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Optimum Time to Add the Feed Additive Availa-ZMC to Reduce the Incidence of Bacterial Chondronecrosis with Osteomyelitis Lameness in Broiler Chickens

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Optimum Time to Add the Feed Additive Availa-ZMC to Reduce the Incidence of Bacterial Chondronecrosis with Osteomyelitis Lameness in Broiler Chickens

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Abstract

Lameness is a health condition threatening the welfare of broiler chickens in the poultry industry. It can be caused by a bacterial infection called Bacterial Chondronecrosis with Osteomyelitis (BCO). Zinpro has created Avalia-ZMC, a feed additive that has been proven to reduce lameness by 20% in wire-flooring pens and 25% in litter-flooring pens (Alrubaye et al., 2020). This experiment focuses on the optimal time to add Availa-ZMC to the diet of the broiler chickens to help reduce the incidence of lameness. This trial took 56 days with 26 pens at the University of Arkansas Poultry Research Farm. At the start of the trial, there were a total of 60 chicks placed in each pen. On day 14, each pen was culled to 50 chicks per pen. The dimensions of the pens were 5*10 feet. To encourage the broiler chicks to move around, the nipple drinker was placed on one side of the pen, and the two feeders were placed on the opposite side. Two pens had wire flooring to induce BCO lameness as the challenge source pens, and the remainder had litter flooring. The chicks in the pens with wire flooring developed lameness first and spread the infection through the air to the other pens (Alrubaye et al., 2020). The experimental design had five different treatment groups T1-T5 using two different diets. The treatments were randomized throughout the 26 pens; diet 1 was the control diet, and diet 2 contained the feed additive Availa-ZMC. Treatment 1 was the control diet and included two wire-flooring pens. Treatment 2 was the negative control diet 1 with litter-flooring pens. Treatment 3 birds were given the diet 2 Availa-ZMC diet. Treatment 4 was diet 1, days 1-28, and diet 2, days 29-56. Treatment 5 was diet 2, days 1-28, and diet 1, days 29-56. Beginning on day 22, the birds were checked for lameness utilizing a broom to walk them. The lame birds were necropsied, and their lesions on the right and left femurs and tibia were examined and recorded. At the end of the

experiment, T3 had the lowest rate of cumulative lameness at 39%. T1 has the highest incidence of cumulative lameness, with 91%.

Introduction

Bacterial chondronecrosis with osteomyelitis (BCO) is a bacterial infection, which used to be referred to as femoral head necrosis, that causes lameness in broiler chickens (Wideman & Prisby, 2013). Some chickens are bred to produce eggs, but broiler chickens are bred for meat production (Chan et al., 2022). Micro-trauma to cartilage cells of the proximal femur or tibial growth plates allows BCO to develop in broiler chickens, allowing bacteria to populate (Wideman & Prisby, 2013). All microbes connected to BCO are unknown, though, after statistical analysis, it has been discovered that many are from the genera *Staphylococcus* (Jiang et al., 2015). The most common isolate of the *Staphylococcus* species is *S. agentis*, found in about 75-80% of BCO lesions (Al-Rubaye et al., 2015). Studies have shown that it is most probable that bacterial species are moved from the respiratory tract or intestinal tract into the blood, then these bacterial species travel through the bloodstream into the bones in the legs (Mandal et al., 2016; Wideman & Prisby, 2013). Typically, BCO is generated on growth plates in the proximal tibia and proximal femur, making the growth plates predisposed to damage (Jiang et al., 2015).

In one year (2018), the United States slaughtered more than 9 billion chickens in exchange for more than 31.7 billion dollars (Chan et al., 2022; USDA NASS, 2018). The welfare of broiler chickens is being compromised by the increased demand for the breeding of broiler chickens (Chan et al., 2022). The consumption of broiler chickens is increasing, which in turn means the demand for more chickens is increasing, which puts the broiler chickens' health at stake.

The pain lameness causes is a concerning condition affecting the well-being of broiler chickens (Granquist et al., 2019). Lameness causes pain in broiler chickens' legs, and the chickens may be unable to walk. Chickens unable to walk cannot move around to get food or water, and they can die due to complications lameness causes, such as starvation (Bradshaw et al., 2002). Another effect of lameness is the cost to the broiler industry. Millions of dollars are lost each year due to lameness. In 2010, the United States poultry industry lost around $80-120 million dollars to lameness (Yousaf et al., 2021). The effects of lameness in the United States are causing up to a 2% loss in broiler flocks (Dunkley, 2007). Losing broiler chickens to lameness results in lost revenue for the commercial broiler industry because infected chickens must be culled. It is essential to find solutions for lameness to improve broiler chickens' well-being and reduce financial loss.

Trials have been completed to induce lameness in broiler chickens. *Staphylococcus Agentis* is an opportunistic bacteria associated with BCO that can induce lameness when isolates of it are added to the water source that the broilers drink from through nipple drinkers (Al-Rubaye et al., 2015). Another way lameness can be induced is when broiler chicken pens have wire flooring instead of litter flooring because the wire flooring causes stress on the legs joints (Wideman et al., 2012).

In this experiment, the feed additive Availa-ZMC was used to determine the best time to add this to the food of broiler chickens to reduce lameness. Availa-ZMC comprises three different minerals: manganese, copper amino acid complex, and organic zinc, which contain the ingredients necessary to provide a balanced diet to broiler chickens (Zinpro, n.d.). Broiler chickens need a well-balanced diet in order to reduce the risk of leg problems (Yousaf et al., 2021). In past experiments, Availa-ZMC has been determined to reduce the incidence of lameness. In this trial, Availa-ZMC was dispersed out at different times depending on the treatment group the broiler chickens were in to determine at which time the cumulative percentage of lameness was the lowest.

Materials and Methods

Trial Set-Up

This trial occurred for 56 days at the University of Arkansas Poultry Research Farm in house A365W. The house contained 26 pens that were sized 5*10 ft. The pens were given numbers 1-26. Every pen had two feeders on one side to provide food, and nipple drinkers on the other side to provide drinking water. Pens 1 and 14 had wire flooring and were control pens to induce lameness. The rest of the pens had litter flooring made of wood shavings. The experiment began with 1560 byproduct males from the Cobb500 broiler breed. On day 1, 60 chicks were placed in each pen. On day 15, the chicks were culled to 50 chicks per pen. For days 1-35, every pen is given a standard feed commercial chick starter in the form of crumbles. For days 35-56, every pen is given a broiler finisher in the form of pellets. The temperature, ventilation, and photoperiod were all set by using computer controls in house A365W. The photoperiod was controlled to be 23 hours of light along with 1 hour of darkness. The thermoneutral temperature target was set for each day. For days 1-3, the temperature was set at 90 °F. For days 4-6, the temperature was decreased to 88 °F. For days 7-10, the temperature is decreased to 85 °F. For days 11-14, the temperature is decreased to 80 °F. For days 14-17, the temperature was decreased to 75 °F. After day 17, the temperature was decreased to 70 °F and set for this temperature until the end of the trial.

Treatment Descriptions

This trial used five different treatment groups. The treatment groups were labeled T1-T5. The treatments were randomized throughout the pens. There were two different diets used in this trial, diet 1 and diet 2. Diet 1 was a control diet that contained basal feed. Diet 2 contained the feed additive Availa-ZMC. Treatment 1 was the control group and received diet 1 for all 56 days. Only two pens received treatment 1, pen 1 and pen 14. These pens contained wire flooring to be used as infection source to induce lameness. Treatment 2 used diet 1 for all 56 days. Treatment 2 was the negative control because it used the diet 1 basal feed, but these pens had litter flooring made of wood shavings. Treatment 3 used the Availa-ZMC diet 2 for all 56 days. Treatment 4 used diet 1 for days 1-28, then was switched to diet 2 for days 29-56. Treatment 5 used diet 2 for days 1-28, then was switched to diet 1 for days 29-56. **Table 1** lists the treatments, group descriptions, and the pens in each treatment group.

Table 1. Treatments detailed descriptions

Treatments		**Group Descriptions**	**Pens**
T1	Diet 1	Wire-flooring pens as infection sources as the positive control	1,14
T2	Diet 1	Basal diet as a negative control	2, 6, 17, 25, 10, 13
T3	Diet 2	Availa-ZMC diet	4, 7, 18, 24, 21, 16
T4	Diet 1-2	Diet 1 (control diet) days 1-28, and Diet 2 (Availa-ZMC diet) days 29-56	5, 12, 22, 26,3, 8
T5	Diet 2-1	Diet 2 (Availa-ZMC diet) days 1-28, and Diet 1 (control diet) days 29-56	9, 11, 15, 19, 20, 23

Assessment of Lameness

Throughout the entire trial, the deaths of the birds were recorded as Sudden Death Syndrome (SDS), Dead on Arrival (DOA), Kinky back (KB), or lame. The date, cause of death, pen number, and treatment were recorded for all deaths of the birds. The birds were assessed for lameness by being prompted to walk with a broom beginning on day 22. There were five brooms used for the five different treatments. If the birds could not walk when prompted by the broom, they were diagnosed as "clinically lame." Once a bird was diagnosed as "clinically lame," the bird was culled or euthanized. Then, the bird was labeled with the treatment and pen number to be necropsied to assess the lesions on the bird's tibia and femur.

The femurs were necropsied first and categorized as Normal (N), Proximal Femoral Head Separation (FHS), Proximal Femoral Head Transitional Degeneration (FHT), or Proximal Femoral Head Necrosis (FHN). The progressions for the femoral lesions can be seen in **Figure 1**. Next, the tibial lesions were categorized as Normal (N), Proximal Tibial Head Necrosis (THN), Tibial Dyschondroplasia (TD), Proximal Tibial Head Necrosis Severe (THNS), or Proximal Tibial Head Caseous (THNC). The progressions for tibial lesions can be seen in **Figure 2**.

On the last day of the trial, day 56, the birds were walked a final time to be diagnosed as lame or healthy. From each treatment, five healthy birds were selected to have their bird weight recorded. The birds were diagnosed as clinically healthy because they showed no signs of lameness. Then, three birds out of the five clinically healthy birds were randomly selected to be necropsied to assess their subclinical lesions. The tibial lesions and the femoral lesions were assessed, and cultures were taken. The samples were extracted for DNA and the Polymerase Chain Reaction was done on the samples.

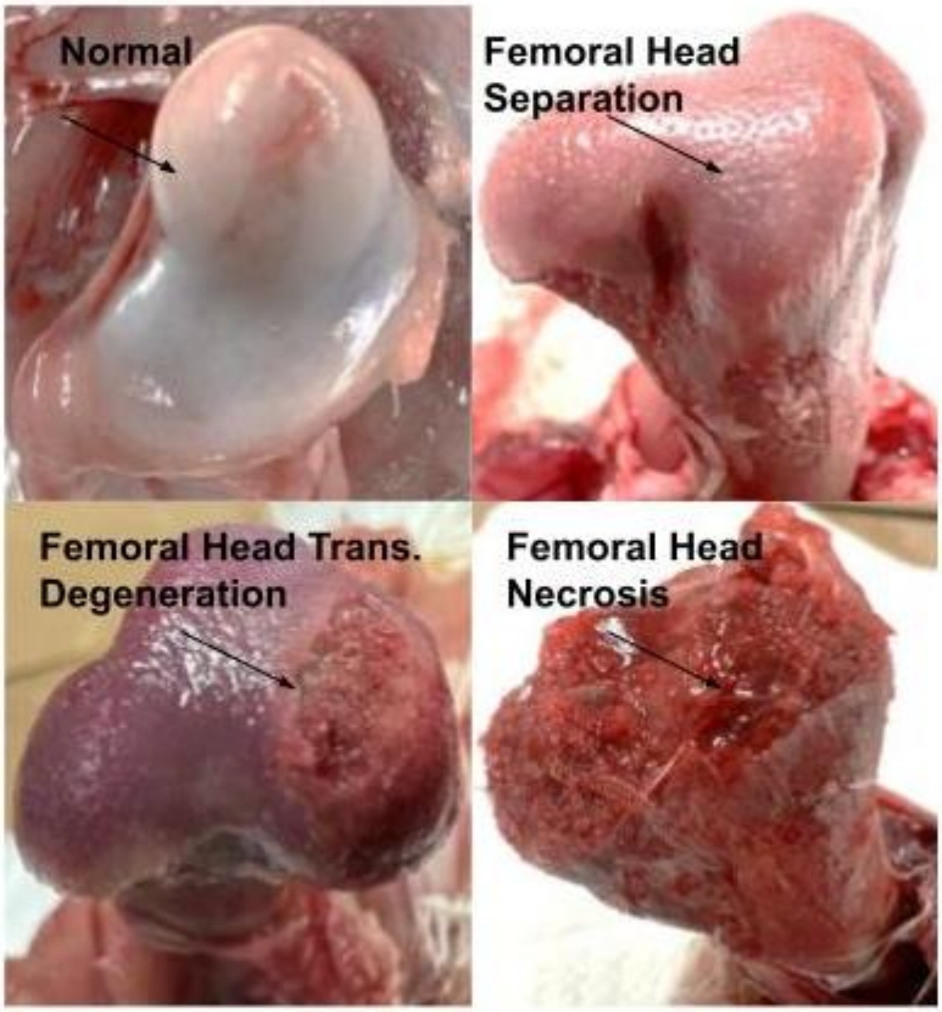

Figure 1. The progression of femoral lesions for lameness caused by BCO. The lesion progressions are shown in order from N, FHS, FHT, and FHN.

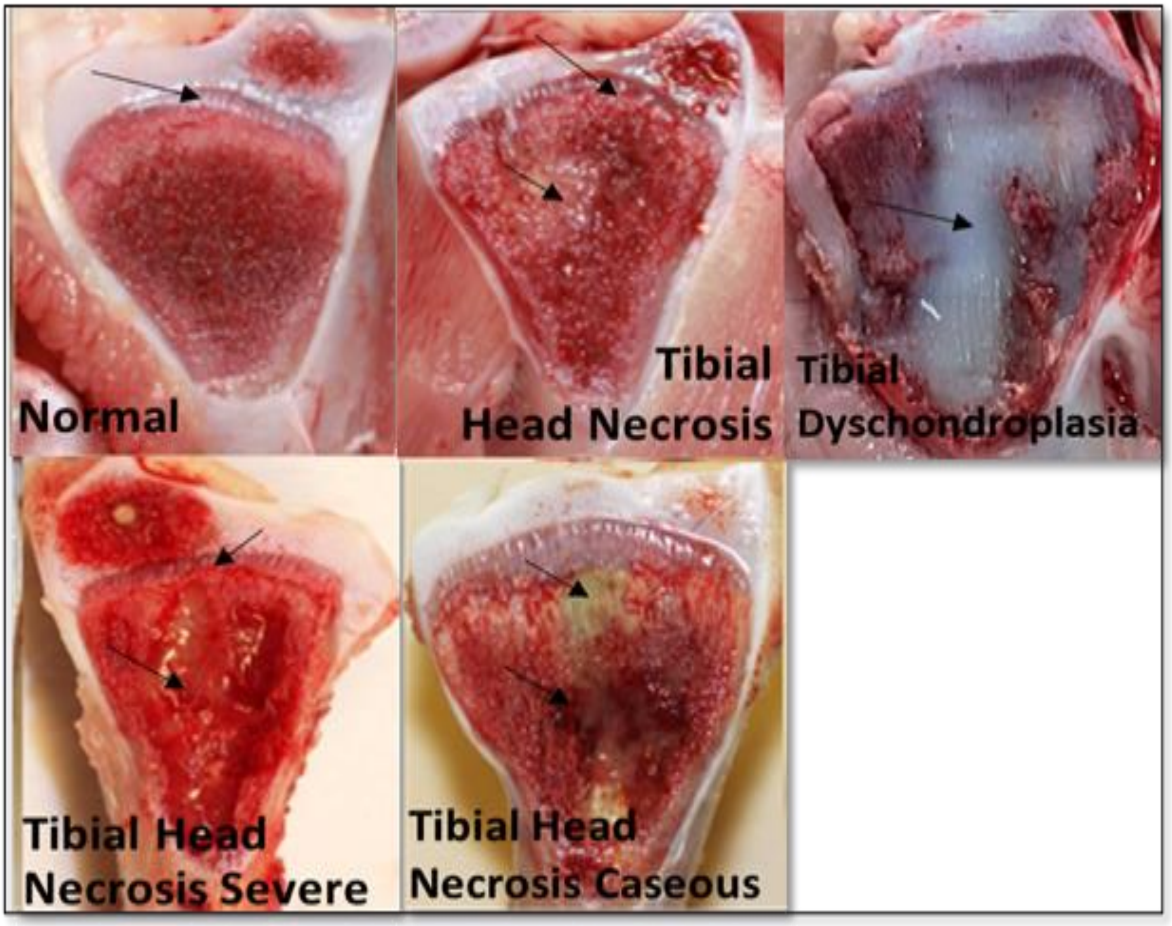

Figure 2. The tibial lesions for lameness caused by BCO (Wideman & Prisby, 2013; Wideman et al., 2013; Wideman Jr et al., 2014; Wideman, 2016).

Results

The cumulative percentage of lameness was evaluated in treatments T2-T5 starting from day 35 to the final day of the trial, day 56. The cumulative percentage of lameness is shown in **Figure 3**. Treatment 1 is not shown because it was the infection source. Treatment 2 had the highest cumulative percentages of lameness. Treatment 3 had the lowest cumulative percentage of lameness. Treatment 5 had the second-lowest cumulative percentage of lameness. Overall, birds that began with diet 1 had the highest cumulative percentage of lameness over birds that began with diet 2.

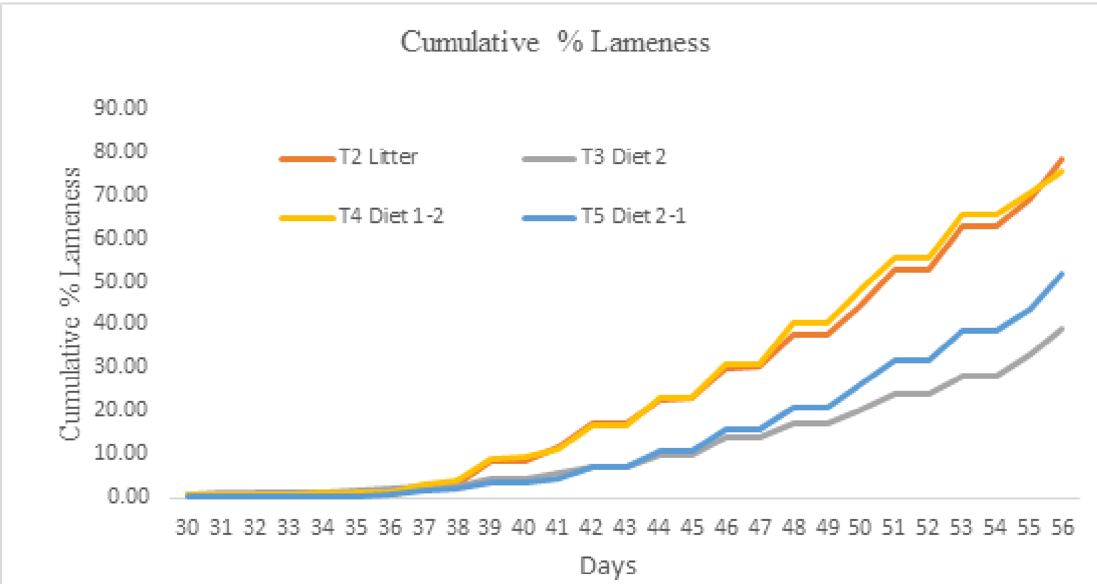

Figure 3. Cumulative percentage of lameness for treatments 2-5

Table 2 shows the cumulative percentage of lameness for days 35, 42, 49, and 56. On day 56, treatment 1 had the highest cumulative percentage for lameness at 91%. On day 56, treatment 3 had the lowest cumulative percentage for lameness at 39%. The difference between the highest cumulative percent for lameness and the lowest on day 56 was 52%.

Table 2. Cumulative percent lameness for specific days

Day	T1	T2	T3	T4	T5
35	4.00	0.67	1.67	1.33	0.33
42	42.00	17.33	7.33	16.67	7.00
49	72.00	37.67	17.33	40.67	20.67
56	91.00	78.00	39.00	75.00	52.00

Table 3 shows the general linear model (GLM) analysis of the cumulative percentage of lameness from the treatments. It was determined that treatment 2 was significantly statistically different from treatment 1 at P=0.0014. There was a significant statistical difference between treatment 2 and treatment 3. There was not a significant statistical difference between treatment 2 and treatment 4 at P=0.436.

Table 3. GLM analysis of the cumulative percent lameness from all treatments

	Diet 1	Diet 2	Diet 1-2	Diet 2-1
P-value	T2	T3	T4	T5
T1	1.40E-03	1.75E-13	3.63E-04	1.13E-09
T2		< 2e-16	4.36E-01*	8.75E-11
T3			< 2e-16	1.57E-03
T4				5.70E-09

Note: (*) means no significant statistical difference

Figure 4 shows the femoral and tibial lesions of birds that were diagnosed clinically lame. Treatment 3 had the lowest number of FHS and FHS cases. None of the treatments had any normal tibial lesions. The tibial lesion severity decreased in treatment 3 and treatment 5. Each treatment group had similar reductions for THN and THNS. THNS was the most recurrent type of tibial lesion.

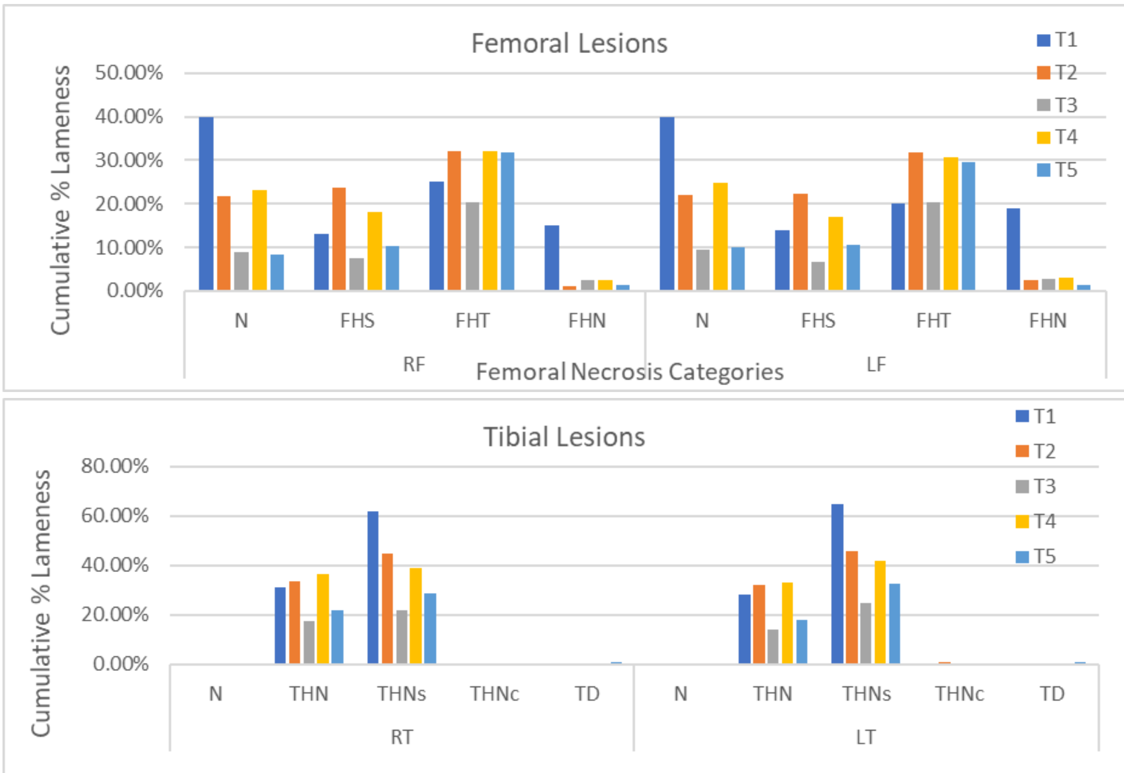

Figure 4. Femoral and Tibial lesions are categorized. The lesions are shown for the femur and the tibia of the lame birds according to the treatment group. The categories for femoral lesions are N, FHS, FHT, or FHN. The categories for tibial lesions are N, THN, THNS, THNC, or TD treatment. RT- Right Tibia, LT- Left Tibia, RF- Right femur, and LF- Left Femur.

The cumulative percentages for lame birds with and without deaths for each treatment are shown in **Table 4**. After the birds were necropsied, it was found that almost all the birds that had died had femoral and tibial lesions. Treatment 3 had the highest number of total deaths, and treatment 4 had the lowest number of total deaths.

Table 4. Lame birds with and without deaths

	T1	T2	T3	T4	T5
% Lame birds with deaths	91.00	78.00	39.00	75.00	52.00
Total death	7	8	9	4	5
% Lame birds without deaths	88.00	76.00	36.00	74.00	50.33

Table 5 shows the percentages for DOA, SDS, KB, lame, and healthy for T1-T5. Treatment 3 had the highest percentage of healthy chickens at 58%, and the highest percentage of DOA at 2%. Treatment 2 had only 19.33% healthy chickens.

Table 5. Percentage of healthy, dead, and lame incidence for each treatment

Percent	T1	T2	T3	T4	T5
DOA	0.00	1.67	2.00	0.67	0.67
SDS	4.00	0.33	1.00	0.67	1.00
KB	3.00	0.67	0.00	0.33	0.00
LAME	91.00	78.00	39.00	75.00	52.00
HEALTHY	2.00	19.33	58.00	23.33	46.33

Note: KB is considered a type of LAME.

In **Table 6**, the number of lame and mortalities were recorded per pen for each treatment group. Treatment 1 had the highest number of lame birds within a single pen at 48 lame birds. Treatment 3 had the lowest number of lame birds within a single pen at 13 lame birds.

Table 6. Lame and mortality count for birds within treatments

Treatments	Lame per pen						Mortality per pen					
T1	48	45					1	4				
T2	42	42	35	40	38	38	2	2	1	0	1	2
T3	18	18	13	22	21	25	1	4	1	0	3	0
T4	37	38	39	35	36	41	1	0	1	0	2	2
T5	24	29	25	26	26	25	1	1	1	0	2	0

The individual weight of the five healthy birds from T2-T5 and the average weight of the five birds per treatment was calculated in **Table 7**. Treatment 4 had the highest average body weight of 4.93kg. Treatment 3 had the lowest average body weight of 4.64kg.

Table 7. Individual and average weight (kg) of five birds from each treatment.		
Treatments	Weight (kg)	Ave (kg)
T2	4.78	4.90
T2	4.84	
T2	5.38	
T2	4.44	
T2	5.24	
T3	4.66	4.64 [a]
T3	3.72	
T3	4.60	
T3	5.54	
T3	4.68	
T4	4.48	4.93 [b]
T4	5.28	
T4	5.08	
T4	5.26	
T4	4.56	
T5	5.02	4.88
T5	4.72	
T5	4.86	
T5	5.12	
T5	4.66	
Note: (a) means the lowest body weight data (b) means the highest body weight data		

The body weight of the clinically healthy birds was analyzed using a T-test analysis as shown in **Table 8**. There were no significant statistical differences.

Table 8. T-test analysis of the weight of seemingly healthy birds from all treatments			
T-test	T3	T4	T5
T2	2.04E-01	4.94E-01	3.81E-01
T3		2.08E-01	2.35E-01
T4			3.91E-01

Only three out of the five healthy birds were necropsied from T2-T5. It can be seen in **Figure 5** that even though the birds were diagnosed as "clinically healthy" many of them had subclinical femoral and tibial lesions.

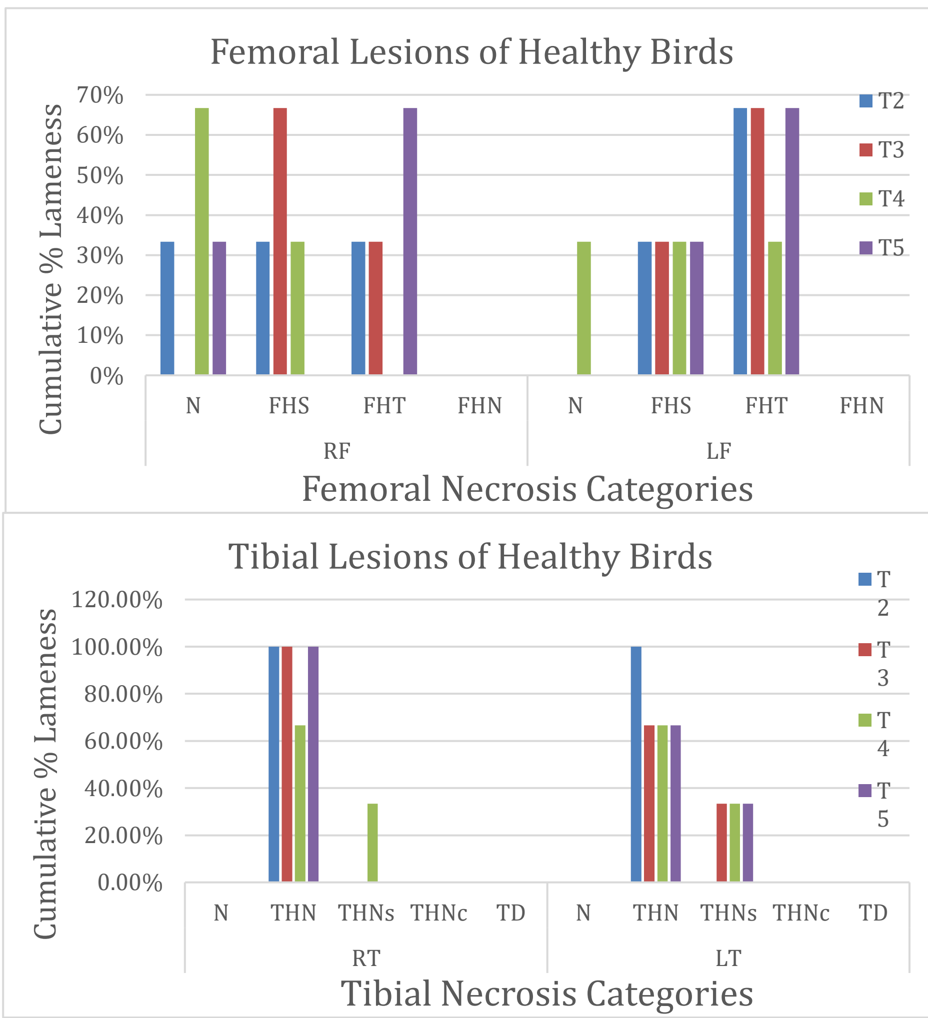

Figure 5. The ratings of tibial and femoral lesions for birds diagnosed as clinically healthy

Discussion

The feed additive Availa-ZMC was successful in lowering the incidence of lameness. The optimal time to add Availa-ZMC would be in the beginning from days 1-28. This is shown in **Figure 3**. For the cumulative lameness percentage in **Figure 3**, treatment 3 had the lowest percentage due to having the Availa-ZMC added to the diet at the beginning of the experiment. Treatment 5 had a similar pattern to treatment 3 in lowering the incidence of lameness because the Availa-ZMC was also added in the first 28 days. While treatment 4 had the Availa-ZMC added from day 28 to the end of the trial, it did not have a large effect on reducing the incidence of lameness. Treatment 4 showed similar patterns of accumulation of lameness as treatment 2 which had only diet 1 the control basal feed. Treatment 1 was not analyzed for cumulative percentage of lameness because it was used as the infection source, so that means the chickens were supposed to become lame in this treatment.

On day 56, treatment 3 had the lowest cumulative percentage of 39% while the other treatments had much higher percentages. This makes sense because treatment 3 had the feed additive Availa-ZMC from the start. Comparing the cumulative percentage of lameness of treatment 1 at 91% and treatment 3 at 39% the Availa-ZMC was able to reduce the incidence of lameness by 52%.

Overall, treatment 3 is the best treatment to use in order to reduce the incidence of lameness. It was found that treatment 3 had the most optimal time to add the dietary supplement. The Availa-ZMC needs to be added at the beginning of the trial to the diets of the broiler chickens to be the most effective. If Availa-ZMC is added to the diet in the middle of the trial, it is not effective in reducing the incidence of lameness in broiler chickens.

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Appendix

Table 1. Treatments detailed descriptions

Treatments		Group Descriptions	Pens
T1	Diet 1	Wire-flooring pens as infection sources	1,14
T2	Diet 1	Basal diet as a negative control	2, 6, 17, 25, 10, 13
T3	Diet 2	Availa-ZMC diet	4, 7, 18, 24, 21, 16
T4	Diet 1-2	Diet 1 (control diet) days 1-28, and Diet 2 (Availa-ZMC diet) days 29-56	5, 12, 22, 26,3, 8
T5	Diet 2-1	diet 2 (Availa-ZMC diet) days 1-28, and Diet 1 (control diet) days 29-56	9, 11, 15, 19, 20, 23

Table 2. Starter Diet 1

Amount:	6050	**Form:**	Crumble
	Diet 1 - Control no algae		
Ingredient Name	**%**	**Total, lb**	**Batch, lb**
Corn - Evonik	57.1208	3,455.8084	1,727.9042
SBM (48%) - Evonik	36.7594	2,223.9437	1,111.9719
Spirulina Algae	-	-	-
Poultry Fat	2.5034	151.4557	75.7279
DL-methionine	0.3398	20.5579	10.2790
L-lysine HCl	0.1966	11.8943	5.9472
L-threonine	0.1131	6.8426	3.4213
Limestone	1.0885	65.8543	32.9271
Dicalcium phosphate	1.0445	63.1923	31.5961
Salt	0.4104	24.8292	12.4146
Sodium bicarbonate	0.0784	4.7432	2.3716
OptiPhos2000 (0.5lb/ton)	0.0250	1.5125	0.7563
Choline chloride (60%)	0.0500	3.0250	1.5125
Tyson 2x Broiler Vit	0.0550	3.3275	1.6638
BioCox60	0.0500	3.0250	1.5125
UofA TM (0.10%; Max = 0.12%)	**0.1000**	6.0500	3.0250
AvilaZMC	**-**	-	-
EDDI	**-**	-	-
Se premix	**-**	-	-
Inert Filler (cellulose or sand)	**0.0651**	3.9386	1.9693
Total	**100.0000**	6,050.0000	3,025.0000

Table 3. Starter Diet 2

Amount:	5200	**Form: Crumble**
	Diet 2 - AvailaZMC no algae	
Ingredient Name	**%**	**Total, lb**
Corn - Evonik	57.1208	2,970.2816
SBM (48%) - Evonik	36.7594	1,911.4888
Spirulina Algae	-	-
Poultry Fat	2.5034	130.1768
DL-methionine	0.3398	17.6696
L-lysine HCl	0.1966	10.2232
L-threonine	0.1131	5.8812
Limestone	1.0885	56.6020
Dicalcium phosphate	1.0445	54.3140
Salt	0.4104	21.3408
Sodium bicarbonate	0.0784	4.0768
OptiPhos2000 (0.5lb/ton)	0.0250	1.3000
Choline chloride (60%)	0.0500	2.6000
Tyson 2x Broiler Vit	0.0550	2.8600
BioCox60	0.0500	2.6000
UofA TM (0.10%; Max = 0.12%)	**0.0400**	2.0800
AvilaZMC	**0.1000**	5.2000
EDDI	**0.0001**	0.0047
Se premix	**0.0250**	1.3000
Inert Filler (cellulose or sand)	**-**	-
Total	**100.0000**	**5,199.9995**

Table 4. Finisher Diet 1

Ingredient Name	%	Total, lb	Batch, lb
Corn - Evonik	64.5540	5,874.4140	1,958.1380
SBM (48%) - Evonik	29.1450	2,652.1950	884.0650
Spirulina Algae	-	-	-
Poultry Fat	3.1730	288.7430	96.2477
DL-methionine	0.3078	28.0098	9.3366
L-lysine HCl	0.2113	19.2283	6.4094
L-threonine	0.0904	8.2264	2.7421
Limestone	0.9942	90.4722	30.1574
Dicalcium phosphate	0.7167	65.2197	21.7399
Salt	0.4249	38.6659	12.8886
Sodium bicarbonate	0.0659	5.9969	1.9990
OptiPhos2000 (0.5lb/ton)	0.0250	2.2750	0.7583
Choline chloride (60%)	0.0467	4.2497	1.4166
Tyson 2x Broiler Vit	0.0300	2.7300	0.9100
BioCox60	0.0500	4.5500	1.5167
UofA TM (0.10%; Max = 0.12%)	**0.1000**	9.1000	3.0333
AvilaZMC	**-**	-	-
EDDI	**-**	-	-
Se premix	**-**	-	-
Inert Filler (cellulose or sand)	**0.0651**	5.9241	1.9747
Total	**100.0000**	**9,100.0000**	**3,033.3333**

Table 5. Finisher Diet 2

Amount:	7800	Form:	Pellet
	Diet 2 - AvailaZMC no algae		
Ingredient Name	**%**	**Total, lb**	**Batch, lb**
Corn - Evonik	64.5540	5,035.2120	2,517.6060
SBM (48%) - Evonik	29.1450	2,273.3100	1,136.6550
Spirulina Algae	-	-	-
Poultry Fat	3.1730	247.4940	123.7470
DL-methionine	0.3078	24.0084	12.0042
L-lysine HCl	0.2113	16.4814	8.2407
L-threonine	0.0904	7.0512	3.5256
Limestone	0.9942	77.5476	38.7738
Dicalcium phosphate	0.7167	55.9026	27.9513
Salt	0.4249	33.1422	16.5711
Sodium bicarbonate	0.0659	5.1402	2.5701
OptiPhos2000 (0.5lb/ton)	0.0250	1.9500	0.9750
Choline chloride (60%)	0.0467	3.6426	1.8213
Tyson 2x Broiler Vit	0.0300	2.3400	1.1700
BioCox60	0.0500	3.9000	1.9500
UofA TM (0.10%; Max = 0.12%)	**0.0400**	3.1200	1.5600
AvilaZMC	**0.1000**	7.8000	3.9000
EDDI	**0.0001**	0.0070	0.0035
Se premix	**0.0250**	1.9500	0.9750
Inert Filler (cellulose or sand)	**-**	-	-
Total	**100.0000**	**7,799.999**	**3,900.000**

Table 6. Experimental Timeline

Age	Date	Day	Comments
1	14 -Dec	Wednesday	Place 60 chicks per pen. All pens on Starter crumble. Temp at 90F
2	15- Dec	Thursday	
3	16 -Dec	Friday	
4	17- Dec	Saturday	Reduce temp to 88 F
5	18- Dec	Sunday	
6	19- Dec	Monday	
7	20- Dec	Tuesday	Reduce temp to 85 F
8	21- Dec	Wednesday	
9	22- Dec	Thursday	
10	23- Dec	Friday	Reduce temp to 80 F
11	24- Dec	Saturday	
12	25- Dec	Sunday	
13	26- Dec	Monday	
14	27- Dec	Tuesday	
15	28- Dec	Wednesday	Reduce temp to 75 F. Collect blood, Liver, Lung, and spleen samples from 5 birds
16	29- Dec	Thursday	
17	30- Dec	Friday	
18	31- Dec	Saturday	Reduce temp to 70 F
19	01- Jan	Sunday	
20	02- Jan	Monday	
21	03- Jan	Tuesday	
22	04- Jan	Wednesday	Begin recording all deaths, lame and infirmed.
23	05- Jan	Thursday	
24	06- Jan	Friday	
25	07- Jan	Saturday	
26	08- Jan	Sunday	
27	09- Jan	Monday	
28	10- Jan	Tuesday	Collect blood, Liver, Lung, and spleen samples from 5 birds.
29	11- Jan	Wednesday	Switch diets in T4 & T5 only.
30	12- Jan	Thursday	
31	13- Jan	Friday	
32	14- Jan	Saturday	
33	15- Jan	Sunday	
34	16- Jan	Monday	
35	17- Jan	Tuesday	Switch all pens to finisher pellets
36	18- Jan	Wednesday	
37	19- Jan	Thursday	
38	20- Jan	Friday	
39	21- Jan	Saturday	

40	22- Jan	Sunday	
41	23- Jan	Monday	
42	24- Jan	Tuesday	Collect blood, Liver, Lung, and spleen samples from 5 birds.
43	25- Jan	Wednesday	
44	26- Jan	Thursday	
45	27- Jan	Friday	
46	28- Jan	Saturday	
47	29- Jan	Sunday	
48	30- Jan	Monday	
49	31- Jan	Tuesday	
50	01- Feb	Wednesday	
51	02- Feb	Thursday	
52	03- Feb	Friday	
53	04- Feb	Saturday	
54	05- Feb	Sunday	
55	06- Feb	Monday	
56	07- Feb	Tuesday	- Weigh 5 birds from each group - Necropsy all birds for lameness category - Gross evaluation of tibia and proximal femur - Culture 2 gross lesions at either tibia or proximal femur (note where culture is taken from) from 2 birds/pen