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Evaluation of Biochar as a Feed Additive in Commercial Broiler Diets

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EVALUATION OF BIOCHAR AS A FEED ADDITIVE IN COMMERCIAL BROILER DIETS

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The School of Animal Sciences

by
Brandon Michael Cheron
B.S., Louisiana State University, 2015
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This thesis is dedicated to my grandmother, Dorothy Lucille Cheron, who passed away before the completion of my master's degree. Without the love and support she had given me over the years I would have not made it to this point in my academic career.

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TABLE OF CONTENTS

ACKNOWLEDGMENTS.....	iii
LIST OF TABLES.....	vi
ABSTRACT.....	vii
CHAPTER	
1. INTRODUCTION.....	1
2. REVIEW OF LITERATURE.....	3
Background.....	3
Effects of feeding biochar to poultry.....	4
Effects of phosphorus and nitrogen runoff on the environment.....	5
Phosphorus in poultry excreta.....	6
Effects of impaired bone development in poultry production.....	7
3. MATERIALS AND METHODS.....	8
Animals and care.....	8
Treatment diets.....	9
Growth and performance.....	14
Bone breaking strength.....	14
Fecal analysis.....	14
Statistical analysis.....	15
4. RESULTS AND DISCUSSION.....	16
Performance data: trial 1.....	16
Fecal and bone analysis: trail 1.....	19
Performance analysis: trial 2.....	19
Fecal analysis: trial 2.....	22
5. SUMMARY, CONCLUSIONS AND FUTURE RESEARCH.....	25
Summary.....	25
Conclusion.....	25
Future research.....	26
REFERENCES.....	27
VITA.....	29

LIST OF TABLES

1. Ingredient composition of treatment diets for 0 to 10 day old broiler chicks.....	10
2. Calculated metabolizable energy (ME) and nutrient analysis values of treatment diets for 0 to 10 day old broilers.....	11
3. Ingredient composition of treatment diets for 0 to 18 day old broiler chicks.....	12
4. Calculated metabolizable energy (ME) values and nutrient analysis of treatment diets for 0 to 18 day old broilers.....	13
5. Least squares means for average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency (G:F), day 0 body weight (BW), day 10 BW, and BBS of 0 to 10 day old broilers.....	17
6. Least squares means for fecal nitrogen (%), phosphorus (%), and dry matter (%) of 0 to 10 day old broilers.....	19
7. Least squares means for broiler weights at 0, 10 and 18 days of age for 0 to 18 day old broilers.....	20
8. Least squares means for average daily gain (ADG), average daily feed intake (ADFI), feed efficiency (G:F), and bone breaking strength (BBS) of 0 10 18 day old broilers	21
9. Least squares means for fecal nitrogen, phosphorus and dry matter content of 0 to 18 day old broilers.....	24

ABSTRACT

Two experiments were conducted to evaluate sugarcane biochar as a feed ingredient in commercial broiler diets. Experiment 1 was conducted for 11 days using 300 male Ross 708 broilers. Broilers were allotted to one of five treatment diets containing 0%, 0.5%, 1.0%, 2%, or 4% biochar. Experiment 2 was conducted for 19 days using 450 male Ross 708 broilers. Chicks were allotted to one of 9 treatment diets containing 0%, 0.25%, 0.5%, 0.75%, 1%, 1.25%, 1.5%, 1.75% or 2% biochar. Broilers and feed were weighed at day 0 and 10 in experiment 1 and day 0, 10 and 18 in experiment 2 to determine average daily gain (ADG), average daily feed intake (ADFI) and feed efficiency (Gain:Feed). The left tibia was collected from each broiler on day 10 (experiment 1) or day 18 (experiment 2) and used to determine bone breaking strength (BBS). Fecal samples were collected on day 10 (experiment 1) or day 10 and 18 (experiment 2) to determine dry matter (DM), nitrogen (N), and phosphorus (P) content. For experiments 1 and 2 treatment had no effect ($P > 0.05$) on ADFI or BBS. Results from day 10 in experiment 1 and day 18 in experiment 2 did not show differences in fecal DM. Differences between treatments in fecal DM content were noted on day 10 of experiment 2. Experiment 1 determined broilers fed 4% biochar had lower ($P < 0.01$) Gain:Feed than broilers fed other diets. No differences were noted in Gain:Feed in experiment 2 for birds fed biochar up to 2%. In experiment 2 birds fed 0.25% or 0.75% biochar on day 10 and 0.25% - 1.25% biochar on day 18 had increased fecal P. Further testing is required to determine the exact effects of feeding biochar on broiler fecal P.

CHAPTER 1 INTRODUCTION

The International Biochar Initiative (2017) defines biochar as, “Solid material obtained from thermochemical conversion of biomass in an oxygen-limited environment.” The biomass used in production of biochar are substances such as bagasse from sugarcane refinement, poultry litter and other biological waste materials that are produced in agricultural systems. It is produced by degradation of the byproduct using gasification or pyrolysis. Biochar is commonly used to improve soil quality for farming or to reduce ammonia levels in livestock production (Doydora, 2009; Gerlach and Schmidt, 2012). Incorporating biochar into crop soil has been shown to improve crop growth (Qian et al., 2015) and enhance the retention of nutrients such as N and P compounds in the soil (Laird et al., 2006; Wang et al., 2015). This effect can reduce the need of fertilizers on crop land, potentially reducing the input and labor costs of farmers, and minimize environmental impact of N and P pollution from agricultural runoff.

Little research has been conducted using biochar as a feed additive in animal production. A previous study used biochar derived from poultry litter as a feed additive in broiler diets. Feeding biochar improved bone mineralization, but reduced bird performance (Evans et al., 2015). The reduction in performance is believed to be due to high levels of arsenic in the biochar. Other forms of biochar, such as sugarcane biochar, have no detectable levels of arsenic and may be less detrimental to bird performance than poultry litter biochar (Control Laboratories, Watsonville, CA)

No trials have been conducted to investigate the effects of feeding biochar to livestock on nutrient digestion or excretion. Poultry litter is a commonly used fertilizer in crop production. Due to the beneficial effects on N and P in soils amended with biochar, it is worth investigating any effects that biochar may have on these nutrients when digested by a live animal. Furthermore, poultry litter biochar may also aid in bone mineralization of broiler chickens as seen in the study by Evans et al. (2015).

While the previous study utilized poultry litter biochar, no research has been conducted to investigate the effects of biochar derived from other biomass sources such as sugarcane. This experiment was designed to investigate the effects of increasing dietary inclusion rates of sugarcane biochar on 1) performance, 2) fecal P and N content and 3) bone breaking strength of Ross 708 broiler chickens.

We hypothesize that the inclusion of sugarcane biochar in commercial broiler diets may increase bone strength and fecal nitrogen or phosphorus concentrations.

CHAPTER 2

A REVIEW OF LITERATURE

Background

Biochar is an ash substance that is produced from the burning of biological material via pyrolysis. This process heats the biological material in an anaerobic environment causing it to decompose into an ash form. This method of decomposition prevents the formation of CO₂ due to the absence of oxygen unlike traditional methods of burning. The carbon that would normally be released as CO₂ is instead sequestered as solid carbon in the biochar (Qian et al., 2015). Lehman et al. (2006) state that up to 50% of the initial carbon content of biological material can be sequestered into biochar as compared to less than 10% when using traditional (aerobic) burning methods. Typically, the biological material used in the production of biochar is the byproduct of some agricultural production, thus converting a waste product into a usable substance with some economic value.

Biochar is most commonly used as a soil amendment for crop production. Biochar serves to improve physical quality of soils as well as aid in nutrient retention of soil amended with biochar. Laird et al. (2010) investigated the leaching of various minerals in fertilized and unfertilized soil amended with biochar. Four treatments of soil amended with biochar at 0, 5, 10 or 20 g kg⁻¹ were placed in soil columns. Manure was incorporated into the top 3cm of soil. Treatments were subjected to leaching using a slow dripping technique. Leachate was analyzed for NO₃-N, NH₄-N, Al, B, Cu, Fe, K, Mn, Na, P, Si and Zn 24 hours prior to the initiation of the leaching process. The results of this study

indicate that addition of biochar significantly reduced nitrogen, phosphorus, magnesium and silicon leaching compared to soils containing no biochar. A similar study by Wang et al. (2015) compared the nutrient retaining abilities of poultry litter biochar amended soils compared to raw poultry litter amended soils. The poultry litter biochar proved to be more effective in retaining nutrients such as N and P in the soil, thus allowing nutrients to be available for uptake by plants for longer periods of time. It also prevents excess nutrients from leaching out of the soil and polluting local water bodies.

Effects of feeding biochar to poultry

Few studies have been conducted to investigate the effects of utilizing biochar as a feed additive in livestock species. In one of these studies, Evans et al. (2015) reported that feeding poultry litter biochar (PLB) as a P replacement to broiler chickens increased bone mineralization of broilers but had a negative impact on weight gain and feed efficiency of the birds. Birds were subjected to a standard commercial, or negative control diet. The positive control and commercial diet were provided with or without poultry litter biochar at 6.89% and 6.16%, respectively. The negative and positive control diets contained 0.23% and 0.45% non-phytate phosphorus, respectively. They found that all diets containing biochar reduced body weight, feed efficiency and feed intake compared to commercial, or negative and positive control diets. Increased bone mineralization as determined by tibiotarsal ash content was noted in birds fed diets containing biochar. The researchers hypothesized that this decrease in performance was due to the high arsenic content of the poultry litter biochar. The biochar used in

the experiment had an arsenic content of 99 ppm. Previous research has defined the maximum tolerable level of arsenic in broiler chickens to be 30 ppm according to The Merck Veterinary Manual (2005).

Effects of phosphorus and nitrogen runoff on the environment

According to the Environmental Protection Agency (EPA, 2017a), the two most concerning pollutants from agricultural runoff are nitrogen (N) and phosphorus (P) compounds. Nitrogen and P are two of the limiting nutrients with regards to plant growth. Because of this, they are frequently used in agricultural systems as fertilizers to promote the growth of crops.

One of the greatest sources of N and P used in agricultural production is fertilizers. Fertilizers produced from livestock manure are high in N and P concentration. The addition of these fertilizers on crop lands results in the eutrophication of water bodies. This process occurs when N and P compounds leach from soil carried by runoff water (Guo et al., 2009). This water carries the excess nutrients as it runs into local water bodies. As with terrestrial plants, P and N are the most limiting nutrients for plant growth in water bodies. The P and N compounds are then readily available for algal growth. The effect from all agricultural runoff sources is compounding and causes a massive amount of nutrient pollution to be leached into water bodies. Algal populations rapidly grow causing an increase in water turbidity. The algae continue to grow until it reaches a high enough concentration and the water body is unable to sustain the algae population. The algae then die and begin to decompose resulting in deoxygenation of the water. As oxygen levels in the water decrease the water is

no longer able to support other aquatic species. The overall effect is the creation of dead zones where aquatic life cannot be sustained. These dead zones have devastating impacts on the ecosystems in and surrounding these bodies of water. Dead zones can have significant economic ramifications as well.

According to the EPA (2017b), the tourism industry loses close to \$1 billion each year due to polluted water bodies. They also state that nutrient pollution results in increased water treatment costs and tens of millions of dollars in losses to the commercial fishing industry.

Phosphorus in poultry excreta

Many common poultry feed ingredients such as corn, soybean meal and other crops have most of the P content bound as phytate. Poultry species have a limited ability to utilize phytate because they do not produce the phytase enzyme that is necessary to break down phytase. Because of this, additional P must be added to the diets, usually in the form of calcium phosphate.

Yi et al. (1996) conducted an experiment to investigate the effects of adding phytase or nonphytate phosphorus to broiler diets on performance, P, Ca and N utilization and P excretion. Treatment diets were formulated to contain 0.27% P with 0%, 0.36%, 0.45% or 0.54% nonphytate P. Treatments were given with or without 350, 700, or 1050 U / kg of phytase enzyme. They found a linear increase in body weight (BW) and feed intake (FI) as phytase levels increased in the 0% to 0.45% nonphytate P diets. They also found that P excretion increased linearly in the diets lacking phytase as nonphytate P levels increased. As phytase levels increased in the diets, the P excretion decreased linearly. The authors

attribute the improved weight and feed intake to the increased availability of P when phytase was added to the P deficient diets. They also attribute the reduced P excretion in diets formulated with phytase to be a result of the birds being able to absorb more P from the feed.

Effects of impaired bone development in poultry production

Commercial strains of broilers have been bred to produce faster growing birds for use in the broiler industry. Selecting for rapid growth in poultry species has increased the efficiency of production of meat type birds. While increased growth rates can reduce the amount of time and resources needed to grow broilers, excessive growth can cause physiological issues with the birds resulting in loss of product. A study by Williams et al. (2000) compared the skeletal development of two Ross broiler strains, a rapid growing broiler strain used for commercial production and a slower growing control strain. Sample birds were collected and euthanized on days 4, 11, 25, 32 and 39 days of age. Upon collection tibiotarsi were dissected and analyzed for ash content and bone density. The authors discovered that bone density of the control strain was higher than the rapid growing strain at all ages. Birds from the control strain had higher periosteal bone density than those of the rapid growing strain. Additionally, birds from the control strain had 5% more endosteal bone than those of the rapid growing strain. The authors determined that the decreased mineralization is most likely due to an inability for the skeletal system to develop in proportion to the muscle growth of the bird. This effect may be due to genetic effects on mineral utilization in the rapid growing strain.

CHAPTER 3

MATERIALS AND METHODS

Animals and care

All methods used in this study were approved by the Louisiana State University Agricultural Center Animal Care and Use Committee.

A total of 300 male Ross x Ross 708 broiler chicks were utilized in experiment 1. The birds were obtained 0 days post hatch from a commercial hatchery (Raeford Farms of Louisiana, Gibson, LA). All birds were wing banded at the initiation of the experiment. Groups of 6 chicks were randomly allotted to cages in one of 3 temperature controlled starter battery cages located in the LSU chick laboratory. Battery cages were kept in one ventilated, negative pressure room. Battery temperature was kept at approximately 35°C for the first week and then lowered to approximately 32°C on day 7. Trial replicates consisted of one cage containing six chicks each. The battery consisted of 24 woven metal wire cages stacked (6 levels) on each end of the battery. Each level was partitioned into two cages with metal wire dividers. A single cage measured 33 x 99 cm (544 cm²/bird) and provided access to one trough feeder and trough waterer. Chicks were subjected to 24 hours of light/day and had ad libitum access to feed and water through the duration of the experiment which was conducted over a period of 11 days. All birds were euthanized via carbon dioxide asphyxiation on day 10 post hatch.

A total of 450 male Ross x Ross 708 broiler chicks were utilized in experiment 2. The birds were obtained day 0 post hatch from Raeford Farms of Louisiana (Gibson, LA). All birds were wing banded at the initiation of the

experiment. Chicks were raised in one of 5 starter battery cages under the same environmental conditions as Trial 1. Trial replicates consisted of one cage containing 5 chicks each (653.4 cm²/bird). Chicks were allowed ad libitum access to feed and water through the duration of the experiment. Chicks were subjected to 24 hours of light/day and had ad libitum access to feed and water through the duration of the experiment which was conducted over a period of 19 days. All birds were euthanized via carbon dioxide asphyxiation on day 18 post hatch.

Treatment diets

In trial 1 each cage of chicks was randomly assigned to one of five treatment diets. Ten replications of each treatment were administered. Diets were provided in mash form. The basal diet was corn-soybean meal based and formulated to meet dietary requirements suggested for Ross 708 broiler strain. The diets were formulated to contain 3000 kcal ME/kg. Dietary treatments were 1) control diet containing 0.0% biochar and 4.0% cornstarch, 2) control with 0.5% inclusion of biochar, 3) control with 1.0% inclusion of biochar, 4) control with 2.0% inclusion of biochar, 5) control with 4.0 % inclusion of biochar. Biochar was added to each treatment diet at the expense of cornstarch to keep the diets isonitrogenous and isocaloric. Ingredient composition and nutrient composition of diets used in trial 1 can be found in Tables 1 and 2, respectively. Nutrient composition of diets in both trials was based on analysis from the University of Missouri (Columbia, MO)

Table 1. Ingredient composition of treatment diets for 0 to 10 day old broiler chicks.

Ingredient	0.0% Biochar	0.5% Biochar	1.0% Biochar	2.0% Biochar	4.0% Biochar
Units	%	%	%	%	%
Corn	44.22	44.22	44.22	44.22	44.22
Soybean meal	42.68	42.68	42.68	42.68	42.68
Soy oil	4.63	4.63	4.63	4.63	4.63
Monocalcium phosphate	1.63	1.63	1.63	1.63	1.63
Limestone	1.17	1.17	1.17	1.17	1.17
Salt	0.50	0.50	0.50	0.50	0.50
Mineral mix ¹	0.25	0.25	0.25	0.25	0.25
Vitamin mix ²	0.25	0.25	0.25	0.25	0.25
Choline chloride ³	0.05	0.05	0.05	0.05	0.05
DL-Met	0.36	0.36	0.36	0.36	0.36
L-Thr	0.06	0.06	0.06	0.06	0.06
BioLys	0.20	0.20	0.20	0.20	0.20
Cornstarch	4.00	3.50	3.00	2.00	0.00
Biochar	0.00	0.50	1.00	2.00	4.00

¹ Provided per kilogram of diet: Cu (copper sulfate), 15 mg; I (calcium iodate), 1.25 mg; Fe (ferrous sulfate•H₂O), 50 mg; manganese (manganese sulfate), 100 mg; Se (sodium selenite), 0.30 mg; Zn (zinc sulfate), 100 mg.

² Provided per kilogram of diet: vitamin A, 8,002.78 IU; vitamin D₃, 3003.8 IU; vitamin E, 25 IU; menadione, 1.5 mg; vitamin B₁₂, 0.02 mg; biotin, 0.1 mg; folic acid, 1 mg; niacin, 50 mg; pantothenic acid, 15 mg; pyridoxine, 4 mg; riboflavin, 10 mg; thiamin, 3 mg.

³ Contains 750,000 mg/kg of choline.

In trial 2 each cage of chicks was randomly assigned to one of nine treatment diets. Ten replications of each treatment were administered. All diets were provided in mash form. Diets in trial 2 were formulated with the same basal diet used in trial 1. Dietary treatments were 1) control diet containing 4.0% cornstarch, 2) control with 0.25% inclusion of biochar, 3) control with 0.5%

Table 2. Calculated metabolizable energy (ME) and nutrient analysis values of treatment diets for 0 to 10 day old broilers.

Composition	0.0% Biochar	0.5% Biochar	1.0% Biochar	2.0% Biochar	4.0% Biochar
ME, kcal/kg*	3000	3000	3000	3000	3000
CP, %	23.54	23.14	24.05	24.52	24.40
Ca, %	1.32	0.91	1.20	1.08	1.17
Non-phytate P, %	0.50	0.50	0.50	0.50	0.50
Lys, %	1.52	1.49	1.59	1.74	1.44
Met, %	0.66	0.57	0.66	0.76	0.59
Cys, %	0.35	0.35	0.36	0.39	0.35
Met+Cys, %	1.01	0.92	1.02	1.15	0.94
Thr, %	0.91	0.89	0.94	0.97	0.91
Arg, %	1.58	1.55	1.60	1.68	1.53
Ile, %	1.11	1.08	1.12	1.16	1.08
Val, %	1.18	1.16	1.19	1.25	1.15
Leu, %	1.91	1.88	1.94	2.00	1.88
His, %	0.62	0.62	0.63	0.66	0.61
Trp, %	0.32	0.33	0.33	0.31	0.31

* Based on formulation estimates not actual dietary analysis

inclusion of biochar, 4) control with 0.75% inclusion of biochar, 5) control with 1.0% inclusion of biochar, 6) control with 1.25% inclusion of biochar, 7) control with 1.5% inclusion of biochar, 8) control with 1.75% inclusion of biochar, 9) control with 2.0% inclusion of biochar. Biochar was added at the expense of cornstarch. The biochar-cornstarch mixture was kept at 4.0% of the diet although biochar was only substituted up to 2.0%. This was done so the diets from trial 1 and trial 2 would remain similar. Ingredient and nutrient composition for diets used in trial 2 can be found in Tables 3 and 4, respectively.

Table 3. Ingredient composition of treatment diets for 0 to 18 day old broiler chicks

Ingredient	0.00% Biochar	0.25% Biochar	0.50% Biochar	0.75% Biochar	1.00% Biochar	1.25% Biochar	1.50% Biochar	1.75% Biochar	2.00% Biochar
Units	%	%	%	%	%	%	%	%	%
Corn	44.22	44.22	44.22	44.22	44.22	44.22	44.22	44.22	44.22
Soybean meal	42.68	42.68	42.68	42.68	42.68	42.68	42.68	42.68	42.68
Soy oil	4.63	4.63	4.63	4.63	4.63	4.63	4.63	4.63	4.63
Monocalcium phosphate	1.63	1.63	1.63	1.63	1.63	1.63	1.63	1.63	1.63
Limestone	1.17	1.17	1.17	1.17	1.17	1.17	1.17	1.17	1.17
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Mineral mix ¹	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin mix ²	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Choline chloride ³	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
DL-Met	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36
L-Thr	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
BioLys	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Cornstarch	4.00	3.25	3.50	3.25	3.00	2.75	2.50	2.25	2.00
Biochar	0.00	0.25	0.50	0.75	1.00	1.25	1.50	1.75	2.00

¹ Provided per kilogram of diet: Cu (copper sulfate), 15 mg; I (calcium iodate), 1.25 mg; Fe (ferrous sulfate•H₂O), 50 mg; manganese (manganese sulfate), 100 mg; Se (sodium selenite), 0.30 mg; Zn (zinc sulfate), 100 mg.

² Provided per kilogram of diet: vitamin A, 8,002.78 IU; vitamin D₃, 3003.8 IU; vitamin E, 25 IU; menadione, 1.5 mg; vitamin B₁₂, 0.02 mg; biotin, 0.1 mg; folic acid, 1 mg; niacin, 50 mg; pantothenic acid, 15 mg; pyridoxine, 4 mg; riboflavin, 10 mg; thiamin, 3 mg.

³ Contains 750,000 mg/kg of choline.

Table 4. Calculated metabolizable energy (ME) values and nutrient analysis of treatment diets for 0 to 18 day old broilers.

Composition	0.00% Biochar	0.25% Biochar	0.50% Biochar	0.75% Biochar	1.00% Biochar	1.25% Biochar	1.50% Biochar	1.75% Biochar	2.00% Biochar
ME, kcal/kg*	3000	3000	3000	3000	3000	3000.00	3000	3000	3000
CP, %	23.85	24.21	23.58	23.26	23.63	24.48	24.84	24.3	24.18
Ca, %	1.19	1.01	0.83	0.88	0.83	1.20	0.88	1.16	1.30
Non-phytate P, %	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Lys, %	1.49	1.42	1.48	1.48	1.45	1.43	1.59	1.56	1.32
Met, %	0.62	0.62	0.66	0.56	0.52	0.57	0.55	0.69	0.56
Cys, %	0.35	0.34	0.36	0.36	0.33	0.34	0.35	0.35	0.31
Met+Cys,%	0.97	0.96	1.02	0.92	0.85	0.91	0.90	1.04	0.87
Thr, %	0.91	0.90	0.92	1.34	0.86	0.89	0.92	0.95	0.84
Arg, %	1.51	1.50	1.55	1.62	1.45	1.50	1.58	1.58	1.37
Ile, %	1.09	1.06	1.07	1.16	1.05	1.10	1.13	1.13	1.02
Val, %	1.15	1.13	1.13	1.23	1.11	1.15	1.20	1.18	1.07
Leu, %	1.86	1.83	1.87	1.94	1.82	1.86	1.91	1.91	1.74
His, %	0.60	0.60	0.60	0.64	0.58	0.60	0.63	0.62	0.56
Trp, %	0.30	0.29	0.29	0.30	0.30	0.31	0.30	0.30	0.29

Growth and performance

In trial 1 broilers and feed were weighed at the initiation of the experiment (day 0 post hatch) and at the termination of the experiment (day 10 post hatch) to determine day 0 body weight (BW), day 10 BW, average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G:F). In trial 2 birds and feed were weighed on days 0, 10 and 18 post-hatch to calculate day 0 BW, day 10 BW, day 18 BW, ADG, ADFI and (G:F)

Bone breaking strength

On day 10 (trial 1) and day 18 (trial 2) the left tibiotarsus was collected from each bird immediately after asphyxiation and analyzed for breaking strength (BBS). Bone breaking strength was measured with a Stable Micro Systems texture analyzer (Model TA-HDi, Hamilton, MA) (trial 1) and Instron tensile tester (Model 5544, Norwood, MA) (trial 2) using A three-point bend rig with a 25kg load capacity. The crosshead speed was set to 5mm/s to avoid splintering of the bone. Bones were stored at - 4°C from the time of collection until the time of analysis. Bones were collected and analyzed in groups of 6 (trial 1) or 5 (trial 2) by repetition.

Fecal analysis

Fecal trays were emptied on day 7 for trial 1 and days 7 and 17 for trial 2 to prevent collection of old feces. Fecal samples were collected by hand from each cage in both experiments. Approximately 100g of feces were taken from each cage for analysis. Fecal samples were stored at - 4°C until time of analysis.

All fecal samples were weighed then heated in a drying oven (Fischer Scientific. Waltham, MA) at 50°C for 24 hours. Total weight and dry weight were used to determine DM content of the feces. After drying, the samples were ground. The ground fecal matter was sent to the Louisiana State University Ag Chemistry Lab (Baton Rouge, LA) for phosphorus and nitrogen analysis.

Statistical analysis

Both trials were completely randomized designs. All data were analyzed using the GLM procedure in SAS (Version 9.4 SAS Inst. Inc., Cary, NC). Treatment means were separated using Fischer's LSD. Treatment means were considered significantly different at $P < 0.05$.

CHAPTER 4

RESULTS AND DISCUSSION

Performance data: trial 1

Least squares means for average daily gain (ADG), average daily feed intake (ADFI), feed efficiency presented as gain: feed ratio (G:F), day 0 body weight (BW), day 10 BW, and bone breaking strength (BBS) for broilers fed one of five treatment diets containing biochar at various inclusion rates are presented in Table 5.

Average daily gain was affected by dietary inclusion rate of biochar ($P < 0.01$). Birds fed the control, 0.5% biochar or 1.0% biochar diets had similar ADG. Birds fed the control, 1.0% biochar or 2.0% biochar diets had similar ADG and birds fed the 2.0% or 4.0% biochar diets had similar ADG. Birds fed the 0.5% biochar diets had higher ADG compared to birds fed 2.0% or 4.0% biochar diets. Average daily feed intake was not affected by dietary inclusion rate of biochar ($P > 0.05$). Feed efficiency was affected by dietary inclusion rate of biochar. Birds fed the control, 0.5%, 1.0% or 2.0% biochar diets had higher G:F than birds fed the 4.0% biochar diet. Results of day 10 BW were similar to the ADG. Birds fed the control, 0.5%, or 1.0% biochar diets had similar day 10 BW. Birds fed the control, 1.0% or 2.0% biochar diets had similar day 10 BW. Bone breaking strength did not appear to be effected by dietary inclusion of biochar.

From the results in Table 5 it can be noted that feeding biochar at the 4.0% level seems to have a negative impact upon broiler performance. Birds fed the 4.0% biochar diet had lowered ADG, G:F ratio and day 10 BW compared to

Table 5. Least squares means for average daily gain (ADG), average daily feed intake (ADFI), and feed efficiency (G:F), day 0 body weight (BW), day 10 BW, and BBS of 0 to 10 day old broilers.

Treatment	ADG (g)	ADFI (g)	G:F (g/g)	Day 0 BW	Day 10 BW (g)	BBS (kg)
1) 0.0% Biochar	20.67 ^{ab}	22.95	0.90 ^a	39.45	246.08 ^{ab}	4.78
2) 0.5% Biochar	21.06 ^a	23.39	0.90 ^a	39.43	250.05 ^a	4.96
3) 1.0% Biochar	20.57 ^{ab}	22.96	0.89 ^a	39.43	245.12 ^{ab}	4.75
4) 2.0% Biochar	19.73 ^{bc}	22.16	0.89 ^a	39.43	236.77 ^{bc}	4.52
5) 4.0% Biochar	18.97 ^c	22.21	0.85 ^b	39.40	229.12 ^c	4.35
P-values (P=)	0.01	0.11	0.01	0.97	0.01	0.09
SEM	0.35	0.38	0.01	0.06	3.52	0.16

^{abc}Means within columns with different superscripts are different (P<0.05).

birds fed diets with lower inclusion rates of biochar or the control. The reduced performance could be due to a binding effect that biochar may exhibit on the nutrients in the digestive tract. Studies have shown that biochar has some ability to bind certain nutrients such as N, P and water in the diet (Laird et al. 2010; Wang et al. 2015). This could lead to lower absorption rates in the small intestine. The ability of biochar to bind water may exacerbate any negative effects of nutrient digestibility. If more water is retained in the intestinal contents as it passes through the small intestine, it may prevent absorption of other nutrients due to dilution and lowered surface area of contact between the epithelium of the small intestine and its digestive contents. It is unclear as to why the birds with higher biochar inclusion rates did not perform as well as the birds fed the control or lower inclusion rate diets. Further testing is required to determine the exact cause of the reduced growth and performance due to higher dietary biochar inclusion.

The data in Table 5 suggest that BBS was not affected by any inclusion rate of biochar. In the study performed by Williams et al. (2000) skeletal growth is rapid between 4 and 18 days of age. Because the birds were euthanized during this period of development, it is possible that the birds were not subjected to the treatments long enough for effects to be seen. This may account for the lack of significant differences in BBS between birds fed different treatments.

Fecal and bone analysis: trial 1

Least squares means of fecal N%, P%, DM%, and BBS for birds fed one of five treatment diets containing various inclusion rates of biochar are presented in Table 6. No effect ($P > 0.05$) was observed on fecal N, P or DM concentrations for birds fed any dietary inclusion rate of biochar.

Table 6. Least squares means for fecal nitrogen (%), phosphorus (%), and dry matter (%) of 0 to 10 day old broilers.

Treatment	Nitrogen (%)	Phosphorus (%)	DM (%)
1) 0.0% Biochar	2.06	1.32	46.83
2) 0.5% Biochar	2.05	0.98	46.11
3) 1.0% Biochar	2.11	1.10	48.41
4) 2.0% Biochar	2.26	1.25	52.31
5) 4.0% Biochar	2.05	1.00	47.42
P-values (P=)	0.83	0.09	0.73
SEM	0.15	0.11	3.42

Performance analysis: trial 2

Least squares means for day 0, 10, and 18 BW for birds fed one of nine treatment diets containing various inclusion rates of biochar are presented in Table 7. No differences in day 0 BW were found between any of the treatment groups ($P > 0.05$). Body weight on day 10 and 18 were not affected by any biochar inclusion rates.

Table 7. Least squares means for broiler weights at 0, 10 and 18 days of age for 0 to 18 day old broilers.

Treatment	Initial weight day 0 (g)	Body weight day 10 (g)	Body weight day 18 (g)
1) 0.00% Biochar	44.00	300.0	705.3
2) 0.25% Biochar	44.02	309.0	713.3
3) 0.5% Biochar	44.02	307.3	705.4
4) 0.75% Biochar	44.06	310.3	686.9
5) 1.0% Biochar	44.06	309.6	719.2
6) 1.25% Biochar	44.08	311.5	698.6
7) 1.5% Biochar	44.06	300.2	712.3
8) 1.75% Biochar	44.08	299.3	675.9
9) 2.0% Biochar	44.10	306.2	657.2
P-values (P=)	0.99	0.89	0.42
SEM	0.12	7.24	19.92

Means were considered different at $P < 0.05$

Least squares means for ADG, ADFI, G:F and BBS for birds fed one of nine treatment diets containing various inclusion rates of biochar are presented in Table 8. Average daily gain, ADFI, G:F and BBS were not affected by any dietary biochar inclusion rate ($P > 0.05$).

These results are consistent with the results obtained from trial 1 presented in Table 5. In the initial trial, we observed that there was a decrease in day 10 weight when biochar was included at 4.0% of the diet. Due to this decrease in performance we determined that the limit of biochar that can be added to the diet without affecting bird performance was 2.0% or less. The data from both trials support our hypothesis that broilers can tolerate dietary biochar inclusion up to the 2.0% level without affecting bird performance.

Table 8. Least squares means for average daily gain (ADG), average daily feed intake (ADFI), feed efficiency (G:F), and bone breaking strength (BBS) of 0 10 18 day old broilers

Treatment	ADG (g)	ADFI (g)	G:F (g/g)	BBS (kg)
1) 0.00% Biochar	36.74	46.11	0.7964	12.100
2) 0.25% Biochar	37.18	46.46	0.8000	12.291
3) 0.5% Biochar	36.75	46.66	0.7883	11.169
4) 0.75% Biochar	35.71	46.02	0.7768	11.275
5) 1.0% Biochar	37.51	47.33	0.7925	12.785
6) 1.25% Biochar	36.36	47.42	0.7675	11.900
7) 1.5% Biochar	37.12	47.32	0.7857	12.525
8) 1.75% Biochar	35.11	45.53	0.7661	10.998
9) 2.0% Biochar	34.06	44.51	0.7624	11.639
P-values (P=)	0.41	0.57	0.57	0.8466
SEM	1.11	1.05	0.015	0.6975

Means were considered different at $P < 0.05$

In the study conducted by Evans et al. (2015) birds fed biochar at 6.2% or 6.9% had reduced performance. This performance reduction was attributed to the arsenic content of the feed. The results from our experiment suggest that the high inclusion rate of biochar may have contributed to this negative impact on bird performance. We believe that high inclusion was not the only factor that contributed to the poor performance; however, it may have compounded the hypothesized negative effect due to arsenic.

Williams et al. (2000) observed rapid bone growth and development between 4 and 18 days of age in both strains of Ross broilers. Therefore, it was expected that the breaking strengths of the bones collected on day 18 in trial 2 would be considerably higher than those collected on day 10 in trial 1. No effect was observed on bone breaking strength for broilers fed biochar at any inclusion level at day 10 or 18 post hatch.

Fecal analysis: trial 2

Least squares means of fecal N%, P% and DM% for day 10 and 18 of birds fed one of nine treatment diets containing various inclusion rates of biochar are presented in Table 9. No effect of dietary biochar inclusion rate was observed for fecal N% on day 10 or 18. Dietary inclusion rate of biochar affected fecal P% on day 10. Birds fed the 0.25%, 0.5%, 0.75%, 1.0%, 1.25%, or 1.5% biochar diets had similar fecal P% on day 10. Birds fed the control, 0.5%, 1.0%, 1.25%, or 1.5% biochar diets had similar fecal P% on day 10. Birds fed the control, 1.5% or 1.75% biochar diets had similar fecal P% on day 10. Birds fed the control, 1.75% or 2.0% diets had similar fecal P% on day 10. Dietary inclusion rate of

biochar also affected day 18 P%. Birds fed the 0.25%, 0.5%, 0.75%, 1.0% or 1.5% biochar diets had similar fecal P% on day 18. Birds fed the control or 1.5% biochar diets had similar fecal P% on day 18. Birds fed 1.75 or 2.0% biochar diets had similar fecal P% on day 18.

From this we can note that on day 10 birds fed the 0.25% or 0.75% biochar diets had higher fecal P% than birds fed the control, 1.75% or 2.0% biochar diets. On day 18 birds fed the 0.5% - 1.25% biochar diets had higher fecal P% than those fed the control diet. The birds fed the 1.75% or 2.0% biochar diets had the lowest fecal P% on day 18. It can be observed that the differences in P% between treatments were consistent between day 10 and day 18.

Differences in the P% became more apparent on day 18. Biochar may have some binding effect upon fecal P which apexes at the 1.25% inclusion rate.

An effect on day 10 fecal DM% by dietary inclusion rate of biochar was observed. Birds fed the control, 0.25%, 0.5%, 0.75%, 1.25%, or 1.5% biochar diets had similar fecal DM%. Birds fed the control, 0.25%, 0.5%, 1.25% or 1.5% biochar diets had similar fecal DM%. Birds fed the control, 0.5%, 1.25%, or 1.75% biochar diets had similar fecal DM%. Birds fed 1.0%, 1.25%, 1.75% or 2% biochar diets had similar fecal DM%. The data show that the birds fed the 1.0% or 2.0% had higher DM% than the birds fed the control diets. No effect was seen on fecal DM on day 18 of the experiment.

Table 9. Least square means for fecal nitrogen, phosphorus and dry matter content of 0 to 18 day old broilers.

Treatment	Day 10			Day 18		
	Nitrogen (%)	Phosphorus (%)	Dry matter (%)	Nitrogen (%)	Phosphorus (%)	Dry matter (%)
1) 0.00% Biochar	1.675	1.933 ^{bcd}	37.28 ^{bcd}	1.604	2.012 ^b	34.13
2) 0.25% Biochar	1.657	2.439 ^a	36.22 ^{cd}	1.62	2.497 ^a	34.38
3) 0.5% Biochar	1.662	2.220 ^{ab}	36.87 ^{bcd}	1.578	2.512 ^a	34.73
4) 0.75% Biochar	1.468	2.537 ^a	34.48 ^d	1.498	2.492 ^a	31.8
5) 1.0% Biochar	1.771	2.132 ^{ab}	42.33 ^a	1.73	2.457 ^a	39.93
6) 1.25% Biochar	1.708	2.376 ^{ab}	37.84 ^{abcd}	1.5	2.430 ^a	32.91
7) 1.5% Biochar	1.532	2.115 ^{abc}	35.68 ^{cd}	1.43	2.222 ^{ab}	32.78
8) 1.75% Biochar	1.716	1.688 ^{cd}	41.28 ^{ab}	1.63	1.398 ^c	38.05
9) 2.0% Biochar	1.787	1.521 ^d	39.94 ^{ab}	1.897	1.309 ^c	44.01
P-values (P=)	0.066	0.0002	0.028	0.651	<0.0001	0.14
SEM	0.074	0.155	1.739	0.161	0.132	3.12

^{abcd} Means within columns with different superscripts are different (P<0.05).

CHAPTER 5

SUMMARY, CONCLUSION AND FUTURE RESEARCH

Summary

Feeding biochar at an inclusion rate of 2.0% or lower to broiler chicks did not affect growth or performance. Feeding biochar at a 4.0% dietary inclusion level negatively affected weight gain of broiler chicks. Feeding biochar at any dietary inclusion rate did not affect bone breaking strength.

Feeding biochar at any inclusion rate did not appear to affect fecal DM. No differences were observed in fecal DM analysis for trial 1. The differences observed in fecal DM on day 10 in trial 2 were inconsistent. In trial 2 there were no observed differences in fecal DM at day 18.

There were no effects on fecal N by dietary inclusion of biochar at any rate. No differences were observed on fecal N at any time points in both trials. The effect of dietary biochar inclusion on fecal P was inconsistent in this study. No effect of dietary biochar inclusion was observed on fecal P% in trial 1. Differences in fecal P were found in trial 2. Lower inclusion levels of biochar seem to have increased fecal phosphorus concentrations with respect to the control. Higher levels of biochar inclusion seem to decrease fecal P levels. Biochar may exhibit some binding effect on fecal phosphorus that apexes at the 1.25% inclusion rate.

Conclusion

Feeding biochar at low levels, between 0.25 and 2.0% of the diet, does not negatively impact the performance of broiler chicks. Further investigation is required to determine if feeding biochar to broilers has any effect on fecal P.

Future research

Investigation into the effects of dietary inclusion of biochar on fecal P in broilers should be investigated further. Total fecal sampling methods may prove more useful in providing more conclusive results.

Although fecal P and N may not be effected by dietary inclusion of biochar, leaching tests should be performed on fecal samples to determine if these nutrients may be less soluble as seen in soil amendment tests using biochar.

Biochar should also be investigated as a litter amendment. The nutrient retention effects of biochar may be better observed in the litter rather than in the intestinal environment of broilers.

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VITA

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