 18	2.27 (8)
C + 0.01% cellobiose	2452 ± 15	2409 ± 15	1.472 ± 0.004[a]	3526 ± 22	1.14 (4)
C + 0.025% cellobiose	2445 ± 12	2402 ± 12	1.464 ± 0.002[ab]	3470 ± 21	2.56 (9)
C + 0.050% cellobiose	2441 ± 11	2399 ± 11	1.474 ± 0.004[a]	3521 ± 15	1.42 (5)

[1]The values are means ± SEM, n = 16 replicate pens for the dietary treatments. [a-b]Values with different superscripts for a given parameter differ, ($P < 0.05$).

Table 4.51. Body weight, body weight gain and feed efficiency of broilers fed a control diet or this diet supplemented with bacitracin methylene disalicylate (BMD) or prebiotics from 0 to 42 days of age[1] (Experiment 7).

Dietary treatments	Body weight	Body weight gain	Feed to gain	Feed Intake	Mortality
		g/bird			% (#)
Control (C)	3260 ± 19	3218 ± 19	1.556 ± 0.005^{bc}	4947 ± 38	2.84 (10)
C + 0.05% BMD	3256 ± 24	3213 ± 24	1.546 ± 0.006^{c}	4858 ± 52	3.98 (14)
C + yeast product	3219 ± 27	3176 ± 27	1.567 ± 0.006^{ab}	4897 ± 53	4.54 (16)
C + 0.01% cellobiose	3233 ± 32	3190 ± 32	1.569 ± 0.006^{ab}	4949 ± 42	1.42 (5)
C + 0.025% cellobiose	3211 ± 14	3169 ± 14	1.563 ± 0.005^{abc}	4851 ± 33	3.12 (11)
C + 0.050% cellobiose	3213 ± 17	3170 ± 17	1.575 ± 0.004^{a}	4965 ± 24	1.70 (6)

[1]The values are means ± SEM, n = 16 replicate pens for the dietary treatments. $^{a-c}$Values with different superscripts for a given parameter differ, ($P < 0.05$).

Table 4.52. Body weight, body weight gain and feed efficiency of broilers fed a control diet or this diet supplemented with bacitracin methylene disalicylate (BMD) or prebiotics from 14 to 42 days of age[1] (Experiment 7).

Dietary treatments	Body weight	Body weight gain	Feed to gain	Feed Intake	Mortality
		g/bird			% (#)
Control (C)	3260 ± 19	2784 ± 18	1.610 ± 0.005^{bc}	4433 ± 36	1.42 (5)
C + 0.05% BMD	3256 ± 24	2777 ± 24	1.600 ± 0.007^{c}	4354 ± 46	3.69 (13)
C + yeast product	3219 ± 27	2750 ± 26	1.620 ± 0.007^{abc}	4391 ± 49	3.69 (13)
C + 0.01% cellobiose	3233 ± 32	2753 ± 31	1.626 ± 0.008^{ab}	4444 ± 38	0.57 (2)
C + 0.025% cellobiose	3211 ± 14	2737 ± 14	1.619 ± 0.006^{abc}	4376 ± 23	2.27 (8)
C + 0.050% cellobiose	3213 ± 17	2737 ± 16	1.635 ± 0.005^{a}	4450 ± 24	1.14 (4)

[1]The values are means ± SEM, n = 16 replicate pens for the dietary treatments. $^{a-b}$Values with different superscripts for a given parameter differ, ($P < 0.05$).

Table 4.53. Body weight, body weight gain and feed efficiency of broilers fed a control diet or this diet supplemented with bacitracin methylene disalicylate (BMD) or prebiotics from 21 to 42 days of age[1] (Experiment 7).

Dietary treatments	Body weight	Body weight gain	Feed to gain	Feed Intake	Mortality
		g/bird			% (#)
Control (C)	3260 ± 19	2259 ± 17	1.669 ± 0.006[ab]	3730 ± 32	0.85 (3)
C + 0.05% BMD	3256 ± 24	2263 ± 22	1.659 ± 0.009[b]	3672 ± 40	3.12 (11)
C + yeast product	3219 ± 27	2248 ± 24	1.676 ± 0.009[ab]	3706 ± 43	3.41 (12)
C + 0.01% cellobiose	3233 ± 32	2241 ± 28	1.686 ± 0.010[ab]	3744 ± 34	0.57 (2)
C + 0.025% cellobiose	3211 ± 14	2228 ± 14	1.679 ± 0.008[ab]	3690 ± 20	1.99 (7)
C + 0.050% cellobiose	3213 ± 17	2231 ± 16	1.697 ± 0.007[a]	3761 ± 22	1.14 (4)

[1]The values are means ± SEM, n = 16 replicate pens for the dietary treatments. [a-b]Values with different superscripts for a given parameter differ, ($P < 0.05$).

Table 4.54. Body weight, body weight gain and feed efficiency of broilers fed a control diet or this diet supplemented with bacitracin methylene disalicylate (BMD) or prebiotics from 35 to 42 days of age[1] (Experiment 7).

Dietary treatments	Body weight	Body weight gain	Feed to gain	Feed Intake	Mortality
		g/bird			% (#)
Control (C)	3260 ± 19	790 ± 13	1.845 ± 0.017	1442 ± 13	0.85 (3)
C + 0.05% BMD	3256 ± 24	782 ± 15	1.840 ± 0.022	1421 ± 15	2.56 (9)
C + yeast product	3219 ± 27	786 ± 18	1.865 ± 0.026	1432 ± 20	2.27 (8)
C + 0.01% cellobiose	3233 ± 32	782 ± 19	1.879 ± 0.026	1429 ± 21	0.28 (1)
C + 0.025% cellobiose	3211 ± 14	767 ± 10	1.889 ± 0.023	1412 ± 9	0.57 (2)
C + 0.050% cellobiose	3213 ± 17	772 ± 12	1.898 ± 0.019	1449 ± 14	0.28 (1)

[1]The values are means ± SEM, n = 16 replicate pens for the dietary treatments.

Table 4.55. Body weight, body weight gain and feed efficiency of broilers from 0 to 8 days of age fed a diet containing bacitracin methylene disalicylate (BMD) or different levels of a product containing a mix of essential oils and acid[1] (Experiment 8).

Dietary treatments	Body weight	Body weight gain	Feed to gain
		g	
BMD at 0.0055%	198 ± 1	156 ± 1	1.201 ± 0.012
Essential oil/acid product 1 at 0.015%	198 ± 1	156 ± 1	1.188 ± 0.008
Essential oil/acid product 1 at 0.02%	199 ± 1	157 ± 1	1.184 ± 0.007
Essential oil/acid product 1 at 0.03%	198 ± 1	156 ± 2	1.199 ± 0.012

[1]The values are means ± SEM, n = 12 replicate pens.

Table 4.56. Body weight, body weight gain and feed efficiency of broilers from 0 to 15 days of age fed a diet containing bacitracin methylene disalicylate (BMD) or different levels of a product containing a mix of essential oils and acid[1] (Experiment 8).

Dietary treatments	Body weight	Body weight gain	Feed to gain
		g	
BMD at 0.0055%	512 ± 4	470 ± 4	1.224 ± 0.007
Essential oil/acid product 1 at 0.015%	512 ± 3	470 ± 3	1.223 ± 0.006
Essential oil/acid product 1 at 0.02%	511 ± 4	469 ± 4	1.223 ± 0.003
Essential oil/acid product 1 at 0.03%	509 ± 4	467 ± 4	1.225 ± 0.005

[1]The values are means ± SEM, n = 12 replicate pens.

Table 4.57. Intestinal characteristics of 15 day old broilers fed a diet containing bacitracin methylene disalicylate (BMD) or different levels of a product containing a mix of essential oils and acid[1] (Experiment 8).

Dietary treatment	Duodenum			Ileum		
	Villi height	Crypt height	Ratio	Villi height	Crypt height	Ratio
	µm	µm		µm	µm	
BMD at 0.0055%	35.14 ± 1.34[b]	2.52 ± 0.11[b]	14.21 ± 0.90	13.91 ± 0.49	2.26 ± 0.09	6.25 ± 0.30
Essential oil/acid product 1 at 0.03%	39.12 ± 1.31[a]	2.84 ± 0.09[a]	13.96 ± 0.64	13.97 ± 0.41	2.45 ± 0.09	5.79 ± 0.29

[1]Values are means ± SEM, n = 12 replicate birds. For each bird, the villi and crypt height was determined as an average from 6 individual sections with one villi and one crypt measured from each section. [a-b]Values with different superscripts for a given parameter differ, (P<0.05).

Table 4.58. Body weight, body weight gain and feed efficiency of coccidiosis vaccinated or unvaccinated chicks fed a control diet or this diet supplemented with antibiotics or essential oils from 0 to 14 days of age[1] (Experiment 9).

Dietary treatment and vaccine status	Body weight	Body weight gain	Feed to gain	Mortality
		g		% (#)
Control (C), vaccinated	425 ± 4	379 ± 4	1.325 ± 0.004[a]	1.79 (3)
C, unvaccinated	423 ± 4	376 ± 4	1.312 ± 0.006[ab]	0.00 (0)
C + 0.002% Virginiamycin, vaccinated	430 ± 4	381 ± 2	1.330 ± 0.007[a]	0.00 (0)
C + 0.1% essential oil mix 2, vaccinated	429 ± 5	382 ± 5	1.318 ± 0.006[ab]	1.79 (3)
C + 0.64% essential oil mix 3, vaccinated	424 ± 3	378 ± 4	1.319 ± 0.007[ab]	1.79 (3)
C + 0.1% essential oil mix 2 and 0.64% essential oil mix 3, vaccinated	435 ± 3	388 ± 3	1.298 ± 0.006[b]	1.79 (3)

[1]The values are means ± SEM, n = 8 replicate pens. [a-b]Values with different superscripts for a given parameter differ, ($P < 0.05$).

Table 4.59. Body weight, body weight gain and feed efficiency of coccidiosis vaccinated or unvaccinated chicks fed a control diet or this diet supplemented with antibiotics or essential oils from 14 to 28 days of age[1] (Experiment 9).

Dietary treatments	Body weight	Body weight gain	Feed to gain	Mortality
		g		% (#)
Control (C), vaccinated	1534 ± 20	1110 ± 17	1.510 ± 0.008	0.00 (0)
C, unvaccinated	1552 ± 11	1129 ± 10	1.494 ± 0.004	0.00 (0)
C + 0.005% BMD and 0.005% salinomycin, vaccinated	1569 ± 12	1139 ± 10	1.507 ± 0.005	0.59 (1)
C + 0.1% essential oil mix 2, vaccinated	1560 ± 15	1131 ± 12	1.499 ± 0.004	0.59 (1)
C + 0.64% essential oil mix 3, vaccinated	1531 ± 12	1107 ± 10	1.496 ± 0.008	0.59 (1)
C + 0.1% essential oil mix 2 and 0.64% essential oil mix 3, vaccinated	1554 ± 7	1119 ± 8	1.508 ± 0.008	0.00 (0)

[1]The values are means ± SEM, n = 8 replicate pens.

Table 4.60. Body weight, body weight gain and feed efficiency of coccidiosis vaccinated or unvaccinated chicks fed a control diet or this diet supplemented with antibiotics or essential oils from 0 to 28 days of age[1] (Experiment 9).

Dietary treatments	Body weight	Body weight gain	Feed to gain	Mortality
		g		% (#)
Control (C), vaccinated	1534 ± 20	1488 ± 20	1.463 ± 0.007	1.79 (3)
C, unvaccinated	1552 ± 11	1505 ± 11	1.448 ± 0.004	0.00 (0)
C + 0.005% BMD and 0.005% salinomycin, vaccinated	1569 ± 12	1520 ± 10	1.463 ± 0.004	0.59 (1)
C + 0.1% essential oil mix 2, vaccinated	1560 ± 15	1513 ± 15	1.453 ± 0.003	2.38 (4)
C + 0.64% essential oil mix 3, vaccinated	1531 ± 12	1484 ± 12	1.451 ± 0.008	2.38 (4)
C + 0.1% essential oil mix 2 and 0.64% essential oil mix 3, vaccinated	1554 ± 7	1507 ± 7	1.453 ± 0.006	1.79 (3)

[1]The values are means ± SEM, n = 8 replicate pens.

Table 4.61. Body weight, body weight gain and feed efficiency of coccidiosis vaccinated or unvaccinated chicks fed a control diet or this diet supplemented with antibiotics or essential oils from 28 to 42 days of age[1] (Experiment 9).

Dietary treatments	Body weight	Body weight gain	Feed to gain	Mortality
		g		% (#)
Control (C), vaccinated	2938 ± 39	1403 ± 22	1.850 ± 0.016	2.38 (4)
C, unvaccinated	3041 ± 44	1489 ± 37	1.795 ± 0.015	0.59 (1)
C + 0.006% salinomycin, vaccinated	3054 ± 36	1485 ± 34	1.817 ± 0.022	1.19 (2)
C + 0.045% essential oil mix 2, vaccinated	3048 ± 49	1488 ± 37	1.793 ± 0.016	0.00 (0)
C + 0.045% essential oil mix 3, vaccinated	2974 ± 38	1443 ± 29	1.817 ± 0.017	0.00 (0)
C + 0.045% essential oil mix 2 and 0.045% essential oil mix 3, vaccinated	3006 ± 22	1452 ± 17	1.819 ± 0.018	1.79 (3)

[1]The values are means ± SEM, n = 8 replicate pens.

Table 4.62. Body weight, body weight gain and feed efficiency of coccidiosis vaccinated or unvaccinated chicks fed a control diet or this diet supplemented with antibiotics or essential oils from 0 to 42 days of age[1] (Experiment 9).

Dietary treatments	Body weight	Body weight gain	Feed to gain	Mortality
		g		% (#)
Control (C), vaccinated	2938 ± 39	2891 ± 39	1.649 ± 0.009	4.17 (7)
C, unvaccinated	3041 ± 44	2994 ± 44	1.618 ± 0.006	0.59 (1)
C + 0.006% salinomycin, vaccinated	3054 ± 36	3005 ± 35	1.635 ± 0.008	1.79 (3)
C + 0.045% essential oil mix 2, vaccinated	3048 ± 49	3001 ± 49	1.620 ± 0.007	2.38 (4)
C + 0.045% essential oil mix 3, vaccinated	2974 ± 38	2928 ± 38	1.630 ± 0.011	2.38 (4)
C + 0.045% essential oil mix 2 and 0.045% essential oil mix 3, vaccinated	3006 ± 22	2960 ± 22	1.631 ± 0.010	3.57 (6)

[1]The values are means ± SEM, n = 8 replicate pens.

Experiment 10

The GE of the control diet was 66 kcal/kg less than the control diet supplemented with 0.3% Termin-8 (**Table 4.63**), the difference representing the energetic contribution of the Termin-8. The TME_N determined for the Termin-8 containing diet was greater (112 kcal/kg) than for the control diet (**Table 4.63**). However, the TME_N/GE ratios (which would factor in the difference in GE between the two diets) were not different from one another.

The application of Termin-8 to the control diet did not reduce the digestibility of amino acids, except for a slight decrease in that of arginine (**Table 4.64**).

Table 4.63. TME_N of control broiler starter diets[1] (Experiment 10).

Diet	GE	TME_N	TME_N/GE
		(kcal/kg)	
Control	3985	3171 ± 21[a]	0.7958 ± 0.0053
Termin-8	4051	3283 ± 22[b]	0.8104 ± 0.0054

[1]The GE values are an average of 3 determinations on each diet while the TME_N values are means ± SEM, n = 5 replicates each consisting of 4 roosters. Both the GE and TME_N values are on as is basis. The dry matter was 88% for both diets.
[a-b]Values with different superscripts for a given diet differ, $P<0.05$.

Table 4.64. Amino acid digestibility coefficients of a broiler starter diets[1] (Experiment 10).

Amino Acid	Diet		P-value
	Control	Termin-8	
		%	
Alanine	85.75 ± 0.43	86.81 ± 0.38	0.103
Arginine	94.17 ± 0.14[a]	93.39 ± 0.18[b]	0.009
Aspartic acid	89.40 ± 0.27	89.25 ± 0.23	0.683
Cysteine	82.23 ± 0.58	82.28 ± 1.08	0.965
Glutamic acid	92.36 ± 0.14	92.39 ± 0.18	0.885
Glycine	48.53 ± 1.57	55.15 ± 4.05	0.166
Histidine	93.12 ± 0.18	93.40 ± 0.11	0.215
Isoleucine	88.56 ± 0.28	88.77 ± 0.38	0.655
Leucine	90.33 ± 0.23	90.47 ± 0.25	0.687
Lysine	91.89 ± 0.26	91.25 ± 0.23	0.108
Methionine	93.71 ± 0.35	93.71 ± 0.34	0.998
Phenylalanine	90.42 ± 0.14	90.73 ± 0.28	0.350
Proline	87.70 ± 0.24	87.85 ± 0.59	0.819
Serine	88.79 ± 0.29	89.17 ± 0.56	0.562
Threonine	86.94 ± 0.44	87.99 ± 0.47	0.143
Tryptophan	95.33 ± 0.27	95.70 ± 0.06	0.217
Tyrosine	89.94 ± 0.34	89.34 ± 0.61	0.413
Valine	86.14 ± 0.42	86.78 ± 0.46	0.332

[1]The values are means SEM, n = 5 replicates each consisting of 4 roosters.
[a-b]Values with different superscripts for a given amino acid differ, ($P < 0.05$).

CHAPTER 5

DISCUSSION

Consumer thoughts about animal protein are shifting. Globally, there is increasing concern by people about the use of antibiotics in animal agriculture and about the welfare of animals in agricultural production systems. This concern and the emerging willingness of some consumers to pay more for products raised to meet their concerns will continue to change the poultry industry as companies strive to meet customer demands. In this industry evolution, feed additives will have an important role to fill. However, the results from the current research indicate that the poultry industry's propensity to use feed additives based on a prevailing assumption that adding them to poultry diets may help bird growth and production and that adding them to the diet does no harm to the bird even if they do not help the bird, is incorrect. A more holistic approach needs to be pursued by the poultry industry as it moves away from the more singular approach that antibiotic utilization offered. Feed additives represented by aluminosilicates, prebiotics, probiotics and essential oils will have a role in this new future along with bird management enhancements, but that role for feed additives needs to be based on sound science and further research.

Azomite

Azomite is an interesting feed additive as its properties as a clay and a source of rare earth elements offers many potential biological benefits in animal production systems. However, the complexity of this product as far as its many potential biological roles is probably leading to variability in the research results being obtained. In the current research, the addition of Azomite

to broiler diets had some consistent positive results, some inconsistent positive results, or no effect.

In experiment 1, the addition of Azomite to a broiler diet at a rate of 0.125, 0.250, or 0.500% decreased the feed to gain ratio of broilers fed these diets relative to the broilers fed the control diet, and the addition of the lowest dose of Azomite also improved body gain. Similar studies with dietary Azomite in aquatic species have also reported decreased feed conversion rate and increased body weight gain (Musthafa et al., 2015; Tan et al., 2014). Azomite is an aluminosilicate-based product that has a relatively high abundance of trace elements, including rare earth elements. Among these elements, cerium and lanthanum are particularly high. He et al., (2009) conducted studies with rare earth elements in broiler chicks, which resulted in increased body weight gain and performance. In order to find out if the tested rare earth elements changed the biochemical properties of the birds, He et al., (2009) measured aspartate aminotransferase (AST), creatine kinase (CK), glucose, total protein, albumin, globulins, phosphorus, calcium, potassium, and sodium. They did not find any differences in these blood parameters.

In a subsequent battery experiment and in a floor pen experiment, the addition of Azomite to broiler diets did not result in enhanced feed utilization or growth. By nature, floor pen experiments are very different from battery brooder experiments, so looking for reasons for differences in the results is more problematic. In the case of the battery brooder experiments, the growth and feed to gain ratio results are not as different as the overall statistical results suggest. Statistical improvements in body weight, body weight gain, and feed to gain ratio existed in the broilers fed the diet supplemented with 0.0625% relative to the control-fed broilers at the end of

the first week of the experiment. Clear trends for improved body weight gain and improved feed to gain ratio existed for this treatment and for the broilers fed the 0.125% Azomite supplement.

It is also interesting to note that the final average bird weights in the second experiment exceeded those of the first experiment with most of this difference occurring in the last 7-day period of the two experiments. The average bird weight in the control group of the first experiment at 21 days was 970 grams, and it was 1005 grams in the second experiment. The 1005-gram average for the control birds in the second experiment exceeds all of the averages from the first experiment while it remains the lowest body weight group in the second experiment. The better growth in the second study may indicate less stress. If Azomite is modulating immunity/stress conditions, there would be less of an opportunity to appreciate differences between treatments in the absence of stressors.

Although the floor pen study with Azomite did not perform like the battery experiments, some important insight was gained. The broilers fed the 0.125% Azomite supplement to the negative control diet had a decreased coefficient of variation in body weight at 49 days of age relative to the broilers fed the negative control diet. Given that the broilers in this treatment numerically weighed more than the control broilers at the end of the experiment, this narrowing of weight variation is probably due to the smaller birds gaining more weight, rather than growth suppression in the larger chickens. The decreased variability in body weight may have come from a reduction of stress, or some other promotion of health. Regardless of the mechanism of action, decreasing body weight variation in broilers is very important in the highly automated poultry industry.

Additionally, as the broilers fed the negative control diet supplemented with Azomite progressed throughout the experiment, the numerical difference between their performance and

that of the broilers fed the negative control diet increased. This is reflected in the differences when comparing inclusion of Azomite in the negative control diet versus not. The broilers fed the negative control diets already were facing a dietary stress and as the broilers grew, the density of animal mass per square foot increased, competition for food and water increased, and the air quality decreases. If, Azomite is mitigating some of the stress response incurred by the birds, then the numerical performance differences observed in this stressful phase of growth would be logical as would the increase in body weight uniformity.

In experiment 1, plasma Alpha-1-acid glycoprotein was measured and it was down-regulated by the presence of dietary Azomite. In avian species, the gastrointestinal tract is one of, if not *the,* most important immune organs. The intestinal epithelial cells, in addition to other immune cells in the gastrointestinal tract, play a vital role in both innate and adaptive immunity (Niewold et al., 2015). Alpha-1-acid glycoprotein (AGP) is a positive acute phase protein released by cells to recruit inflammatory cells. The decrease in AGP may indicate that the immune system is being modulated by Azomite. Alternatively, Najafi et al., 2016 reported an increase in serum AGP in broiler chicks after feed-restricting them for 30 hours. This finding suggests that this biomarker may be indicative of stress in addition to immunological activity. Although there were no intended stressors in this study, environmental stressors are always present. Dampening the energy-costly immune/stress processes would allow for more host resources to be used for growth and production, similar to the theory of antibiotic growth promotion by Niewold (2007).

Though only one immune system parameter was tested in the course of this experiment, the lower values of this acute phase protein fits well with results reported in aquatic species. Tan et al., (2014) observed a resistance to hypoxic stress in shrimp fed diets with Azomite inclusions (2 g/kg and 4 g/kg). In the same research, they also observed shrimp had increased innate immune

parameters, including superoxide dismutase and lysozyme. Testing other indicators of stress and inflammation in future studies with poultry may help further explain how Azomite is modulating avian physiology.

The consistent calcium and phosphorus digestibility results from experiment 1 and experiment 3 suggest that Azomite increases the absorption of calcium and phosphorus. The increased absorption of calcium and phosphorus observed in the present research raises some interesting questions. The first of these inquiries is how the absorption of these minerals might be increased. Bolukbasi et al., (2016) conducted research in laying hens fed cerium oxide and had similar results. With this specific rare earth element, they suggested that the improvements in mineral absorption may occur due to interactions with the calcium transporter in the intestine. This argument is augmented in the current study because of the apparent metabolizable energy data. The apparent metabolizable energy was not different between groups. A difference in energy utilization would be more likely to occur in tandem with the changes observed in mineral absorption if the mechanism was decreased passage rate or improved intestinal villi morphology. Though these mechanisms cannot be ruled out, they seem less likely. In a study describing the increased caloric efficiency induced by clay products (Quisenberry, 1968), the researcher used higher aluminosilicate inclusions than the current study. The lowest inclusion that Quisenberry used was a 2.5% inclusion, which is fivefold higher than the inclusion that was analyzed for digestibility in the current study. Alternatively, Azomite may be altering the intestinal microflora in such a way as to create the reported mineral absorption rates. Beneficial bacteria are thought to work in a variety of ways including competitive exclusion of pathogenic bacteria, regulation of the immune system, and increasing nutrient availability (Ajuwon, 2016). If Azomite is acting

as a prebiotic to improve the beneficial intestinal microbial population, it could help explain the reported improvements.

In addition to understanding why more of these minerals are being absorbed with the Azomite inclusion, it will be necessary to determine if this increased digestibility translates into meaningful biological improvements for the bird. In the present research, tibia ash weights were not increased in the broilers fed the control and 0.500% Azomite treatments (data not shown), indicating that increased calcium digestibility did not likely increase overall bone density in the Azomite fed birds. However, this was not surprising given that the dietary level of calcium and phosphorus exceeded requirements and would have supported optimum bone formation. Performing a future study with a marginally calcium/phosphorus deficient diet would help detect the potential benefit of Azomite increasing calcium and phosphorus digestibility values.

Despite some obvious differences, aquaculture and poultry production have much in common. Both poultry and fish are efficient meat-producing animals that have similar monogastric physiology and well-defined environmental parameters for optimal growth and development. Research in aquaculture has reported that prawns performed better in earthen ponds that were treated with Azomite prior to filling them with water and stocking the crustaceans (Azomite International, Black Tiger Shrimp). With this, they reported increased phytoplankton and zooplankton populations in the Azomite treated ponds. This implies that the Azomite may be "feeding" the ecosystem, and subsequently, the freshwater prawns. Similarly, in future studies it would be interesting to look at chickens placed on fresh litter that does not have a robust, pre-existing microflora. Having pre-existing microflora in the litter has been reported to aide in chick growth and development, most likely due to the early microbial seeding of the intestine (Maurer et al., 2013). With fresh litter, there could be added intestinal stress due to the

absence of these organisms. Perhaps Azomite could help the microbial populations to establish themselves more quickly and thus decrease the stress in this early, yet critical, phase of growth.

The current research clearly indicates that much further research is needed before the actual utility of adding Azomite as a feed additive in broiler production is proven. However, preliminary results from the current research, and that of others, suggests that Azomite may improve nutrient utilization and act as an immune/stress modulator.

Probiotics

Wang et al., (2016) reported that *B. subtilis* inclusion in broiler rations increased weight gain in the last two weeks of the six-week experiment. The current research did not support this improvement in weight gain in the finisher phase for any of the 3 *B. subtilis* products tested. In fact, none of the 3 products effected body weight or body weight gain in broilers in the current research.

Reis et al., (2017) reported that the *Bacillus subtilis* strain they tested increased economic efficiency. The *Bacillus* in their research increased digestibility, which they attributed, in part, to enzyme secretion. The effect the 3 tested *B. subtilis* probiotics had on the feed to gain ratio in the current research was variable. The lowest dose of *B. subtilis* product 1 improved the feed to gain ratio in broilers during the starter period and then had no effect for the rest of the experiment. *B. subtilis* product 2 had no effect on the feed to gain ratio in broilers at any time point during experiment 6. In contrast, during the starter phase of experiment 5, addition of *B. subtilis* product 2 or 3 to a diet containing wheat and rye resulted in broilers with an increased feed to gain ratio relative to broilers fed an un-supplemented control diet. This increased feed to gain ratio persisted through 0-28 days of age in the broilers fed a diet supplemented with *B. subtilis* product 3. Previous experiments have suggested that *B. subtilis* strains help reduce pathogens and the

consequence of these pathogens in the host (Fritts et al., 2000; Hayashi et al., 2018). The reduced feed efficiency in the starter phase for all 3 *B. subtilis* products may be due to an immune response stimulated by the products. The energy used in this response would reduce the available nutrient pool that could have been utilized for growth.

Unlike experiment 4, no antibiotics or anticoccidial drugs were used in experiments 5 and 6. Despite this alteration, the *B. subtilis* inclusions in the negative control diet did not improve the performance over the 42-day experiment. The necrotic enteritis reported via gross necropsy indicates that there was a challenge present in both of these experiments. Previous research reported that *B. subtilis* may be effective in helping the host to repress infection (Hayashi, et al., 2018). The performance data and the mortality of the current research does not support these findings as the *B. subtilis* treatments did not have reduced incidence of mortality nor necrotic enteritis in experiment 5 and 6.

Some *Bacillus subtilis* strains have been reported to incite the immune system and reduce the presence of pathogens. This species of bacteria has been reported to increase macrophage recruitment and decrease bacteremia (Hayashi et al., 2018). There is a fine line when it comes to inflammatory-modulation. Inducing too little inflammation may not lead to any changes due to the dietary inclusion, while inducing too much inflammation can decrease the growth and feed efficiency (Niewold et al., 2015). Excessive inflammation can cause intestinal epithelial cell disruption and leaky tight junctions in the intestine. This breakdown increases the likelihood of infection, especially for *Clostridium perfringens*, and other pathogenic and opportunistic pathogens, which often present clinical disease in association with *Eimeria* replication. Though this *Bacillus* might otherwise be protective, it may be pushing the host's immune system too

hard. Instead of finding the right balance of immune stimulation, perhaps in the current research it was compromising the intestine and encouraging necrotic enteritis.

In experiment 4, in the starter period *B. licheniformis* product 1 reduced growth and increased feed conversion compared to *B. licheniformis* product 2 and *B. subtilis* product 1. Niewold (2007) theorized that though antibiotics had been studied extensively in their efficacy against microbial control, improvements in performance might be due to modulation of inflammation and muscle catabolism. Some have reported that the *Bacillus* bacteria suppresses stress and inflammation, while others have reported resistance to bacterial insults (Deng et al., 2012; Knap et al., 2010). As Niewold suggested with antibiotics, perhaps the mechanism by which these *Bacillus* products are acting is through regulation of inflammatory processes. Given the reduced growth the *Bacillus licheniformis* product 1 group, it may have induced a strong inflammatory reaction, which caused the broilers to divert nutrients away from growth and development.

In previous studies, probiotics have been implicated in helping poultry cope with experimentally-induced heat stress (Ashraf et al., 2013; Deng et al., 2012). These studies suggested that the probiotics used in their experiments might have been acting to reduce insults from heat stress by improving intestinal morphology, especially at the level of the duodenum and the ileum. The temperature in these studies did not exceed 36°C (96.8°F). In the current research in experiment 4 there was an acute heat stress event when the electricity to the building was lost. The environmental temperature spiked to 99°F, which is above the thresholds of the previous studies. The outage also resulted in a complete loss of ventilation which exacerbated the heat stress. Although over 15% of the birds died from each treatment, the power outage did not elucidate differences between groups in terms of subsequent performance. This suggests that the

probiotic strains utilized in this experiment did not have a profound modulating effect on acute stress susceptibility or the recovery from it. Perhaps mild or moderate chronic stress is needed in order to observe improvements with these bacterial strains.

Recycled litter from several previous flocks was utilized for the 3 experiments involving probiotics. This litter would have likely had diverse microflora. The presence of this microflora may be taking away from some of the efficacy of the *Bacillus* probiotics by acting as a probiotic reservoir for all of the dietary treatments. Experiments documenting broiler performance as a result of this probiotic on fresh shavings should help distinguish efficacy by decreasing the chickens' access to probiotic bacteria in the environment.

Prebiotics

Between experiments 6 and 7 a total of 4 prebiotic products were examined in broilers. Two were yucca-based products (Magni-Phi and Micro-Aid), another was a yeast product, and the final product was a disaccharide (cellobiose). None of the tested products improved broiler performance except for weight gain in the starter period for the broilers fed Magni-Phi. But, even this benefit was associated with a negative as the mortality rate was 5.1% in the Magni-Phi treatment versus 3.5% in the control treatment.

Dietary supplementation with the yeast product and cellobiose had negative effects on broiler performance. The addition of the yeast product to the diet of broilers from 0 to 21 days of age decreased body weight and body weight gain and increased the feed to gain ratio in these broilers relative to the broilers fed the control diet. Feeding the highest does of cellobiose from day 0 to 42 increased the feed to gain ratio of the broilers fed it relative to those fed the un-supplemented control diet. As suggested in previous research, fermented yeast derivatives are hypothesized to prime the immune system (Gao et al., 2008; Roto et al., 2017). The current

research may support this hypothesis, even though no immune parameters were obtained during the course of the experiment. Given that there were no differences in growth and feed conversion ratio between the control diet and the antibiotic (BMD) supplemented diet, there was not a clinical bacterial challenge in the current experiment. Without sufficient challenge, immunological stimulation may have partitioned energy away from the birds that would otherwise have been used for growth and development. This could explain why the yeast product had higher feed to gain values and lower body weight gain in the first three weeks of the experiment. Under more challenging conditions, the product may have been able to have increased performance compared to the control group.

If the prebiotic products help the host cope with protozoan challenges as suggested by Lensing et al., (2012), the use of Coban (monensin) throughout the cellobiose and yeast product experiment may have also put these products at a disadvantage. Monensin has a long history of helping control coccidiosis in the poultry industry by inhibiting potassium transport, increasing sodium ion influx, and inhibiting sporozoite cell invasion (Chapman et al., 2008). This monovalent carboxylic ionophore is produced by a species of *Streptomyces* and also has activity against many bacteria, especially gram-positive bacteria (Butaye, 2003). By adding this coccidiostat, it may have situated the dietary treatments on a more equal playing field and thus resistance to a variety of pathogens would be less appreciated across diets.

The yucca products appeared to be fulfilling different roles in the current research as the broilers fed these 2 products differed consistently from one another throughout the experiment. Broilers fed Magni-Phi had greater body weights and weight gains than those fed Micro-Aid, but broilers fed Micro-Aid had lower mortality and fewer cases of necrotic enteritis than those fed Magni-Phi. Thus, Micro- Aid may be acting as described by Crevens et al., (2015), to help the

host resist necrotic enteritis. With a mild challenge, this stimulation of the immune system might have acted to reduce growth and feed efficiency in this group of broilers. Others have reported that yucca products decrease *Eimeria* oocyst in the stool per gram of feces (Galli et al., 2018). A reduction in the coccidiosis challenge should lead to improved growth and efficiency as well as decreased mortality due to necrotic enteritis and the birds in this experiment were not given a coccidiostat.

As the current research and the cumulative research of others indicates, prebiotic feed additives are not simply replacing antibiotics. Each prebiotic should be assessed individually under a host of different conditions. The current research suggests that none of the tested prebiotics were beneficial and can be detrimental when administered to broilers under well managed and controlled conditions. However, this does not mean that these prebiotics might not be useful in some situations or when incorporated in combination with other feed additives.

Essential oils

Overall, none of the essential oil mixtures utilized inhibited broiler performance. In the first experiment (Experiment 8) that compared a dietary supplementation of BMD to an essential oil mix. In this experiment, the chicks were not significantly stressed. The diet was formulated to meet energetic and amino acid requirements. The diet was corn and soybean-based without inclusion of products such as rye, wheat or barley with higher non-starch polysaccharide content. Meat and bone meal which is reported to have higher bacterial loads (Jones, 2011), was also not included in the diet. Additionally, Coban (monensin), which was included in the basal diet, served to reduce potential stress induced by *Eimeria* species.

In the absence of these challenges, there were no differences in growth and performance of the chicks between the experimental groups. The chicks fed a diet supplemented with the

0.03% essential oil and acid mix 1 had increased villi height measurements and increased crypt depth lengths as compared to the group fed a diet supplemented with BMD. Previous research suggests that does not promote intestinal villi development and may result in decreased villi heights (Jayaraman et al., 2017). This finding is somewhat counterintuitive in that decreased surface area of the intestinal villi might result in an animal with depressed growth and feed utilization. Even so, antibiotics typically promote performance even though the villi are shorter.

In the current research, only the broilers receiving BMD and the highest dietary dose of essential oil and acid mix 1 were sampled for intestinal morphology. Therefore, it cannot be determined whether intestinal development may have improved linearly with the dose of essential oil/acid blend 1 or whether other inclusion rates may have enhanced villi and crypt development even more. Future research is needed to explore consequences of different inclusions.

In the second essential oil experiment, the broilers were challenged with a coccidiosis vaccine. The broiler industry often uses coccidiosis vaccines in order to prepare the intestines of young chickens for future protozoal insults. This priming with live coccidia oocysts helps minimize the effects of this ubiquitous disease. Coccidiosis vaccine was administered via spray application at the hatchery. Inoculation of the parasite took place soon after the application, as the chicks pecked the oocyst-laden liquid off of themselves and one another. The unvaccinated control broilers that were not sprayed would have avoided this early contact with oocysts.

During the starter phase of the experiment the broilers fed the combination of both essential oil mixes had a lower feed to gain ratio than the vaccinated control broilers and the vaccinated broilers supplemented with virginiamycin. After the starter phase this improvement was not seen. This early difference could have been due to morphological changes in the

intestine, as was documented in the first essential oil experiment. Unfortunately, no intestinal samples were obtained in the second experiment, thus these changes cannot be confirmed. In subsequent studies, it would be useful to take intestinal samples to document histological or microbiota changes in the intestine. Future studies should also pair performance data with inflammatory markers to see if these products may be enhancing the immune response, as has been suggested in previous research (Farhadi et al., 2017; Habibi et al., 2015).

The degree that the vaccine ended up stressing the birds in this experiment is difficult to assess. Statistically for the overall experimental period there were no differences in body weight, body weight gain, or feed to gain ratio. However, the unvaccinated controls weighed and gained over 100 grams more than the vaccinated controls and had feed conversion ratio of 1.618 versus 1.649. In addition, the mortality rate was 4.17% versus 0.59% for the vaccinated and unvaccinated controls. Thus, while there was no challenge based on statistics, there might have been one in reality.

In this experiment, straight run chicks were used which reflects industry practice in raising broilers, but introduces experimental variability. In addition, only 8 replicate pens per treatment were utilized in this experiment. Although the essential oil experiments were presented as experiments 8 and 9 in this dissertation, they were actually completed as experiments 1 and 2, respectively, and influenced the rest of the research completed with Azomite, prebiotics and probiotics, in that, all subsequent research utilized only male broilers and had a minimum of 12 replicates per treatment.

Formaldehyde

While the other research dealing with Azomite, probiotics, prebiotics and essential oils focused on optimizing bird health and growth through immune enhancement and intestinal

physiology and microbiota manipulations, the focus of the formaldehyde research was to eliminate pathogens in the diet prior to consumption. Short term conditioning and pelleting of feed have been successful in reducing the levels of *Salmonella* in feed (Jones, 2011). However, complete elimination of *Salmonella* in feed and the emergence of thermotolerant strains of *Salmonella* have necessitated the use of extended heat treatment processes and the concurrent application of chemical preservatives (Boroojeni et al., 2014). Ruano et al., (2001) reported that formaldehyde exposure for greater than 3 hours was effective in eliminating several viral, bacterial, and fungal pathogens even in the presence of organic matter. Formaldehyde is considered an effective antimicrobial feed preservative because it eliminates *Salmonella* in feed at low inclusion rates and prevents recontamination post pelleting (Wales et al. 2010). However, there is concern that the addition of formaldehyde to poultry diets may have a negative impact on protein digestibility (Sica et al., 2016), especially when the treated diets are subjected to the heat of conditioning and pelleting.

The results from the current research indicate that a concern about decreased energy or amino acid availability when formaldehyde is applied to diets using an air atomizing liquid application system is likely unwarranted. The application of Termin-8 to the control diet did not reduce its TME_N or the digestibility of amino acids values, except for a slight decrease in arginine digestibility. The minimal reduction in the digestibility of arginine likely indicates an interaction between arginine and formaldehyde. Arginine and lysine are the 2 amino acids that have amine groups attached to the terminal epsilon carbon and formaldehyde is known to form a more stable bond between these secondary amine groups (Metz et al., 2004). In addition, arginine has a higher cross-linking capability in the presence of formaldehyde than any other amino acid. Arginine's enhanced ability to bind formaldehyde, or link to other molecules in the

presence of formaldehyde, may explain why it was the only amino acid to have a slight decrease in digestibility when Termin-8 was applied to the feed.

While understanding that interactions exist between Termin-8 and arginine may be valuable from an academic standpoint, in a practical feeding situation, the decrease in arginine digestibility would not affect bird performance, especially given that dietary arginine levels are typically well in excess of the requirement.

The findings of this experiment indicate that when formaldehyde is applied using an air atomizing liquid application system, it does not decrease the overall digestible amino acid or metabolizable energy content of the diet. However, further research is necessary to determine if this method of formaldehyde application to poultry diets also does not alter the activity of dietary supplemental enzymes or probiotics, as previous research indicates the direct application of formaldehyde during the mixing of diets inhibits the activity of added dietary phytase and carbohydrase enzymes (Sriperm et al., 2014).

Summary-

In veterinary medicine, canine patients present frequently with gastrointestinal distress. This can be incited by a number of factors including pathogens, stress, or dietary indiscretion. As an initial therapy, veterinarians often prescribe metronidazole and probiotics. Although it seems counterintuitive to give both an antibiotic and a bacterial culture, the current mindset is, "the probiotic might help and it should not hurt."

The poultry industry currently has a similar mindset, in that, they believe many of the additives being used will not hurt. But, what if some of these additives do hurt? In the current research, some of the additives studied caused broilers to have decreased body weight gain and feed efficiency during phases of the production cycle. The conclusion is not that these products

are bad and should not be used. Rather, these products need to be better understood for proper utilization. Although none of the feed additives resulted in larger or more efficient broilers at 42 or 49 days of age in the current research, perhaps they would show better results under different dietary or challenge conditions.

Just as antibiotics are not suited to all conditions, feed additives are not either. Some of these feed additives have been chosen and selected for based on their very specific properties including, antibacterial properties and enzyme excretion. As suggested by the succession of experiments conducted, they are not helpful under all conditions and they can even be harmful in certain phases of development. Thus, instead of maintaining a mindset that these products need to replace antibiotics, it would be best if additives were viewed as intestinal health promoting entities that need to be used temporally and decisively based on their specific characteristics. Some of these products may be better suited during certain phases of the broiler production cycle while others might be better utilized if dosed only at the start of pathogenic insults. These products need to be considered as helpful tools for promoting health as part of the larger one-health mindset.

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