EVALUATION OF A RICE/SOY FERMENTATE ON BROILER PERFORMANCE, LITTER CHARACTERISTICS, AND FECAL ODORANT VOLATILIZATION

A Thesis

by

MALLORI PAIGE WILLIAMS

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2012

Major Subject: Poultry Science

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Approved by:

Chair of Committee,	Jason T. Lee
Committee Members,	Craig Coufal
	Tryon Wickersham
	Tri Duong
Head of Department,	John B. Carey

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ABSTRACT

Evaluation of a Rice/Soy Fermentate on Broiler Performance, Litter Characteristics, and Fecal Odorant Volatilization.

(August 2012)

Mallori Paige Williams, B.S., Texas A&M University

Chair of Advisory Committee: Dr. Jason Lee

The objective of this research was to determine the effect of a rice/soy fermentate when included in broiler diets and spray applied as a litter amendment on broiler performance, litter characterization, and ammonia and odorant volatilization. A series of three experiments were conducted to evaluate the effectiveness of the fermentate to reduce ammonia and odor compound volatilization when spray-applied to on recycled broiler litter. In experiment 1, spray-applying the two fermentate products did not affect ammonia volatilization; however the methodology was verified, as reductions were observed in the positive control. In experiment 2, spray application of the rice/soy fermentate did not have any impact on litter characteristics or average broiler body weight. However spray application of the rice/soy fermentate significantly reduced ($P<0.05$) observed mortality at the conclusion of the experiment. In experiment 3, spray application of the two fermentate products on fresh pine shavings following two activation times did reduce ammonia volatilization; although significant ($p < 0.05$)

differences were observed in carbon and nitrogen content on day 43 and nitrogen content on day 35.

Two experiments were conducted to evaluate the effectiveness of two fermented rice/soy products on volatilization of fecal odor compound volatilization and performance parameters when included in broiler diets. In experiment 1, the addition of fermentate B at 900 g/ton increased ($p < 0.05$) d 21 body weight. The inclusion of both fermentates (A and B) resulted in significant decreases ($p < 0.05$) in multiple volatile organic compounds, strongly associated with odor related to poultry. In experiment 2, the addition of fermentate B at 900 g/ton resulted in a significant increase ($p < 0.05$) in d 14 body weight. Inclusion of both rice/soy fermentates (A and B) significantly increased ($p < 0.05$) carcass weights. Additionally, significant reductions ($p < 0.05$) were observed in day 21 and 42 fecal pH with both fermentates (A and B). Taken in totality, these data demonstrate the ability of a rice/soy fermentate to alter litter nutrient content and intestinal environment resulting in increased nitrogen sequestering, reduced digest pH, reduce odorant volatilization, increased early bird weight, and reduce early mortality.

DEDICATION

I would like to dedicate this to my family. You are the most important thing in my life and always will be. Without the love and support you have given me throughout the years, none of this would have been possible, and I cannot thank you enough for that.

To my parents, you have made me the person I am today, and without your love and support none of this would be possible…

To my sisters, Britni and Halei, you have forever been there to provide not only support, but friendship that only a sister can supply…

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I would also like to thank my family and friends for their unconditional love and support throughout the years. Without you none of this would be possible.

Lastly, I would like to thank God, You are the strength of my heart.

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CHAPTER I

INTRODUCTION

Agricultural producers in the United States are increasingly concerned with odors and gases generated and emitted from their operations. Poultry litter is a valuable fertilizer source that contains high levels of phosphorous (P), potassium (K), nitrogen (N), and trace minerals providing nutrients required for crop growth (Kelleher et al., 2002). However, ammonia and odor volatilization from poultry litter are perceived as indicators of airborne pollutants. Emissions originate from a variety of sources including; poultry production facilities, manure/litter storage units, and land application of manure/litter (Choi et al., 2010). The most common types of nuisance complaints associated with poultry production are land application of manure/litter and surface area of the storage piles. Due to the increasing concerns, sustainable use of poultry litter and manure has made the implementation of improved waste management methodologies a priority (Cook et al., 2011).

Odors are generated by anaerobic decomposition of livestock and poultry waste such as manure. Odor volatilization is attributed to absorption and metabolism of non-absorbable byproducts by microorganisms in the gastrointestinal tract or litter (Chavez et. al., 2004). In anaerobic environments, the decomposition of organic compounds results in odorous volatile compounds that are metabolic intermediates or end products

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This thesis follows the style of Poultry Science.

of microbial processes (Zhu et al., 2000). Between 80 and 200 odorous compounds are produced from the decomposition of manure.

To ensure nutritional requirements are met and maximize growth performance, livestock and poultry are fed diets that often contain a surplus of N. Nitrogen that is not deposited into animal protein is excreted in urine and feces of swine and cattle and in the uric acid by poultry. Ammonia (NH_3) is formed from the microbial breakdown of nitrogenous wastes, such as undigested proteins and excretory uric acid in poultry manure (Atapattu et. al., 2008). The N in uric acid is quickly converted into ammonia by hydrolysis, mineralization, and volatilization (Ritz et at., 2004). Nitrogen concentrations in litter are typically higher in poultry manure than in other animals; therefore, poultry litter has a high potential for ammonia volatilization (Kithome et al., 1999).

Concerns regarding ammonia concentrations within poultry housing, emissions from poultry operations, and potential environmental effects from emissions have emphasized the need to identify strategies for reduced ammonia (NH_3) volatilization from poultry operations (Coufal et. al., 2006). Ammonia emissions from broiler litter not only causes environmental problems, but are also detrimental to the health, welfare, and performance of poultry (Atapattu et. al., 2008). Many studies have demonstrated that high levels of NH_3 on farms reduces feed efficiency (Charles and Payne, 1966; Caveny et al., 1981), growth rate (Reece et al., 1979, 1980, 1981; Moore et al., 1999) and egg production (Deaton et al., 1984), damages the respiratory tract (Nagaraja et al., 1983), and impairs immune responses (Nagaraja et al., 1984). Recently, interest has increased in non-chemical litter amendments such as biological treatments. Biological treatments include a variety of enzymes, enzyme inhibitors, nutrients and/or microorganisms. Addition of biological amendments to water, feed or litter enhances or alters the native microbial population with the goal of increasing NH_3 degradation rates, preventing the formation of toxic compounds, improving feed efficiency and/or competitively excluding undesirable microorganisms (Cole et al., 2006; Patterson and Burkholder, 2003; Shah et al., 2007). Ammonia volatilization from poultry manure is increased by microbial activity (Kim and Patterson, 2003). BiOWiSH™ is a microbial biocatalyst produced through a rice/soy fermentation process that contains a consortium of naturally occurring yeast and bacteria. This fermentate is currently used internationally to reduce ammonia volatilization, odorants, and improve growth performance. The objective of this research was to determine the effect of a rice/soy fermentate when included in broiler diets or spray applied as a litter amendment on broiler performance, litter characterization, and ammonia and odorant volatilization.

CHAPTER II

LITERATURE REVIEW

Ammonia and Odor Production in Agriculture

Livestock provides significant benefits to today's economy; however, odor and ammonia emissions from livestock facilities can detrimentally effect bird performance, impact producer welfare, and negatively impact the environment. Animal operations are a source of airborne pollutants including gases, odor, dust, and microorganisms. Sources of these contaminants from production sites include buildings, feed mills, manure storage, land application of litter (manure), mortality compost structures, and a variety of other smaller emission sources. The most common source of ammonia and odor emissions from poultry production is the land application of manure and the surface area of the manure storage. Poultry manure and litter have been recognized as a source of odor and nuisance; however, poultry litter is a valuable source of nutrients, fertilizer, required for crop growth. Sustainable use of poultry litter and manure has made the implementation of improved animal manure and litter management methodologies a priority (Cook et al., 2011).

Understanding Odor and Ammonia Emissions

Livestock and poultry odors are generated by anaerobic decomposition of livestock waste such as manure. Odor volatilization is attributed to absorption and metabolism by microorganisms of non-absorbable byproducts in the gastrointestinal tract or litter (Chavez et. al., 2004). In anaerobic environments, the decomposition of organic compounds results in production of odorous volatile compounds that are metabolic intermediates or end products of microbial processes (Zhu, 2000). As manure decomposes, as many as 80 to 200 odorous compounds can be produced. Some of the principle classes of odorous compounds include amines, sulfides, volatile fatty acids, indoles, skatoles, mercaptans, alcohols, and carbonyls (Choi et al., 2011). All of these groups can result from the partial decomposition of manure. Composition of broiler excreta is related to the composition of the diet (Sutton et al., 2002), thus dietary formulation influences odor volatilization. Breakdown of protein proceeds to proteoses, peptones, peptides, amino acids and finally, to ammonia and volatile organic acids such as formic, acetic, propionic, and butyric acids. Carbohydrates in animal waste include sugars, starch, and cellulose. Starch and cellulose are broken into glucose units as the first step of decomposition. Under anaerobic conditions, these glucose units are broken into alcohols, aldehydes, ketones, and organic acids. Lipids are esters of the tri-hydroxy alcohol called glycerol. Bacteria use fats as a source of energy, hydrolyzing them first to long-chain fatty acids and alcohols. These acids undergo further breakdown in which acetic acid is cleaved from the original acid. Kreis (1978) identified 17 different volatile compounds associated with odor from poultry waste (table 2-1).

Table 2-1. Volatile organic compounds with descriptors and odor thresholds.

Volatile Organic Compound	Description	Odor Threshold
		ppm
Ammonia	Pungent; Irritating	1.5 ŧ
Carbon Dioxide	Odorless	74,000 ¥
		ppb
Propionic Acid	Body Odor; Vomitus	5.7 ŧ
Butyric Acid	Body Odor; Vomitus	0.19 ŧ
Isobutyric Acid	Rancid; Butter; Cheese	1.5 ŧ
Valeric Acid	Foul	0.037 ŧ
Isovaleric Acid	Fatty Acid; Sweat; Buttery	0.078 ŧ
Indole	Piggy; Mothball; Burnt; Musty	0.3 ŧ
Skatole	Outhouse; Fecal	0.0056 ŧ
Dimethyl Sulfide	Decayed Cabbage	3 ŧ
Dimethyl Disulfide	Repulsive	2.2 ŧ
Hydrogen Sulfide	Rotten Egg	0.41 ŧ
Methanethiol	Rotten Cabbage	0.07 ŧ
Ethanethiol	Garlic Odour	0.0087 ŧ
Propanethiol	--	--
Methane	--	--

ŧ – Nagata, Y. Measurement of Odor Threshold by Triangle Odor Bag Method. In: *Odor Measurement Review*. Office of Odor, Noise and Vibration. Environmental Management Bureau. Ministry of the Environment, Government of Japan.

¥ - Occupational Health and Environmental Safety, 2010. *2010 Respirator Selection Guide*. Retrieved from website: http://multimedia.3m.com.

Ammonia (NH_3) is formed from the breakdown of nitrogenous wastes, such as undigested proteins and excretory uric acid, in poultry manure by microorganisms (Atapattu et. al., 2008). To ensure nutritional requirements are met, livestock and poultry are fed a high protein diets that many times contains a surplus of N. Nitrogen not deposited in the animal is excreted in the urine and feces of swine and cattle and in uric acid by poultry. Poultry litter has a high potential for ammonia volatilization because the N concentrations in litter are higher than in the manure of other animals

(Kithome et al., 1999). The N in uric acid can be converted to ammonia by hydrolysis, mineralization, and volatilization (Ritz et at., 2004). Microbial degradation of uric acid in the litter is the primary source of NH_3 formation and *Bacillus pasteurii* is one of the primary uricolytic bacteria that facilitate NH_3 formation (Ritz et al., 2004). For optimum growth, these bacteria require a pH around 8.5. Decomposition requires uric acid, water, and oxygen to react giving off ammonia and carbon dioxide. Decomposing uric acid into ammonia involves several enzymes, including uricase and urease. Uricase converts uric acid into allantoin, which is then converted into glyoxylate and urea. Addition of moisture, urease hydrolyzes urea into ammonia and carbon dioxide. Formation of ammonia continues with the microbial breakdown of manure under both aerobic and anaerobic conditions (Ritz et al., 2004).

Factors directly affecting degradation of N into ammonia are pH, temperature, and moisture level of the litter (Elliot and Collins, 1982; Carr et. al., 1990). These factors have a direct effect on the living environment of the microorganisms that facilitate the conversion of uric acid to ammonia. Ammonia production is negligible when litter pH is less than 7.0, increasing continuously as the pH approaches 7.0, and is high when pH is 8.0 or greater (Ritz et al., 2004). High temperatures in poultry rearing facilities increase bacterial activity significantly increasing ammonia production (Ritz et al., 2004). Litter moisture is directly affected by relative humidity. Previous research has found that as the relative humidity increased from 45 to 75%, ammonia levels became more variable and generally increased (Weaver and Meijerhof, 1991). While each of these factors can impact ammonia production, the two most important variables affecting ammonia volatilization in commercial facilities are temperature and moisture (Coufal et al., 2006).

Regulatory and Environmental Concerns

Poultry litter is a valuable fertilizer source that provides nutrients necessary for crop growth. However, a problem currently facing the poultry industry is managing high levels of ammonia (NH_3) in poultry houses. It has been reported that over 50% of poultry manure N might be volatilized in the form of NH_3 (Sims and Wolf, 1994). Battye et al. (1994) reported that approximately 80% of NH_3 in the United States comes from agricultural production.

Ammonia volatilization from poultry litter can lead to several environmental problems, including direct toxic effects on vegetation, atmospheric N deposition, leading to eutrophication and acidification of sensitive ecosystems, and to the formation of secondary particulate matter in the atmosphere effecting human health, atmospheric visibility and global radiative balance (Sutton et at., 2009). Fertilization of vegetation by ammonia occurs the same way as applying fertilizer to the soil. Atmospheric ammonia deposits on the leaf or soil surface at the base of the plant and is absorbed by the plant. This can impact changes in plant growth and could potentially have a toxic effect on vegetation. Soil acidification occurs when ammonia reaches the soil surface and reacts with water in the soil which is converted into ammonium (NH_4^+) and absorbs to soil. Ammonium eventually disassociates into nitrite (NO_2^-) or nitrate (NO_3^-) by nitrifying bacteria, releasing hydrogen ions into the soil. Surplus of hydrogen ions eventually leads to the formation of an acidic soil environment. Surface waters may be affected by acidification and eutrophication. Eutrophication is a result of nutrient pollution from deposition into natural waters (Dillon and Molot, 1989). This usually promotes excessive plant growth and decay and is likely to cause severe reductions in water quality. Changes in the ecosystems due to ammonia deposition occur through toxic effects on vegetation with ammonia uptake, soil acidification, and eutrophication (Sutton et al., 2009). When these changes occur, the natural balance of the ecosystem is disrupted.

The Clean Air Act establishes a threshold emission limit of 100 tons/yr of any air pollutant. Ammonia is regulated in the Clean Air Act as a precursor to particulate formation (Miles et al., 2006). The comprehensive Environmental Response, Compensation and Liability Act and the Emergency Planning and Community Right-to-Know Act have reporting requirements of 100 lb of NH_3/d or 18.3 tons/yr. This is a level that could potentially affect large animal production facilities.

Ammonia emissions from agricultural operations can be detrimental to the health of producers. Even at low levels, exposure to ammonia can irritate the respiratory tract and eye. As a result of these problems, the Federal Occupational Safety and Health Administration (OSHA) permissible worker exposure limit for ammonia is 50 parts per million (ppm) over an 8-hour period and the American Conference of Governmental Industrial Hygienists (ACGIH) has established a short-term (15-min) exposure limit of 35 ppm.

Bird Performance, Health and Welfare

Ammonia (NH_3) emissions from poultry litter are detrimental to the health, welfare, and performance of birds. Many studies have shown that high NH_3 levels in broiler houses potentially reduces feed efficiency, growth rate, egg production, damages the respiratory tract, and impairs immune responses. An experiment was conducted by Miles et al. (2004), to determine the effects of atmospheric ammonia on the performance of modern broilers. Broilers were exposed to ammonia levels of 0, 25, 50, and 75 ppm of aerial ammonia from 0 to 4 weeks of age. Final body weight was depressed by 6 and 9% for the 50 and 75 ppm concentrations of ammonia when compared with 0 ppm. Mortality was also significantly greater at the 75 ppm ammonia concentration increasing to 13.9% compared with 5.8% for 0 ppm. These data suggest that broilers exposed to concentrations greater than 25 ppm of atmospheric ammonia experienced a reduction in growth performance parameters and experienced increased levels of mortality.

A similar study was conducted by Wang et al. (2010) evaluated ammonia concentrations of 0, 13, 26, and 52 ppm. Body weights and feed efficiency were significantly depressed when broilers were exposed to ammonia levels at 52 ppm compared to 0 ppm. Host immune repose to ammonia levels was also evaluated and identified reduced Newcastle Disease Virus (NDV) hemagglutination inhibition antibody titers when exposed to the 26 and 52 ppm ammonia as compared to 0 ppm. The results of this experiment suggest that high atmospheric ammonia levels reduced growth performance and immunological response of broiler chickens.

An experiment conducted by Beker et al. (2004) evaluated the effects of ammonia concentration on broiler performance, tracheal lesions, conjuctival lesion, ascites incidence, hematocrit (HCT), blood uric acid (BUR), and blood urea nitrogen (BUN). Final body weight, feed consumption, and body weight gain were not significantly affected as ammonia concentration increased from 0 to 60 ppm. This experiment indicates a higher tolerance to ammonia by broilers prior to body weight decreases. In contrast, gain to feed ratio was significantly depressed at 60 ppm ammonia. Right ventricular weight, HCT, tracheal lesions, and pulmonary lesions increased with age to 21 d but were not affected by atmospheric ammonia. These data vary from other similar experiments; however, broiler performance related to feed to gain ration was lowered when exposed to high levels of atmospheric ammonia.

Effects of atmospheric ammonia at 0, 25, and 50 ppm in relation to time on broiler cockerels were studied by Caveny et al. (1981). Average body weights, feed efficiencies, air sac scores, and paired-lung and bursa of Fabricius weights were determined at 28, 40, and 49 days of age. Feed efficiency at 49 days of age was significantly affected by ammonia levels as broilers exposed to 50 ppm ammonia through 49 d were least efficient. Broiler subjected to 50 and 25 ppm ammonia from 1 to 28 days and adjusted to 25 ppm ammonia from 29 to 49 days and broilers exposed to 50 and 25 ppm ammonia groups from 1 to 28 days and reduced to 0 ppm ammonia from 29 to 49 days exhibited increased feed to gain ratios compared to control broilers. Average body weights, air sac scores, lung weights, and bursa of Fabricius weights as a percentage of live body weight, carcass grades, and condemnations were not significantly affected by treatment. The results of this experiment indicated that ammonia levels of 25 and 50 ppm from day of age can adversely affect feed efficiency in market broilers.

Two trials were conducted by Miles et al. (2005) to determine ocular responses to ammonia in broiler chickens. Sixty male commercial broilers were placed in each of eight environmentally controlled chambers receiving 0, 25, 50, or 75 ppm aerial ammonia from 1 to 28 days. Birds exposed to 25 ppm ammonia gas developed ocular abnormalities but at a slower rate when compared with birds exposed to 50 and 75 ppm. Birds exposed to higher concentrations (50 and 75 ppm) also developed more severe lesions. Lymphocytes and heterophils were seen in the iris at 49 days in ammonia-exposed birds even when ammonia exposure terminated at 28 days. The lower ammonia concentrations resulted in abnormalities that were slight when compared with those seen at the higher ammonia concentrations. Results of these experiments indicate that ocular responses in broiler chickens are affected by atmospheric ammonia.

Detrimental effects of ammonia in poultry production have been evident for years. Numerous field studies have shown how ammonia levels as low as 25 ppm affect bird health and performance. Ammonia levels above 25 ppm in the poultry house can damage the bird's respiratory system and allow infectious agents to become established, leading to declining flock health and performance. Additionally, body weight, feed-efficiency, and condemnation rate may be higher in birds exposed to high ammonia concentrations.

Broilers do not perform to their genetic potential in a poor environment. The quality of the in house environment is directly related to litter quality. The litter environment is ideal for bacterial proliferation and ammonia production (Ritz et al., 2004). Wet litter is the primary cause of ammonia emissions which is one of the most serious performance and environmental factors affecting broiler production as evidenced by decreased performance and bird health (Miles et al., 2005; Caveny et at., 1981; Beker et al., 2004).

Mitigation Strategies to Reduce Gaseous Emissions from Poultry Operations

Concerns regarding gaseous emissions within poultry housing, from poultry operations, and the potential environmental effects emphasize the need for research to mitigate ammonia (NH_3) and odor volatilization from major poultry operations (Coufal et. al., 2006). Studies have shown that various litter amendments reduce ammonia levels in the poultry house and improve bird performance and health (Shah et al., 2007). Use of improved feed management practices, selective feed ingredient use, precision diet formulation, and dietary electrolyte balance reduce nutrient excretion and subsequent odor and gas emissions from livestock manure (Sutton et al., 2002).

Dietary

Composition of freshly excreted manure is primarily influenced by diet composition (Sutton et al., 2002). In addition, feed management practices can influence the efficiency of nutrient utilization in poultry and livestock operations. Since the broiler is the initial source of nutrient excretion, diet manipulation is a feasible solution to control excess nutrient excretion and odor emission to minimize environmental impact (Sutton et al., 2002).

Broiler diets are rich in dietary crude protein (CP), and N not digested is deposited onto litter as waste (Gates, 2000). Dietary CP is approximately 6.25% nitrogen (N), and undigested CP in the diet is broken down by microorganisms into ammonium (NH_4) and ammonia (Gates, 2000). Several investigators have examined the effects of diet manipulation on litter equilibrium ammonia gas concentration in broiler facilities (Ferguson et al., 1998). Reducing the CP has shown to reduce NH_3 gas concentration by about 30% (Ferguson et al., 1998). Gates (2000) also reported on the effects reduced CP has on volatilization of ammonia from broiler litter.

Broilers are fed rations that contain about 17 to 23% CP throughout the growout period. An efficient way to reduce the N excretion in broilers is to reduce the CP and add supplemental synthetic amino acids in the diet (Chavez et al., 2004a). Addition of synthetic amino acids allows producers to meet amino acid requirements with a lower CP diet. A series of investigations by Chavez et al. (2004a, 2004b, and 2004c) indicate that supplemental methionine sources significantly influenced odor production, odor volatile concentrations, and may result in the production of different odor-related compounds in broiler excreta.

Adjusting dietary composition has shown to decrease the amount of ammonia that is lost from laying-hen facilities. Inclusion of feed ingredients with high concentrations of fiber in laying hen diets lowered ammonia emission (Roberts et al., 2007). Dietary inclusion of 10% corn dried distillers grains plus solubles (DDGS), 7.3%

wheat middlings, or 4.8% soybean hulls lowered ammonia emission from laying hen manure (Roberts et al., 2007). Manipulation of livestock diets to alter excretion composition, and thus the odor of excretions has shown to be an effective methodology in poultry operations.

Chemical

When broilers are raised on litter, amendments can be used to reduce the ammonia concentrations in the houses and improve productivity. Typically, poultry litter consists of wood shavings, rice hulls, or peanut hulls. Organic nitrogen (N) and uric acid in the excreta and spilled feed are converted to ammonia and ammonium by the microbes in the litter (Shah et al., 2007). Ammonium can bind to the litter and also dissolve in water. A portion of the ammonium will be converted to ammonia, depending on litter characteristics including moisture, temperature, and pH of the litter. Typically, untreated poultry litter has a pH of 8 and at this pH a large percentage of the inorganic N in litter is in the ammonia form. This form can become gas and volatilize from the litter. Ammonia production is most abundant during high temperatures and very alkaline litter conditions (Shah et al., 2007).

Acidifiers are used to create acidic litter conditions with a pH less than 7, resulting in more of the ammoniacal-N being retained as ammonium rather than volatilized as ammonia. Acidity creates unfavorable conditions for bacteria and enzymes that contribute to ammonia formation from the degradation of nitrogenous waste. Chemical amendments including aluminum, calcium, and iron can greatly reduce ammonia volatilization from poultry litter (Moore and Miller, 1994: Moore et al., 1995, 2000; Shreve et al., 1995, 1996; Burgess et al., 1998).

A study conducted by Moore et al. (2000), compared ammonia levels in broiler houses treated with Al+Clear ($Al_2(SO_4)_3$;Alum) to control houses. Ammonia concentrations during the first three weeks were 6-20 ppm in the treated houses, compared to the control houses with ammonia concentrations at 28-43 ppm. Performance parameters including body weight and feed conversion were improved in the alum-treated houses compared to the control houses due to lower ammonia levels during the early stages of growth. Similar results were observed with regards to reducing ammonia concentrations in broiler houses treated with alum (Moore et al., 1995; Do et al., 2005). Alum was also effective in lowering litter pH by adding a source of acidity or hydrogen ions, which react with ammonia to form ammonium (NH_4^+) (Burgess et al., 1998; Moore et al., 2003).

McWard and Taylor (2000) conducted a broiler growout trial to evaluate the impact of Poultry Guard on ammonia levels and broiler performance. Poultry Guard-treated pens exhibited ammonia levels between 12 and 20 ppm compared with 60 to 85 ppm in the control pens during the first 28 days. For the remainder of the study, treated pens lowered ammonia concentrations approximately 20 ppm compared to the untreated pens. Improved growth performance, increased carcass quality, reduced breast blisters, foot-pad dermatitis, and air-sac lesions were observed in the birds reared in pens treated with Poultry Guard as compared to untreated controls.

Pope and Cherry (2000) evaluated the impact of Poultry Litter Treatment (PLT;$NaHSO_4$) on ammonia levels and bacterial loads in broiler houses. During the first three weeks of growout, PLT-treated houses were observed to have reduced ammonia concentrations of 6, 18, and 11ppm, as compared to untreated control with ammonia concentrations of 62, 28, and 20 ppm respectively. A similar study was conducted by Terzich et al. (1998) to evaluate the effect of PLT on ammonia levels, body weight, respiratory-tract lesions, and death due to ascites in broilers reared on used litter. PLT treated litter resulted in ammonia levels ranging from 5 to 22 ppm, compared with the untreated controls ranging between 53 and 115 ppm throughout the duration of the trial. Treatment of litter with PLT resulted in increased body weights, fewer respiratory lesions, and a lower rate of death due to ascites.

Acidifiers effectively reduce ammonia levels in poultry houses improving in-house air quality. Applications have been shown to be effective in suppressing ammonia levels below 25 ppm and may last from 3 to 4 weeks after application, however, others have shown that ammonia suppression may last as long as up to 7 weeks. This mechanism works by reducing the pH of the litter to suppress ammonia volatilization. As long as the pH of the litter stays relatively low (less than 7), excessive ammonia emissions can be prevented. Reducing ammonia levels in poultry houses not only improves bird performance and health but may also prove to be beneficial to worker health. The poultry industry has effectively used acidifiers as a management strategy to limit the production of ammonia in poultry facilities.

Biological

Ammonia is produced in poultry facilities when uric acid present in the urine and organic nitrogen are converted to ammonium by the microbes in the litter and feces. Supplementing water, feed or litter with biologicals enhances or alters microbial populations with the goal of increasing NH_3 degradation rates, preventing the formation of toxic compounds, improving feed efficiency, and competitively excluding undesirable microorganisms (Cole et al., 2006; Patterson and Burkholder, 2003; Shah et al., 2007; Awad et al., 2006). Biological treatments include blends of enzymes, enzyme inhibitors, nutrients, and/or microorganisms. These treatments allow microbes to work in suboptimal conditions in the litter or improve the conditions of the litter to enhance performance of the microbes and/or the enzymes.

Inhibiting the enzyme activity (urease) in the feces or litter is a potential strategy to reduce ammonia production. Ammonia is the by-product of a 5-step enzymatic degradation of uric acid (Singh et al., 2009). Urease inhibitors have been used as a soil additive to reduce urease activity when urea-based fertilizers are applied. The mechanism of these inhibitors is to inhibit urease activity which converts urea to ammonia, thus decreasing the loss of N as ammonia. There are limited studies on the effectiveness of a urease inhibitor when used in poultry facilities; however, an experiment done by Singh et al. (2009) indicated that a commercially available urease inhibitor resulted in a significant reduction in ammonia concentration from layer feces that was allowed to accumulate on a layer of broiler litter over time. However, inhibitors at this time may be too costly and too easily broken down to be practical or economical to growers (McCrory and Hobbs, 2001).

Beneficial microbes, enzymes, and metabolic stimulants have also been reported as being effective in reducing ammonia levels in poultry houses (Shah et al., 2007). A study compared ammonia concentrations from litter treated with a metabolic stimulant vs. un-treated litter (Shah et al., 2007). Average ammonia concentrations from chambers containing the metabolic stimulant were reduced by 61% compared to the control. A similar study was conducted by DeLaune et al. (2004) using a microbial mixture to determine the effects on ammonia volatilization from poultry litter. In this experiment, the highest ammonia emission rates were reported from the microbial treatments. Researchers that have evaluated biologicals for reduction of ammonia volatilization from poultry litter have had variable success (DeLaune et al., 2004; Shah et al., 2007). Therefore, a better understanding for the use of biological amendments as an alternative strategy to reduce ammonia volatilization from poultry litter has become a priority.

BiOWiSH is a microbial biocatalyst produced through a proprietary rice/soy fermentation process that contains a consortium of naturally occurring yeast and bacteria. This product is currently used internationally as a strategy to reduce ammonia and odorant volatilization from poultry facilities and improve growth performance in poultry. The objective of this research program was to determine the effect of this rice/soy fermentate when included in broiler diets and spray applied as a litter amendment on broiler performance, litter characterization, and ammonia and odorant volatilization.

CHAPTER III

THE EFFECT OF THE SPRAY APPLICATION OF A RICE/SOY FERMENTATE ON THE AMMONIA VOLATILIZATION POTENTIAL OF POULTRY LITTER

Introduction

Poultry litter is a valuable fertilizer source containing high levels of phosphorous (P), potassium (K), nitrogen (N), and trace minerals necessary for crop growth (Kelleher et al., 2002). However, ammonia and odor emissions from poultry litter are perceived as indicators of airborne pollutants. Sources of these emissions originate from poultry production facilities, manure/litter storage units, and the land application of the manure/litter (Choi et al., 2010). The most common types of nuisance complaints associated with poultry production are the land application of manure/litter and the surface area of storage piles. Sustainable use of poultry litter and manure has made the implementation of improved animal manure and litter management methodologies a priority (Cook et al., 2011).

Concerns regarding ammonia concentrations within poultry housing, emissions from poultry operations, and the potential environmental effects from these emissions have emphasized the need for research to identify strategies to reduce ammonia (NH_3) volatilization from major poultry operations (Coufal et. al., 2006). Poultry litter has a high potential for ammonia volatilization because the nitrogen (N) concentrations in litter are typically higher than in the manure of other animals (Kithome et al., 1999).

Sims and Wolf (1994) reported that over 50% of poultry manure N might be volatilized in the form of NH_3. Large quantities of ammonia are released in the environment annually, and animal feeding operations account for 50 to 70% of the total release in the United States (NRC, 2003; Miles et al., 2011). Ammonia volatilization from poultry litter can lead to several environmental problems, including direct toxic effects on vegetation, atmospheric nitrogen deposition, leading to eutrophication and acidification of sensitive ecosystem, and to the formation of secondary particulate matter in the atmosphere effecting human health, atmospheric visibility and global radiative balance (Sutton et at., 2009). Ammonia emissions from broiler litter not only cause environmental concerns, but are also detrimental to the health, welfare, and performance of birds (Atapattu et. al., 2008). Many studies have demonstrated that high levels of NH_3 on farms could reduce feed efficiency, growth rate, and egg production (Charles and Payne, 1966; Reece et al., 1979, 1980, 1981; Caveny et al., 1981; Deaton et al., 1984; Moore et al., 1999;), damage the respiratory tract (Nagaraja et al., 1983), and impair immune responses (Nagaraja et al., 1984). Therefore, reduction in ammonia volatilization from poultry litter is important to maintain human and animal health, as well as reduce potential negative environment impacts.

Recently, interest has increased in non-chemical litter amendments such as biological treatments, including enzymes, enzyme inhibitors, nutrients and/or microorganisms. These treatments allow microbes to work in suboptimal conditions in the litter or improve the conditions of the litter to enhance performance of the microbes and/or the enzymes. The addition of biological amendments to water, feed or litter enhances or alters the native microbial population with the goal of increasing NH_3 degradation rates, preventing the formation of toxic compounds, improving feed efficiency and/or competitively excluding undesirable microorganisms (Cole et al., 2006; Patterson and Burkholder, 2003; Shah et al., 2007). BiOWiSH is a microbial biocatalyst produced through a rice/soy fermentation process that contains a consortium of naturally occurring yeast and bacteria. A series of three experiments were conducted to evaluate the effectiveness of two rice/soy fermentates to reduce ammonia and odor volatiles when spray-applied to broiler litter.

Materials and Methods

For experiment 2 and experiment 3, broiler chicks were provided age appropriate supplemental heat and given access to feed and water *ad libitum*. All animal care procedures were conducted in accordance with an Animal Use Protocol approved by the Texas A&M University laboratory animal care committee. Prior to chick placement, grow out facilities were thoroughly cleaned and disinfected. Used litter was used for bedding material for experiment 2 and fresh pine shavings were used for experiment 3. Pens were equipped with one 30 lb tube feeder and nipple drinkers.

Experiment 1

This experiment was conducted to evaluate the effect of two rice/soy fermentate products (fermentate A and fermentate B) on ammonia volatilization from broiler litter under simulated housing conditions. Used litter was obtained from two commercial broiler operations and transported to the TAMU Poultry Research Center. The litter from the two facilities differed according to age. One operation had reared 1 broiler flock (new) prior to use in the trial while the other operation had reared 5 flocks (old) prior to use. Each litter source was homogenized and allocated into 14 plastic containers with an area of 0.28 sq. m and approximately 15.24 cm deep. Litter was added to a depth of 10.16 cm. Prior to treatment, 140 ml of distilled water was spray applied to each container to increase the moisture content of the litter to replicate similar moisture levels observed in poultry rearing facilities. Moisture was added on a daily basis to the litter to simulate fecal moisture deposition (Table 3-1) and the litter in each container was raked daily to simulate broiler movement. The experiment consisted of seven total litter treatments including a negative control without any litter amendment applied, a positive control which had Poultry Litter Treatment (PLT) ($NaHSO_4$) applied, and two fermentate products at different application rates (Table 3-2). The fermentate was applied to the litter per manufacturer's recommendations which included an activation period of one hour in water prior to spray application. Fermentate B was spray applied 72 hr prior to bird placement and fermentate A and PLT was applied 24 hr prior to bird placement. The combination treatment included equal concentrations of fermentate A and B. Each treatment was replicated in four identical containers.

Table 3-1. Moisture applied to litter to simulate fecal moisture deposition.

Age (days)	Daily Average[1] Feed Intake (g/bird)	Daily Fecal[2] Output (g/bird)	Daily Moisture[3] Content of Fecal Output (g/bird)	Daily Moisture[4] Applied to Litter (g/3.5 birds)
1	13	14.95	11.96	83.72*
2	15	17.25	13.8	96.6*
3	18	20.7	16.56	115.92*
4	21	24.15	19.32	135.24*
5	23.5	27.03	21.62	151.34*
6	25	28.75	23	161*
7	26.5	30.48	24.28	169.96*
8	32	36.8	29.44	103.04
9	37	42.55	34.04	119.14
10	41.5	47.73	38.18	133.63
11	46.5	53.48	42.78	149.73
12	52.5	60.38	48.3	169.05
13	58.5	67.28	53.82	188.37
14	65.5	75.33	60.26	210.91
15	72	82.8	66.24	231.84
16	78	89.7	71.76	251.16
17	83	95.45	76.36	267.26
18	88	101.2	80.96	283.36
19	93	106.95	85.56	299.46
20	98	112.7	90.16	315.56
21	103	118.45	94.76	331.66
22	108	124.2	99.36	347.76
23	113	129.95	103.96	363.86
24	117	134.55	107.64	376.74
25	121	139.15	111.32	389.62
26	124.5	143.18	114.54	400.89
27	128	147.2	117.76	412.16
28	131.5	151.23	120.98	423.43
29	135.5	155.83	124.66	436.31
30	138.5	159.28	127.42	445.97
31	141	162.15	129.72	454.02
32	144.5	166.18	132.94	465.29
33	147.5	169.63	135.7	474.95
34	151.5	174.23	139.38	487.83
35	155	178.25	142.6	499.1

[1]Daily average feed intake was determined from *Commercial Poultry Nutrition, 3rd Edition* (Leeson and Summers).
[2] The weight of fresh manure output is 115 percent of the total dry feed intake. Daily fecal output was calculated by taking daily feed intake, and multipling it by 1.15 to get daily fecal output.
[3]Fresh manure is approximately 80 percent water. Daily moisture content was determined by multiplying daily fecal output by 0.8 to get an estimate of daily moisture content of fecal output.
[4]Plastic containers with an area of 0.28 sq. m, would house 3.5 birds to get industry densities of 0.07 sq. m./bird. Therefore daily moisture content was multiplied by 3.5 to determine moisture content per 3.5 birds.
*Moisture content was doubled the first 7 days to mimic half house brooding, which is a common practice used in the industry.

A SAM IV MAX ammonia gas monitor (OI Analytical, College Station, TX) was used to determine ammonia volatilization from the litter using a static chamber method after being placed on the litter for a 24 minute period. A SAM IV MAX is a gas monitor that obtains multiple readings every six minutes. This monitor is highly accurate which decreases the variability of ammonia concentration. A period of 24 minutes was used because a preliminary experiment indicated that saturation in the static chamber occurred. Ammonia measurements were taken prior to litter treatment to establish baseline measurements and again 1 day prior to mimicked bird placement and on day 0, 3, 7, 14, 21, 28, and 35d post bird placement. The litter containers were stored uncovered in a poultry rearing facility and environmental temperatures were maintained at levels necessary for chick rearing dependent on bird age.

Table 3-2. Treatment descriptions of the spray application of one of two fermentates (A[1] and B[2]) on new and used litter in plastic litter boxes. Fermentate B was spray applied 72 hr prior to bird placement and fermentate A and PLT was applied 24 hr prior to bird placement.

Litter Treatments		
Trt	Treatment Description	Fermentate 2 kg/946 L /929 sq m
1	Negative Control (NC)[4]	--
2	Positive Control (PC)[5]	
3	Fermentate A	5
4	Fermentate B	5
5	Fermentate A	25
6	Fermentate B	25
7	Fermentate A + B[6]	10

[1] BiOWiSH Odor – BiOWiSH Technologies, Naperville, IL, 60563
[2] BiOWiSH Aqua – BiOWiSH Technologies, Naperville, IL, 60563
[4] Negative Control
[5] Positive Control, Poultry Litter Treatment (PLT) ($NaHSO_4$), Jones-Hamilton Co., Walbridge, Ohio
[6] 1:1 ratio of fermentate A and B

Experiment 2

This experiment was designed to evaluate the effect of a rice/soy fermentate when spray applied on recycled litter from 6 previous flocks on broiler performance, litter characterization, and ammonia and odorant volatilization during a 46 d trial. The experiment consisted of 12 total pens (2.74 x 3.66 m) with 6 untreated pens serving as control pens and 6 pens treated with the fermentate, 48 hr prior to chick placement. Each pen was spray-applied according to the manufacturer's specifications of 2 kg of product diluted in 227 L of water for a 12 hours activation period to be for application on 929 sq. m. Pens were set-up to replicate commercial broiler production conditions in a cross-ventilated house. Used litter was acquired from a local integrator and placed in all pens at a depth of 12.7 cm at the initiation of the study.

Day-old broiler chicks were placed at a density of 0.07 sq. m./bird and reared for 46 d (1,620 birds total). A standard industry type feeding program was utilized consisting of a 4 phase dietary program. All animal care procedures were conducted in accordance with an Animal Use Protocol approved by the Institutional Animal Care and Use Committee at Texas A&M University. All chicks on day 1, 14, and 46 were weighed to determine average body weights. Feed consumption throughout the experiment was recorded for each pen for each dietary phase, and daily mortality was recorded. Average feed consumption, feed conversion, body weight and mortality were analyzed.

Ammonia measurements were taken 2 days prior to chick placement, day 0, 3, 7, 14, 21, 28, 35, and 45. A SAM IV MAX gas monitor (OI Analytical, College Station, TX) was used to evaluate ammonia volatilization from the litter using a static chamber method after a 24 minute period. Odorant volatilization from litter was assessed via a lateral flow wind tunnel method. The wind tunnel had a sampling port for the collection of air samples. The top of the lateral flow wind tunnel is a 0.635 cm thick plexiglass with four 0.953 cm holes for air outlet where samples were collected. The flush gas inlet is a 5.08 x 5.08 cm steel tube with ten 0.318 cm holes spaced 2.54 cm apart. Compressed breathing air was used as the flush gas at a flow rate of 8 L/min. Air samples were collected on d 14 and 28 in sorbent tubes and transported to West Texas A&M University Olfactometry Laboratory for analysis via gas chromatography/mass spectrometry (GC/MS). Thermal desorption tube samples were analyzed using a PAL® autosampler and an Agilent® 6890 GC/MS. The GC was equipped with forte BP5 5% Phenyl / 95% dimethyl polysiloxane 0.53mm ID x 30m and a forte BP20 (polar) Polyethylene Glycol 0.53 mm ID x 25m. Samples were automatically desorbed at 280°C and injected into the GC/MS. The column oven was ramped from 40°C to 240°C at 8°C per min for a total run time of 38 minutes. On d 46, cake litter was removed from the pens and weighed to determine caked litter weight.

Litter samples were collected from each pen prior to litter treatment, chick placement, and on a weekly basis during the 46-day grow-out. From the litter samples collected, moisture percentage and pH measurements were determined. Moisture was determined with triplicate 10 gram samples of litter dried at 105 ºC for 24 hrs. Duplicate 12 gram samples of litter were diluted in 60 mLs of water for pH determination per pen.

Experiment 3

This experiment was designed to evaluate the effectiveness of two rice/soy fermentate to prevent ammonia volatilization during a 7 week growout when applied to fresh pine shavings following two activation times. The trial consisted five total treatments with seven replicates per treatment for a total of 35 replicate pens. Forty-one broilers were housed in each replicate pen at a stocking density of 0.079 sq. m./bird. Broilers were fed an industry dietary program consisting of a starter, grower, finisher, and withdrawal diet. Body weight gain and feed conversion were determined at the completion of the 43 day trial. The application rate of each product was 1 kg diluted in 208 L of water and applied to 929 sq. m. Two activation times (30 minutes and 18 hrs) were investigated with each fermentate product (A and B). Prior to spray application of each treatment, samples of each solution as well as the water source were plated for microbial recovery including TCA (Total Plate Count Agar), MRS, and PDA (Potato dextrose agar). TCA is a microbiological growth medium commonly used to assess or to monitor "total" or viable bacterial growth of a sample. MRS was designed to favor the luxuriant growth of *Lactobacilli.* PDA is the most widely used medium for growing fungi and bacteria which attack living plants or decay dead plant matter. Application of the products to the litter took place 24 hr prior to chick placement.

Ammonia concentration was determined on day 14, 21, 35, and 43 post chick placement. A SAM IV Max gas monitor was used to determine ammonia volatilization of the litter using a static chamber method after a 24 minute period. On day 43, all caked litter was removed from the pens and weighed. Litter moisture and pH were determined on days corresponding with ammonia volatilization measurements throughout the experiment. For moisture determination, triplicate 10 gram samples of litter were weighed into aluminum pans and dried at 105 ºC for 24 hr while pH was determined by mixing a 12 gram sample of litter in 60 mL of water and determined with a calibrated pH meter. At the completion of the first flock, caked litter was removed and weighed the day after flock removal on day 43. To simulate the placement of a second flock, the house sat idle for a 10 day period following cake removal. Ten days post-live haul, ammonia volatilization was determined and a second application of the rice/soy fermentate took place matching the initial treatment of flock 1. Three days post application (mimic of chick placement), ammonia volatilization was determined.

Statistical Analysis

All data were subjected to a one-way Analysis of Variance (ANOVA) using the General Linear Model (SPSS, V 18.0). Means were deemed significantly different at $P \leq 0.05$ and separated using Duncan's Multiple Range Test when appropriate.

Results

Experiment 1

The spray-application of the two fermentate products did not result in any observed reductions in ammonia volatilization compared to the negative control throughout the duration of the trial (Table 3-3 and Table 3-4). The application of PLT (positive control) reduced measured ammonia concentrations on day 0, 3, and 7.

Following day 7, differences were not observed between any of the treatment groups. The effectiveness of the positive control treatment in ammonia reduction had a similar time line regardless of litter age. The results of this experiment confirm the ability of the static chamber method used in this study to identify treatments effective in reducing ammonia; however, the bucket method simulated broiler rearing may not be the most appropriate way to evaluate a microbial litter amendment targeted at ammonia reduction.

Table 3-3. Ammonia concentration (ppm) from litter (new) following 1 broiler flock spray-applied with one of two fermentates (A[1] and B[2]) in a static flux chamber after a 24 minute period of time. Fermentate B was spray applied 72 hr prior to bird placement and fermentate A and PLT was applied 24 hr prior to bird placement.

Ammonia Concentration (ppm)										
Trt	Conc[3]	d -3	d -1	d 0	d 3	d 7	d 14	d 21	d 28	d 35
NC[4]	--	76.25	41.81	42.88[a]	60.00[a]	39.19[a]	52.00	35.68	52.87	22.06
PC[5]			41.63	8.13[b]	14.38[b]	8.31[b]	37.38	44.06	44.81	30.94
A	5		40.50	57.81[a]	60.75[a]	35.75[a]	49.07	37.13	45.00	19.81
B	5		47.56	52.50[a]	68.31[a]	39.88[a]	51.19	40.63	38.40	17.75
A	25		40.50	52.56[a]	63.00[a]	39.94[a]	54.63	40.63	46.94	15.44
B	25		42.19	56.00[a]	58.50[a]	44.31[a]	57.13	39.56	51.38	18.79
A + B[6]	10	91.47	63.19	56.63[a]	63.75[a]	40.67[a]	58.56	37.88	49.63	19.13
SEM		2.40	2.52	6.91	7.91	5.94	6.48	4.29	4.61	4.35

[a,b] Means in columns with different superscripts differ significantly at $p < 0.05$.
[1] BiOWiSH Odor – BiOWiSH Technologies, Naperville, IL, 60563
[2] BiOWiSH Aqua – BiOWiSH Technologies, Naperville, IL, 60563
[3] Concentration of fermentate - kg/946 L. /929 sq m
[4] Negative Control
[5] Positive Control, Poultry Litter Treatment (PLT) ($NaHSO_4$), Jones-Hamilton Co., Walbridge, Ohio
[6] 1:1 ratio of fermentate A and B

Table 3-4. Ammonia concentration (ppm) from litter (old) following 6 broiler flocks spray-applied with one of two fermentates (A[1] and B[2]) in a static flux chamber after a 24 minute period of time. Fermentate B was spray applied 72 hr prior to bird placement and fermentate A and PLT was applied 24 hr prior to bird placement.

Ammonia Concentration (ppm)										
Trt	Conc[3]	Initial	d -1	d 0	d 3	d 7	d 14	d 21	d 28	d 35
NC[4]	--	66.80	39.44	38.27[a]	42.19[a]	36.56[a]	50.75	33.06	39.63	62.53
PC[5]			44.44	9.75[b]	13.80[b]	11.25[b]	29.38	43.25	51.07	63.19
A	5		46.81	57.54[a]	55.80[a]	42.25[a]	49.75	36.81	40.00	76.31
B	5		37.50	58.63[a]	59.20[a]	43.81[a]	54.87	38.88	37.40	92.87
A	5		41.56	53.73[a]	53.81[a]	40.88[a]	55.25	33.75	35.38	91.50
B	5		41.88	51.73[a]	50.79[a]	41.88[a]	54.50	30.75	37.44	75.63
A + B[6]	10	74.13	52.06	51.94[a]	49.13[a]	39.50[a]	58.53	32.44	38.00	87.38
SEM		6.03	3.36	7.89	10.78	7.17	3.96	3.75	3.62	2.82

[a,b] Means in columns with different superscripts differ significantly at $p < 0.05$.
[1] BiOWiSH Odor – BiOWiSH Technologies, Naperville, IL, 60563
[2] BiOWiSH Aqua – BiOWiSH Technologies, Naperville, IL, 60563
[3] Concentration of fermentate - kg/946 L. /929 sq m
[4] Negative Control
[5] Positive Control, Poultry Litter Treatment (PLT) ($NaHSO_4$), Jones-Hamilton Co., Walbridge, Ohio
[6] 1:1 ratio of fermentate A and B

Experiment 2

The goal of this experiment was to manipulate the native microbial populations in the litter with the addition of a rice/soy fermentate to decrease NH_3 degradation rate and competitively exclude undesired microorganisms. The addition of the microbial biocatalyst fermentate did not impact the ammonia concentration potential of the litter throughout the entire trial. Additionally, no effects of fermentate application were observed on odorant emission, litter pH, or litter moisture content (Table 3-5). No differences were observed on d 14 or d 46 performance measurements including average broiler body weight and cumulative feed conversion ratio; however the spray application of the rice/soy fermentate prior to chick placement resulted in a significant reduction ($p<0.05$) in cumulative mortality (Table 3-6). The majority of the observed reduction in mortality took place during the first 7 d of grow out. This suggest that the microbial biocatalyst fermentate may have had a possible probiotic type effect on the birds, however, further research is necessary to identify the mechanism of action and determine the type of conditions necessary to observe this desired effect. Although the concentrations of the odorants were not impacted with the addition of the fermentate, the odor profile of the litter were similar with regard to concentrations between 14 (Table 3-7) and 28 (Table 3-8) days of age with acetic acid, butyric acid, isobutyric acid, and hexanoic acid being present in high concentrations while 4-ethyl-phenol, 2-aminoacetophenome, indole, and skatole being present in low concentrations.

Table 3-5. Litter characteristics including, ammonia concentration (ppm), pH, and moisture of litter following spray application of a rice/soy fermentate[1]. Two kg of product diluted in 227 L of water for a 12 hours activation period to be for application on 929 sq. m.

Litter Characteristics									
Treatment	Day -2	Day 0	Day 3	Day 7	Day 14	Day 21	Day 28	Day 35	Day 45
	Ammonia Concentration (ppm)								
Control	53.89	53.50	22.56	12.88	15.33	59.56	81.94	108.17	243.61
Fermentate	48.11	60.00	24.72	14.42	16.78	62.50	79.28	119.33	246.11
SEM	1.93	5.27	2.53	0.95	0.96	4.00	5.69	9.74	7.68
	pH								
Control	8.12	7.90	8.48	8.46	8.36	8.49	8.67	8.56	8.53
Fermentate	8.00	8.25	8.39	8.56	8.38	8.45	8.71	8.62	8.51
SEM	0.07	0.10	0.05	0.02	0.02	0.03	0.03	0.04	0.04
	Moisture (%)								
Control	22.78	15.69	13.13	13.95	17.84	25.65	27.70	28.34	24.41
Fermentate	22.08	17.16	14.52	14.33	18.72	26.81	26.00	27.93	24.97
SEM	0.74	0.56	0.60	0.36	0.53	0.66	0.62	0.73	1.00

[1] BiOWiSH Aqua – BiOWiSH Technologies, Naperville, IL, 60563

Table 3-6. Average body weight (BW), feed conversion ratio (FCR), mortality of broilers and caked litter weight following litter spray application of a rice/soy fermentate[1] prior to bird placement. Two kg of product diluted in 227 L of water for a 12 hours activation period to be for application on 929 sq. m.

	BW (kg)		FCR (feed:gain)		Mortality (%)	Cake Weight (kg/pen)
Treatment	d 14	d 46	d 1-14	d 1-46	d 1-46	d 46
Control	0.396	2.77	1.26	1.80	7.6[a]	62.04
Fermentate	0.396	2.76	1.27	1.80	2.5[b]	60.49
SEM	0.002	0.03	0.01	0.02	1.0	3.82

[a,b] Means in columns with different superscripts differ significantly at $p < 0.05$.

[1] BiOWiSH Aqua – BiOWiSH Technologies, Naperville, IL, 60563

Table 3-7. Profile of odorant compounds collected from a lateral flow wind tunnel covering litter material spray applied with a rice/soy fermentate[1] 14 days following chick placement after a 20 minute collection period ± SE. Two kg of product diluted in 227 L of water for a 12 hours activation period to be for application on 929 sq. m.

Volatile Organic Compounds (µg/m³)		
	Control	Fermentate
Acetic Acid	245.5 ± 59.3	258.9 ± 27.8
Propionic Acid	12.24 ± 2.26	15.46 ± 3.02
Isobutyric Acid	338.5 ± 178.5	239.7 ± 50.0
Butyric Acid	218.1 ± 19.1	175.2 ± 13.8
Isovaleric Acid	11.01 ± 0.584	14.07 ± 1.30
Valeric Acid	73.87 ± 4.44	70.56 ± 8.03
Hexanoic Acid	257.6 ± 83.4	267.4 ± 67.9
Phenol	16.62 ± 4.18	19.78 ± 5.75
Para-cresol	3.45 ± 1.39	1.70 ± 0.478
4-ethyl-phenol	1.00 ± 0.404	0.436 ± 0.138
2-aminoacetophenome	0.570 ± 0.232	0.342 ± 0.146
Indole	0.121 ± 0.046	0.053 ± 0.018
Skatole	0.012 ± 0.001	0.017 ± 0.004

[1] BiOWiSH Aqua – BiOWiSH Technologies, Naperville, IL, 60563

Table 3-8. Profile of odorant compounds collected from a lateral flow wind tunnel covering litter material spray applied with a rice/soy fermentate[1] 28 days following chick placement after a 20 minute collection period ± SE. Two kg of product diluted in 227 L of water for a 12 hours activation period to be for application on 929 sq. m.

Volatile Organic Compounds (µg/m³)		
	Control	Fermentate
Acetic Acid	289.2 ± 48.8	207.9 ± 35.9
Propionic Acid	15.92 ± 6.50	30.54 ± 7.11
Isobutyric Acid	312.2 ± 41.05	179.0 ± 68.24
Butyric Acid	119.7 ± 15.5	107.6 ± 11.5
Isovaleric Acid	20.45 ± 1.30	22.85 ± 1.86
Valeric Acid	69.28 ± 10.7	72.93 ± 26.8
Hexanoic Acid	151.7 ± 42.5	214.0 ± 55.9
Phenol	17.91 ± 4.50	17.85 ± 0.94
Para-cresol	3.42 ± 0.434	4.78 ± 1.32
4-ethyl-phenol	0.627 ± 0.147	1.65 ± 0.544
2-aminoacetophenome	0.861 ± 0.339	1.61 ± 0.466
Indole	0.074 ± 0.012	0.203 ± 0.134
Skatole	0.068 ± 0.021	0.039 ± 0.015

[1] BiOWiSH Aqua – BiOWiSH Technologies, Naperville, IL, 60563

Experiment 3

Increasing the activation time of the product from 30 minutes to 18 hours increased the recovery of the microrganisms between 1 and 2 log values indicating that an increased activation time may result in increased and effectiveness of the product (Table 3-12). However, spray application of either rice/soy fermentate product at either activation time did not result in a reduction ($p > 0.05$) in ammonia concentration potential of the litter as measured via a static chamber method at any time throughout the trial (Table 3-10). Additionally, no differences were observed with regard to litter pH, litter moisture, or broiler performance characteristics (Table 3-9 and Table 3-10). However, significant differences were observed in nutrient content of the litter on days 35 and 43 with the application of the fermentate to the litter (Table 3-11). Application of fermentate B activated for 18 hr increased ($p<0.05$) nitrogen content in the litter on day 35 when compared to the control while increases ($p<0.05$) in nitrogen content of the litter were observed on day 43 with the application of both fermentate products at both activation times compared to the control. Carbon content was reduced in ($p<0.05$) with the application of fermentate B on day 43. These alteration in nitrogen and carbon concentrations associated with fermentate application also resulted in a decreased ($p<0.05$) in the carbon:nitrogen ratio.

Table 3-9. Average body weight (BW), feed conversion ratio (FCR), mortality and caked litter weight (CW) of 43 day old broilers reared on litter spray applied with one of two rice/soy fermentates (A[1] and B[2]) at two activation times (30 min and 18 hr) prior to chick placement. One kg of product diluted in 208 L of water and applied to 929 sq. m.

Trt	Activation Time	BW (kg)	FCR	Mortality (%)	CW (kg)
Control	--	2.37	1.76	7.6	1.66
A	18 h	2.35	1.76	9.4	2.44
A	30 m	2.36	1.75	7.3	2.03
B	18 h	2.29	1.79	9.8	2.47
B	30 m	2.32	1.77	10.5	1.90
SEM		0.02	0.01	0.8	1.10

[1] BiOWiSH Odor – BiOWiSH Technologies, Naperville, IL, 60563
[2] BiOWiSH Aqua – BiOWiSH Technologies, Naperville, IL, 60563

Table 3-10. Litter characteristics including ammonia concentration (ppm), pH, and moisture of litter spray applied with one of two rice/soy fermentates (A[1] and B[2]) at two activation times (30 min and 18 hr) prior to chick placement. One kg of product diluted in 208 L of water and applied to 929 sq. m. Following removal of the flock, the fermentate was reapplied (re-app) to mimic consecutive flock placement.

Litter Characteristics						
Trt	Activation Time	d 14	d 21	d 35	d 43	Re-app
	Ammonia Concentration (ppm)					
Control	--	15.62	71.1	252.9	390.3	105.58
A	18 h	17.93	94.5	282.4	335.4	127.29
A	30 m	17.50	77.0	277.1	417.4	132.75
B	18 h	15.71	66.0	253.8	429.9	132.00
B	30 m	17.54	73.2	252.7	397.4	137.21
SEM		0.7	2.6	8.1	13.1	5.1
	pH					
Control	--	7.02	7.07	8.48	8.61	
A	18 h	7.32	6.79	8.32	8.51	
A	30 m	7.12	6.46	8.31	8.56	
B	18 h	7.11	6.53	8.35	8.68	
B	30 m	6.97	6.96	8.42	8.48	
SEM		0.16	0.17	0.04	0.03	
	Moisture (%)					
Control	--	29.01	37.44	36.20	33.28	
A	18 h	34.64	41.43	35.791	31.99	
A	30 m	31.09	43.09	35.23	31.30	
B	18 h	29.04	42.04	34.00	29.78	
B	30 m	30.76	38.01	34.29	31.82	
SEM		1.09	1.05	0.72	0.93	

[1] BiOWiSH Odor – BiOWiSH Technologies, Naperville, IL, 60563
[2] BiOWiSH Aqua – BiOWiSH Technologies, Naperville, IL, 60563

Table 3-11.Carbon percentage, nitrogen percentage, and C:N ratio of litter spray applied with one of two rice/soy fermentates (A[1] and B[2]) at two activation times. One kg of product diluted in 208 L of water and applied to 929 sq. m.

	Litter Characteristics		
TRT	Activation Time	d 35	d 43
	Carbon (%)		
Control	--	42.03	41.39[a]
A	18 h	41.88	41.25[ab]
A	30 m	42.30	40.81[ab]
B	18 h	42.53	40.64[b]
B	30 m	42.08	41.21[ab]
SEM		0.09	0.09
	Nitrogen (%)		
Control	--	2.57[b]	2.58[c]
A	18 h	2.58[ab]	2.73[b]
A	30 m	2.54[b]	2.74[b]
B	18 h	2.69[a]	2.79[b]
B	30 m	2.52[b]	2.95[a]
SEM		0.02	0.03
	C:N		
Control	--	16.35[ab]	16.12[a]
A	18 h	16.29[ab]	15.19[b]
A	30 m	16.74[a]	14.97[bc]
B	18 h	15.85[b]	14.64[bc]
B	30 m	16.88[a]	14.17[c]
SEM		0.12	0.13

[a-c] Means in columns with different superscripts differ significantly at $p < 0.05$.

[1] BiOWiSH Odor – BiOWiSH Technologies, Naperville, IL, 60563

[2] BiOWiSH Aqua – BiOWiSH Technologies,Naperville, IL, 60563

Table 3-12. Microbial recovery including, TCPA (Total Plate Count Agar), MRS, and aPDA (Potato dextrose agar) ± SE.

Trt	Activation Time	TCPA	MRS	aPDA
Water	--	--	--	--
A	18 h	$2.90x10^5 \pm 6.88x10^4$	$1.08x10^5 \pm 2.81x10^4$	$3.28x10^4 \pm 3.50x10^3$
A	30 m	$1.45x10^3 \pm 2.16x10^2$	$4.06x10^4 \pm 1.61x10^4$	$2.90x10^2 \pm 1.53x10^1$
B	18 h	$5.53x10^4 \pm 6.50x10^3$	$1.43x10^5 \pm 5.30x10^4$	$7.13x10^3 \pm 5.25x10^3$
B	30 m	$1.44x10^4 \pm 8.27x10^3$	$6.44x10^4 \pm 3.60x10^4$	$2.93x10^2 \pm 4.98x10^1$

Discussion

Ammonia (NH_3) emissions from poultry litter are detrimental to the health, welfare, and performance of birds. Many studies have shown that high NH_3 levels in broiler houses could potentially reduce feed efficiency, growth rate, egg production, damage respiratory tract, and impair immune responses (Miles et al., 2004, 2005; Wang et al., 2010; beker et al., 2004; Caveny et al., 1981). Studies have shown that various litter amendments reduce ammonia levels in the poultry house and improve bird performance and health (Shah et al., 2006). In experiment 2, the addition of the rice/soy fermentate as a litter amendment did not improve body weight gain or feed conversion ratio of the birds, however a significant reduction in mortality was observed in the birds reared in pens spray-applied with the rice/soy fermentate.

Ammonia is formed from the breakdown of nitrogenous wastes, such as undigested proteins and excretory uric acid, in poultry manure by microorganisms. Factors that control the formation of ammonia are pH, temperature, and moisture contents of the litter (Elliot and Collins, 1982; Carr et al., 1990). Reece et al. (1979) demonstrated that pH of broiler litter influences ammonia levels in poultry rearing facilities, such as, as the pH of litter decreases the amount of ammonia loss decreases. Aluminum sulfate [Al2(SO4)3] and numerous other acidic compounds are effective in lowering litter pH and reducing ammonia concentration in commercial broiler houses (Moore et al., 1995). Chemical litter amendments such as Alum, Poultry Litter Treatment (PLT), and Poultry Guard have been found to reduce ammonia emission and increase the performance of poultry (McWard and Taylor, 2000). Similarly, in experiment 1 a significant reduction in ammonia concentration was observed with the addition of PLT confirming the ability of the static chamber methodology to effectively measure differences in ammonia concentration. Although chemical amendments have been shown effective in reducing ammonia concentration, the interest in the use of microbial based litter amendments is increasing.

Supplementation of litter with biologicals enhances microbial population with the goal of increasing NH_3 degradation rates, preventing the formation of toxic compounds and competitively excluding undesirable microorganisms (Cole et al., 2006; Patterson and Burkholder, 2003; Shah et al., 2007). Researchers that have evaluated biologicals for reduction of ammonia volatilization from poultry litter have had variable success (DeLaune et al., 2004; Shah et al., 2007). Similarly, the addition of the rice/soy fermentate in reducing ammonia concentration was not effective although differences were observed in litter nutrient content at the conclusion of the trial in Experiment 3. Therefore, a better understanding for the use of microbial litter amendments as an alternative strategy to reduce ammonia volatilization is a priority to determine the effectiveness.

Anaerobic decomposition of animal wastes is the primary cause of odors (Kreis, 1978). As manure decomposes it produces between 80 and 200 odorous compounds including ammonia (NH_3), volatile organic compounds (VOCs), and hydrogen sulfide (H_2S). Some of the principle classes of these odorous compounds include amines, sulfides, volatile fatty acids, indoles, skatoles, phenols, mercaptans, alcohols, and carbonyls (Powers, 2003). Kreis (1978) identified 17 different volatile compounds associated with odor from poultry waste. The current study evaluated 13 different volatile compounds associated with odor from poultry waste. Although the addition of the rice/soy fermentate did not reduce any of the odorants, variations in concentrations among the identified odorant compounds were observed.

The results of the current work do not indicate the addition of either rice/soy fermentate as a litter amendment is effective at ammonia volatilization reduction, however, the application of the fermentates did increase livability and increase litter nitrogen concentration indicating possible bird health benefits and nitrogen sequestering. Further research and development are still needed in order to improve the knowledge of microbial litter amendments and determine the optimum conditions under which they are effective.

CHAPTER IV

EVALUATION OF A RICE/SOY FERMENTATE ON PERFORMANCE AND VOLATILIZATION OF ODORANTS FROM FRESH FECAL MATERIAL WHEN INCLUDED IN BROILER DIETS

Introduction

Poultry production operations are a source of airborne pollutants including gases, odors, dust and microorganisms. Poultry producers in the United States are becoming increasingly concerned over the odors and gases that are generated and emitted from their operations. Due to the continuous expansion of suburbs into rural areas, nuisance complaints from neighbors are increasing. Local units of government as well as state and federal regulatory agencies have begun to enforce air quality standards to control nuisance complaints. Types of nuisances include odors, noise, dust, flies, rodents, etc. The most abundant type of nuisance originating from poultry operations is odor emissions.

The predominate source of odors are generated by the anaerobic decomposition of livestock waste such as manure. As manure decomposes, between 80 and 200 odorous compounds are produced. Odor is a mixture of many of these compounds at low levels. Complex combinations of gases are perceived differently than each gas is perceived individually. Odor volatilization is attributed to the metabolism by microorganisms of non-absorbable byproducts in the gastrointestinal tract (Chavez et al., 2004b). This microbial growth is dependent on temperature, pH, moisture, nutrient availability, and atmospheric conditions.

Since the broiler is the initial source of nutrient excretion, diet manipulation can be a feasible solution to control excess nutrient excretions and odor emissions that will have a major impact to minimize environmental issues (Sutton et al., 2001). Odor mitigation has been achieved through the supplementation of various methionine sources (Chavez et al., 2004a, 2004b, and 2004c), and ammonia mitigation with the inclusion of dried distillers grains plus solubles (Wu-Haan et al., 2010), wheat middlings, and soybean hulls (Roberts et al., 2007). The objective of these studies was to evaluate the effectiveness of a rice/soy fermentate which contains a consortium of naturally occurring yeast and bacteria to mitigate odor volatilization when included in broiler diets.

Materials and Methods

Two experiments were conducted to evaluate the effectiveness of two fermented rice/soy products (BiOWiSH Technologies, Naperville, IL, 60563) on volatilization of odor compounds from fresh fecal material and growth performance when included in broiler diets. For each of the following experiments, broiler chicks were provided age appropriate supplemental heat and given access to feed and water *ad libitum*. All animal care procedures were conducted in accordance with an Animal Use Protocol approved by the Texas A&M University laboratory animal care committee. Prior to chick placement, grow out facilities and battery units were thoroughly cleaned and disinfected. Fresh pine shavings and used litter were used at a 50:50 ratio for bedding material in the growout facilities. Pens were equipped with one 30 lb tube feeder and nipple drinkers.

Experiment 1

This experiment was designed to determine the effect of feeding a rice/soy fermentate to broilers on growth performance and odorant volatilization from fresh broiler fecal material. This experiment consisted of 360 1-d old Cobb 500 male chicks that were weighed and randomly allotted to battery pens and dietary treatment based on body weight upon arrival to research farm. The basal starter diet was corn and soybean meal-based (Table 4-1) and was separated into five equal batches.

The rice/soy fermentate was added at four specified treatment levels to one of the five batches to obtain four experimental treatments and a control. The four experimental treatment groups contained the inclusion of two different rice/soy fermentate products at 300, 600, and 900 g/ton for fermentate A and fermentate B at 900 g/ton. Each of the five treatment consisted of six replicate pens with each replicate containing 12 birds. Average broiler body weight and feed consumption were determined on d 7, 14, and 21.

Table 4-1. Dietary formulation and calculated nutrient concentrations of straight-run market broilers fed non-medicated diets with the inclusion of one of two different rice/soy fermentates.

Basal Experimental Diets	
Ingredient	**Starter Diet (%)**
Corn	58.38
Soybean Meal	34.50
Fat Blended	2.81
Limestone	1.57
Salt	0.46
Bifos 16/21p	1.56
Lysine HCL	0.17
DL-Met 98	0.26
Vitamins[1]	0.25
Trace Min[2]	0.05
Calculated Nutrient Concentration	
Protein	22.00
Calcium	0.95
Phosphorus	0.70
Available Phos.	0.45
Methionine	0.59
TSAA	0.95
Threonine	0.82
Crude Fat	5.36
Lysine	1.30
Sodium	0.20

[1] Vitamin premix added at this rate yields 11,023 IU vitamin A, 3,858 IU vitamin D_3, 46 IU vitamin E, 0.0165 mg B_{12}, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadione, 1.75 mg folic acid, 7.17 mg pyroxidine, 2.94 mg thiamine, 0.55 mg biotin per kg diet. The carrier is ground rice hulls.

[2] Trace mineral premix added at this rate yields 149.6 mg manganese, 125.1 mg zinc, 16.5 mg iron, 1.7 mg copper, 1.05 mg iodine, 0.25 mg selenium, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil.

On day 12, manure pans were thoroughly cleaned and ammonia concentration determined following a 48 hr collection period on day 14 of age. On day 14, pans were removed and 1 kg of fecal material was placed in a static chamber for 20 minutes at which time ammonia concentration was determined using a Drager CMS gas analyzer with ammonia chips that range from 2-50 ppm. On day 20, manure pans in the batteries were again thoroughly cleaned, and manure was collected for 24 hours. After 24 hours (on day 21 of age), manure pans were moved to a clean, well-ventilated area for odorant assessment via lateral flow wind tunnel method. The wind tunnel had a sampling port for the collection of air samples. The top of the lateral flow wind tunnel is a 0.635 cm thick plexiglass with four 0.953 cm holes for air outlet where samples were collected. The flush gas inlet is a 5.08 x 5.08 cm steel tube with ten 0.318 cm holes spaced 2.54 cm apart. Compressed breathing air was used as the flush gas at a flow rate of 8 L/min. Odor samples were collected by sorbent tubes and transported to West Texas A&M University (WTAMU) Olfactometry Laboratory for analysis via gas chromatography/mass spectrometry. Thermal desorption tube samples are analyzed using a PAL® autosampler and an Agilent® 6890 GC/MS. The GC is equipped with forte BP5 5% Phenyl / 95% dimethyl polysiloxane 0.53mm ID x 30m and a forte BP20 (polar) Polyethylene Glycol 0.53 mm ID x 25m. Samples were automatically desorbed at 280°C and injected into the GC/MS. The column oven is ramped from 40°C to 240°C at 8°C per minute for a total run time of 38 minutes.

Following odor collection, fecal material was placed in a static chamber for ammonia concentration determination following a 20 minute period. Ammonia concentration in the static chamber was determined using a SAM IV MAX gas monitor (OI Analytical, College Station, TX). Hydrogen sulfide was also measured following the 20 minute period in the static chamber using a Jerome meter. Following ammonia and hydrogen sulfide concentration measurement, two 12 g samples per replicate pen were diluted in 60 mLs of distilled water for pH determination in duplicate per replicate pen. Moisture loss, and carbon and nitrogen content were determined from the fecal samples collected on day 21. Triplicate 10 gram samples of fresh fecal material were weighed into aluminum pans and dried at 105ºC for 24 hrs for moisture analysis. All three dried samples were combined and ground using a Wiley mill equipped with an 80 mesh screen. Ground samples were then analyzed for carbon and nitrogen content on a dry matter basis by combustion method using an Elementar Vario Max analyzer.

Experiment 2

This experiment was designed to determine the effect of dietary inclusion of rice/soy fermentates on broiler growth performance, fecal pH, and odorant volatilization from fresh broiler fecal material during a complete growout. This experiment consisted of 1,020 day old straight-run Cobb 500 chicks that were weighed and randomly allotted to floor pens and dietary treatment based on body weight upon arrival to the research farm. The basal starter, grower, and finisher diets were corn and soybean meal-based (Table 4-2). Each dietary phase was mixed as one large basal diet and separated into three equal batches. The inclusion of the two fermentate products were added at the specified treatment level to one of the three batches to obtain two experimental treatments and a control. The two fermentates were included at a rate of 900 g/ton.

Table 4-2. Dietary formulation and calculated nutrient concentrations of straight-run market broilers fed non-medicated diets with the inclusion of one of two different rice/soy fermentates.

Basal Experimental Diets			
Ingredient	**Starter (%)**	**Grower (%)**	**Finisher (%)**
Corn	58.35	65.21	68.48
Soybean Meal	34.50	29.22	24.68
Fat Blended	2.84	1.93	2.90
Limestone	1.57	1.41	1.59
Salt	0.46	0.33	0.22
Bifos 16/21p	1.56	1.40	1.29
Sodium Bicarbonate	--	0.11	0.26
Lysine HCL	0.16	0.15	0.12
DL-Met 98	0.26	0.24	0.16
Vitamins[1]	0.25	0.25	0.25
Trace Min[2]	0.05	0.05	0.05
Calculated Nutrient Concentration			
Protein	22.00	20.00	18.00
Calcium	0.95	0.85	0.89
Phosphorus	0.70	0.66	0.62
Available Phos.	0.45	0.41	0.38
Methionine	0.59	0.54	0.44
Lysine	1.30	1.15	1.00
TSAA	0.95	0.88	0.75
Threonine	0.82	0.62	0.67
Crude Fat	5.38	4.69	5.73
Sodium	0.20	0.18	0.18

[1] Vitamin premix added at this rate yields 11,023 IU vitamin A, 3,858 IU vitamin D_3, 46 IU vitamin E, 0.0165 mg B_{12}, 5.845 mg riboflavin, 45.93 mg niacin, 20.21 mg d-pantothenic acid, 477.67 mg choline, 1.47 mg menadione, 1.75 mg folic acid, 7.17 mg pyroxidine, 2.94 mg thiamine, 0.55 mg biotin per kg diet. The carrier is ground rice hulls.

[2] Trace mineral premix added at this rate yields 149.6 mg manganese, 125.1 mg zinc, 16.5 mg iron, 1.7 mg copper, 1.05 mg iodine, 0.25 mg selenium, a minimum of 6.27 mg calcium, and a maximum of 8.69 mg calcium per kg of diet. The carrier is calcium carbonate and the premix contains less than 1% mineral oil.

Each treatment consisted of ten replicate pens with each pen containing 34 birds at placement. Broilers were reared on a 50:50 ratio of recycled litter to fresh pine shavings. The diets did not contain any medications throughout the trial; therefore birds were vaccinated with a coccidiosis vaccine (Coccivac®-B) on day of age as a coccidiosis preventative. The dietary program consisted of three dietary phases, starter (1-14 days), grower (15-28 days), and finisher (29-42 days). Body weights and pen feed consumptions were determined on days of dietary changes including 14, 28, and 41. Following day 41 bulk weights, 5 male and 5 female broilers per replicate (300 total birds) were subjected to an 8 hr feed withdrawal period and processed at the TAMU broiler processing facility to obtain carcass weights and carcass yield data.

On day 20, 3 male broilers per floor pen were placed in grower battery units to collect fresh fecal material for a period of 24 hours. On day 40, 2 male broilers per floor pen were placed in grower battery units to collect fresh fecal material for a period of 24 hours. After the collection period, manure pans were moved to a clean, well-ventilated area for volatilized odorant collection via flux chamber method as previously described. Following odor collection, two 5 g samples per replicate pen were diluted in 25 mLs of distilled water for pH determination in duplicate per replicate pen.

Statistical Analysis

For both experiments, all data were analyzed via a One-way Analysis of Variance using the General Linear Model. Means were deemed significantly different at $p \leq 0.05$ and separated using a Duncan's Multiple Range Test.

Results

Experiment 1

The addition of fermentate B at 900 g/ton resulted in an increased ($p<0.05$) body weight beginning as early as day 7 with a 3.5% increase. Increased body weight gain continued throughout the remainder of the experiment with a significant increase ($p \leq 0.05$) in day 21 body weight with a 5% increase (Table 4-3). The inclusion of both fermentates (A and B) resulted in decreases ($p \leq 0.05$) in multiple evaluated volatile organic compounds that have been strongly associated with odor, including 4-ethyl phenol, para-cresol, indole, skatole, valeric acid, isovaleric acid and hexanoic acid (Table 4-4). These odorants have a variety of descriptors and may vary in offensiveness. One of the less offensive odorant would be isovaleric acid that has a descriptor of buttery and sweat. The more offensive odorants such as 4-ethyl phenol, para-cresol, indole, skatole, valeric acid, and hexanoic acid have descriptors of horse manure, barnyard, mothball, outhouse, foul, and goat-like odor, respectively. No significant differences were observed with regard to feed conversion ratio, feed consumption, mortality, fecal pH, moisture percentage, ammonia and hydrogen sulfide emissions, or nitrogen and carbon levels (Table 4-3, Table 4-4, and Table 4-5); however, multiple observations including reduced fecal pH and numerical decreases in feed conversion indicate support for further investigation with a larger sample size. These data suggest the ability of a rice/soy fermentate inclusion to reduce odorant concentration from fresh fecal material and increased early body weight with dietary inclusion of the fermentate B product supporting the idea of further investigation into performance and odor profiles in a full scale grow out trial taking broilers to market age.

Table 4-3. Weekly average body weights (BW), feed conversion ratio (FCR), and feed consumption of battery reared male broilers fed one of two rice/soy fermentates (A[1] and B[2]) at varying dietary concentrations.

		BW (g)			FCR (Feed:Gain)			Feed Consumption (g/bird/day)		
Ingredient	Conc (g/ton)	d 7	d 14	d 21	d 1-7	d 1-14	d 1-21	d 1-7	d 1-14	d 1-21
Control	0	161.0	450.8	909.7^{b}	1.29	1.29	1.36	21.6	37.4	55.8
A	300	161.9	456.5	906.2^{b}	1.22	1.26	1.36	21.0	37.2	55.5
A	600	159.2	452.7	908.6^{b}	1.26	1.28	1.36	21.1	37.1	55.6
A	900	162.3	454.6	908.7^{b}	1.25	1.27	1.33	20.8	36.0	53.3
B	900	167.2	470.4	946.4^{a}	1.22	1.26	1.35	21.3	37.6	56.5
SEM		1.2	3.0	5.2	0.01	0.01	0.01	0.2	0.3	0.4

[a,b] Means in columns with different superscripts differ significantly at $p < 0.05$.
[1] BiOWiSH Odor – BiOWiSH Technologies, Naperville, IL, 60563
[2] BiOWiSH Aqua – BiOWiSH Technologies, Naperville, IL, 60563

Table 4-4. Fecal characteristics of 21 day old battery reared male broilers fed one of two rice/soy fermentates (A[1] and B[2]) at varying dietary concentrations.

Ingredient	Concentration (g/ton)	pH	Moisture Percentage	Nitrogen Content	Carbon Content	C:N
Control	0	6.58	69.65	4.35	41.02	9.45
A	300	6.48	69.14	4.44	40.73	9.26
A	600	6.58	68.48	4.28	40.83	9.59
A	900	6.38	68.25	4.52	40.73	9.04
B	900	6.33	69.12	4.42	40.98	9.35
SEM		0.06	0.32	0.03	0.05	0.08

[1] BiOWiSH Odor – BiOWiSH Technologies, Naperville, IL, 60563
[2] BiOWiSH Aqua – BiOWiSH Technologies, Naperville, IL, 60563

Table 4-5. Profile of odor compounds (µg/m³) ± SE collected off of 1 kg of fresh fecal material from 21 day old battery reared male broilers fed one of two rice/soy fermentates (A[1] and B[2]) at varying dietary concentrations, following a 20 minute collection period in a lateral flow wind tunnel.

Volatile Organic Compounds (µg/m³)					
Ingredient	Control	A	A	A	B
Concentration	0	300	600	900	900
Acetic Acid	106.4 ± 16.3	80.96 ± 20.4	76.75 ± 7.8	85.83 ± 15.3	69.40 ± 18.0
Propionic Acid	8.99 ± 3.1	14.50 ± 7.7	5.70 ± 3.5	3.76 ± 1.3	14.74 ± 4.2
Isobutyric Acid	47.53 ± 2.5	10.18 ± 6.8	37.51 ± 19.4	26.81 ± 9.4	65.85 ± 19.8
Butyric Acid	132.8 ± 3.1	75.06 ± 21.9	84.82 ± 21.9	74.65 ± 17.0	120.3 ± 21.8
Isovaleric Acid	24.02 ± 2.9	16.29 ± 4.3	6.30 ± 3.1	13.79 ± 2.7	15.72 ± 5.8
Valeric Acid	68.68 ± 13.1[a]	22.99 ± 10.9[b]	18.74 ± 2.4[b]	26.62 ± 4.2[b]	29.35 ± 4.8[b]
Hexanoic Acid	260.4 ± 24.7[a]	104.2 ± 20.5[b]	124.8 ± 20.0[b]	103.6 ± 7.7[b]	162.1 ± 20.3[b]
Phenol	9.17 ± 0.91	7.24 ± 1.9	7.59 ± 1.2	9.47 ± 0.87	6.93 ± 0.67
Para-cresol	4.94 ± 0.15[a]	1.70 ± 0.29[b]	2.55 ± 0.41[b]	2.65 ± 0.51[b]	1.96 ± 0.13[b]
4-ethyl-phenol	13.52 ± 0.95[a]	1.65 ± 0.44[c]	2.15 ± 0.48[bc]	4.10 ± 1.3[b]	1.70 ± 0.37[c]
2-aminoacetophenome	0.04 ± 0.01	0.16 ± 0.12	0.06 ± 0.02	0.06 ± 0.01	0.03 ± 0.01
Indole	0.08 ± 0.02[a]	0.02 ± 0.01[b]	0.04 ± 0.02[b]	0.02 ± 0.003[b]	0.02 ± 0.01[b]
Skatole	0.15 ± 0.004[a]	0.07 ± 0.03[b]	0.06 ± 0.01[b]	0.04 ± 0.01[b]	0.04 ± 0.01[b]
Hydrogen Sulfide[3]	0.14 ± .022	0.19 ± .015	0.18 ± .018	0.30 ± .080	0.16 ± .021
Ammonia Concentration[4]					
Day 14	45.6 ± 22.7	32.3 ± 9.50	20.3 ± 5.80	44.8 ± 12.0	27.0 ± 6.60
Day 21	26.5 ± 4.7	31.7 ± 11.8	31.2 ± 10.5	29.0 ± 5.3	25.8 ± 9.5

[a-c] Means in columns with different superscripts differ significantly at $p < 0.05$.
[1] BiOWiSH Odor – BiOWiSH Technologies, Naperville, IL, 60563
[2] BiOWiSH Aqua – BiOWiSH Technologies, Naperville, IL, 60563
[3]Hydrogen sulfide was measured following the 20 minute period in the static chamber using a Jerome meter
[4]Fecal ammonia emissions in a static flux chamber over a 20 minute period of time ± SE of broilers fed one of two rice/soy fermentates (A[1] and B[2]) at varying dietary concentrations. Day 14 measurements were taken using a Drager CMS gas analyzer with ammonia chips that range from 2-50 ppm. Day 21 measurements were taken using a SAM IV MAX ammonia monitor (OI Analytical).

Experiment 2

The addition of fermentate B at 900 g/ton resulted in a significant increase ($p \leq 0.05$) in day 14 body weight (Table 4-6). At the conclusion of the trial, inclusion of fermentate B did result in the highest average body weight although did not test to be significant ($p < 0.05$). The inclusion of the fermentates did not result in an observed difference in feed conversion during the starter or finisher period of the trial. However, during the grower period of the trial, inclusion of the fermentates did increase feed conversion ratio although no difference was observed at the conclusion of the trial in cumulative feed conversion ratio (Table 4-6). The inclusion of both rice/soy fermentates (A and B) significantly increased ($p \leq 0.05$) processing data including, individual live weights and carcass weights; however, no difference was observed with regards to carcass yield percentage (Table 4-7). Fecal pH on day 21 was significantly reduced with the inclusion of both fermentate products (A and B) when compared to the pH of the control (table 4-7). Day 42 fecal pH was significantly reduced with the inclusion of fermentate B when compared to the control. No significant differences were observed in odorant concentrations on days 21 or 42 with the inclusion of either rice/soy fermentate product (Tables 4-8 and 4-9).

Table 4-6. Body weight and feed conversion ratio (FCR) of straight-run market broilers fed non-medicated diets with the inclusion of one of two different rice/soy fermentates (A[1] and B[2]).

BW (kg)				FCR Feed:Gain				
Diet	d 14	d 28	d 41	Starter	Grower	Finisher	d 1-28	d 1-42
Control	0.39[b]	1.25	2.35	1.46	1.69[b]	2.24	1.62[b]	1.89
A	0.40[b]	1.25	2.37	1.47	1.73[a]	2.25	1.65[a]	1.92
B	0.41[a]	1.27	2.38	1.44	1.75[a]	2.21	1.65[a]	1.90
SEM	0.002	0.01	0.01	0.01	0.01	0.01	0.01	0.01

[a,b] Means in columns with different superscripts differ significantly at $p < 0.05$.
[1] BiOWiSH Odor – BiOWiSH Technologies, Naperville, IL, 60563
[2] BiOWiSH Aqua – BiOWiSH Technologies, Naperville, IL, 60563

Table 4-7. Processing parameters and fecal pH of straight-run market broilers fed non-medicated diets with the inclusion of one of two different rice/soy fermentates (A[1] and B[2]).

Processing Data				Fecal pH	
Diet	Live Wt (g)	Carcass Wt (g)	Carcass Yield (%)	d 21	d 42
Control	2383[b]	1745[b]	73.3	6.04[a]	6.70[a]
A	2452[a]	1796[a]	72.6	5.24[b]	6.00[ab]
B	2447[a]	1790[a]	73.1	5.47[b]	5.25[b]
SEM	16	12	0.2	0.12	0.21

[a,b] Means in columns with different superscripts differ significantly at $p < 0.05$.
[1] BiOWiSH Odor – BiOWiSH Technologies, Naperville, IL, 60563
[2] BiOWiSH Aqua – BiOWiSH Technologies, Naperville, IL, 60563

Table 4-8. Profile of odor compounds (µg/m³) collected off of 500 g of fresh fecal material from 21 day old broilers fed one of two rice/soy fermentates (A[1] and B[2]), following a 20 minute collection period in a lateral flow wind tunnel.

Volatile Organic Compounds				
Ingredient	Control	A	B	
Concentration	0	900	900	SEM
Acetic Acid	479.6	576.1	445.0	55.3
Propionic Acid	79.9	29.6	30.2	18.6
Isobutyric Acid	98.8	59.6	412.4	121.8
Butyric Acid	12.9	33.3	44.6	11.1
Isovaleric Acid	13.0	6.0	10.9	2.7
Valeric Acid	24.7	20.1	18.4	3.5
Hexanoic Acid	3.9	4.7	7.7	1.6
Phenol	24.1	39.5	42.4	5.8
Para-cresol	23.8	52.2	42.2	5.8
4-ethyl-phenol	5.8	15.7	4.0	2.1
Indole	4.8	7.5	5.1	1.0
Skatole	3.4	3.8	3.8	0.5

[1] BiOWiSH Odor – BiOWiSH Technologies, Naperville, IL, 60563
[2] BiOWiSH Aqua – BiOWiSH Technologies, Naperville, IL, 60563

Table 4-9. Profile of odor compounds (µg/m³) collected off 500 g of fresh fecal material from 42 day old broilers fed one of two rice/soy fermentates (A[1] and B[2]), following a 20 minute collection period in a lateral flow wind tunnel.

Volatile Organic Compounds				
Ingredient	Control	A	B	
Concentration	0	900	900	SEM
Acetic Acid	637.8	1160.2	1409.3	154.2
Propionic Acid	163.2	213.1	587.1	83.0
Isobutyric Acid	24.9	22.2	68.2	11.1
Butyric Acid	232.0	484.8	568.8	92.1
Isovaleric Acid	69.7	21.2	24.7	18.5
Valeric Acid	71.4	48.8	52.8	16.0
Hexanoic Acid	138.5	68.0	58.5	30.1
Heptanoic Acid	15.3	19.9	21.5	1.9
Phenol	59.6	65.9	61.0	2.5
Para-cresol	90.8	84.7	86.4	6.4
4-ethyl-phenol	49.9	42.3	37.0	3.6
Indole	19.2	15.9	13.9	2.1
Skatole	18.6	2.2	2.1	4.1

[1] BiOWiSH Odor – BiOWiSH Technologies, Naperville, IL, 60563
[2] BiOWiSH Aqua – BiOWiSH Technologies, Naperville, IL, 60563

Discussion

The composition of freshly excreted manure is directly related to the original composition of the diet. Diet manipulation is a potential strategy to reduce fecal odorants in poultry production. Odor volatilization is attributed to the microbial degradation of manure by microorganisms in the gastrointestinal tract. This microbial growth and degradation is affected by temperature, pH, moisture, nutrient availability, and atmospheric conditions. In experiment 1, the inclusion of both fermentates (A and B) resulted in decreases ($p \leq 0.05$) in multiple volatile organic compounds that have been strongly associated with animal manure odor, including 4-ethyl phenol, para-cresol, indole, skatole, valeric acid, isovaleric acid, and hexanoic acid. These odorants have a variety of descriptors and may vary in offensiveness. Isovaleric acid has a descriptor of buttery, whereas odorants including 4-ethyl-phenol, para-cresol, indole, and skatole have descriptors of horse manure, barnyard, musty, outhouse, and fecal. In experiment 2, the inclusion of both rice/soy fermentate products in broiler diets significantly reduced ($p \leq 0.05$) fecal pH at day 21 and 42 days of age. A reduced gut pH is beneficial in improving energy and nutrient utilization (Gonzalez-Alvarado et al., 2007). Pathogenic bacteria entering the gastrointestinal tract via the feed have a greater chance of being inactivated by the highly acidic environment (Naughton and Jensen, 2001).

The contrasting results of the two experiments may be due to the different rearing environments. In experiment 1, male broilers were reared in batteries whereas in experiment 2, broilers were reared in floor pens containing recycled litter. A study by Torok et al. (2009) was conducted to determine if litter type influences gut microbiota and performance in broilers. Microbial profiling was done to investigate changes in cecal bacterial communities associated with litter material and age. At both ages evaluated, the microbiota of chickens raised on used litter was significantly ($p < 0.05$) different from that of chickens raised on any other litter materials, except on softwood shavings at day 28 of age. This suggests that the type of litter material can influence colonization and development of cecal microbiota in chickens. Broilers in experiment 2 could potentially have a different microbial population because they were reared on used litter resulting in the reduction in pH without the observed reduction in odorant concentration. It may be necessary to adjust dietary concentration to account for the exposure to environmental bacteria based on rearing conditions in order to observe the desired effect.

Supplementing water, feed or litter with biologicals enhances or alters microbial population with the goal of preventing the formation of toxic compounds, improving feed efficiency, and competitively excluding undesirable microorganisms (Cole et al., 2006; Patterson and Burkholder, 2003; Shah et al., 2007). In experiment 1, the addition of fermentate B at 900 g/ton resulted in an increased body weight beginning as early as day 7 with a 3.5% increase and continued throughout the remainder of the study with a significant increase ($p \leq 0.05$) in day 21 body weight with a 5% increase. In experiment 2, the addition of rice/soy fermentate B at 900 g/ton resulted in a similar increase ($p \leq 0.05$) in early body weight however; the observed difference was not significant on d 28 or 42 although increasing in live weight and carcass weight were observed in the sample of processed broilers. The inclusion of fermentate B at 900 g/ton in both experiments resulted in significant increases in body weight at the early stages of growth possibly indicating a probiotic type effect.

Combined, the results of these two experiments indicate the ability of a rice/soy fermentate product to increased early broiler body weight, alter pH, and reduce volatile organic compound volatilization from fresh fecal material possibly through the alteration of the microbial populations that are responsible for the degradation of manure.

CHAPTER V

CONCLUSION

The livestock industry provides significant benefits to today's economy; however livestock and poultry producers in the United States are becoming increasingly concerned over the odor and gases that are generated and emitted from their animal operations. Odor and ammonia emissions from livestock facilities can possibly result in detrimental effects to animal performance, provide potential concerns for producer welfare, and potentially result in negative environmental issues. Poultry manure and litter have been recognized as a source of odor and potential nuisance, however, poultry litter is a valuable source of nutrients that can be used as a fertilizer providing nutrients for crop growth. Because of the growing concerns, there is a need to improve animal manure and litter management methodologies. This research program focused upon managing odor and ammonia volatilization through the use of a rice/soy fermentate as a feed ingredient and a spray applied litter amendment.

Concerns regarding ammonia concentrations within poultry housing, emissions from poultry operations, and the potential environmental effects from these emissions have emphasized the need for research to identify strategies to reduce ammonia volatilization from large poultry operations (Coufal et. al., 2006). Recently, interest has increased in non-chemical litter amendments such as biological treatments, including enzymes, enzyme inhibitors, nutrients and/or microorganisms. The fermentate product evaluated in this research program to help control ammonia volatilization from poultry litter contains a variety of microorganisms and enzymes. Spray application of both rice/soy fermentate products did not result in a reduction in ammonia concentration potential of the litter as measured via a static chamber method however; mortality was significantly reduced in the birds reared in pens with used litter treated with the spray application of the rice/soy fermentate. No differences were observed with regard to litter pH, litter moisture, or broiler performance characteristics, however, significant differences were observed in nutrient content of the litter on days 35 and 43 with the application of the fermentate to new litter. The results of the current work do not indicate the addition of either rice/soy fermentate as a litter amendment is effective at ammonia volatilization reduction, however, the application of the fermentates did increase livability and increase litter nitrogen concentration indicating possible bird health benefits and nitrogen sequestering.

Livestock and poultry odors are generated by the anaerobic decomposition of livestock waste such as manure. The composition of broiler excreta is related to the composition of the diet (Sutton et al., 2002), thus dietary formulation respective of nutrient profiles can influence odor volatilization. The inclusion of both fermentates (A and B) resulted in decreases ($p \leq 0.05$) in multiple volatile organic compounds that have been strongly associated with odor, including 4-ethyl phenol, para-cresol, indole, skatole, valeric acid, isovaleric acid, and hexanoic acid. These odorants have a variety of descriptors and vary in offensiveness with several of these odorants being classified as offensive including 4-ethyl phenol, para-cresol, indole, skatole, valeric acid, and hexanoic acid have descriptors of horse manure, barnyard, mothball, outhouse, foul, and goat-like odor, respectively. The inclusion of both rice/soy fermentate products in broiler diets significantly reduced ($p \leq 0.05$) fecal pH at day 21 and 42 days of age. A reduced gut pH is beneficial in improving energy and nutrient utilization (Gonzalez-Alvarado et al., 2007). Pathogenic bacteria entering the gastrointestinal tract via the feed have a greater chance of being inactivated by the highly acidic environment (Naughton and Jensen, 2001). Additionally, the addition of fermentate B at 900 g/ton resulted in an increased early broiler body weight beginning as early as day 7 with a 3.5% increase and continued throughout the remainder of the study with a significant increase ($p \leq 0.05$) in day 21 body weight with a 5% increase. An increased in feed withdrawal body weight and carcass weight was also observed with the dietary inclusion of the fermentates in the floor pen trial. This effect may be a result of the biologicals enhancing or altering the microbial population with the goal of preventing the formation of toxic compounds, improving feed efficiency, and competitively excluding undesirable microorganisms (Cole et al., 2006; Patterson and Burkholder, 2003; Shah et al., 2007). The inclusion of fermentate B at 900 g/ton in both experiments resulted in significant increases in body weight at the early stages of growth possibly indicating a probiotic type effect.

Combined, these data indicate the ability of a rice/soy fermentate product to increased early broiler body weight and liveability, reduce fecal pH, and reduce volatile organic compound volatilization from fresh fecal material possibly through the alteration of the microbial populations that are responsible for the degradation of manure.

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VITA

Name: Mallori Paige Williams

Address: 560 Hwy 202
Refugio, Texas 78377

Email Address: mpw032007@tamu.edu

Education: B.S., Poultry Science, Texas A&M University, 2010