EVALUATION OF THE EFFECTIVENESS OF AN ENGINEERED BIOCARBON IN BROILER PRODUCTION

by

HACI OMER BAYIR

(Under the Direction of Justin Fowler)

ABSTRACT

Biocarbon (Bio-C) is a carbon-rich, porous solid product produced by the thermal decomposition of carbonaceous biomass, such as wood, bamboo or nut shells, in the absence of air or with limited air. Bio-C has been reported to absorb urea, ammonia, ammonium, and fat and fat-soluble compounds, bind and ameliorate the harmful effects of toxins, and has been added in animal feeds to promote growth performance. The purpose of this study was to evaluate the effectiveness of an engineered Bio-C as a topical application to the litter (at rates of 0, 50 and 100 lbs/1000 ft^2 Bio-C, with 50 lbs/1000 ft^2 sodium bisulphate (PLT) as a positive control treatment) on ammonia (NH_3) volatilization (Exp-1), the effects of Bio-C as a feed supplement (at concentrations of 0, 0.2, 0.6 and 1.0 % of the diets) for reducing NH_3 volatilization (Exp-2), to evaluate the effect of Bio-C as a feed supplement (at concentrations of 0, 0.1, 0.2 and 0.4 % of the diets) for improvement of growth performance (Exp-3), and to evaluate the efficacy of Bio-C (0.4 % of the diet) in mitigating the effects of a 0.5 mg/kg dose of aflatoxins (AF) in Exp-4. Neither topical application to the litter (Exp-1) nor the addition of Bio-C in the feed (Exp-2) had an effect on NH_3 generation or volatilization compared to the control treatments. BW for birds fed 0.2 and 0.6% Bio-C were significantly higher than the control treatment at the end of the Exp-2. However, when birds were reared for a full six weeks at a 0.1, 0.2 or 0.4% of Bio-C dose range, performance parameters were not affected in Exp-3. The results of Exp-4 showed that the performance and relative organ weight of birds receiving 0.5 mg/kg AF were not significantly different compared to the control group at any point during the 21-day trial. No main effects were seen on the performance parameters by the inclusion of 0.4% Bio-C in the diet. In total, the data suggest that Bio-C does not appear to have an impact on NH_3 control or growth performance. In addition, supplementation of Bio-C has no effect on broiler performance when fed in diets free of AF or containing up to 0.5 mg/kg AF.

INDEX WORDS: ammonia, aflatoxin, biocarbon, feed additive, broiler

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DEDICATION

To my family whose encouragement, support and love has driven me to pursue my dreams.

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CHAPTER 1

INTRODUCTION AND REVIEW OF RELATED LITERATURE

INTRODUCTION

The United States has the largest poultry industry in the world, and the combined value of production sales from broilers, eggs, turkeys was $38.7 billion in 2016. Broiler production constitutes 67% of the poultry industry, producing about 8.7 billion birds annually [1]. National per capita consumption of poultry meat in the U.S. is considerably higher than that of beef or pork, and broiler meat comprises the largest part of it. Georgia, Alabama, Arkansas, North Carolina and Mississippi are the top five broiler producing states, respectively [1, 2].

A contract production system between independent farmers and integrated companies is the predominate form of the broiler industry in the U.S. Under this system, the birds, feed, health care or technical assistance are supplied by the companies that coordinate this system from the hatchery to the time of harvest; whereas the land, building, equipment, daily care and management of the birds are provided by the farmers. These farmers are paid based on the performance of their flocks and are rewarded if greater flock performance than average is achieved. Therefore, optimal bird care and greater efficiency are the vital part of the system [3].

Broilers are raised in large, open structure houses known as growout houses, and floor of the houses are covered with bedding material such as wood chips, rice hulls, or peanut shells. Litter is defined as the mixture of these bedding material with excreta of birds, spilled feed and water, and feathers [4]. According to estimation by the U.S. Egg and Poultry Association (2018), a typical broiler chicken produces approximately 1 kg litter (composition of bedding material, feces, feed, water and feather) for a 47-day growing period. The major portion of litter produced by poultry has been applied to agricultural land as a fertilizer [5, 6]. Because the availability and expense of bedding material, and the concern of environmental contamination, litter can be reused for multiple flocks, which is now the standard practice in the U.S. industry [4, 6, 7].

NH_3 emissions have become a major concern for the industry because of this reuse of the same litter for several flocks [8, 9]. NH_3 volatilization from broiler litter has potential negative impacts on the performance, welfare, and health of birds, environment and human health, as well as reductions in the nutrient value of the litter as a fertilizer [10-12]. There are common strategies to mitigate NH_3 release from poultry litter, including ventilation, dietary manipulation and manure management [10, 13]. Acidifying agents, odor and moisture absorbents, and microbial and enzyme inhibitors as manure and litter management are the most common methodologies used in the industry.

Aflatoxins (AF) are highly toxic and carcinogenic fungal metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus*. The common forms of AF are AFB_1, AFB_2, AFG_1 and AFG_2, however, the most toxic form is AFB_1. There is no animal species that are immune to the detrimental effects of these toxins once they contaminate feed. When exposed, AF may induce "aflatoxicosis", a syndrome that results in decreased feed intake and weight gain, immunosuppression, reduced egg production, increased relative liver and kidney weights, impaired serum biochemistry, and eventually mortality [14, 15]. Therefore, efforts have been made and varied systems have been developed to detoxify grains and animal feeds that have been contaminated with AF. Once produced, there is no way to eliminate AF completely from the feeds, so efforts have been made to minimize their effects through binding or sequestration.

Biocarbon (Bio-C), which has high porosity and high internal surface area, generally refers to the carbonaceous residue of wood, cellulose, coconut shells or other by-products left after heating organic matter in the absence of oxygen [16]. This odorless and tasteless black powder has been reported to absorb urea, ammonia, ammonium, and fat and fat-soluble compounds, has been added in animal feed to promote growth performance, as well as bind and ameliorate the harmful effects of toxins [17]. Therefore, the overall objective of this study was to evaluate the effectiveness of an engineered Bio-C in broiler production. The specific objectives being:

1) To evaluate the effectiveness of a topical application of Bio-C to broiler bedding on NH_3 volatilization.
2) To evaluate the effectiveness of Bio-C as a feed supplement on NH_3 volatilization and on growth performance parameters.
3) To evaluate the effect of Bio-C as a feed supplement for growth performance improvements during a full 6-week growout period.
4) To evaluate the efficacy of an engineered Bio-C in young broilers during an aflatoxin exposure.

REVIEW OF RELATED LITERATURE

Ammonia in Poultry

Birds excrete nitrogen (N) in the form of uric acid and undigested protein in fecal waste. Around 50 percent of N in broiler manure is uric acid that can be converted to NH_3 through hydrolysis, mineralization, and volatilization [10, 12, 18]. NH_3 is mainly generated through microbial degradation of uric acid by the urease and uricase enzymes. Through the enzyme uricase, uric acid is converted to allontoin, which is next converted to either glyoxalic acid or urea [19]. With the addition of moisture, urea is further broken down into NH_3 and CO_2 by urease, then these products are released into the atmosphere [10, 20]. This entire process is affected by temperature, moisture, pH, nitrogen content of the litter, stocking density and age of birds [10, 21].

Effects of ammonia on broilers

Atmospheric NH_3 in broiler house has harmful effects on the health and performance of birds. These effects have widely been reported in the literature. Birds are often exposed to 50 ppm NH_3 in the industry, increasing up to 200 ppm if the house is poorly ventilated [7]. When exposed to NH_3 ranging from 25 to 125 ppm, feed intake, feed efficiency and body weight gain, and thus overall body weight has been shown to decrease in broilers [7, 22-25]. Kling and Quarles [22] exposed male leghorn chicks to NH_3 at concentrations of 0, 25 or 50 ppm from 4th to 8th week of the experiment, and growth performance, weight of lung and bursa of Fabricius were determined at 4, 5, 6 and 8 weeks of age. The authors reported that growth performance was significantly lower in birds exposed to NH_3. Caveny and Quarles [24] studied the effects of NH_3 at 0, 25 and 50 ppm on broiler cockerels for a 7-week period and indicated that birds exposed to 50 ppm NH_3 for 7 weeks had depressed feed efficiency compared to the control.

Anderson and Beard [26] reported that birds exposed to 20 ppm NH_3 for over 6 weeks developed respiratory tract damage and become susceptible to Newcastle disease. Birds developed keratoconjunctivitis when exposed to over 45 ppm NH_3, resulting in difficulty locating feed and water [10, 27]. Other problems related to long time NH_3 exposure are delay in reaching sexual maturity, histopathologic changes in spleen, liver and kidneys [7].

Effects of ammonia on humans

High NH_3 levels in broiler house operations may have negative effects on farm workers who spend up to 8 to 10 hours in a day in the house. Exposure to as low as 20 ppm NH_3 can cause irritation of the respiratory tract, eyes and throat, increased susceptibility to secondary infections, and cough [12, 26, 28-30]. Anderson and Beard [26] found discomfort, excessive nasal and lacrimal secretion and coughing when workers were exposed to 50 ppm NH_3 for several hours while weighing turkeys. Pedersen and Selig [29] reported that exposure to concentration lower than 5000 ppm for a few minutes causes pain to the eyes, pain when swallowing, and a slight cough. In addition, when NH_3 reacts with acidic gases (NO_X, SO_X), it contributes to particulate matter (PM), which refers to particles with diameter 2.5 µm or less, in the atmosphere. Long term exposure to such small PM has been associated with asthma, chronic cough, bronchitis, chest illness and premature death [10, 30, 31].

Effects of ammonia on the environment

Ammonia volatilization from poultry litter has detrimental effects on the environment. It is estimated that 50 to 80 percent of total human-related ammonia in the U.S. comes from animal feeding operations, and poultry accounts for about 27 percent [32]. Some of the major negative effects of ammonia on the environment are eutrophication of water sources, soil acidification and aerosol formation [12, 33]. Furthermore, ammonia is a precursor for particulate matter (PM) that contributes to the formation of haze, resulting in reduced natural visibility [34].

Mitigation of ammonia volatilization

Since built-up litter has become the standard practice in the U.S. industry, controlling and mitigating NH_3 in broiler houses is a necessity due to these effects on broiler production, and health and the environmental concerns. Ritz and Fairchild [10] suggested that NH_3 concentration in the house should be lower than 25 ppm. Under current practice of commercial production, however, this number is extremely low and difficult to achieve. There have been a number of attempts to control NH_3 levels in broiler houses, including ventilation, dietary manipulation and manure management.

Ventilation removes excess moisture, NH_3, dust and odors, brings fresh air into the house, thus improving air quality [35]. Therefore, reduced NH_3 concentration in the house can be accomplished by adequate ventilation. Carr and Wheaton [36] showed that increased ventilation rate decreases NH_3 concentration. However, Elliott and Collins [37] reported that increased ventilation rate causes higher NH_3 emissions, and increases energy costs, especially in cold weather.

Birds excrete N in the form of uric acid and undigested protein, then about 50 percent of this fecal waste is converted to NH_3. One method to reduce N in the excreta and control NH_3 is to lower the dietary crude protein level with supplementation of synthetic amino acids [38]. Moran Jr. and Bushong [39] reported that reduced dietary crude protein caused a reduction of N content of the litter without effecting bird performance at 6 weeks of age. Ferguson and Gates [40] studied the effect of diets with reduced crude protein and supplemental amino acids on bird performance, N excretion and NH_3 gas concentration for a 6-week period. The authors found that

NH_3 concentration declined by 31 % and litter N was reduced about 16 % by reducing crude protein level from 215g/kg to 196g/kg in the diet. Besides reduction of crude protein in the diet, the feed can be formulated based on amino acid requirements rather than total crude protein. Therefore, feed additives or enzymes can be used for optimum feed efficiency and minimum nutrient excretion, and phase feeding could be taken into consideration [38].

Litter amendments such as acidifiers, absorbents, microbial and enzyme inhibitors and other chemicals as a part of a manure management plan are the most widely accepted methodologies to reduce the formation and release of NH_3 in broiler facilities [10, 41]. Acidifiers such as Al+Clear, Poultry Litter Treatment, and Poultry Guard lower the pH of the litter and convert volatile NH_3 into non-volatile ammonium (NH_4^+), thus reducing NH_3 volatilization and improving bird health and productivity [41-44]. Kim and Patterson [45] tested zinc and copper to inhibit enzyme activity and reduce microbes in the litter and found that those minerals decreased the activity of uricase, the growth and number of microbes, resulting in reduced NH_3 volatilization. Naturally occurring clay products such as clinoptilolite (natural zeolite) act as ion-exchangers and absorb moisture of the litter, thus inhibiting microbial activity and formation of NH_3. Nakaue and Koelliker [46] reported that litter moisture and NH_3 were lowered by application of clinoptilolite to the litter.

Aflatoxins in poultry

The contamination of agricultural harvests all over the world by mycotoxins has received a great degree of attention over the decades [47]. Mycotoxins are secondary metabolites produced by various fungal species (*Aspergillus, Fusarium or Penicillium*). Although there are many forms of mycotoxins, aflatoxins (AF) are one of the most commonly studied when it comes to poultry [48-50].

Aflatoxins (AF) are highly toxic and carcinogenic fungal metabolites produced by *Aspergillus flavus* and *Aspergillus parasiticus.* Fluorescing under UV light, AF are named according to their blue or green fluorescence: AFB_1 and AFB_2 (for blue), AFG_1 and AFG_2 (for green). In addition, AFM_1 and AFM_2 are metabolites of AFB_1 and B_2 that are found in milk or urine. Of these, AFB_1 is considered the most carcinogenic and one of the most prevalent worldwide [15, 51, 52].

The occurrence of AF in agricultural feed commodities, such as corn, sorghum, wheat and peanuts, is quite common all over the world, and these crops are at a high risk. Crop susceptibility can be increased due to drought stress or insect damage, leading to AF contamination prior to harvest, plus warm and humid conditions favoring fungal colonization and AF production. Contamination of crops can occur during harvest, transport or poor storage conditions [47, 50, 53, 54].

Livestock, including poultry, are exposed to AF by consuming contaminated agricultural commodities in the diet. There is no animal species that are immune to detrimental effects of these toxins, including humans. Increased awareness and considerable attention have been given to AF in poultry since 1960 when Turkey X disease was observed. This disease caused the death of over 100,000 turkeys in England. The causative agent was later discovered that a peanut meal imported from Brazil was contaminated with AF [55].

Research over the past 40 years have shown the adverse effects of AF on production parameters, resulting in economic losses for the poultry industry. Beyond its mutagenic, carcinogenic and immunosuppressive effects, dietary exposure to AF can impair feed intake, weight gain, feed efficiency, depress growth rate, cause increased liver size, paleness of the liver, and reduce reproductive performance and egg production [15, 56-62]. However, severity of syndrome called ‘aflatoxicosis’ depends on many factors, including animal species, age, source and dose of the AF fed, and exposure time.

There are several methods to prevent and control the growth of the fungi and the production of toxin. The most convenient method at the preharvest of agricultural commodities is to prevent contamination by using resistant crops, fungicides and pesticides. Harvesting the crops or grains at the proper stage of maturity also helps to prevent the contamination. Postharvest methods include rapid drying of crops, proper storage conditions and the use of chemicals [48, 63]. As it relates to animal production, one of the most promising strategies to reduce the adverse effects of mycotoxins has been the addition of toxin binders, such as biocarbon [64, 65], hydrated sodium calcium aluminosilicate (HSCAS) [59, 66] or bentonite [67] into contaminated feed.

Biocarbon and its properties

Biocarbon (Bio-C) is defined as a carbon-rich, porous solid product produced by the thermal decomposition of carbonaceous biomass, such as wood, bamboo or nut shells. The process of the production of Bio-C is called pyrolysis in which biomass is heated in an airtight oven or kiln to a temperature between 200 to 900 ºC with limited or no air until no further volatiles are emitted.

The carbon content of Bio-C is more than 50 % of the dry weight of the biomass, depending on the material used. It also has a highly-porous structure, high surface area and various functional groups such as carboxyl, hydroxyl and phenolic groups.

Biocarbon products vary significantly in chemical composition, carbon stability, and quality and yield characteristics depending on the source of carbonaceous materials used and the production conditions. Volatile compound content, ash content, pH, water holding capacity, bulk density, pore volume and specific surface can be used to evaluate the quality and yield of Bio-C. However, there are main factors that play an important role on the yield and quality of Bio-C, including the temperature of the pyrolysis process and the nature of the biomass. Increasing temperature decreases its yield, however its carbon content increases proportionally to temperature. The surface area and pore size of Bio-C are also affected by the temperature. Bio-C with more pores and increased surface area can be obtained with increasing temperature since increased temperature removes the moisture and the volatile matters from the biomass surface. Regarding the nature of biomass, the moisture content and composition (cellulose, lignin etc.) of biomass significantly affect the yield. High moisture content and high lignin, for example, result in high yields and fixed carbon content [68-70]

Bio-C has been used extensively in the agroeconomic system for a range of environmental benefits, including carbon sequestration and mitigation of climate change, and as a sorbent for contaminants in water and soil [70]. Regarding its use in animal production, activated charcoal or carbon, which is a type of Bio-C that is treated or activated with O_2, CO_2 or chemicals to increase microporosity and surface area, was the most commonly used carbonaceous material in the past. Bio-C has been reported to absorb urea, ammonia, ammonium, and fat and fat-soluble compounds, bind and ameliorate the harmful effects of toxins, and has been added in animal feeds to promote growth performance [17, 64, 71].

Biocarbon on ammonia volatilization

Oya and Iu [72] examined deodorization performance of charcoal particles loaded with orthophosphoric acid (H_3PO_4) against NH_3 and trimethylamine and showed improved deodorization performance for NH_3 of woody charcoal when it was loaded with H_3PO_4.

Asada and Ishihara [73] studied the relationship between the carbonizing temperature of bamboo carbide and removal effect of harmful gases and odorants and found that NH_3 sorption has been shown to be greater in charcoal produced at lower (400 and 500°C) than at higher temperature. In another study, Asada and Ohkubo [74] indicated that low temperature-char removed greater NH_3 compared to those produced at high temperature in aqueous solutions. However, they also reported that better sorption was achieved when charcoal was treated with sulfuric acid.

Tsutomu and Takashi [75] compared the removal efficiencies for NH_3 and amine gases in the bags between woody charcoal and activated carbon carbonized at 500 ºC at two temperatures, 5 and 20 ºC and showed that woody charcoal had higher removal efficiency for those gases compared to that of commercial activated carbon.

A study was conducted by Thu and Koshio [76] to examine the effects of dietary bamboo charcoal (BC) supplementation on growth performance, body composition, and ammonia nitrogen excretion of juvenile Japanese flounder, *P. olivaceus*. The authors indicated that the concentration of ammonia nitrogen excretion in fish fed BC diets was significantly lower than the fish fed the control diet.

Steiner and Das [77] investigated the effects of biochar on NH_3 volatilization during co-composting of poultry litter and indicated that the addition of biochar in the poultry litter reduced NH_3 emissions by up to 64 %.

Ritz and Tasistro [78] evaluated surface-applied char on the reduction of NH_3 volatilization from broiler litter and found that surface application of peanut hull char was not effective in reducing NH_3 concentration in broiler production. However, they also showed that application of char treated with sulfuric acid resulted in significant reduction in NH_3 concentration.

The effectiveness of zeolite and biochar on the reduction of NH_3 emission during pretreatment process of chicken litter in open and closed conditions was investigated by Gorliczay and Tamás [79]. They reported that the addition of zeolite and biochar reduced the amount of produced NH_3 significantly, however there was no significant difference between the two additives.

The effects of various ratios of wood derived biochar added to the mixture of poultry manure with wheat straw (as a bulking agent) on NH_3 emissions in gaseous and liquid phases during laboratory scale composting was examined by Janczak and Malińska [80]. The results of the study indicated that the accumulated NH_3 emissions decreased with the increasing biochar rate. 30 % and 44 % reduction in gaseous ammonia emissions were observed by 5 and 10 % inclusion of biochar in the poultry manure, respectively.

Chen and Liao [81] evaluated the efficacy of biochar produced from different feedstocks (cornstalk, bamboo, wood, layer manure and coir) on the physico-chemical properties of the compost and NH_3 and methane emissions during composting of layer manure. The results showed that the addition of various biochars reduced the NH_3 and methane emissions. Moreover, cornstalk biochar treatment had the lowest NH_3 and methane productions, and it had lower NH_3 and methane emissions compared to the other treatments.

Biocarbon on growth performance

Proudfoot and Lamoreux [82] evaluated the performance of commercial broiler genotypes on fish meal diet with a charcoal supplement. Birds received activated charcoal at a concentration of 0.5 % for 21 days prior to slaughter. They found that there was a slight but not significant increase in body weight and the proportion of Grade A carcass weight in birds receiving the activated charcoal in their diet.

Kutlu [83] evaluated effects of dietary wood charcoal on 1-week-old male broiler for 6-week-period and found that the birds received wood charcoal supplementation showed higher feed intake, weight gain and feed conversion ratio than those did not received during first three weeks. In addition, the birds received 5 % charcoal in their diet indicated the highest feed intake, weight gain and feed conversion ratio. However, the results showed that wood charcoal in the feed had no effect on any of the above variables at the end of the study. Kutlu and Unsal [84] also studied effects of dietary wood charcoal on 1-week-old broilers for 6-week-period. It was found that birds received 2.5 % wood charcoal in starter and finisher diets had higher feed intake, improved body weight gain and feed conversion ratio during at the end of the study. The same author [71] investigated effects of dietary wood (oak) charcoal on broiler for a six-week-period and layers for a seven-week-period. They revealed that dietary wood charcoal significantly increased feed intake, body weight gain, and improved feed conversion ratio up to 28 days of age in broilers. In addition, dietary supplementation of wood charcoal had no significant effect on parameters related to growth performance and egg quality, but decreased number of cracked eggs in layers.

Samanya and Yamauchi [85] studied effects of dietary charcoal powder including wood vinegar compounds (CWVC) on morphological changes of the intestinal villi on 130-day-old male Single Comb White Leghorn chickens for 4-week-period and reported that the birds fed with 1 and 3 % CWVC tended to have higher body weight gain and feed conversion ratio. The results also stated that birds received 1 % CWVC in the diet had higher intestinal villus height, epithelial cell area and cell mitosis compared to the other groups.

The effect of hardwood charcoal on the performance of 204 one-day-old heavy Big 6 tom turkey was examined by Majewska and Pyrek [86] for 18-week-period. The results revealed that the birds fed the diet supplemented 0.3 % charcoal had significantly heavier body weight compared to the control treatment. Turkeys received charcoal in the diet consumed around 6.5 % less feed per kg gain than those in the control. In another study by the same author Majewska and Pudyszak [87], the effect of hardwood charcoal on the performance of broiler chickens was investigated. The data of the experiments indicated that the addition of 0.3 % charcoal in the diet improved body weight and feed conversion ratio by 3.5 % and 2 %, respectively.

The growth performance and intestinal villus histological alterations were examined by adding CWVC to the diet by Mekbungwan and Yamauchi [88] by using 24 male piglets for 30 days. It was reported that piglets received 1 and 3 % CWVC in their diet tended to gain more daily body weight while consuming less feed, and feed efficiency was also improved for these groups. Piglets received CWVC by 1 and 3 % (respectively) in the diet tended to have highest villus height, cell area and cell mitosis numbers whereas 5 % CWVC group showed almost similar results compared to the control.

An experiment was conducted by Ayanwale and Lanko [89] to evaluate the growth performance, nutrient utilization, egg quality characteristics and cost-benefit values of feeding different levels activated sheabutter tree charcoal to laying hens. Birds were fed until they were 22 weeks of age with diets containing 0, 10, 20, 30 or 40 % levels of charcoal. They found that feeding activated sheabutter charcoal had no effect on the performance parameters measured at the 3 stages among treatments.

Van and Mui [90] conducted two experiments to investigate the effect of including charcoal and of different processing methods of foliage from *Acacia mangium* in the diet on intake and performance of goats. In experiment 1, the goats were fed diets contained 0, 0.5, 1, or 1.5 g bamboo charcoal per kg body weight. The results revealed that supplementation of bamboo charcoal in the diet had no effect on total dry matter intake of the diet. Feeding at a rate of 0.5 or 1 g/kg BW charcoal resulted in increased crude protein digestibility compared to the control, however, it was not significantly different in charcoal treatments. In experiment 2, there were six treatments with or without 0.5 g charcoal/kg BW. No significant difference was found in dry matter intake in goats fed diets with or without bamboo charcoal. However, the goats received charcoal in their diets grew faster compared to those did not receive charcoal in the diet.

Two experiments were conducted to evaluate the effects of dietary supplementation of activated charcoal mixed with wood vinegar (AC) on broiler performance and antibiotic residue in eggs by Sung and You [91]. The data of the experiments showed that feed intake, body weight gain and feed conversion ratio were not significantly different among treatments, and no significant difference was found in relative weight of breast meat, leg and liver by the addition of AC in the diet.

The effect of feeding citrus wood charcoal on the growth performance parameters of broiler chicks was examined by Bakr [92]. The birds fed with diets supplemented with 0, 2, 4, and 8 % citrus wood charcoal of the ration dry matter, and the study lasted for 42 days. The data indicated that the birds received 2 % charcoal in their diet had higher body weights compared to the control at the first week of feeding with charcoal. They concluded that addition of citrus wood charcoal has considerable improvement in feed intake, body weight gain and feed conversion ratio up to 29 days of age.

Odunsi and Oladele [93] conducted a study to investigate whether dietary wood charcoal (WC) would affect growth performance, hematology and carcass characteristics, and to examine whether vegetable oil (VO) supplementation has an effect on similar parameters in broiler chickens. The four diets contained 0, 2.5, 5 or 7.5 % WC, and two diets contained 5 or 7.5 % WC with 1.5 % VO. They reported that feed intake was improved in birds received 5% WC with or without VO whereas birds received 7.5 % WC had the least consumption. Body weight gain and feed conversion ratio was significantly better in control birds compared to birds fed diets supplemented with WC without VO at the end of the experiment.

Ruttanavut and Yamauchi [94] investigated effects of dietary bamboo charcoal powder including vinegar liquid on growth performance and histological intestinal change in Aigamo Ducks for a 7-week-period. The results showed that the growth performance tended to improve with increasing dietary bamboo charcoal powder and bamboo vinegar solution (up to 1%) although feed intake, weight gain and feed efficiency were not significantly different. Moreover, the intestinal villus height, villus area, epithelial cell area and cell mitosis in all intestinal segments tended to increase with increasing dietary bamboo charcoal powder and bamboo vinegar solution.

Majewska and Mikulski [95] examined to determine whether silica grit is a necessary feed additive for turkeys and whether it can be replaced by charcoal or hardwood ash. They reported that turkeys fed a diet supplemented with 0.3 % charcoal had significantly higher body weight compared to other treatments after 10 weeks of rearing. After 20 weeks of rearing, the highest body weight was observed in turkeys receiving charcoal and hardwood ash. Rearing efficiency index (REI) was also higher in turkeys receiving charcoal and hardwood ash than the control. Moreover, turkeys fed a diet with charcoal had the lowest mortality rate among treatments.

Effects of dietary bamboo charcoal powder including vinegar liquid (SB) on 48 154-day-old White Leghorn hens performance and intestinal histological changes were studied by Yamauchi and Ruttanavut [96] for 33-week-period. The results indicated that feed intake, body weight gain, feed efficiency, and mean egg weight were not significantly different among the birds fed with or without SB. The birds fed 0.5 and 1.0 % SB tended to have higher total egg production. Moreover, there was increasing trend of intestinal villus height, cell area, and cell mitosis number in the birds received SB in the diet.

Thu and Koshio [76] studied the effects of dietary bamboo charcoal (BC) supplementation on growth performance, body composition, and ammonia nitrogen excretion of juvenile Japanese flounder (*Paralichthys olivaceus*) in a 50-day feeding trial. The diets contained 0, 0.25, 0.5, 1, 2 and 4 % BC. Based on the results, weight gain, specific growth rate, feed efficiency ratio and protein efficiency ratio were significantly higher in fish fed 0.5 % BC diet compared to the control. In addition, 0.5 % BC group had lower feed conversion rates than those of the other BC treatments, suggesting a potential quadratic response.

The effects of activated charcoal (AC) on apparent total tract nutrient digestibility and taste preference in cows was studied by Erickson and Whitehouse [97]. In the first experiment, cows fed diets containing about 60 % poor-quality corn silage with 0, 20, or 40 g of AC that were mixed into the diet during p.m. feeding. The results showed that DMI and apparent total-tract nutrient digestibility of NDF (non-digestible fiber), hemicellulose, CP (crude protein), and milk fat content were increased in cows received AC in their diet. In experiment 2, cows were fed 0, 20 or 40 g of AC per day with using good-quality silage, and no difference in apparent total-tract nutrient digestibility or milk composition were found. In experiment 3, cows received

0, 10, 20, 40 or 80 g/d AC to determine taste preference, which resulted in increased AC level decreased the preference for that particular feed.

Kana and Teguia [98] evaluated charcoal from maize cob (charcoal A) or seed of *Canarium schweinfurthii* Engl (charcoal B) using 3-week-old chicks for four-week-period. The authors found that birds fed the range of 0.2-0.6 % of either charcoal A or B had significantly higher final body weight as compared to control groups. However, over 0.6% charcoal in the feed depressed overall body weight and weight gain. The result also indicated that birds fed 0.2% charcoal showed best growth performance among treatments.

The effects of bamboo charcoal including vinegar liquid (BV) on growth performance and meat quality in Wenchang broilers was examined by Cai and Jiang [99]. The birds fed diets supplemented with 0.5 % BV or not, for 3 weeks. The authors revealed that there was a slight improvement in weight gain in BV supplemented group even though there was no statistically significant difference among treatments.

Jiya and Ayanwale [100] conducted a 56-day feeding trial to evaluate the effect of activated coconut charcoal on the performance and nutrient digestibility of broilers. They reported that adding 2 % activated coconut shell charcoal in the starter diet increased feed intake and final body weight. It is suggested, however, that activated coconut shell charcoal can be included in the finisher diet up to 0.5% inclusion level for better performance. In another study, Jiya and Ayanwale [101] also reported that 0.5% activated charcoal supplementation increased relative organ weights, reduced cholesterol level of the broiler meat as a result or efficient mineral intake and nutrient utilization due to the absorptive effect of the activated charcoal.

Chu and Kim [102-103] investigated the effects of bamboo charcoal on growth performance in fattening pigs in two different experiments. The results from the first study indicated that fattening pigs fed diet with bamboo charcoal or bamboo vinegar supplementation had better final growth performance. It was also found that at a range from 0.3 to 0.6 % charcoal supplementation to the diet resulted in increased growth performance.

The effect of sea tangle and charcoal (STC) supplementation on growth performance and meat characteristics of ducks was examined by Islam and Ahmed [104]. The ducks fed diets supplemented with 0, 0.1, 0.5, 1 % STC, and antibiotic, and the experiment lasted 3 weeks. They reported that no significant difference was observed in daily feed intake, weight gain or feed efficiency among treatments on a weekly basis.

Oso and Akapo [105] studied the effects of unpeeled cassava (*Manihot esculenta Crantz*) root meal (UCRM) supplemented with or without charcoal in broilers for 6-week-period. The results indicated that birds fed with charcoal supplementation (6 gr/kg) had higher cumulative live weight, weight gain and feed intake. Apparent crude protein digestibility was the highest for chicks fed control diet (with or without 6gr/kg charcoal).

Rattanawut [106] assessed the combined effect of bamboo charcoal powder and bamboo vinegar liquid (BCV) on growth performance and intestinal morphology by using 4-week-old Betong chickens for 16-week-period. He found that the treatments consisted of 1% BCV had the best growth performance although there was no significant difference for feed intake, body weight gain and feed efficiency among treatments. In addition, the birds fed 1.0 % BCV had the highest jejunal villus height and jejunal-villus area.

Quaiyum and Jahan [107] studied effects of dietary bamboo charcoal (BC) on ammonia reduction, growth performance and intestinal histological alterations of *Pangasius hypophthalmus* for a 50 days period. It was revealed that weight gain value and feed conversion ratio were higher in those received from 0.5 to 2 % BC in the diets than the control. Furthermore, increased villus height and consequently decreased lumen areas were seen with increasing dietary BC level even though there was no significant difference among treatments with or without BC supplementation.

Boonanuntanasarn and Khaomek [108] examined the effects of dietary activated charcoal (AC) on health status, intestinal morphology and fillet geosmin content of Nile tilapia prior to harvesting. AC was supplemented into diets at levels of 0, 10, 20, and 30 g per kg of the dry diet. They found that final weight, weight gain, specific growth rate and feed conversion ratio were not significantly different among treatments.

Biocarbon as a mycotoxin binder

Ademoyero and Dalvi [109] investigated the efficacy of activated charcoal and other agents in the reduction of hepatotoxic effects of a single dose AFB_1 in chickens. Activated charcoal (200 mg/kg), GSH (200 mg/kg), cysteine (200 mg/kg), selenium (as sodium selenite, 2 mg/kg), beta-carotene (25 mg/kg), and fisetin (25 mg/kg) were administered orally by stomach tube either alone or in combination with AFB_1 (6 mg/kg, in oil) to treatments. The results showed that the oral administration of these agents simultaneously with AFB_1 reduced the liver injury caused by AFB_1 in chickens.

The effect of aflatoxicosis by purified AFB_1 and its reversal by activated charcoal, phenobarbital and reduced glutathione was examined by Dalvi and McGowan [110]. Day-old Hubbard X Hubbard broilers maintained on a diet containing 0, 2.5, 5, 10 mg AFB_1 for 8 weeks. The author observed reductions in weight gain and feed intake at 2.5 and 5 mg, however changes were evident at 10 mg contaminations. Birds receiving 10 mg AFB_1 and activated charcoal (0.1% in the feed) or either reduced glutathione (0.05%) or phenobarbital (0.05%) in their drinking water showed an improvement in feed intake and weight gain over the birds receiving

10 mg AFB_1 alone. In addition, the simultaneous presence of these agents with AFB_1 considerably prevented the inhibitory effect of AFB_1 on the microsomal cytochrome P-450 and benzphetamine N-demethylase activity. The author concluded that these agents were able to provide moderate protection against AFB_1-induced liver injury.

Rotter and Frohlich [111] examined the absorption ability of activated charcoal to ochratoxin A (OA) *in vitro*, and to reduce the toxic effects of OA in a feeding study. They reported that activated charcoal was able to remove OA from the solution. However, the addition of activated charcoal in OA contaminated diets appears to be an impractical method of reducing OA toxicity to chicks that are continuously consuming OA.

The amelioration of aflatoxicosis in broiler and Leghorn chickens was examined by Kubena and Harvey [112] by feeding a hydrated, sodium calcium aluminosilicate (HSCAS) or activated charcoal (AC). AC or HSCAS at a concentration of 0.5 % of the diet were added into diets containing no AF, 5 mg of AF per kg, or 7.5 mg of AF per kg. The results indicated that AF reduced body weight gains in both broilers and leghorns. A significant increase in the relative weights of the liver, kidney, proventriculus, and gizzard as well as significant increases in activity of serum gamma glutamyl transferase; also, significant decreases in the relative bursa weights as well as the concentrations of serum total protein and albumin were found in leghorns when they were fed 5 mg of AF per kg of the diet with or without charcoal. The author summarized that HSCAS can modulate the toxicity of AF in the chicken; however, adding activated charcoal to the diet did not appear to have an effect on the toxicity of AF.

The effect of activated charcoal on the toxicity of 0.5 mg AFB_1 in feed was investigated by Jindal and Mahipal [64] by using 60 broiler chicks fed from 1 to 42 days of age. The performance of birds and various biochemical parameters were assessed. The author showed that the feeding of activated charcoal with AF reduced the inhibitory effect of AF on body weights and feed intake. In addition, the results also showed improvement in the serum aspartate aminotransferase, alkaline phosphatase, total proteins, calcium and phosphorus levels when the AF-contaminated diets contained activated charcoal.

Edrington and Sarr [113] investigated the effect of hydrated, sodium calcium aluminosilicate (HSCAS), acidic HSCAS, and activated charcoal (AC) on reduction of urinary excretion of AFM_1 in turkey poults in the first experiment, and to evaluate the ability of two types of AC to modify aflatoxicosis when added to AF-contaminated diets of turkey poults in the second experiment. In the first experiment, at a concentration of 0.75 mg AFB_1 per kg was orally dosed via gelatin capsule while 0.75 mg AF per kg was included into the diets in the second experiment. The results showed that AC decreased AFM_1 excretion in the first experiment whereas no protective effects from AF toxicity were found in the second one.

The effect of two activated carbons (CACl and CAC2) and a hydrated sodium calcium aluminosilicate (HSCAS) on carryover of AFB_1 from feed to AFM_1 in milk was determined by Galvano and Pietri [114] by using 12 late-lactation Friesian cows that fed a basal diet containing AFB_1, with or without the absorbents at 2 % rate. There was a reduction in both feed and milk AF concentration during the week the cows received the absorbents in their diet. However, feed intake, body weight, and milk production and composition were not affected by the addition of activated carbons in the diet. In another study, Galvano and Pietri [115] conducted in vitro tests to evaluate the efficacy of 17 activated carbons (ACs) in binding AFB_1 from solutions, and compare them with an HSCAS. The finding of the experiment demonstrated that ACs with high methylene blue index (MBI) and low surface acidity have greater affinity for AFB_1 in vitro than HSCAS and are potential protective agents against aflatoxicosis.

To evaluate the effectiveness of a superactivated charcoal (SAC) in alleviating mycotoxicosis, two experiments were also conducted by Edrington and Kubena [116]. A total of 432 male broiler chicks (216 per experiment) were maintained diets containing 4 mg AF or 6 mg T-2 toxin/kg of diet, with or without 0.5 % SAC, from 1 to 21 days of age. The findings of the experiments indicated that body weight gain was decreased by AF and T-2 toxin, and relative weight of liver, kidney and spleen were increased by AF in the feed. The addition of SAC in the AF-contaminated diet resulted in BW gains that were intermediate between gains of chicks fed AF and those of controls. The authors concluded that the addition of dietary SAC is moderately effective in alleviating the toxicity of AF, but little benefit was observed when T-2 toxin was fed to growing broiler chicks.

The effectiveness of 19 activated carbons (ACs), a hydrated sodium calcium aluminosilicate (HSCAS) and sepiolite (S) on in vitro affinity in binding ochratoxin (OA) and deoxynivalenol (DON) from solution were tested by Galvano and Pietri [117]. The authors also tested relationships between absorption ability and physicochemical parameters of ACs (surface area, iodine number, methylene blue index). The results indicated that ACs have high *in vitro* affinity for chemically different mycotoxins, and they could be a potential multi-mycotoxin-sequestering agent.

Rao and Chopra [118] examined the carry-over of AFB_1 into milk as AFM_1 in early-lactation goats fed a diet contaminated with AFB_1 over 14 days. Feed contained no adsorbent, or 1 % dry matter feed intake of sodium bentonite or activated charcoal. They reported that AFM_1 excretion in milk was reduced, and percent carry-over was significantly declined by adsorbent supplementation to diet without affecting milk composition parameters. In another study, Rao and Chopra [119] also showed that there was an improvement in weight of goats fed a AF-contaminated diet supplemented with charcoal or bentonite.

Teleb and Hegazy [120] investigated kaolin and activated charcoal for their ability to reduce the detrimental effects of AF in broilers. Birds fed diets containing 0 or 0.03 mg AF per kg, with 0.5 % kaolin or activated charcoal for 45 days. The authors showed that supplementation of kaolin and activated charcoal to the AF contaminated diet reduced the mortality rate and improved body weight gain and efficiency of feed utilization.

The toxicity of fumonisin B_1 (FB) from culture material with and without activated carbon was evaluated by Piva and Casadei [121] by using 56 weanling piglets. The diets contained 2 or 30 mg FB, with or without activated charcoal at 1 % of the diet. The findings of the experiment showed that the dietary supplementation of activated carbon had no beneficial effect when added alone or in combination to the FB-contaminated diet.

Denli and Okan [66] tested different absorbents, including HSCAS, diatomite, and activated charcoal for their ability to alleviate the harmful effects of AFB_1. Broiler chicks were fed diets containing these absorbents at 0.25 % feed dry matter. On day 42, blood serum parameters and liver weights and histology were measured. They found that the HSCAS was able to limit the toxic effects of AF on liver whereas activated carbon had no significant effect on neither blood serum nor liver parameters.

Kana and Teguia [122] evaluated the effect of plant charcoal from *Canarium schweinfurthii* (charcoal A) and maize cob (charcoal B) in the diet on aflatoxin B_1 toxicosis in broiler chickens. Birds received diets containing 0 or 0.022 mg FB_1, supplemented with either 0.2, 0.4, or 0.6% of charcoal A (A0.20, A0.40 and A0.60 respectively) or charcoal B (B0.20, B0.40, and B0.60 respectively). The author concluded that 0.2 % of *Canarium schweinfurthii* charcoal and 0.6% of maize charcoal could be used as feed additives to absorb AFB_1 and promote growth performance of broiler chickens.

Al Masad and Thalij [123] studied activated charcoal for alleviation of toxicity and induced stress of AF and T-2 toxin on immune response system by using 160 male broiler chicks fed from 1 to 21 days of age. The feed contained 2.5 mg AF or 4 mg/kg T-2 toxin, or in combination. They found that the addition of activated charcoal at a concentration of 0.4% to the feed in the diet was responsible for reduction the toxic effects on all parameters when AF or T-2 toxin present individually or in combination.

Jayasri and Kumar [124] examined the combined effect of AF and OA (Ochratoxin A) on kidney function parameters in broilers and their amelioration using adsorbents. 1 mg AF and 2 mg OA were mixed with the diet, and 0.4 % activated charcoal and 0.2 % lyophilized yeast culture were used to test their efficacy in ameliorating the combined toxicity. A total of 128 one day old broiler chicks were reared for 42 days. Based on the findings of this study, the addition of activated charcoal at 0.4% partially ameliorated the effect on combined toxicity where as a combination of activated charcoal at 0.4% and yeast culture at 0.2 % has complimentary effect in ameliorating the combined toxicity in broilers. The author, however, also reported that combination of activated charcoal and yeast culture could not completely ameliorate the combined toxicity in broilers.

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CHAPTER 2

EVALUATION OF AN ENGINEERED BIOCARBON ON REDUCTION OF AMMONIA VOLATILIZATION AND ON GROWTH PERFORMANCE PARAMETERS IN BROILER PRODUCTION[1]

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SUMMARY

Ammonia (NH_3) volatilization from the house litter can have detrimental effects on bird health and performance, worker's health and the environment. Carbonized biomass (biocarbon or charcoal) has been suggested to reduce NH_3 generation and volatilization, by applying it directly to the litter or adding it into the feed. The purpose of this study was to evaluate the effectiveness of an engineered biocarbon (Bio-C) as a topical application to the litter on NH_3 volatilization (Exp-1), the effects as a feed supplement for reducing NH_3 volatilization (Exp-2), and to evaluate the effect of Bio-C as a feed supplement for improvement of growth performance (Exp-3). Neither topical application to the litter (Exp-1) nor the addition of Bio-C in the feed (Exp-2) had an effect on NH_3 generation or volatilization compared to the control treatments. BW for birds fed 0.2 and 0.6% Bio-C were significantly higher than the control treatment at the end of the 28 days (Exp-2). However, when birds were reared for a full six weeks, performance parameters were not affected by any of Bio-C treatments in Exp-3. The effect of carbonized biomass on alleviating the effects of toxin exposure are well-known, however it does not appear to have as strong an impact on NH_3 control or growth performance.

Key words: biocarbon, ammonia, feed additive, broiler

DESCRIPTION OF PROBLEM

Broilers are typically raised in large, open structure houses with a floor that is covered with a bedding material such as wood shavings, rice hulls, peanut shells, and crushed corn cobs. Litter is defined as the mixture of these bedding materials with birds' excreta, spilled feed, water and feathers [1]. In the US, because of the availability and expense of bedding materials and the cost of labor, used litter is recycled for multiple flocks in the same house [2]. NH_3 emission can become a major concern as the same litter is used over the course of several flocks, and it is a problem that has received attention in the literature [3, 4].

Birds excrete excess N in the form of uric acid, which is converted into NH_3 by hydrolysis, mineralization, and volatilization by several microbial enzymes [5], and this process is affected by the moisture, temperature, pH of the litter, stocking density, and age of birds [6, 7]. Elevated atmospheric NH_3 concentration in the house can have negative effects on bird health, worker's health, and the environment. During the first weeks of the rearing, it is crucial to maintain a low concentration of NH_3 in the house. Birds in commercial houses are often exposed to elevated NH_3, which can exceed 50 ppm if there is inadequate ventilation [2]. Continuous exposure to levels as low as 20 ppm NH_3 can decrease feed intake and feed efficiency, resulting in decreased overall growth performance [8, 9]. When exposed to high levels of NH_3, birds develop respiratory disorders, keratoconjunctivitis, have a difficulty locating feed and water, and can become susceptible to secondary infections [2, 7, 10]. Elevated NH_3 levels in the house may also have negative effects on worker's health. Exposure to over 20 ppm NH_3 may cause irritation of the respiratory tract, eyes, throat, increased susceptibility to secondary infections and cough [11, 12].

With the reuse of litter over several flocks in commercial broiler houses being the common practice, generation and release of NH_3 increases relative to houses that replace with fresh litter for each flock. Adequate ventilation reduces NH_3 concentration in the houses. Additionally, due to heating of the house, farmers often reduce ventilation rates to cut heating expenses, especially in cold weather [13]. Use of litter amendment products, such as acidifying agents [14], microbial and enzyme inhibitors [15], and absorbents [16] have commonly been used to control NH_3 generation, however those products typically have short-term efficacy and begin to decline in the first two weeks of rearing period [8]. These situations have driven the industry to try different litter management strategies, in addition to ventilation, to control NH_3 in broiler houses over the entire grow-out.

One such strategy is the inclusion of pyrolyzed carbonaceous material (biocarbon) applied to the litter, or alternatively into the feed. Biocarbon, which has high porosity and high internal surface area, generally refers to the carbonaceous residue of wood, cellulose, coconut shells or other by-products left after heating organic matter in the absence of oxygen [17]. This odorless and tasteless black powder has high water holding capacity, can absorb urea, ammonia, ammonium, and fat and fat-soluble compounds, as well as bind and ameliorate the harmful effects of toxins [18, 19]. Therefore, the aim of this study was to evaluate the effectiveness of an engineered biocarbon (Bio-C) at reducing NH_3 volatilization when applied either directly to the litter or added into the feed, and for an effect on growth performance in broilers.

MATERIALS AND METHODS

The experiments were conducted at the University of Georgia Poultry Research Center, with research protocols approved by the Institutional Animal Use and Care Committee.

Exp-1 and Exp-2:

Exp-1 and Exp-2 each consisted of four treatments, with four replicates per treatment. Used broiler litter was used as the bedding material with each pen. In Exp-1, the four treatments were 0 (control), 50, and 100 lbs/1000 ft^2 of Bio-C on the litter, and a positive control treatment of 50 lbs/1000 ft^2 sodium bisulphate (PLT). Bio-C and PLT were spread manually onto the litter before placement of chicks. In Exp-2, Bio-C was incorporated into the feed at 0 (control), 0.2, 0.6 and 1.0 % of the total diet. A total of 1760 Cobb-500 male chicks were obtained on day-of-hatch and randomly distributed among treatment pens (55 bird/pen, 0.75 ft^2/bird). The birds were fed a standard broiler diet for 21 days in Exp-1 and for 28 days in Exp-2, with feed and water provided *ad libitum*. Titanium dioxide (TiO_2, 0.2%) was used as an indigestible marker. Live performance data were recorded at 0, 7, 14, and 21 days of age for both experiments and on day 28 for Exp-2, and mortality was recorded daily. Ileal digesta were collected from 3 birds per pen for energy digestibility at day 21. All samples were dried at 100˚C for 24 h and then ground. Feed and digesta samples were sent to the University of Georgia Agricultural and Environmental Services Laboratories (Feed and Environmental Water Laboratory; Athens, GA) for gross energy and TiO_2 determination.

NH_3 equilibrium concentration in parts per million was measured at day 1, 5, 8, 12, 15, and 21 after the application of the products by using a static-chamber system following the procedure described by [4]. In this procedure, the NH_3 produced during a 24-h period was collected into the air in an inverted container settled on the litter was trapped in a beaker containing 25 mL of 0.1 N H_2SO_4. NH_3 was measured colorimetrically as ammonium (NH_4^+-N) by the salicylate-hypochlorite method [20]. The milligrams of ammonium collected in the beakers were transformed into the millimoles of NH_3 released per square meter of floor space and reported as per day for data analysis. NH_3 concentration in parts per million (ppm) was also measured by using a MSA Chillgard RT Ammonia Monitor, which employs photoacoustic spectroscopy for trace gas detection. Approximately 150 grams of litter from the pens was collected at each NH_3 sampling period, then submitted to the University of Georgia Agricultural and Environmental Services Laboratories for pH analysis.

Exp-3:

This experiment consisted of four treatments, with eight replicates per treatment. Bio-C was incorporated into the feed at 0 (control), 0.1, 0.2 and 0.4 % of the total diet. A total of 800 Cobb-500 male birds were obtained on day-of-hatch and randomly distributed among treatment pens (25 birds/pen, 0.75 ft^2/bird). The birds were fed standard broiler started crumble (0 to 21), pelleted grower (21 to 35) and pelleted finisher (35 to 42) diets. Feed and water were provided *ad libitum*. Titanium dioxide (TiO_2, 0.5%) was used as an indigestible marker. Live performance data were recorded weekly, and mortality was recorded daily for 42 days. Ileal digesta for energy digestibility and jejunum tissues for histology analysis were collected from 3 randomly selected birds per pen on both day 21 and 42. After collection, all jejunum tissues were immediately placed in 10% neutral buffered formalin. After fixation, tissues were cut into cassettes and routinely processed overnight. Samples were then embedded in paraffin, sectioned at 4 microns, placed on slides, and then stained with hematoxylin and eosin and cover slipped. Villus height and crypt depth were measured using methods by [21] and the ImageJ software (National Institutes of Health) at the Poultry Diagnostic and Research Center Histology Laboratory (Athens, GA).

Data were analyzed using one-way ANOVA via SPSS, with significant differences ($P \leq 0.05$) separated by Duncan's Multiple Range Tests.

RESULTS AND DISCUSSION

Exp-1:

The topical application of Bio-C to the litter did not have an effect on NH_3 concentration (Figure 2.1) or growth performance (Table 2.5) compared to the control. In the 50 lbs/1000 ft^2 of Bio-C treatment, there was a 0.03 difference in FCR ($p = 0.02$) compared to other treatments. Ritz and Tasistro [22] also found similar results. The authors reported that topical application of peanut hull char to the litter had no effect on reduction of NH_3 volatilization or on the birds' performance parameters. Also, in this study, the PLT treatment had significantly lower NH_3 concentration when compared to other treatments at day 1, but there were not any significant differences thereafter. Litter pH (Table 2.4) was significantly lowered by PLT application to the litter on day 1, 5 and 15, whereas Bio-C had no effect on litter pH at any sampling period. PLT is known to lower litter pH and provide an ionic effect that enhances acidification, converting litter ammonium into ammonium sulfate, reducing NH_3 generation and volatilization [23]. In addition, when litter pH is below 7, NH_3 release is negligible. Release does not become significant until litter pH is near 7 and reaches high levels at pH 8 and above [24]. Even though PLT application significantly reduced litter pH compared to both control and Bio-C on day 1, 5 and 15, it was below 7 only on day 1 of the experiment, which explains similar NH_3 volatilization results.

Exp-2:

Neither NH_3 concentrations (Table 2.6) nor litter pH (Table 2.8) were significantly different between the control treatment and the feed-applied Bio-C treatments at any time point. Body weight for treatments 0.2 and 0.6 % Bio-C were significantly higher than the control treatment at day 21. Results on day 28 also showed that birds receiving 0.2 and 0.6 % Bio-C had significantly higher body weight and cumulative weight gain. However, Yamauchi and Ruttanavut [25] conducted an experiment on forty-eight white leghorn hens fed a diet containing 0 (control), 0.5, 1.0 and 1.5% bamboo charcoal powder and a bamboo-vinegar compound liquid in the feed and reported that growth performance did not differ when compared to the control group. Also, we found no significant differences for ileal digestible energy (IDE) or feed conversion ratio, therefore, the mechanism behind the difference in BW at 0.2 and 0.6% was investigated further in Exp-3.

Exp-3:

Body weight, weight gain and feed conversion ratio (Table 2.11) were not significantly different among treatments throughout the 42 days of age. IDE was significantly lower in 0.1% and 0.4% treatments when compared to the control and 0.2% treatments on both day 21 and 42. This reduction in IDE may be explained by the inclusion of Bio-C in the diet having a negative effect on the absorption of fat and fat-soluble nutrients. Kutlu and Unsal [26] found that adding charcoal impaired lipid absorption, which could reduce energy available for optimum growth in broilers when they fed sixty-four 1-week-old chicks for six weeks with diets containing 0, 2.5, and 5 % wood charcoal. There was also no significant difference in the jejunal villus height, crypt depth or villus height to crypt depth ratio at either of the sampling periods. Kutlu [27] fed sixty-four one-week-old broiler chicks with diets containing 0 (control), 2.5, 5, and 10 % of wood charcoal for a six-week period and reported that bird's performance was not affected by the inclusion of the charcoal at the end of the experiment. Carbonized biomass is well-known as an absorptive agent, showing benefit in toxin-challenged animals [28-31], however, in this study, we failed to find any growth-promoting effects under standard grow-out conditions.

CONCLUSIONS AND APPLICATIONS

1. Neither surface application of Bio-C nor addition of Bio-C into the feed were effective in reducing ammonia in broiler production.
2. Bio-C, as a feed additive to broiler diets, had no effect on growth performance parameters.
3. Since the reuse of litter over several flocks in commercial broiler houses is common, new strategies or applications for the entire grow-out period to control NH_3 are needed.

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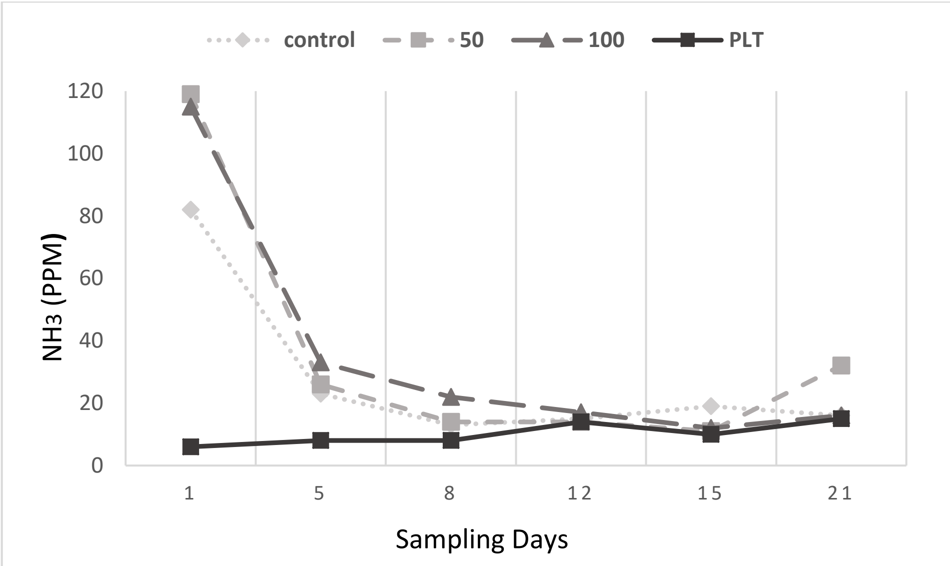
Table 2.1. Ingredient and nutrient composition of the diets in Exp-1, Exp-2 and Exp-3 (as-fed basis)

	Amount			
	Exp-1 and Exp-2	Exp-3		
Item	Starter	Starter	Grower	Finisher
Ingredient (% of diet)				
Corn, grain	57.47	55.93	61.71	65.48
Soybean meal, 48% CP	35.25	36.84	31.90	28.28
Soybean oil	2.63	3.15	2.58	2.75
Limestone	1.59	1.53	1.48	1.42
Dicalcium phosphate	1.39	1.50	1.41	1.31
Salt	0.49	0.44	0.39	0.32
DL-methionine	0.21	0.20	0.21	0.12
L-Lysine-HCI	0.14	0.08	-	-
Vitamin premix[1]	0.35	0.25	0.25	0.25
Mineral premix[2]	0.08	0.08	0.08	0.08
Calculated composition				
ME, kcal/kg	3050.00	3080.00	3100.00	3150.00
CP, %	21.41	22.00	20.00	18.50
Crude fat, %	4.86	5.31	4.93	5.19
Ca, %	0.95	0.95	0.90	0.85
Available P, %	0.45	0.48	0.45	0.43
Lys, %	1.19	1.31	1.11	1.01
Met, %	0.53	0.56	0.55	0.44

[1]Supplied per kilogram of diet: vitamin A, 5511 IU; vitamin D3, 1102 ICU; Vitamin E, 11.02 IU; vitamin B12, 0.01 mg; Biotin, 0.11 mg; Menadione, 1.1 mg; Thiamine, 2.21 mg; Riboflavin, 4.41 mg; d-Pantothenic Acid, 11.02 mg; Vitamin B6, 2.21 mg; Niacin, 44.09 mg; Folic Acid, 0.55 mg; Choline, 191.36 mg.

[2]Supplied per kilogram of diet: Mn, 107.2 mg; Zn, 85.6 mg; Mg, 21.44 mg; Fe, 21.04; Cu, 3.2 mg; I, 0.8 mg; Se, 0.32 mg.



control: no litter treatment application

50: 50 lbs/1000 ft^2 of Bio-C was applied on the litter

100: 100 lbs/1000 ft^2 of Bio-C was applied on the litter

PLT: 50 lbs/1000 ft^2 of PLT was applied on the litter

N: 220 birds/treatment (55 birds/replicate)

Figure 2.1. Effect of topical application of Bio-C and PLT to the litter on NH_3 concentration (ppm) in Experiment-1

Table 2.2. Effect of topical application of Bio-C and PLT to the litter on NH_3 concentration (ppm) in Experiment-1.

sampling days	Treatments					
	[1]control	[2]50	[3]100	[4]PLT	[5]SEM	P-value
1	82±40[b]	119±36[b]	115±53[b]	6±4[a]	9.50	0.04
5	23±7	26±14	33±20	8±6	3.24	0.10
8	13±3	14±8	22±17	8±5	2.44	0.33
12	15±3	14±8	17±12	14±5	1.98	0.92
15	19±10	11±5	12±5	10±2	1.52	0.24
21	16±8	32±29	16±9	15±7	4.07	0.43

[a-b]Means with different superscripts within a column differ significantly ($P \leq 0.05$).

[1]control: no litter treatment application

[2]50: 50 lbs/1000 ft^2 of Bio-C was applied on the litter

[3]100: 100 lbs/1000 ft^2 of Bio-C was applied on the litter

[4]PLT: 50 lbs/1000 ft^2 of PLT was applied on the litter

[5]SEM: Standard error of mean

N: 220 birds/treatment (55 birds/replicate)

Table 2.3. Effect of topical application of Bio-C and PLT to the litter on NH_4-N concentration (ppm) in Experiment-1

	Treatments					
sampling days	[1]control	[2]50	[3]100	[4]PLT	[5]SEM	P-value
1	434±109[b]	528±186[b]	607±139[b]	41±47[a]	37.91	<0.01
5	183±94	227±182	227±157	34±48	32.80	0.18
8	99±76	125±125	159±144	33±17	25.77	0.40
12	94±59	71±62	133±155	107±95	25.15	0.85
15	196±227	94±121	117±159	63±63	27.34	0.38

[a-b]Means with different superscripts within a column differ significantly ($P \leq 0.05$).

[1]control: no litter treatment application

[2]50: 50 lbs/1000 ft^2 of Bio-C was applied on the litter

[3]100: 100 lbs/1000 ft^2 of Bio-C was applied on the litter

[4]PLT: 50 lbs/1000 ft^2 of PLT was applied on the litter

[5]SEM: Standard error of mean

N: 220 birds/treatment (55 birds/replicate)

Table 2.4. Effect of topical application of Bio-C and PLT to the litter on litter pH in Experiment-1

sampling days	Treatments					
	[1]control	[2]50	[3]100	[4]PLT	[5]SEM	P-value
1	8.3±0.2[b]	8.3±0.2[b]	8.5±0.1[b]	5.8±2.2[a]	0.28	0.02
5	8.1±0.1[b]	8.1±0.2[b]	8.2±0.1[b]	7.1±0.5[a]	0.07	<0.01
8	8.1±0.3	7.9±0.5	8.2±0.3	7.6±0.3	0.87	0.08
12	8.4±0.3	8.1±0.3	8.2±0.4	8.0±0.4	0.09	0.48
15	8.6±0.2[b]	8.2±0.3[ab]	8.4±0.4[ab]	7.9±0.3[a]	0.07	0.03

[a-b]Means with different superscripts within a column differ significantly ($P \leq 0.05$).

[1]control: no litter treatment application

[2]50: 50 lbs/1000 ft^2 of Bio-C was applied on the litter

[3]100: 100 lbs/1000 ft^2 of Bio-C was applied on the litter

[4]PLT: 50 lbs/1000 ft^2 of PLT was applied on the litter

[5]SEM: Standard error of mean

N: 220 birds/treatment (55 birds/replicate)

Table 2.5. Effect of topical application of Bio-C and PLT to the litter on bird's performance in Experiment-1

variables	treatments					
	[1]control	[2]50	[3]100	[4]PLT	[5]SEM	P-value
Day 0 to 7						
BW (kg/bird)	0.18±0.01	0.18±0.01	0.17±0.01	0.18±0.01	<0.01	0.43
WG (kg/bird)	0.13±0.00	0.13±0.01	0.13±0.01	0.14±0.01	<0.01	0.73
FCR	1.05±0.02	1.08±0.02	1.04±0.03	1.04±0.02	<0.01	0.12
FI (kg/bird)	0.14±0.00	0.14±0.01	0.14±0.00	0.14±0.01	<0.01	0.59
Mortality (%)	0.00±0.00	0.46±0.91	0.91±1.05	0.00±0.00	0.17	0.25
Day 0 to 14						
BW (kg/bird)	0.48±0.01	0.48±0.01	0.47±0.01	0.48±0.01	<0.01	0.59
WG (kg/bird)	0.43±0.01	0.43±0.01	0.42±0.01	0.43±0.00	<0.01	0.61
FCR	1.46±0.02	1.51±0.02	1.49±0.02	1.47±0.04	<0.01	0.09
FI (kg/bird)	0.63±0.01	0.65±0.01	0.63±0.01	0.64±0.01	<0.01	0.17
Mortality (%)	0.47±0.95	1.42±1.83	0.91±1.05	0.46±0.93	0.31	0.67
Day 0 to 21						
BW (kg/bird)	0.92±0.03	0.91±0.01	0.92±0.02	0.92±0.01	<0.01	0.90
WG (kg/bird)	0.86±0.03	0.87±0.01	0.88±0.01	0.87±0.01	<0.01	0.76
FCR	1.49±0.02[a]	1.53±0.01[b]	1.50±0.01[a]	1.49±0.02[a]	<0.01	0.02
FI (kg/bird)	1.30±0.02	1.32±0.02	1.31±0.02	1.31±0.03	<0.01	0.69
Mortality (%)	0.47±0.95	1.42±1.83	0.91±1.05	1.38±0.92	0.31	0.68

[a-b]Means with different superscripts within a column differ significantly ($P \leq 0.05$).

[1]control: no litter treatment application

[2]50: 50 lbs/1000 ft^2 of Bio-C was applied on the litter

[3]100: 100 lbs/1000 ft^2 of Bio-C was applied on the litter

[4]PLT: 50 lbs/1000 ft^2 of PLT was applied on the litter

[5]SEM: Standard error of mean

N: 220 birds/treatment (55 birds/replicate)

Table 2.6. Effect of Bio-C as a feed additive on NH_3 concentration (ppm) in Experiment-2

sampling days	Treatments					
	[1]control	[2]0.2	[3]0.6	[4]1.0	[5]SEM	P-value
1	56±11	64±30	73±27	74±46	7.72	0.83
5	21±7	26±19	27±15	21±7	3.74	0.90
8	18±10	22±15	30±32	18±11	4.80	0.79
12	7±2	11±6	10±6	7±3	1.13	0.67
15	13±10	11±11	10±6	11±7	2.19	0.97
21	68±73	56±55	89±73	65±73	17.37	0.92

[1]control: basal diet only

[2]0.2: 0.2% of Bio-C was added in the diet

[3]0.6: 0.6% of Bio-C was added in the diet

[4]1.0: 1.0% of Bio-C was added in the diet

[5]SEM: Standard error of mean

N: 220 birds/treatment (55 birds/replicate)

Table 2.7. Effect of Bio-C as a feed additive on NH_4-N concentration (ppm) in Experiment-2

	Treatments					
sampling days	[1]control	[2]0.2	[3]0.6	[4]1.0	[5]SEM	P-value
1	300±55	315±168	387±138	395±171	35.30	0.71
5	191±114	190±164	148±67	181±153	32.58	0.96
8	114±96	153±126	231±227	154±143	38.42	0.75
12	42±30	65±84	77±86	26±25	15.87	0.68
15	179±263	142±252	267±361	166±176	67.77	0.92
21	725±713	780±764	978±833	599±793	194.30	0.92

[1]control: basal diet only

[2]0.2: 0.2% of Bio-C was added in the diet

[3]0.6: 0.6% of Bio-C was added in the diet

[4]1.0: 1.0% of Bio-C was added in the diet

[5]SEM: Standard error of mean

N: 220 birds/treatment (55 birds/replicate)

Table 2.8. Effect of Bio-C as a feed additive on litter pH in Experiment-2

	Treatments					
sampling days	[1]control	[2]0.2	[3]0.6	[4]1.0	[5]SEM	P-value
1	8.4±0.1	8.4±0.1	8.4±0.1	8.4±0.1	0.02	0.96
5	8.2±0.1	8.2±0.2	8.2±0.2	8.2±0.2	0.04	0.96
8	8.1±0.2	8.3±0.1	8.0±0.3	8.2±0.2	0.05	0.22
12	8.1±0.3	8.1±0.4	8.1±0.4	8.1±0.2	0.82	1.00
15	8.2±0.4	7.7±0.6	8.0±0.7	7.9±0.7	0.15	0.78
21	8.6±0.4	8.7±0.5	8.7±0.3	8.4±0.6	0.12	0.80

[1]control: basal diet only

[2]0.2: 0.2% of Bio-C was added in the diet

[3]0.6: 0.6% of Bio-C was added in the diet

[4]1.0: 1.0% of Bio-C was added in the diet

[5]SEM: Standard error of mean

N: 220 birds/treatment (55 birds/replicate)

Table 2.9. Effect of Bio-C as a feed additive on bird's performance in Experiment-2

variables	[1]control	[2]0.2	[3]0.6	[4]1.0	[5]SEM	P-value
	treatments					
Day 0 to 7						
BW (kg/bird)	0.17±0.01	0.18±0.00	0.18±0.01	0.17±0.01	<0.01	0.20
WG (kg/bird)	0.13±0.01	0.14±0.01	0.13±0.01	0.13±0.01	<0.01	0.22
FCR	1.07±0.02[b]	1.06±0.01[b]	1.00±0.05[a]	1.06±0.03[b]	<0.01	0.01
FI (kg/bird)	0.14±0.01	0.14±0.00	0.14±0.01	0.14±0.01	<0.01	0.73
Mortality (%)	0.00±0.00	0.00±0.00	1.37±1.74	0.46±0.91	0.27	0.22
Day 0 to 14						
BW (kg/bird)	0.45±0.01	0.52±0.11	0.46±0.01	0.45±0.01	0.01	0.27
WG (kg/bird)	0.41±0.01	0.48±0.10	0.42±0.02	0.41±0.02	0.01	0.23
FCR	1.51±0.03	1.49±0.02	1.50±0.03	1.54±0.03	<0.01	0.07
FI (kg/bird)	0.62±0.02	0.63±0.01	0.64±0.02	0.63±0.01	<0.01	0.31
Mortality (%)	0.46±0.93	0.00±0.00	2.82±2.43	0.46±0.91	0.35	0.06
Day 0 to 21						
BW (kg/bird)	0.91±0.02[b]	0.95±0.01[a]	0.94±0.03[a]	0.93±0.01[ab]	<0.01	0.04
WG (kg/bird)	0.86±0.02[b]	0.91±0.01[a]	0.90±0.03[a]	0.88±0.01[ab]	<0.01	0.01
FCR	1.51±0.01	1.49±0.04	1.49±0.02	1.50±0.01	<0.01	0.68
FI (kg/bird)	1.30±0.03	1.43±0.19	1.35±0.03	1.33±0.02	0.02	0.31
Mortality (%)	0.92±1.06	9.87±19.74	2.82±2.43	0.92±1.06	2.50	0.56
Day 0 to 28						
BW (kg/bird)	1.47±0.04[b]	1.55±0.02[a]	1.54±0.06[a]	1.51±0.01[ab]	<0.01	0.03
WG (kg/bird)	1.43±0.04[b]	1.51±0.02[a]	1.50±0.06[a]	1.46±0.01[ab]	<0.01	0.03
FCR	1.67±0.04	1.65±0.07	1.61±0.02	1.63±0.03	0.01	0.31
FI (kg/bird)	2.39±0.06	2.61±0.35	2.42±0.06	2.38±0.04	0.05	0.29
Mortality (%)	1.39±1.80	11.19±22.40	2.82±2.43	0.92±1.06	2.83	0.56

[a-b]Means with different superscripts within a column differ significantly ($P \leq 0.05$).

[1]control: basal diet only

[2]0.2: 0.2% of Bio-C was added in the diet

[3]0.6: 0.6% of Bio-C was added in the diet

[4]1.0: 1.0% of Bio-C was added in the diet

[5]SEM: Standard error of mean

N: 220 birds/treatment (55 birds/replicate)

Table 2.10. Effect of topical application of Bio-C and PLT to the litter (in Exp-1) and effect of Bio-C as a feed additive (in Exp-2 and Exp-3) on ileal digestible energy

	treatments					
Exp-1	[1]control	[2]50	[3]100	[4]PLT	[5]SEM	P-value
IDE[1] (d-21)	2882±135	2836±209	2832±222	2971±61	42.43	0.64
Exp-2	[6]control	[7]0.2	[8]0.6	[9]1.0		
IDE (d-21)	2948±47	2838±159	2897±61	2931±52	23.07	0.38
Exp-3	[10]control	[11]0.1	[12]0.2	[13]0.4		
IDE (d-21)	2689±188[a]	2290±293[b]	2663±219[a]	2300±312[b]	45.63	<0.01
IDE (d-42)	2705±119[a]	2436±87[b]	2654±116[a]	2388±159[b]	22.06	<0.01

[a-b]Means with different superscripts within a column differ significantly ($P \leq 0.05$).

[1]IDE, Ileal Digestible Energy

[1]control: no litter treatment application

[2]50: 50 lbs/1000 ft^2 of Bio-C was applied on the litter

[3]100: 100 lbs/1000 ft^2 of Bio-C was applied on the litter

[4]PLT: 50 lbs/1000 ft^2 of PLT was applied on the litter

[5]SEM: Standard error of mean

[6]control: basal diet only

[7]0.2: 0.2% of Bio-C was added in the diet

[8]0.6: 0.6% of Bio-C was added in the diet

[9]1.0: 1.0% of Bio-C was added in the diet

[10]control: basal diet only

[11]0.1: 0.1% of Bio-C was added in the diet

[12]0.2: 0.2% of Bio-C was added in the diet

[13]0.4: 0.4% of Bio-C was added in the diet

Table 2.11. Effect of Bio-C as a feed additive on bird's performance in Experiment-3

variables	treatments					
	[1]control	[2]0.1	[3]0.2	[4]0.4	[5]SEM	P-value
Day 0 to 7						
BW (kg/bird)	0.19±0.00	0.19±0.00	0.20±0.00	0.19±0.00	<0.01	0.73
WG (kg/bird)	0.15±0.00	0.15±0.00	0.15±0.00	0.15±0.00	<0.01	0.71
FCR	1.31±0.11	1.33±0.07	1.42±0.28	1.39±0.11	0.03	0.49
FI (kg/bird)	0.20±0.01	0.20±0.01	0.22±0.04	0.21±0.02	<0.01	0.32
Mortality (%)	0.85±1.21	0.00±0.00	0.50±0.93	0.50±0.93	0.16	0.31
Day 0 to 14						
BW (kg/bird)	0.54±0.00	0.54±0.01	0.54±0.01	0.54±0.01	<0.01	0.98
WG (kg/bird)	0.50±0.01	0.50±0.01	0.50±0.01	0.50±0.01	<0.01	0.98
FCR	1.31±0.08	1.29±0.04	1.30±0.10	1.32±0.08	0.01	0.89
FI (kg/bird)	0.66±0.04	0.65±0.03	0.65±0.04	0.66±0.03	<0.01	0.90
Mortality (%)	1.10±1.21	0.60±1.14	0.25±0.71	0.25±0.71	0.20	0.83
Day 0 to 21						
BW (kg/bird)	0.99±0.02	0.98±0.02	0.96±0.04	0.96±0.04	<0.01	0.37
WG (kg/bird)	0.94±0.02	0.940.02	0.92±0.05	0.92±0.04	<0.01	0.36
FCR	1.44±0.03	1.44±0.02	1.44±0.09	1.46±0.05	0.01	0.87
FI (kg/bird)	1.36±0.05	1.35±0.05	1.35±0.05	1.34±0.04	<0.01	0.82
Mortality (%)	1.10±1.21	0.60±1.14	1.00±1.07	0.75±1.04	0.20	0.80
Day 0 to 28						
BW (kg/bird)	1.75±0.02	1.75±0.04	1.71±0.12	1.71±0.11	0.02	0.62
WG (kg/bird)	1.71±0.02	1.71±0.04	1.67±0.12	1.67±0.11	0.02	0.62
FCR	1.48±0.05	1.48±0.01	1.47±0.07	1.51±0.06	<0.01	0.55
FI (kg/bird)	2.73±0.10	2.75±0.09	2.73±0.19	2.74±0.12	0.02	0.99
Mortality (%)	1.10±1.21	1.10±1.21	1.48±1.28	1.10±1.21	0.22	0.90

Day 0 to 35						
BW (kg/bird)	2.54±0.07	2.54±0.08	2.45±0.14	2.46±0.18	0.02	0.33
WG (kg/bird)	2.50±0.07	2.50±0.08	2.41±0.14	2.42±0.18	0.02	0.33
FCR	1.81±0.06	1.86±0.08	1.89±0.11	1.88±0.16	0.02	0.54
FI (kg/bird)	4.17±0.16	4.19±0.16	4.04±0.30	4.04±0.21	0.04	0.39
Mortality (%)	1.49±1.28	1.74±1.21	1.58±1.44	1.10±1.21	0.23	0.80
Day 0 to 42						
BW (kg/bird)	3.24±0.13	3.22±0.15	3.09±0.21	3.07±0.27	0.04	0.22
WG (kg/bird)	3.19±0.13	3.18±0.15	3.04±0.21	3.03±0.27	0.04	0.22
FCR	1.94±0.05	1.98±0.08	2.04±0.14	2.01±0.15	0.02	0.33
FI (kg/bird)	5.50±0.26	5.39±0.42	5.34±0.47	5.31±0.34	0.07	0.77
Mortality (%)	2.23±1.09	3.05±2.28	2.82±1.47	1.88±1.22	0.28	0.44

[1]control: basal diet only

[2]0.1: 0.1% of Bio-C was added in the diet

[3]0.2: 0.2% of Bio-C was added in the diet

[4]0.4: 0.4% of Bio-C was added in the diet

[5]SEM: Standard error of mean

N: 200 birds/treatment (25 birds/replicate)

Table 2.12. Effect of Bio-C as a feed additive on jejunal morphology in Experiment-3

variables	treatments				[5]SEM	P-value
	[1]control	[2]0.1	[3]0.2	[4]0.4		
day-21						
VH[6] (μm)	1445.53±135.02	1451.93±126.25	1462.19±89.08	1431.88±106.55	20.44	0.96
CD[7] (μm)	192.06±15.96	190.35±23.53	195.03±13.80	194.77±32.25	3.99	0.97
VH: CD[8] (μm)	7.54±0.57	7.69±0.76	7.51±0.32	7.46±0.79	0.11	0.90
day-42						
VH (μm)	1774.08±166.62	1847.70±66.96	1807.27±113.85	1849.81±146.95	22.85	0.60
CD (μm)	269.69±54.76	253.96±26.10	275.23±84.19	264.09±38.41	9.78	0.89
VH: CD (μm)	6.75±1.04	7.36±0.95	6.98±1.60	7.12±1.06	0.21	0.78

[1]control: basal diet only

[2]0.1: 0.1% of Bio-C was added in the diet

[3]0.2: 0.2% of Bio-C was added in the diet

[4]0.4: 0.4% of Bio-C was added in the diet

[5]SEM: Standard error of mean

[6]VH, Villus height; [7]CD, Crypt depth; [8]VH: CD, Villus height to crypt depth ratio

N: 200 birds/treatment (25 birds/replicate)

CHAPTER 3

THE EFFICACY OF AN ENGINEERED BIOCARBON IN YOUNG BROILERS DURING AN AFLATOXIN EXPOSURE[1]

[1]H. O. Bayir, J. D. Liu, J. Fowler, and C. W. Ritz. To be submitted to The Journal of Applied Poultry Research

ABSTRACT

This study was conducted to evaluate the efficacy of a carbonaceous biomass (Bio-C) in mitigating the effects of a 0.5 mg/kg dose of aflatoxins (AF). A total of 192 Cobb-500 male broilers were obtained on the day of hatch and randomly allocated to one of 32 treatments pens (6 birds/pen). Birds were fed a broiler starter mash diet containing either 0 or 0.5 mg/kg AF, with or without 0.4% of Bio-C, resulting in 4 treatments arranged as a 2x2 full-factorial. Pen weights and feed consumed were recorded at 0, 7, 14, and 21 days of age, and mortality was recorded daily. On day 21, three birds from each pen were killed by cervical dislocation and the liver, kidney, and spleen were removed and weighed for relative organ weight assessment. Data were analyzed as a 2x2 full-factorial for AF level and Bio-C using the GLM procedures of SPSS, with a significance defined at $p \leq 0.05$. The results showed that the performance of birds receiving 0.5 mg/kg AF were not significantly different compared to the control group at any point during the 21 day trial. No main effects were seen on the performance parameters by the inclusion of Bio-C in the diet. There was also no AF/Bio-C interaction. The relative weights of the liver, kidney, and spleen were not significantly different whether birds were fed 0.5 mg/kg AF or 0.4% Bio-C. These data suggest that supplementation of Bio-C has no effect on broiler performance when provided in diets either free of AF or containing up to 0.5 mg/kg.

Key words: aflatoxin, biocarbon, binder, broiler

INTRODUCTION

Agricultural crops, such as corn, sorghum, rice and wheat are major feed sources for poultry worldwide. The risk of these crops being contaminated with mycotoxins has drawn great attention due to the hazardous implications that mycotoxins have and the negative economic impact that they cause the poultry industry [1, 2]. Aflatoxins (AF) are highly toxic and carcinogenic fungal metabolites produced by the *Aspergillus flavus* and *Aspergillus parasiticus* species. Although the forms of AF can range from AFB_1, AFB_2, AFG_1 and AFG_2, the most commonly produced and most toxic is AFB_1. There is no animal species that are immune to the detrimental effects of these toxins once they have contaminated the feed. When exposed, AF induces "aflatoxicosis", a syndrome that results in decreased feed intake and weight gain, immunosuppression, reduced egg production, increased relative liver and kidney weights, impaired serum biochemistry, and eventually mortality [3-5]. Therefore, efforts have been made and varied systems have been developed in order to detoxify grains and animal feeds that have been contaminated with AF. Once produced, there is no effective way to eliminate AF completely from the feeds, instead efforts have been made to minimize their effects through binding or sequestration [6, 7].

One of the promising and cost-effective ways to ameliorate the toxicity of AF found in the animal diet is the addition of non-nutritive adsorbent materials. One such feed additive is carbonized biomass (biocarbon or charcoal) [8-11]. Biocarbon (Bio-C) , which has a high porosity and high internal surface area, typically refers to the carbonaceous residue of wood, cellulose, coconut shells or other by-products left after heating the organic matter in the absence of oxygen [12]. This odorless and tasteless black powder has been shown to ameliorate the harmful effects of AF [11, 13-16]. Therefore, the aim of this study was to evaluate the efficacy of an engineered Bio-C when added into the feed for protection against the toxicity of AF in young broilers.

MATERIALS AND METHODS

The experiment was conducted at the University of Georgia Poultry Research Center, with research protocol approved by the Institutional Animal Use and Care Committee.

In vitro adsorption test:

The procedure as described by Grant and Phillips [17] was used, with some minor changes: a working solution was prepared at a concentration of 88 mg/kg (AFB_1) in acetonitrile and then diluted with deionized water to the treatment concentrations of 0.4, 1.6, 3.2, 6.4, 9.6 and 16 mg. A 0 mg blank solution was also prepared using deionized water. The concentration of the treatment solutions was verified by measuring the absorbance of the 365 nm AFB_1 peak in a scan (200-800 nm wavelength) with a UV-visible- spectrophotometer.

To each of the 5 mL of AFB_1 test solution tubes (with the concentrations 0.0, 0.4, 1.6, 3.2, 6.4, 9.6 and 16 mg), 10 mg of the Bio-C was added (0.2%). The concentrations were obtained by dilution of the working solution with deionized water. Each of the samples in this assay were prepared and analyzed in duplicate. After 24 hours of shaking at 200 motions/min on an orbital shaker, the samples were centrifuged at 51,000 g for 30 min and the amount of absorbed AFB_1 was determined, measuring the AFB_1 absorbance of the supernatant at 365 nm (in water-acetonitrile solvent) with UV-visible spectrophotometry. Also, to construct a standard curve, an AFB_1- solution at the concentrations of 0, 0.4, 1.6, 3.2, 6.4, 9.6 and 16 mg were measured.

In vivo study:

The experimental design consisted of 4 treatments, arranged as a 2x2 factorial: T1 (control with no AF and no Bio-C), T2 (0.4% Bio-C), T3 (0.5 mg AF/kg of diet), and T4 (0.5 mg AF/kg of diet plus 0.4% Bio-C), with eight replicates per treatment. A total of 192 Cobb-500 male chicks were obtained on day-of-hatch and randomly distributed among treatment pens (6 birds/pen). The birds were fed a standard broiler starter diet in mash form for 21 days, and all birds were allowed *ad libitum* access to feed and water with 24 h light. Live performance data were recorded at 0, 7, 14, and 21 days of age and mortality was recorded daily. At the end of the experiment, three birds from each pen were randomly selected and killed by cervical dislocation, and liver, kidney and spleen were removed and weighed for relative organ weight assessment.

AF for the treatment diets was produced by the inoculation of *Aspergillus flavus* on rice as described by Shotwell and Hesseltine [18]. The rice was dried and ground into a powder form, diluted with corn meal to a concentration of 0.5 mg/kg, and then added into the diets to provide the desired AF concentration.

Data were analyzed as a 2x2 full-factorial for AF level, Bio-C inclusion, and their interaction using the GLM procedures of SPSS, with significance defined at $p \leq 0.05$. Where significant, means were separated using Duncan's Multiple Range Tests.

RESULTS AND DISCUSSION

In vitro adsorption test:

The adsorbed amount of AFB_1 over the increasing test doses is shown in Figure 3.1. The results were fit to a quadratic-ascending broken-line model with an R^2 value of 0.91. At the 0.4 mg/kg dose, Bio-C adsorbed 29% of the AFB_1 in solution. At 1.6 mg/kg, 70% of the AFB_1 in solution was adsorbed by the Bio-C. At 3.2, 6.4, 9.6 and 16 mg/kg dose, Bio-C adsorbed 61%, 35%, 40% and 30% of the AFB_1 in solution, respectively. The broken-line model showed a peak adsorption capacity for the 0.2% Bio-C in solution of 4.93 mg/kg AFB_1. Overall, Bio-C adsorbed an average of 44% of AFB_1 in solution of the range of doses (0.4, 1.6, 3.2, 6.4, 9.6 and 16 mg/kg). Galvano and Pietri [19] evaluated the efficacy of 17 different activated carbons (ACs), such as exhausted olive residues, peach stones, almond shells and commercial activated carbons, in binding AFB_1 in solution. The authors used 5 ml of a 0.004 mg/ml aqueous solution of AFB_1 and 2 mg of an AC and found that the adsorption abilities of these 17 ACs were ranged from 44.47% to 99.82%.

In vivo study:

Table 3.2 shows growth performance parameters of birds fed 0 or 0.5 mg/kg AF contaminated diets with or without Bio-C supplementation on day 0 to 7. BW, WG, FCR and FI showed no difference in birds fed a diet containing 0.5 mg/kg AF compared to the 0 mg control group. The addition of Bio-C at 0.4 % of the diet did not show any improvement on those parameters (except for FI), as indicated by the AF x Bio-C interaction. Even though there was a slight increase in FI with the inclusion of the Bio-C, the results were significant only at a p-value of 0.07. A total of three birds died during the first week, one in T2 and two in T3, therefore mortality was not significantly different among treatments.

Table 3.3 shows growth performance parameters of birds fed 0 or 0.5 mg/kg AF contaminated diets with or without Bio-C supplementation on day 0 to 14. The presence of 0.5 mg/kg AF in the diet did not impair any of the performance parameters compared to the control group. Statistical analysis also indicated that the birds receiving 0.4% Bio-C in the diet showed no changes in any of the performance parameters.

Table 3.4 describes performance parameters of birds fed 0 or 0.5 mg/kg AF contaminated diets with or without Bio-C supplementation throughout the 21 days of age. Over the entire 21 day feeding period, birds fed the AF contaminated diet showed no effects on their performance parameters. When 0.4% Bio-C was included in the diets, the performance parameters were also not significantly different among treatments (whether they contained 0 or 0.5 mg/kg AF). There was no mortality among any of the treatments during the second or third week.

One of the common indicators of AF exposure is depressed bird performance. The results of this experiment indicate that 0.5 mg/kg AF in the diet did not depress body weight or feed conversion after three weeks. Likewise, Verma and Johri [20] reported no significant effects on performance parameters when birds fed 0.5 mg/kg AF contaminated diets for 7 weeks. However, the authors observed depressed body weights, reduced feed consumption and impaired feed efficiency when birds were given 1 and 2 mg/kg of AF in the diets. Magnoli and Texeira [21] fed birds with 0 and 0.1 mg/kg AF contaminated diets for 33 days and found no significant differences on performance parameters among treatments. Miller and Wyatt [22] fed broiler chicks with 0, 1.25, 2.5 and 5 mg/kg AF containing diets for three weeks. The authors found that 1.25 and 2.5 mg/kg AF in the diets had no effect on body weights, however, body weight was reduced by 5 mg/kg AF in the diets. Contrary to the results from present experiment, Bintvihok and Kositcharoenkul [23] found impaired growth performance in birds fed diets contaminated with 0.05 and 0.1 mg/kg AF. However, in that study, birds were fed AF-contaminated diets for six weeks. Moreover, Huff and Kubena [24] fed broiler chickens with diets containing 0, 1.25, 2.5 and 5 mg/kg AF for three weeks and reported that growth performance was depressed only by 2.5 and 5 mg/kg AF in the diet compared to the control at the end of the study. In the current study, body weights between the 0 mg and the 0.5 mg treatment only varied by 4.2%. In most studies in the literature in which BW reduction was reported, the dose of AF in the feed was greater than the dose used in this study. However, the dose selected for this study was still 25x the FDA's advisory action level (0.02 mg/kg). This was done to better reflect a realistic contamination scenario. In addition, since body weight gain and feed intake did not significantly differ, feed conversion ratios were not significantly different among treatments. The inclusion of 0.4% Bio-C in the diet did not have a beneficial effect on growth performance when either added alone or in combination to AF-contaminated diets in the present study. Kubena and Harvey [25] and Edrington and Sarr [26] studied the efficacy of activated charcoal to ameliorate the toxic effect of AF (7.5 and 0.75 mg/kg, respectively) in broilers and turkeys and indicated no improvements in birds' performance at 0.5% charcoal dose in the diets in both study. However, an improvement has been observed in performance parameters of birds fed AF-contaminated diets with an inclusion of activated charcoal by [11, 13], who fed doses of AF ranging from 0.5 to 10 mg/kg. The reason behind these conflicting results are likely due to differences in the duration of feeding, composition of the feed, the dose and source of AF fed, particular species or strain of the birds and bird age. Further, Bio-C (or, in some cases, activated charcoal) from different sources are known to have different absorption capacities or physical characteristics. So, the particulars of each product need to be taken into account.

The effects of experimental diets on relative organ weights are presented in Table 3.5 (grams per 100 g of body weight). The results found that relative liver, kidney and spleen weights were not affected by dietary AF, and the inclusion of Bio-C in the diet did not show any effect on those values as indicated by the lack of an AF x Binder interactions. In contrast, Kubena and Harvey [25] fed birds with 5 mg/kg AF-contaminated diet and reported increased relative weight of the liver and kidney. Further, Edrington and Sarr [26] fed turkey poults with diets contaminated with 0.75 mg AF and found decreased liver and increased kidney and spleen weight. However, both authors did not find any beneficial effect with the inclusion of an activated charcoal into the diet at 0.5% level. Also, in both of those studies, the concentration AF in the diets were higher than the concentration used in the present experiment. It is possible that though the dose used here (of 0.5 mg) is 25-times the FDA action level for aflatoxins, the concentration was still low enough to not affect the relative organ weights or the growth of the birds to any statistically significant degree.

In conclusion, feeding a 0.5 mg/kg AF-contaminated diet did not impair the performance of broilers, nor the relative organ weights, after three weeks. Further, the dietary supplementation of Bio-C (at 0.4%) had no beneficial effect when it was added either in the control diet or the diet containing AF over the rearing period. Based on the results of the present experiment and previous reports, the toxicity of AF on birds appears both dose- and time-dependent, and Bio-C shows a degree of variation in its ability to alleviate the toxic effects of AF in poultry.

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Table 3.1. Ingredient and nutrient composition of the experiment (as-fed basis)

Item	Amount
Ingredient (% of diet)	
Corn, grain	57.47
Soybean meal, 48% CP	35.25
Soybean oil	2.63
Limestone	1.59
Dicalcium phosphate	1.39
Salt	0.49
DL-methionine	0.21
L-Lysine-HCI	0.14
Vitamin premix[1]	0.35
Mineral premix[2]	0.08
Calculated composition	
ME, kcal/kg	3050.00
CP, %	21.41
Crude fat, %	4.86
Ca, %	0.95
Available P, %	0.45
Lys, %	1.19
Met, %	0.53

[1]Supplied per kilogram of diet: vitamin A, 5511 IU; vitamin D3, 1102 ICU; Vitamin E, 11.02 IU; vitamin B12, 0.01 mg; Biotin, 0.11 mg; Menadione, 1.1 mg; Thiamine, 2.21 mg; Riboflavin, 4.41 mg; d-Pantothenic Acid, 11.02 mg; Vitamin B6, 2.21 mg; Niacin, 44.09 mg; Folic Acid, 0.55 mg; Choline, 191.36 mg.

[2]Supplied per kilogram of diet: Mn, 107.2 mg; Zn, 85.6 mg; Mg, 21.44 mg; Fe, 21.04; Cu, 3.2 mg; I, 0.8 mg; Se, 0.32 mg.

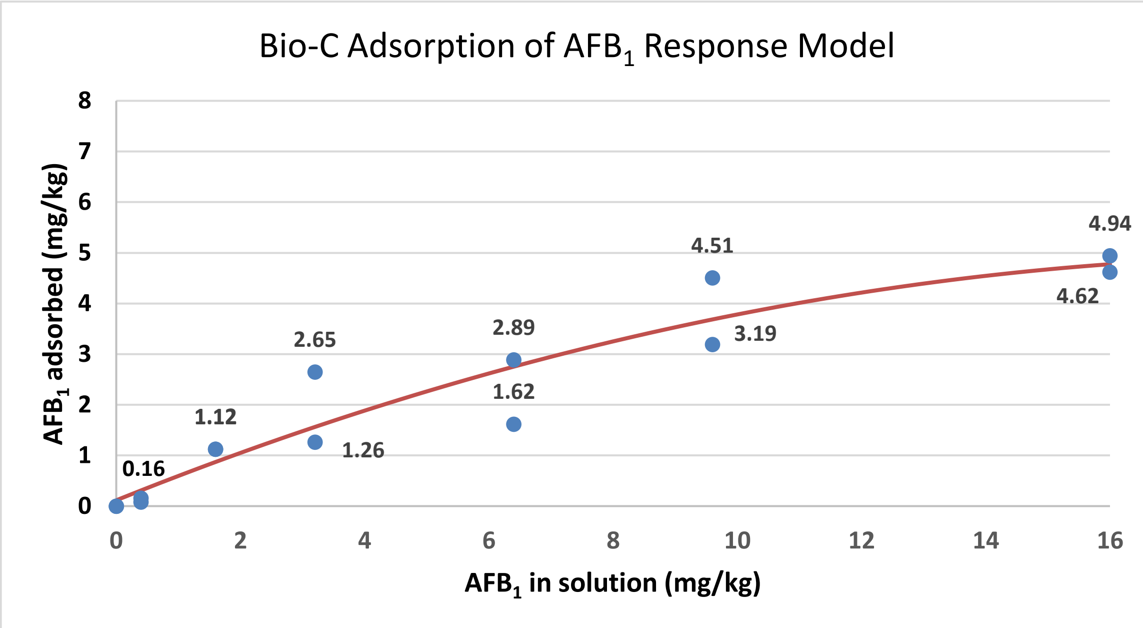

Figure 3.1. Broken-line (with a quadratic-ascending segment) model for the adsorbed amount of AFB_1 in solution over the increasing test doses

Table 3.2. Effect of Bio-C on growth performance parameters of birds fed with diets containing 0 or 0.5 mg AF per kg of diet (day 0 to 7)

	Variables				
Treatments	BW (g/bird)	WG (g/bird)	FCR	FI (g/bird)	Mortality (%)
AF (mg)					
[1]0	139±13	100±13	1.19±0.07	119±13	0.00±0.00
[2]0.5	132±22	92±18	1.21±0.09	111±17	6.25±4.38
Binder					
[3]Control	140±12	101±12	1.21±0.06	122±12	2.08±2.10
[4]Bio-C	146±9	107±9	1.18±0.04	126±10	0.00±0.00
P-Value					
AF	0.84	0.84	0.72	0.60	0.40
Bio-C	0.17	0.16	0.72	0.07	0.40
AF x Bio-C	0.22	0.19	0.39	0.23	0.10
[5]SEM	2.69	2.66	0.01	2.32	1.21

[1]0: Basal diet only (no AF and Bio-C)

[2]0.5: Basal diet plus 0.5 mg/kg AF

[3]Control: Basal diet plus 0.4% Bio-C

[4]Bio-C: Basal diet plus 0.5 mg/kg AF and 0.4% Bio-C

[5]SEM: Standard error of mean

N: 48 birds/treatment (6 birds/replicate)

Table 3.3. Effect of Bio-C on growth performance parameters of birds fed with diets containing 0 or 0.5 mg AF per kg of diet (day 0 to 14)

	Variables				
Treatments	BW (g/bird)	WG (g/bird)	FCR	FI (g/bird)	Mortality (%)
AF (mg)					
[1]0	377±35	338±35	1.27±0.08	428±35	0.00±0.00
[2]0.5	376±45	336±45	1.24±0.04	418±46	6.25±4.38
Binder					
[3]Control	386±32	347±32	1.26±0.04	439±36	2.08±2.10
[4]Bio-C	396±24	357±24	1.25±0.05	447±22	0.00±0.00
P-Value					
AF	0.74	0.73	0.31	0.98	0.40
Bio-C	0.25	0.24	0.88	0.13	0.40
AF x Bio-C	0.66	0.63	0.55	0.46	0.10
[5]SEM	6.22	6.19	0.01	6.37	1.21

[1]0: Basal diet only (no AF and Bio-C)

[2]0.5: Basal diet plus 0.5 mg/kg AF

[3]Control: Basal diet plus 0.4% Bio-C

[4]Bio-C: Basal diet plus 0.5 mg/kg AF and 0.4% Bio-C

[5]SEM: Standard error of mean

N: 48 birds/treatment (6 birds/replicate)

Table 3.4. Effect of Bio-C on growth performance parameters of birds fed with diets containing 0 or 0.5 mg AF per kg of diet (day 0 to 21)

	Variables				
Treatments	BW (g/bird)	WG (g/bird)	FCR	FI (g/bird)	Mortality (%)
AF (mg)					
[1]0	810±70	770±70	1.31±0.19	1001±66	0.00±0.00
[2]0.5	844±96	804±96	1.24±0.16	987±66	6.25±4.38
Binder					
[3]Control	854±48	815±48	1.25±0.14	1008±72	2.08±2.10
[4]Bio-C	868±82	828±82	1.26±0.16	1030±37	0.00±0.00
P-Value					
AF	0.37	0.38	0.55	0.86	0.40
Bio-C	0.21	0.21	0.72	0.27	0.40
AF x Bio-C	0.70	0.71	0.51	0.41	0.10
[5]SEM	13.47	13.45	0.03	10.93	1.21

[1]0: Basal diet only (no AF and Bio-C)

[2]0.5: Basal diet plus 0.5 mg/kg AF

[3]Control: Basal diet plus 0.4% Bio-C

[4]Bio-C: Basal diet plus 0.5 mg/kg AF and 0.4% Bio-C

[5]SEM: Standard error of mean

N: 48 birds/treatment (6 birds/replicate)

Table 3.5. Effect of Bio-C as a feed additive on relative organ weights in AF exposed birds on day 21 (g/100 g of body weight)

Treatments	Variables		
	RLW[1]	RKW[2]	RSW[3]
AF (mg)			
[4]0	2.51±0.16	0.55±0.05	0.11±0.02
[5]0.5	2.48±0.22	0.55±0.07	0.12±0.01
Binder			
[6]Control	2.46±0.27	0.54±0.04	0.12±0.01
[7]Bio-C	2.52±0.28	0.55±0.06	0.12±0.02
P-Value			
AF	0.89	0.74	0.66
Bio-C	0.91	0.79	1.00
AF x Bio-C	0.56	0.65	0.20
[8]SEM	0.04	0.01	<0.01

[1]Relative liver weight; [2]Relative kidney weight; [3]Relative spleen weight

[4]0: Basal diet only (no AF and Bio-C)

[5]0.5: Basal diet plus 0.5 mg/kg AF

[6]Control: Basal diet plus 0.4% Bio-C

[7]Bio-C: Basal diet plus 0.5 mg/kg AF and 0.4% Bio-C

[8]Standard error of mean

N: 48 birds/treatment (6 birds/replicate)

CHAPTER 4

CONCLUSION

CONCLUSION

Ammonia (NH_3) emission have become a major problem for poultry industry in the U.S since the same litter is used for multiple flocks. NH_3 generation and volatilization from broiler litter is detrimental to bird health and performance. The negative effects of NH_3 on birds have been well studied in the literature. When exposed to NH_3 over 25 ppm, feed intake, feed efficiency, body weight gain and overall body weight have been shown to decrease in broilers. Moreover, birds can develop respiratory tract disease and keratoconjunctivitis and become susceptible to secondary infections. There are several methods to control NH_3 generation and volatilization from poultry litter in broiler facilities, including ventilation, dietary manipulation and manure and litter management; however, the most commonly used one is manure and litter management. Carbonized biomass (biocarbon or charcoal) has been suggested as a method to reduce NH_3 generation and volatilization and may potentially have a positive effect on bird growth performance when applied on the litter or added into the feed.

In chapter 2, the efficacy of an engineered biocarbon (Bio-C) on NH_3 volatilization and growth performance parameters were investigated in three experiments.

In Exp-1, Bio-C was applied on the litter topically at rates of 0, 50 and 1000 lbs/1000 ft^2, and a positive control treatment of 50 lbs/1000 ft^2 sodium bisulphate (PLT). The results indicated that topical application of Bio-C to the litter had no effect on reduction of NH_3 volatilization, litter pH, and performance parameters compared to the control. NH_3 concentration was significantly lowered by PLT application to the litter on the first sampling day; however, no significant differences were found among treatments thereafter.

In Exp-2, Bio-C was incorporated into the feed at 0, 0.2, 0.6 and 1.0 % of the total diet. Results from this experiment showed that Bio-C had an effect on neither NH_3 concentration nor litter pH. However, birds received 0.2 and 0.6 % Bio-C in their diets had significantly higher body weight and cumulative weight gain at the end of the 28 days rearing period. Further, there is no significant difference in ileal digestible energy (IDE) and FCR.

In Exp-3, Bio-C was incorporated into the feed at 0, 0.1, 0.2 and 0.4 % of the total diet and growth performance parameters were evaluated in a full 6 week growout period. The result showed that body weight, weight gain and feed conversion ratio were not affected by the addition of any Bio-C concentration throughout the 6 weeks. However, ileal digestible energy (IDE) was found significantly lower in 0.1% and 0.4% treatments when compared to the control and 0.2% treatments on both day 21 and 42.

Aflatoxins (AF) are one of the common mycotoxins in agricultural commodities, such as corn, sorghum and wheat, which are extensively used as feed sources for poultry worldwide, causing serious risks to poultry. Many studies from the past have reported the adverse effects of AF on performance parameters, liver and kidney functions, immune functions and egg production. Therefore, when the field strategies to prevent fungal species to produce the toxin in the crop is not enough, efforts should be made to minimize the effects of AF from the feed in cost-effective ways. One such method to ameliorate the toxicity of AF found in the poultry diet is the addition of non-nutritive adsorbent agents. Bio-C has been shown to ameliorate the harmful effects of AF when added in the feed in the literature.

In chapter 3, the effectiveness of an engineered Bio-C, as a feed additive, on the toxicity of 0.5 mg/kg AF was examined in a 21 days *in vivo* trial (Exp-4). In addition, the adsorption capacity of Bio-C for AF from aqueous solution was investigated *in vitro*. The results from the *in vitro* experiment revealed that an average of 44% of AF in aqueous solution was absorbed by Bio-C. The results from the *in vivo* trial showed that there was no significant difference in the performance of birds among treatments throughout the 21 days. No change in the performance parameters were found by the addition of Bio-C in the feed. Also, no significant differences were determined in the relative weights of the liver, kidney, and spleen when birds were fed 0.5 mg/kg AF or 0.4% Bio-C.

Overall, in the current experiments, the efficacy of an engineered Bio-C on NH_3 volatilization, growth performance parameters and mitigation of AF toxicity have been studied. Based on the results of the experiments, the topical application and dietary addition Bio-C have no effect on NH_3 volatilization. However, we did find some evidence of improvements to body weight and weight gain in birds receiving 0.2 and 0.6% Bio-C in their diets. Therefore, the mechanism behind these changes was further investigated. When birds received Bio-C in the diets for a full six-week rearing period (at doses ranging from 0.1% - 0.4%), there were no improvements in performance parameters, energy digestibility, or morphology of the small intestine found among treatments.

Further, when birds were fed 0.5 mg/kg AF, there was no main effect for Bio-C on any of the variables measured (nor any AF and Bio-C interaction). In the *in vitro* analysis, however, we did find that Bio-C has some binding affinity for AFB_1. When in solution, 0.2% Bio-C adsorbed (on average) 44% AF in solution over a range of 0.4 – 16 mg/kg, with a peak adsorption capacity of 4.93 mg/kg.

Collectively, based on the results from the present research, both a litter application and a feed supplementation of Bio-C were not effective at reducing ammonia volatilization nor was it effective at improving any performance parameters of birds. Even when we found improved body weight and weight gain from Exp-2, it was evident that there was no impact on intestinal morphology, energy digestibility, feed conversion, or growth rate. In addition, when Bio-C was provided in diets with or without an aflatoxin contamination (0.5 mg/kg), there was no effect on bird performance parameters. Since the reuse of the same litter over multiple flocks in broiler facilities is the common practice in the U.S. and mycotoxin contamination of feedstuffs is a key problem worldwide, new methods or applications to control NH_3 for entire grow-out period and safe and effective strategies to mitigate the toxic effects of AF in poultry are required.