Investigating Benefits of Supplementing Broiler Feed with Broiler Litter Biochar



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Executive Summary

In areas of high livestock density, manure and bedding used as a soil amendment can contribute towards a soil nutrient surplus. This surplus can increase the potential for nutrients to leach into the environment. Due to the current nutrient surplus in the Lower Mainland, B.C.'s poultry sector is working hard to find cost-effective alternatives to the land application of broiler chicken litter. One such alternative, thanks to broiler litter's high wood content, could be to heat the broiler litter in a pyrolysis unit to produce biochar.

Biochar is a fine-grained, highly porous substance with a high surface area per unit of volume. Used for centuries as a soil amendment, more recently biochar has been proposed as a feed additive to improve the digestive process of broilers. By helping in the grinding process and providing a habitat for beneficial microorganisms, it is claimed that biochar can increase broiler weight gain and/or improve feed conversion by reducing nutrient loss in excrement.

A 35 day floor pen study was carried out in which 288 one-day old male broiler chicks were randomly allotted to 24 pens. Each pen was fed one of three treatments; feed supplemented with 0% (T1), 0.5% (T2) and 1% (T3) biochar made from broiler litter. Over the course of the study, broiler weight gain, feed consumption, feed conversion ratio and health were measured.

The results of the study suggested that supplementing broiler chicken feed with 0.5% or 1% biochar has no statistically significant impact on broiler chicken weight gain, feed consumption, feed conversion ratio or health. While it is unknown as to why there was no impact, one reason could be the composition of the broiler litter biochar. As such, use of a different feedstock, such as clean wood, could have had different results.



Introduction to B.C.'s Poultry Sector

Poultry production is an economic cornerstone of B.C.'s agriculture sector. According to the 2012 B.C. Agri-Food Industry Year in Review, B.C.'s poultry farmers raise and market over 180 million kilograms of chickens and turkeys, generating almost \$421 million a year in total farm cash receipts.¹ At any one time during the year, B.C.'s Fraser Valley is home to almost 90% of the chickens and turkeys in B.C. These chickens and turkeys are estimated to produce over 200,000 tonnes of litter a year.

Broiler litter at the end of a grow-out cycle is a mixture of droppings, bedding material, spilled feed and water; the proportions of which vary depending on barn management. Although many different bedding materials are available, most poultry farmers in B.C. use wood shavings, typically a mixture of spruce, pine and fir. At the end of each production cycle, when broilers are shipped for processing, litter is removed from the barn and storage for subsequent use as fertilizer through land application.

Broiler litter contains useful amounts of phosphorus, nitrogen and potassium, as well as trace elements of zinc, manganese and copper. While nutrient content can vary considerably between both sources and batches, broiler litter can be the basis for very productive pasture and agricultural production. In many areas around the world, broiler litter is seen as a valuable source of locallyavailable crop nutrients.

While a valuable source of crop nutrients, there can be a risk of nutrient accumulation in soils with regular application of high rates of broiler litter over the long-term. Phosphorus, zinc and copper, although essential plant nutrients found in broiler litter, can accumulate in soils over time causing nutrient imbalances. Nitrogen, in the form of urea and uric acid, can create ammonia which can contribute to the formation of air pollutants that impact regional air quality. Nitrogen can also impact water quality by polluting groundwater and, as with phosphorus, cause the eutrophication of surface water.

With a relatively low moisture content of 30% - 40%, it is possible to transport broiler litter to nutrient deficient areas for land application. However, on average, a tonne of broiler litter contains less than 200lbs of nitrogen, phosphate and potash, while a tonne of commercial fertilizer contains over 700lbs. Due to its low nutrient content to weight ratio (when compared to commercial fertilizer), it is often uneconomical to transportation broiler litter over long distances. While this distance depends heavily upon transportation costs and the cost of commercial fertilizer, a good rule of thumb is that broiler litter should be used within 50km - 100km of the source.

Currently there are more soil nutrients available in B.C.'s Lower Mainland than are needed for the crops that are grown or that the land can sustain. Several reports over the past few years have concluded that nutrient surpluses from livestock farming have existed in the Lower Mainland for many years. These surpluses are causing nutrients to build-up in soils and increasing the potential

¹ <u>http://www.agf.gov.bc.ca/stats/YinReview/Agrifood-YIR-2012.pdf</u>



for nutrients to leach into the environment. Due to this surplus, B.C.'s poultry sector has been working hard for many years to find cost-effective alternatives to the land application of broiler litter in the Lower Mainland.

What is Biochar?

Similar to blackened charcoal found at the bottom of extinguished fires, biochar is a form of black carbon created by heating organic material (feedstock) such as wood waste, straw or litter, at a high temperature in an oxygen-free or low oxygen environment. The technical term for this process is called pyrolysis. While traditional charcoal is one example of biochar produced from wood, today the term biochar is much broader than this, encompassing black carbon produced from any feedstock.

Used for centuries as a soil amendment, biochar is a fine-grained, highly porous substance with a high surface area per unit of volume (Figure 1). When applied to soils, and due to its ability to persist with very little biological decay, biochar's high surface area and porosity acts as a catalyst for plant growth by retaining water and providing a habitat for beneficial microorganisms to flourish. Biochar is also used as a natural filter and can be consumed to help adsorb toxins.

Figure 1: Images of Biochar at the Macro (left) and Micro (right) Scale



More recently, the use of biochar beyond that of a soil amendment has started to grow. By helping in the grinding process and providing a habitat for beneficial microorganisms in the digestive system of broilers, it is claimed that the consumption of biochar can increase uptake of foodstuffs and the energy contained within them. Increased uptake can result in increased weight gain and/or improved feed conversion by reducing nutrient loss in excrement.

Biochar also helps to keep broiler's digestive systems healthy. Furthermore, due to its absorptive properties, it is claimed that biochar can reduce the moisture content and odour of broiler



droppings, improving overall barn environment. However, despite its potential benefits not all biochar is created equal, as both the chemical and physical properties of biochar can vary significantly depending upon the type of feedstock used.

Biochar as a Broiler Feed Supplement

Several research projects have been conducted over the past decade to better understand the benefits of biochar as a feed supplement for broilers. Gerlach and Schmidt (2012) found biochar deactivated toxins already in the digestive system, positively activating intestinal flora and vitality. Teleb *et al.* (2004) found a diet supplement of 0.5% biochar made from locally available wood overcame the detrimental effects of feeding broilers 30 ppb aflatoxin² by showing reduced mortality rates and improved growth rates when compared to those fed 30 ppb aflatoxin.

Kutlu *et al.* (2000), Kana *et al.* (2010), Jiya *et al.* (2013) and Prasai (2013) all found significant increased growth rates and higher final body weights for broilers fed diets supplemented with 0.2% - 0.6% biochar made from oak, maize cob, seed of Canarium, coconut shell and locally available wood. However, these and other studies also found that too much biochar in the diet can be deleterious. Odunsi *et al.* (2007), Kana *et al.* (2010) and Jiya *et al.* (2013) all found depressed growth rates and final body weights for broilers fed diets supplemented with 2% or more biochar.

Doydora *et al.* (2011), Ritz (2011) and Prasai (2013) found that when used as a feed additive for broilers, biochar made from pine chips, peanut hulls and locally available wood significantly reduced the amount of ammonia and phosphorus in droppings, therefore requiring smaller land area on which to spread the litter. Furthermore, the internet is abound with anecdotal reports that speak to the benefits of using biochar as a feed supplement for broilers, including dryer excreta, reduced odour, reduced water runoff from litter piles during rainfall events, and reduced nitrogen burning after the field application of litter.

Research

Research Justification

Due to the current nutrient surplus from livestock farming, cost-effective alternatives to the land application of broiler litter in the Lower Mainland are required. One such alternative, thanks to broiler litter's high wood content, could be to heat the broiler litter in a pyrolysis unit to produce biochar. This biochar, an inert highly porous black substance with high surface area per unit of volume, could then be used as a supplement in broiler feed. To date no known study has investigated the effects of supplementing broiler feed with biochar made from broiler litter.

Initially, while the thought of supplementing broiler feed with biochar made from broiler litter may seem strange, it should be noted that the idea of feeding poultry litter to livestock is not new.

² Aflatoxin, a group of closely related extremely toxic chemicals that are produced by *Aspergillus flavus* and *Aspergillus parasiticus*, can occur as natural contaminants in poultry feed.



According to Jeffrey *et al.* (1998) and Bolan *et al.* (2010), feeding poultry litter to dairy and beef cattle is often seen as an effective litter disposal option, while processed poultry litter has been used as a feed ingredient for almost 40 years in the U.S. Furthermore, by heating broiler litter to over 500°C for 30 minutes, pyrolysis is a very effective disinfection technique and ensures that biochar is free of pathogens.³

The potential benefits of supplementing broiler feed with broiler litter biochar are two-fold. Firstly, creating demand for broiler litter biochar in the Lower Mainland will provide an alternative to land application; thereby helping to reduce nutrient overloading. Secondly, increased weight gain and/or improved feed conversion will provide economic benefit to B.C.'s broiler farmers by enabling them to sell their birds at a higher weight and/or reduce feed portions.

B.C.'s broilers require 3.3Kg - 3.6Kg feed per bird during their 5 - 6 week production cycle. According to past research, supplementing broiler feed with <1% biochar is sufficient to achieve increased weight gain and/or improved feed conversion. Due to the small amount of biochar needed, if broiler feed was supplemented with 0.5% - 1% biochar, each chicken would only require 16.5 - 36 grams of biochar during its production cycle. At an estimated cost of \$600 - \$1,000/tonne of biochar⁴, the additional cost of feeding B.C.'s broilers 16.5 - 36 grams of biochar would only increase feed costs by 1c - 3.6c/broiler.

If supplementing broiler feed with 0.5% - 1% biochar, as past research suggests, does increases broiler weight gain by 5% - 10%, the market value of each broiler would increase by 10c - 20c (more than the cost of the biochar). Furthermore, if all 85 million broilers produced in the Lower Mainland were fed 0.5 - 1% biochar, this would create demand for 1,403 - 3,060 tonnes of biochar. Roughly 10% of the broiler litter in the Lower Mainland would be needed to make this amount of biochar.

Research Objective

The objective of this research was to determine if supplementing broiler feed with 0.5% or 1% broiler litter biochar increases broiler weight gain and/or improves feed conversion when compared to broilers fed commercial feed. If it can be shown that broiler litter biochar does increase weight gain and/or improve feed conversion, this could provide a cost-effective alternative to the land application of broiler litter in the Lower Mainland.

Study Design

This study was constructed as a replicated floor pen design in 6 blocks of 4 pens each. Treatments were randomly assigned to pens within each block. Because there were 4 pens in each block, the fourth pen was randomly assigned one of the 3 treatments so that one treatment per block was

³ Despite this, it should be noted that biochar is currently not on the Canadian Food Inspection Agency's list of approved feed ingredients for livestock in Canada.

⁴ The cost of biochar depends on feedstock costs and quality. Furthermore, the cost of biochar is expected to change over time as pyrolysis technologies improve. Due to these many variables, the estimated cost of biochar varies greatly.



duplicated in that block. The extra treatment per block was stratified so that they were evenly distributed through all blocks.

Blocking of pens was done to account for potential variation associated with the position of the pens in the barn, and their position relative to heating and ventilation. Table 1 shows the experimental design. The sample size allowed for detection of a 5% difference in final weight with a 0.1 Kg standard deviation at $p \le 0.05$.

Table 1: Experimental Design

Treatment No.	Biochar Inclusion Rate	No. Pens per Treatment	No. Birds per Pen	
T1	0 %	8	12	
T2	0.5%	8	12	
Т3	1.0%	8	12	

Biochar

Broiler litter was taken from a commercial broiler barn in the Fraser Valley and dried to 15% moisture content before being delivered to Diacarbon's pyrolysis unit in Agassiz. Once processed at 550°C for 30 minutes, the resulting biochar was transported to Ritchie-Smith Feeds in Abbotsford where it was incorporated into commercial starter, grower and finisher broiler feed.

Experimental Diets

The experimental diets were formulated to meet the nutritional requirements of commercial broilers. Three diets, starter and grower crumbles and finisher pellets, were manufactured prior to the start of the trial. The pellets were subjected to a durability test to determine if the biochar negatively impacted pellet quality. This test showed no difference in quality between pellets with and without biochar. Once complete, the nine different feeds - starter, grower and finisher feed supplemented with 0% (T1), 0.5% (T2) and 1% (T3) biochar by mass - were delivered to S.J. Ritchie Research Farms Ltd in Abbotsford for the floor pen study.

Despite the biochar causing slight discolouration in the feed and a small change in nutritional value, it was decided to not add any colouring or non-nutritional filler to the feed with 0% biochar (T1). The reason for this decision was that this research was undertaken to determine the impacts of supplementing broiler feed with broiler litter biochar when compared to commercial broiler feed. As such, colouring the 0% biochar feed (T1) and adding filler to alter its nutritional value would defeat the purpose of this research.

Floor Pens

The 35 day floor pen study was carried out using mini-pens (3ft by 4ft) assembled in the middle of a 14,000ft² poultry barn. The experimental broilers were the same age as the 17,000 broilers placed



in the barn. This configuration allowed the trial broilers to be exposed to the type of conditions commonly found in B.C.'s commercial poultry operations.

The pens were assembled in blocks of 4 each, with each of the 6 blocks being separated from each other by approximately 6 feet. Each pen contained a single 16-inch tube feeder and two nipple drinkers. The concrete floor was bedded with approximately 4 inches of soft wood sawdust. Heat was provided to the whole barn through gas-fired brooders and the floor pens were placed so that they were in close proximity to the heaters.

Broilers

17,000 straight-run Ross 308 broiler chicks were purchased from a commercial hatchery and delivered to the S.J. Ritchie Research barn. Broilers were place in the barn according to standard practices. From this available pool, 288 male chicks, identified by feather sexing, were selected and allocated to pens according to the study design. Only healthy, robust chicks were selected for this study.

Procedures

Chicks were arbitrarily placed into pens according to the study design. Chicks were individually weighed at placement and the weights recorded. Any chick that was significantly lighter or heavier that the chicks in the experimental group were not used for this study. Broilers were subsequently weighed individually once weekly and the weights recorded. At the end of the study, all experimental broilers were humanely euthanized and destroyed.

All feed placed into the feeders was weighed and the weight recorded. At each weighing, once weekly, any feed remaining in the feeders was weighed back and replaced. When feed was changed from starter to grower or grower to finisher, all feed weighed back was removed and destroyed. Mortalities were removed from the pens when found; the dead birds were weighed and a post mortem examination performed to determine cause of death.

Statistical Analysis was done using the General Lineal Model procedure in Statistix v.9 (Statistix Software, Tallahassee, Florida). Means were separated by Least Significant Difference procedure.

Results

Tables 2 and 3 show average broiler weight gain, feed consumption and feed conversion ratio (Appendix A and B for data by pen), and mortalities (Appendix C for mortality analysis) for broilers fed T1, T2 and T3. To determine the significance of these results an analysis of variance was carried out using a Randomized Block Design with the 3 treatments (T1, T2 and T3) and 8 blocks as the test parameters, and average start weight, final weight, feed consumption, and feed conversion ratio as the dependent variables. This analysis showed there was no significant difference among the three treatments for the four dependent variables (Appendix D).



To confirm these findings, a pairwise comparison using least significant difference for each variable was created to tabulate the mean results. This analysis was further repeated as a complete randomized design, removing the block parameter (as there was no significant difference among the blocks), to see if this had any impact on the results. The pairwise comparison showed that there were no significant pairwise differences among the means (Appendix E).

Treatment	Average Placement WT. (grams) ^a	Average 7 Day WT. (grams)	Average 14 Day WT. (grams)	Average 21 Day WT. (grams)	Average 28 Day WT. (grams)	Average 35 Day WT. (grams) ^b
T1 (0% biochar)	42.75	197.00	472.66	1,025.66	1,751.6	2,608.89
T2 (0.5% biochar)	42.15	194.84	477.85	1,020.19	1,714.99	2,561.16
T3 (1% biochar)	42.97	199.28	467.81	996.80	1,696.83	2,536.59

Table 2: Average Broiler Weight Gain by Treatment

a – Values within the column are not significantly different (*p*=0.368)

b – Values within the column are not significantly different (*p*=0.574)

Table 3: Average Feed Consumption, Feed Co	onversion Ratio and Mortalities by Treatment
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Treatment	Average Feed Consumption (grams) ^a	Average Feed Conversion Ratio ^b	Mortalities #	Mortality %
T1 (0% biochar)	48,415	1.56	1	1.04%
T2 (0.5% biochar)	47,004	1.58	3	3.13%
T3 (1% biochar)	47,499	1.63	4	4.17%

a – Values within the column are not significantly different (*p*=0.772)

b – Values within the column are not significantly different (*p*=0.387)

Discussion

Once the feed pen trial was completed, laboratory analysis was performed on the broiler litter biochar (Table 4). The reason for this analysis was twofold. Firstly, to see if there was anything in the biochar that might have mitigated the supposed benefits of increased weight gain and/or improved feed conversion. Secondly, to determine if the broiler litter biochar made for this study was similar to broiler litter biochar recently made in a small-scale pyrolysis unit and analysed by the B.C. Ministry of Agriculture.



Table 4 shows that the levels of aluminum (AI) and boron (B) in each of the three biochar samples made for this study were high; the average AI level was 3,791ug/g, while the average B level was 44ug/g. While the high level of AI was surprisingly, of perhaps more importance was the high level of B, as at this level B is found to be toxic to plants. Further scrutiny of the laboratory analysis shows that the level of iron (Fe) varied greatly, from 4,119ug/g to 2,866ug/g. It is unknown what could have caused this variation.

The laboratory analysis also showed that while there were differences between the broiler litter biochar used in this study and that analysed by the B.C. Ministry of Agriculture, these differences were within the range of differences observed between the batches of biochar analysed by the B.C. Ministry of Agriculture. Furthermore, the total organic carbon:hydrogen ratio, a reflection of biochar stability and "completeness" of the pyrolysis process, for both the broiler litter biochar used in this study and the biochar analysed by the B.C. Ministry of Agriculture were similar.

Based on these results, it was determined that any observed differences between the biochar used in this study and that analysed by the B.C. Ministry of Agriculture should be attributed to differences in broiler litter used, and not to the pyrolysis process. This determination is important as it implies that if broiler litter biochar were to be made on a commercial scale, each batch of biochar could be different from the next if the broiler litter comes from different barns.

	Microwave Digestion (HNO ₃) - ICP-OES									
Sample	Al ug/g	B ug/g	Ca %	Cu ug/g	Fe ug/g	K %	Mg %	Mn ug/g		
1	3735	44	3.92	400	3448	3.358	0.865	679		
2	3758	43	4.00	400	4119	3.380	0.874	668		
3	3880	45	3.86	399	2866	3.322	0.876	655		
Average	3791	44	3.93	400	3478	3.35	0.872	667		

Table 4: Laboratory Analysis of Broiler Litter Biochar

Microwave Digestion (HNO ₃) - ICP-OES					Anions - Hot H2O Extraction -HPLC			
Sample	Na ug/g	P %	S %	Zn ug/g	F ⁻ ppm	Cl ⁻ ppm	NO2 ⁻ ppm	Br ⁻ ppm
1	5007	2.102	0.477	643	<0.01	6725	<0.01	<0.01
2	5082	2.091	0.491	623	<0.01	6720	<0.01	<0.01
3	4910	2.167	0.483	671	<0.01	6603	<0.01	<0.01
Average	5000	2.120	0.484	646	<0.01	6683	<0.01	<0.01



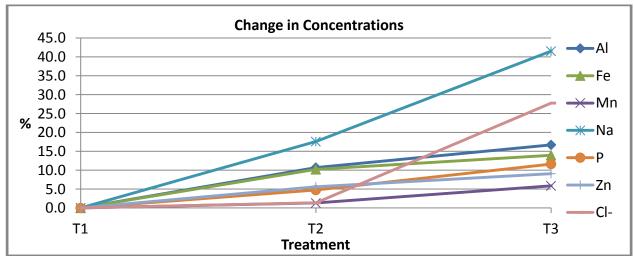
Anions - Hot H2O Extraction -HPLC			Inorganic	рН	Total C, N and H			
Sample	NO3 ⁻ ppm	PO4 ⁻ ppm	SO4 ⁻ ppm	C %	рН 1:2 Н2О	C %	N %	H %
1	<0.01	9491	5476	0.60	10.54	56.1	2.66	2.20
2	<0.01	9913	5263	0.94	10.54	57.4	2.84	2.13
3	<0.01	9505	5141	0.61	10.53	54.5	2.67	2.01
Average	<0.01	9636	5293	0.72	10.54	56.0	2.72	2.11

Table 5, which shows laboratory analysis of the litter from the floor pens (Appendix F for full analysis) shows obvious trends between increased concentrations of Aluminum (Al), Iron (Fe), Manganese (Mn), Sodium (Na), Zinc (Zn), Phosphorous (P) and Chlorine (Cl) and increased biochar. Of these increases, the most dramatic was Na which was 17.6% higher in litter from T2 pens when compared to T1 pens, and 41.5% higher in litter from T3 pens when compared to T1 pens.

Table 5: Laboratory Analysis of Broiler Litter

Treatment	Al ug/g	Fe ug/g	Mn ug/g	Na ug/g	Zn ug/g	P %	Cl ⁻ ppm
T1 (0% biochar)	447.8	627.4	387.9	2,694.8	425.0	1.04	4,080.1
T2 (0.5% biochar)	495.7	691.4	393.0	3,168.5	448.9	1.08	4,135.6
T3 (1% biochar)	522.6	715.0	410.6	3,813.4	463.6	1.16	5,213.4

Graph 1: Increasing Concentrations Found in Broiler Litter





Conclusion

From the results of this study it can be concluded that supplementing broiler feed with broiler litter biochar had no statistically significant impact on broiler weight gain, feed consumption, feed conversion ratio or health.

One reason as to why supplementing broiler feed with biochar had no significant impact could be because broiler litter was used. In studies that found significant increases in growth rates from supplementing broiler feed with biochar, different feedstocks were used to make the biochar; including oak, pine, coconut shells, corn cobs, peanut hulls and seed of Canarium. It is therefore possible that had an alternative feedstock been used (such as pine instead of broiler litter) there may have been a statistically significant impact on weight gain and/or feed conversion.

A second reason as to why supplementing broiler feed with broiler litter biochar had no significant impact could be because of the nutritional value of the biochar. Blake and Hess (2014) found that due to its high calcium and phosphorus content, broiler litter ash could be used as a replacement for dicalcium phosphate in broiler feed without any negative consequences. It is therefore possible that the supplementation of broiler litter biochar resulted in the broilers being feed too high levels of certain nutrients. Feeding broilers excess nutrients may negate possible positive benefits that the biochar might have had. This might go some way to explain the high levels of sodium and chlorine found in the litter of broilers fed T3.

While it is possible that broiler litter biochar could be used as a nutritional supplement in broiler feed, it should be noted that the biochar made for this study was quite different from the biochar analysed by the B.C. Ministry of Agriculture. Furthermore, the iron content in the biochar for this study varied greatly (from 2,866ug/g - 4,119ug/g). These differences, which were attributed to the feedstock, suggest that if broiler litter biochar were used as a supplement in broiler feed, every single batch of biochar would have to be carefully analysed and assessed to determine its nutrient value before it could be added to broiler feed as a nutritional supplement.



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Appendix

Appendix A: Broiler Weight Gain by Pen

		Average	Average	Average	Average	Average	Average	Average
Pen	Treat-	Placement	7 Day	14 Day	21 Day	28 Day	35 Day	Daily WT.
#	ment	WT.	WT.	WT.	WT.	WT.	WT.	Gain
		(grams)	(grams)	(grams)	(grams)	(grams)	(grams)	(grams)
1	T1	41.38	201.75	479.00	999.17	1,745.00	2,659.17	75.98
2	Т3	42.75	204.17	471.58	975.00	1,710.00	2,524.17	72.12
3	T2	42.42	197.00	456.50	989.17	1,704.17	2,555.83	73.02
4	T1	42.17	197.08	446.50	995.83	1,735.83	2,534.17	72.40
5	Т3	43.25	193.50	475.00	999.17	1,714.17	2,585.83	73.88
6	T2	40.17	194.25	507.25	996.67	1,586.67	2,345.00	67.00
7	T1	42.58	200.67	505.00	1,066.67	1,776.67	2,645.00	75.57
8	Т3	41.83	202.08	483.17	1,007.50	1,675.00	2,485.00	71.00
9	T1	41.58	204.25	502.75	1,087.50	1,869.17	2,718.33	77.67
10	T2	40.92	195.17	484.42	1,025.00	1,683.33	2,502.50	71.50
11	T2	41.17	207.33	486.25	1,097.27	1,895.45	2,716.36	77.61
12	Т3	41.75	201.17	463.50	957.50	1,605.00	2,497.50	71.36
13	Т2	42.00	187.58	458.83	969.17	1,634.17	2,439.17	69.69
14	T1	42.25	189.58	463.58	1,029.17	1,741.67	2,589.17	73.98
15	Т3	44.04	192.17	457.92	994.17	1,728.33	2,577.50	73.64
16	Т2	45.17	189.58	471.27	999.09	1,730.00	2,627.27	75.06
17	T1	44.13	193.75	424.50	949.17	1,608.33	2,462.50	70.36
18	Т3	44.46	192.58	471.25	1,021.67	1,766.67	2,631.82	75.19
19	T2	43.13	193.64	468.09	1,031.82	1,783.64	2,668.18	76.23
20	T1	42.71	200.33	502.92	1,095.00	1,842.50	2,749.17	78.55
21	Т2	42.25	194.17	490.17	1,053.33	1,702.50	2,635.00	75.29
22	Т3	42.42	205.58	476.42	1,036.36	1,775.45	2,600.91	74.31
23	Т3	43.29	203.00	443.60	983.00	1,600.00	2,390.00	68.29
24	T1	45.17	188.58	457.00	982.73	1,693.64	2,513.64	71.82
Ave	erage	42.62	197.04	472.77	1,014.21	1,721.14	2,568.88	73.40



Appendix B: Feed Consumption, Feed Conversion Ratio and Mortalities by Pen

Pen #	Treat- ment	7 Day FCR	14 Day FCR	21 Day FCR	28 Day FCR	35 Day FCR	Total Feed Consumption (grams)	Mortalities #
1	T1	0.71	1.18	1.35	1.43	1.53	48,890	0
2	Т3	0.90	1.23	1.41	1.48	1.59	48,040	0
3	T2	0.94	1.29	1.32	1.46	1.56	47,860	0
4	T1	0.88	1.28	1.36	1.45	1.58	47,990	0
5	Т3	0.83	1.20	1.37	1.47	1.56	48,370	0
6	T2	1.00	1.15	1.35	1.48	1.60	44,970	0
7	T1	0.87	1.20	1.34	1.46	1.55	49,170	0
8	Т3	0.84	1.16	1.36	1.50	1.59	47,560	0
9	T1	0.87	1.18	1.25	1.41	1.52	49,480	0
10	T2	0.81	1.15	1.29	1.44	1.55	46,670	0
11	T2	0.89	1.24	1.37	1.45	1.57	46,761	1
12	Т3	0.89	1.25	1.35	1.60	1.67	50,190	0
13	T2	0.80	1.14	1.33	1.49	1.61	47,200	0
14	T1	0.80	1.19	1.30	1.50	1.63	50,780	0
15	Т3	0.82	1.22	1.34	1.46	1.58	48,920	0
16	T2	0.81	1.23	1.36	1.49	1.59	45,830	1
17	T1	0.93	1.29	1.39	1.52	1.54	45,480	0
18	Т3	0.79	1.16	1.30	1.43	1.65	47,770	1
19	T2	0.84	1.18	1.37	1.49	1.61	47,240	1
20	T1	0.94	1.24	1.33	1.45	1.56	51,370	0
21	T2	0.97	1.22	1.33	1.52	1.57	49,500	0
22	Т3	0.87	1.15	1.36	1.47	1.59	45,630	1
23	Т3	1.19	1.74	1.56	1.65	1.82	43,510	2
24	T1	0.87	1.24	1.42	1.51	1.60	44,158	1
Ave	erage	0.88	1.23	1.35	1.48	1.59	47,639	N/A



Appendix C: Mortality Analysis

Pen #	Treatment	Age (days)	Weight (grams)	Pathological Findings	Tentative Morphological diagnosis
19	T2	7	74	 Culled (cervical dislocation) Dehydrated, off feed Litter in gizzard No other significant lesions 	Starve out
16	T2	9	217	 Off feed Purulent arthritis in hock joints Swollen liver 	Bacterial arthritis, multi-systemic bacterial infection
23	Т3	9	259	 Crop full of feed Well flebarn No significant lesions 	Acute death syndrome
23	Т3	12	418	 Feed and litter mixed in gizzard Congested lungs Swollen kidneys, liver, spleen Pale/toneless gastrointestinal tract 	Multi-systemic bacterial infection
11	T2	14	269	 Culled (cervical dislocation) Litter-filled gizzard No significant lesions 	Cull
24	T1	14	155	- Severe fibrinous peritonitis	Multisystemic bacterial infection
22	Т3	19	349	- Polyserositis	Multisystemic bacterial infection
11	Т3	35	2,299	 Feed in crop/gizzard Gas-distended gastrointestinal tract Mucoid enteritis Mild tenosynovitis 	Mucoid enteritis, tenosynovitis



Appendix D: Analysis of Variance

Randomized Complete Block AOV Table for Average Start Weight

Source	DF	SS	MS	F	Р
Block	5	16.7860	3.35719		
Trt	2	2.4885	1.24427	1.08	0.3638
Error	16	18.4702	1.15439		
Total	23	37.7447			
Grand Mean		42.623	CV 2.52		
Relative Efficiency,		RCB 1.49			

Means of Average Start Weight for Treatment

Treatment	Mean	
А	42.998	
В	42.682	
С	42.190	
Observations	per Mean	8
Standard Erro	0.3799	
Std Error (Diff	of 2 Means)	0.5372

Randomized Complete Block AOV Table for Average Final Weight

Source	DF	SS	MS	F	Р
Block	5	36871	7374.3		
Trt	2	14110	7054.9	0.58	0.5737
Error	16	196185	12261.5		
Total	23	247166			
Grand Mean		2568.9	CV 4.31		
Relative Efficie	ncy,	RCB 0.84			

Means of Average Final Weight for Treatment

-		
Treatment	Mean	
А	2546.1	
В	2603.8	
С	2556.8	
Observations p	per Mean	8
Standard Error	39.150	
Std Error (Diff	of 2 Means)	55.366



Randomized Complete Block AOV Table for Average Feed Consumption

Source	DF	SS	MS	F	Р
Block	5	80499	16099.8		
Trt	2	12758	6378.8	0.26	0.7715
Error	16	387047	24190.4		
Total	23	480303			
Grand Mean		4065.3	CV 3.83		
Relative Efficie	ncy,	RCB 0.86			

Means of Average Feed Consumption for Treatment

Treatment	Mean	
А	4085.8	
В	4078.0	
С	4031.9	
Observations	per Mean	8
Standard Erro	54.989	
Std Error (Diff	of 2 Means)	77.766

Randomized Complete Block AOV Table for Feed Conversion Ratio

Source	DF	SS	MS	F	Р
Block	5	0.01515	0.00303		
Trt	2	0.00636	0.00318	1.01	0.3870
Error	16	0.05052	0.00316		
Total	23	0007203			
Grand Mean		1.5837	CV 3.55		
Relative Efficie	ency,	RCB 0.94			

Means of Feed Conversion Ratio for Treatment

Treatment	Mean	
А	1.6067	
В	1.5670	
С	1.5774	
Observations p	oer Mean	8
Standard Error	0.0199	
Std Error (Diff	of 2 Means)	0.0281



Appendix E: Pairwise Comparison Tables

LSD All-Pairwise Comparisons Test of Average Start Weight for Treatment					
Treatment	Mean	Homogeneous Groups			
А	42.998	A			
В	42.682	A			
С	42.190	А			
Alpha	0.05	Standard Error for Comparison	0.5372		
Critical T Value	2.120	Critical Value for Comparison	1.1388		
Error term used:	Error, 16 DF				
There are no signif	icant pairwise	differences among the means			

LSD All-Pairwise Comparisons Test of Average Final Weight for Treatment				
Treatment	Mean	Homogeneous Groups		
А	2603.8	А		
В	2556.8	А		
С	2546.1	А		
Alpha	0.05	Standard Error for Comparison	55.366	
Critical T Value	2.120	Critical Value for Comparison	117.37	
Error term used:	Error, 16 DF			
There are no signif	ficant pairwise	differences among the means		

LSD All-Pairwise C	omparisons Te	st of Average Feed Consumption j	for Treatment
Treatment	Mean	Homogeneous Groups	
А	4085.8	А	
В	4078.0	А	
С	4031.9	А	
Alpha	0.05	Standard Error for Comparison	77.766
Critical T Value	2.120	Critical Value for Comparison	164.86
Error term used:	Error, 16 DF		
There are no signi	ficant pairwise	differences among the means	

LSD All-Pairwise Comparisons Test of Feed Conversion Ratio for Treatment				
Treatment	Mean	Homogeneous Groups		
А	1.6067	А		
В	1.5774	А		
С	1.5670	А		
Alpha	0.05	Standard Error for Comparison	0.0281	
Critical T Value	2.120	Critical Value for Comparison	0.0596	

```
Error, 16 DF
Error term used:
There are no significant pairwise differences among the means
```



	Microwave Digestion (HNO ₃) - ICP-OES							
Sample	Al ug/g	B ug/g	Ca %	Cu ug/g	Fe ug/g	K %	Mg %	Mn ug/g
T1	475	35	1.69	230	642	2.225	0.589	397
T1	420	36	1.45	224	609	2.107	0.545	377
T1	447	34	1.60	224	632	2.188	0.588	389
Т2	532	36	1.83	192	728	2.200	0.555	419
Т2	464	33	1.57	168	646	2.063	0.488	356
Т2	491	35	1.66	187	701	2.139	0.527	404
Т3	532	36	1.75	186	733	2.129	0.578	400
Т3	524	35	1.85	191	712	2.177	0.596	412
Т3	512	37	1.87	195	700	2.229	0.612	420

Appendix F: Laboratory Analysis of Broiler Litter

Microwave Digestion (HNO ₃) - ICP-OES					Anions - Hot H2O Extraction -HPLC			
Sample	Na ug/g	P %	S %	Zn ug/g	F ⁻ ppm	Cl ⁻ ppm	NO2 ⁻ ppm	Br ⁻ ppm
T1	2710	1.046	0.492	428	<0.1	4000	<0.1	<0.1
T1	2629	0.991	0.506	408	<0.1	3885	<0.1	<0.1
T1	2746	1.070	0.513	439	<0.1	4355	<0.1	<0.1
T2	3065	0.996	0.481	406	<0.1	4064	<0.1	<0.1
T2	3185	1.089	0.507	458	<0.1	4212	<0.1	<0.1
T2	3256	1.170	0.520	482	<0.1	4131	<0.1	<0.1
Т3	3747	1.126	0.519	452	<0.1	5597	<0.1	<0.1
Т3	3881	1.184	0.535	474	<0.1	4809	<0.1	<0.1
Т3	3813	1.158	0.518	464	<0.1	5234	<0.1	<0.1



Anions	- Hot H2O	Extraction	n -HPLC	Total C, N, S and H				
Sample	NO3 ⁻ ppm	PO4 ⁻ ppm	SO4 ⁻ ppm	C %	N %	S %	H %	
T1	<0.1	6783	5654	46.2	4.44	0.52	6.13	
T1	<0.1	6909	6742	46.4	4.68	0.53	6.09	
T1	<0.1	6787	7012	47.0	4.68	0.50	6.14	
Т2	<0.1	5325	5994	47.9	5.02	0.50	6.09	
Т2	<0.1	5600	5725	47.9	5.11	0.52	6.10	
Т2	<0.1	4872	6128	47.5	5.20	0.53	6.09	
Т3	<0.1	5332	6474	47.7	4.38	0.54	6.23	
Т3	<0.1	5559	6526	47.3	4.38	0.51	6.06	
Т3	<0.1	6232	6975	47.2	4.75	0.50	6.03	

Mehlich III Extractable/Available Elements (1 g litter / 25 ml extractant)									
Sample	Al mg/Kg	B mg/Kg	Ca mg/Kg	Cu mg/Kg	Fe mg/Kg	K mg/Kg	Mg mg/Kg	Mn mg/Kg	
T1	54.5	22.1	6729	119	235	17967	4849	362	
T1	49.1	21.3	6129	109	211	17002	4272	321	
T1	56.0	22.8	6836	122	249	17925	4854	373	
Т2	43.2	22.2	7896	98	228	18076	4550	377	
Т2	47.9	22.2	8017	105	257	17386	4331	390	
Т2	43.7	21.1	7699	99	229	17388	4435	365	
Т3	34.8	22.2	7971	93	232	17781	4820	365	
Т3	31.4	22.0	7756	89	208	17474	4569	345	
Т3	35.0	22.3	8014	94	232	18158	5006	368	



Mehli	рН				
Sample	Na mg/Kg	P mg/Kg	S mg/Kg	Zn mg/Kg	рН 1:1 Н2О
T1	2287	8825	3406	327	7.00
T1	2174	7746	3152	284	7.02
T1	2259	8785	3350	318	7.00
T2	2671	9455	3369	341	7.03
Т2	2586	8977	3256	354	7.05
T2	2618	9113	3258	325	7.05
Т3	3235	8992	3352	320	7.05
Т3	3156	8666	3305	313	7.05
Т3	3268	9268	3443	322	7.07