12-2022

Evaluation of the Selected Nuproxa Feed Additives for Protection against Lameness and Improving the Wellbeing of Broilers in the Lameness Challenge Model

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Evaluation of the Selected Nuproxa Feed Additives for Protection against Lameness and Improving the Wellbeing of Broilers in the Lameness Challenge Model

An Honors Thesis submitted in partial fulfillment of the requirements of Honors Studies in Biological Sciences

By

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Fall 2022

Biological Sciences

J. William Fulbright College of Arts and Sciences

The University of Arkansas

Acknowledgments

I would like to thank Dr. Alrubaye for allowing me to be a part of his research team and for being my honors thesis advisor. Without his patience and dedication to his work and students I would not have been able to have this enriching experience. Additionally, I would like to thank Khawla Alharbi and Andi Asnayanti for helping me along the way as well as the rest of the research team. I would also like to thank the members of my defense committee Dr. Tameka Bailey, Dr. Timothy Kral, and Dr. Fiona Davidson for taking the time to review my thesis. Lastly, I would like to thank the J. William Fulbright College of Arts and Sciences and the Honors College for giving me the opportunity to complete this thesis.

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Abstract

Bacterial Chondronecrosis with Osteomyelitis (BCO) is a form of bacteria that causes lameness in broiler chickens and is a common concern within the poultry industry. This easily transmissible disease can quickly take over entire livestock of chickens leading to an animal welfare issue. Due to this, it is a pressing issue that must be solved in order to protect the wellbeing of broiler chickens and ensure viable livestock. Nuproxa is a company that specializes in chicken feed in which we used to test different amounts of Panbonis, a dietary supplement, in its effectiveness in protecting broiler chickens against BCO lameness. We conducted this experiment for 56 days with seven different treatments including four different diets. Treatments 1 and 2 were our positive and negative controls, respectively. Treatment 1 included the administration of diet 1, with no Panbonis content, as well as wire flooring within the pen. The wire flooring is known to rapidly induce lameness within broiler chickens, therefore acting as our index case in the spread of BCO. Treatment 2 also included the administration of diet 2 but used the same litter flooring as the other pens. Treatments 3 through 7 used varying diets with differing Panbonis concentration and used the basal litter flooring. Each treatment had four pens, except for the positive control (treatment 1) which were placed at the front of the house, leading to a total of 26 pens. After day 35, we began to check all of the pens for incidences of lameness, cull, and necropsy each day to record the progression and spread of BCO lameness for each diet. When completing each necropsy, we checked the left and right femurs as well as the left and right tibias and labeled them with a series of lesions associated with lameness. After day 56 we completed our recordings of lameness per pen. It was found that treatments 1 and 2 had the most cases of lameness with incidences of 81% and 74% respectively. Treatment 4 was found to have the least cases of lameness with an incidence of 34%. Treatment 4 used only diet 3 which

consisted of 100 g/ton of Panbonis and is recognized as the best treatment for significantly decreasing the infection rate of BCO lameness in broiler chickens.

Introduction

Bacterial Chondronecrosis with Osteomyelitis (BCO) is a pressing animal welfare issue within the poultry industry that is found to be a leading cause of lameness in broiler chickens (Alrubaye et al., 2020, McNamee et al. 2000). Once these chickens become lame, they must be culled due to their lack of ability to walk to get food and water (Bradshaw et al. 2002). Mobility issues are most commonly tied to the risk factor of growth rate, stress associated with rapid growth rate can cause the bones to break under the chicken's own body weight (Knowles et al. 2008). Male broiler chickens have been found to have weaker legs than their female counterparts because they have a more rapid growth rate than females (Hancock et al., 1995). Lameness also leads to other animal welfare issues, such as cleanliness which then causes them to be unfit for human consumption (Granquist et al. 2019). This leads to the loss of millions of dollars, in fact leg issues typically cost the poultry industry 80 to 120 million dollars each year (*Bacterial Chondronecrosis with Osteomyelitis – Lameness*, 2022). These issues will continue to worsen if a solution is not found due to the ever-growing population and demand for meat. Currently, over 66 billion chickens are slaughtered each year for consumption and this number is projected to increase with our population (Hartcher et al., 2019).

The bacteria that cause BCO is able to enter the bloodstream through disruptions in the tight junction barrier along the epithelial lining of the intestines. Stress has been found to be a leading cause in the translocation of bacteria into the bloodstream, which broiler chickens encounter frequently (Ando et al., 2020). (Lara et al., 2013, Rostagno, 2020). Once the bacteria are in the bloodstream, it colonizes at the growth plates of the tibia and femur, causing its disruption and eventual incidence of lameness (Wideman, 2013). The stress put on the leg bones due to the rapid rate of growth of broiler chickens causes osteochondrosis clefts at the growth plates, which allows the bacteria to accumulate faster and accelerate the process (Wideman, 2016). Femoral head necrosis, a result of BCO, has been shown to substantially weaken the femur, allowing it to break easily and cause lameness (Madhwal et al. 2020). Wire flooring in opposition to wood shaving litter flooring, significantly increases the rate at which this occurs, due to the added stress on the femurs and tibias of the broiler chickens (Al-rubaye et al., 2017, Wideman et al., 2012).

Compared to healthy chickens, lame chickens have been found to have a multitude of bacteria that can be associated with BCO lameness, but the most common bacteria have been found to be a part of the genera *Staphylococcus* (Jiang et al., 2015, Mandal et al. 2016). For example, *Staphylococcus agnetis* is an agent that has been found to assist in the induction of BCO lameness (Al-rubaye et al., 2015). *Escherichia coli* (*E. coli*) has also been found to be a cause of BCO due to its presence in the bone marrow of lame broiler chickens (Gaußmann et al., 2018, Wijesurendra, D. S. et al. 2017). Antibiotic treatment of these bacteria has been found to be ineffective due to the location of the lesions and formed antibiotic resistance (Szafraniec et al. 2022). For these reasons the solution to BCO induced lameness must be solved using alternatives to antibiotics. In turn, it has been found that probiotics and supplements can help decrease permeability within the intestines and increase bone strength, preventing lameness (McCabe et al. 2018). Panbonis is a supplement commonly used in chicken feed that is an active form of vitamin D3 and increases metabolism of calcium and phosphorus (*Panbonis,* 2022). Due to these reasons, we tested the effect of different concentrations of Panbonis in chicken feed on the incidence of lameness in broiler chickens in a controlled environment.

Materials and Methods

Animal Care and Welfare

All animal experiments were approved by the University of Arkansas Institutional Animal Care and Use Committee under protocols 15043, 16073, 16073-1, and 17067.

Lameness Trials

This experiment was conducted over 56 days for Nuproxa in the A365W house. There was a total of 26 pens and each pen housed 56 chicks at the start of the trial for a total of 1456 byproduct males Cobb500. All pens were culled to 50 birds per pen on day 14. Each pen was 5x10 square feet and each contained two feeders at one end and a water supply at the other with nipples for the chicks to drink from. The house was temperature controlled and was set to have 23 hours of light and 1 hour of darkness each day. This was done to control for both heat and light environmental stressors. On day 1 the temperature was set to 90°F. On day 4 the temperature was reduced to 88°F. On day 7 the temperature was reduced to 85°F. On day 10 the temperature was reduced to 80°F. On day 15 the temperature was reduced to 75°F. On day 18 the temperature was reduced to 70°F and remained at that temperature for the rest of the trial.

Treatment Details

There were seven different treatments composed of a variety of four diets. Treatment 1 posed as our positive control and used diet 1, basal feed which contained no Panbonis. Treatment 1 was also the only treatment where the pens used wire-flooring instead of wood-shaving litter. There were only two pens for treatment 1 stationed at the front of the house to serve as the infection source, all other treatments had four pens each. There were huge fans at the end of the house (opposite of the treatment 1 pens) which functioned to spread the bacteria from the infection source to the other pens via the air. Treatment 2 was our negative control and also used diet 1 but had wood-shaving litter. Treatment 3 used diet 2, which had a Panbonis content of 50 g/ton. Treatment 4 used diet 3, which had a Panbonis content of 100 g/ton. Treatment 5 used diet 4, which had a Panbonis content of 200g/ton. Treatment 6 used diet 1 for the first 28 days and then was switched to diet 3. Treatment 7 used diet 3 for the first 28 days and then was switched to diet 1. All pens received standard commercial chick starter feed (crumbles) from d1-34 and broiler finisher feed (pellets) from d35-56. **Table 1** below explains each treatment and what treatment each pen had.

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
T3	Diet 2	Panbonis 50 g/ton	4, 7, 18, 24
T4	Diet 3	Panbonis 100 g/ton	3, 8 ,20, 23
T5	Diet 4	Panbonis 200 g/ton	10, 13, 21,16
T6	Diet 1-3	Basal diet on d1-28 and Panbonis 100g/ton on d29-56	5, 12, 22, 26
T7	Diet 3-1	Panbonis 100g/ton on d1-28 and basal diet on d29-56	9, 11, 15, 19

Necropsy and Lameness Evaluation

On day 21, all birds were coerced to walk daily by using a broom to walk the birds from one side of the pen to the other to pick out any lame birds. Any bird found to be reluctant or unable to walk were diagnosed as "clinically lame" and euthanized and necropsied. We began to record all the deaths and necropsied the dead birds to record lesions on both femurs and tibia. During the trial, each bird recorded as dead-on arrival (**DOA**), sudden death syndrome (**SDS**), or lame, the date, pen number, and treatment were all recorded, and the bird was necropsied. On day 56, we checked for lameness one last time and necropsied all the remaining lame birds. Then five birds were selected from each treatment and weighed. We then conducted a gross evaluation of the tibia and proximal femurs of each bird. We lastly selected two healthy birds from each pen and took two cultures from gross lesions at either tibia or proximal femur to assess bone marrow bacterial growth yielding a total of 104 samples.

During each necropsy both tibias and femurs were evaluated and labeled based on their lesions. Femoral lesions were described as Normal (**N**), Femoral Head Separation (**FHS**), Femoral Head Transitional (**FHT**), or Femoral Head Necrosis (**FHN**) as shown in **figure 1**. Tibial lesions were described as Normal (**N**), Tibial Head Necrosis (**THN**), Tibial Head Necrosis Sever (**THNS**), Tibial Head Necrosis Caseous (**THNC**), or Tibial Dyschondroplasia (**TD**) as shown in **figure 2**.

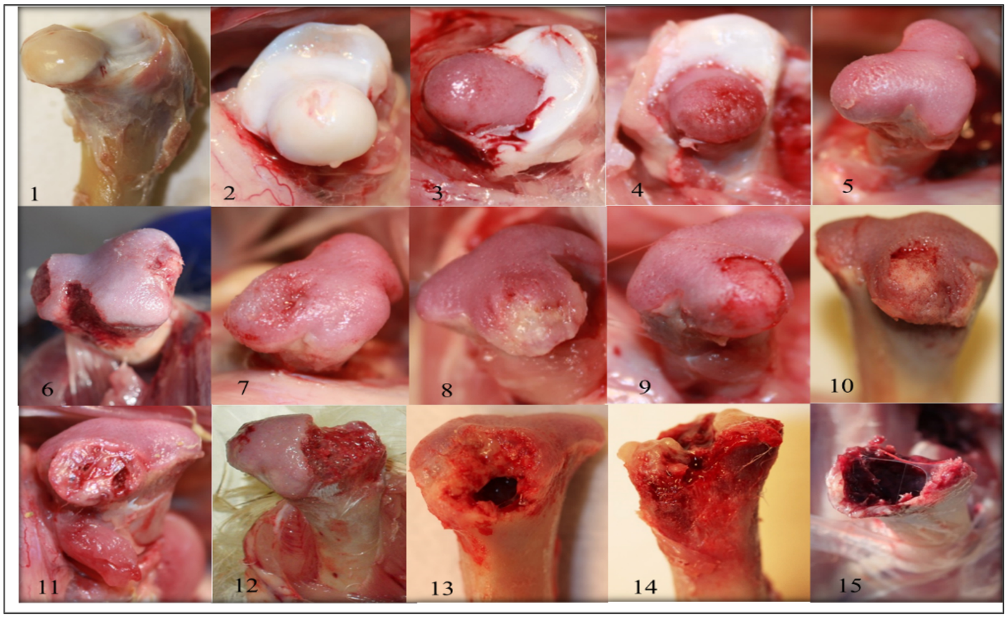

Figure 1. Femoral lesions for BCO lameness progression categorizations: 1-2 = N; 3-5 = FHS; 6-10 = FHT; 11-15 = FHN (Wideman Jr et al., 2014; Wideman et al., 2013; Wideman & Prisby, 2012)

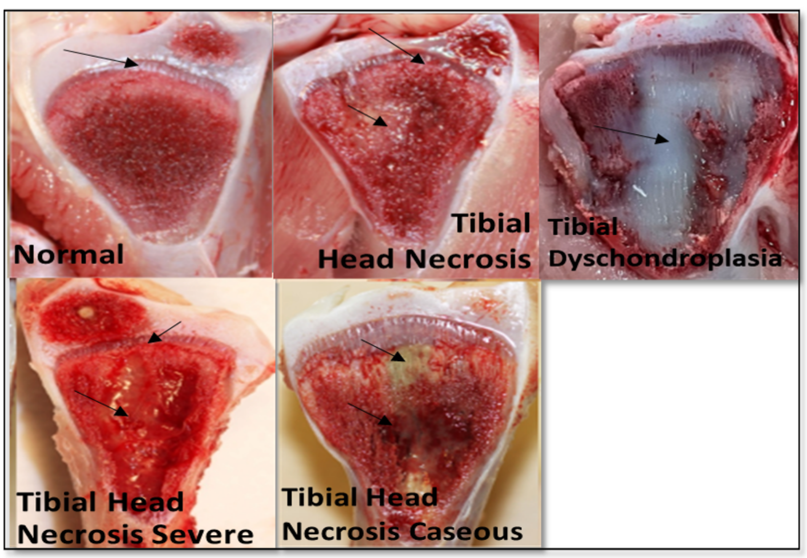

Figure 2. Left to right, top down: Tibial lesions for BCO lameness progression categorizations: Normal, Tibial Head Necrosis, Tibial Head Necrosis Severe, and Tibia Dyschondroplasia (Wideman & Prisby, 2012; Wideman et al, 2013; Wideman Jr et al., 2014; Wideman, 2016)

Results

The cumulative percent lameness by treatment group is shown in **figure 3.** This graph shows us that a significant number of cases of lameness appeared after day 40. It also shows that wire-flooring, present in treatment 1, yielded the largest percent of lameness. Treatment 2, which had litter flooring, is shown to have the second highest percentage of lameness. Diet 3 (treatment 4) and Diet 3-1 (treatment 7) are shown to have the smallest percentages of lameness.

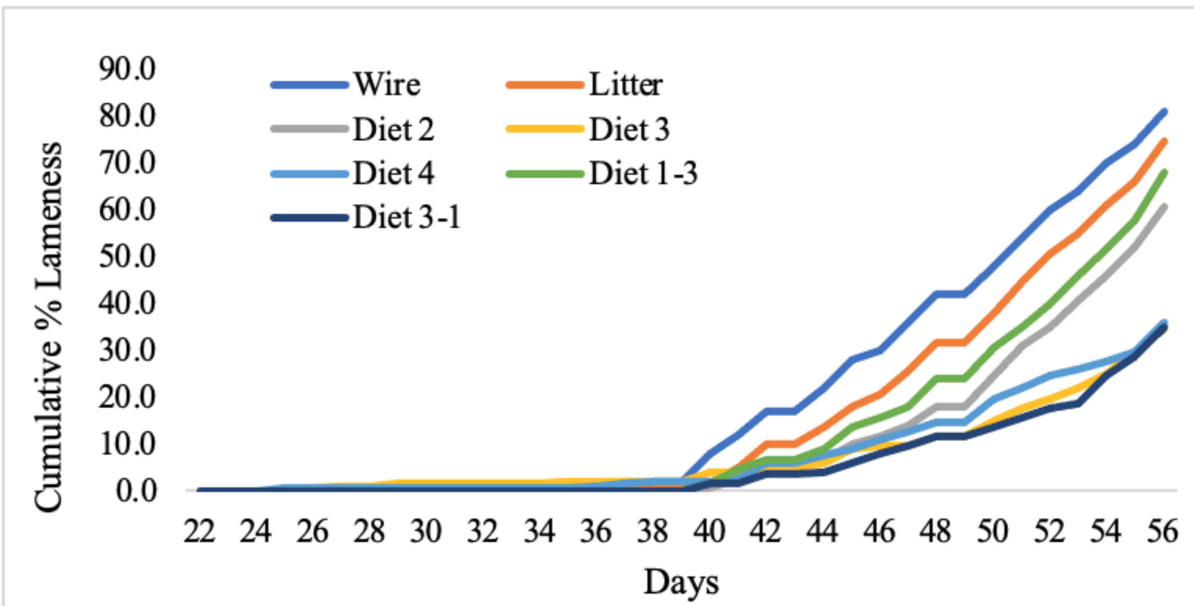

Figure 3. Cumulative percent lameness by treatment group

The general linear model analysis of the cumulative percent lameness by treatment group shown in **table 2.** demonstrates that there was no significant difference between the percent lame in treatment 1 and treatment 2 at = 1.53E-01. There is also shown to be no significant difference between treatment 4 and treatment 7 at = 9.12E-01. However, a significant difference was found between treatment 1 and treatment 4 at *P*= 5.67E-13 and between treatment 1 and treatment 7 at *P*= 8.98E-13.

	Litter	Diet 2	Diet 3	Diet 4	Diet 1-3	Diet 3-1
P-value	T2	T3	T4	T5	T6	T7
T1	1.53E-01*	2.40E-04	5.67E-13	2.36E-12	8.93E-03	8.98E-13
T2		2.57E-03	4.83E-15	2.73E-14	1.06E-01*	9.59E-15
T3			2.43E-07	7.85E-07	1.65E-01*	4.12E-07
T4				8.14E-01*	1.54E-10	9.12E-01*
T5					4.94E-10	9.01E-01*
T6						2.97E-10
Note: (*) means no significant statistical difference						

Table 2. GLM analysis of the cumulative percent lameness from all treatments

As shown in **figure 4**, the number of femoral head necrosis (FHN) cases was lowest in treatments 5, 6, and 7 but was highest in treatment 4. Tibial Head Necrosis Severe (THNS) was the most common tibial lesion and had the most cases for treatment 1 and the least number of cases for treatments 4 and 7. There were also similar patterns shown for Tibial Head Necrosis (THN). There was no pattern relating BCO lesion severity regarding right or left femoral and tibial lesions.

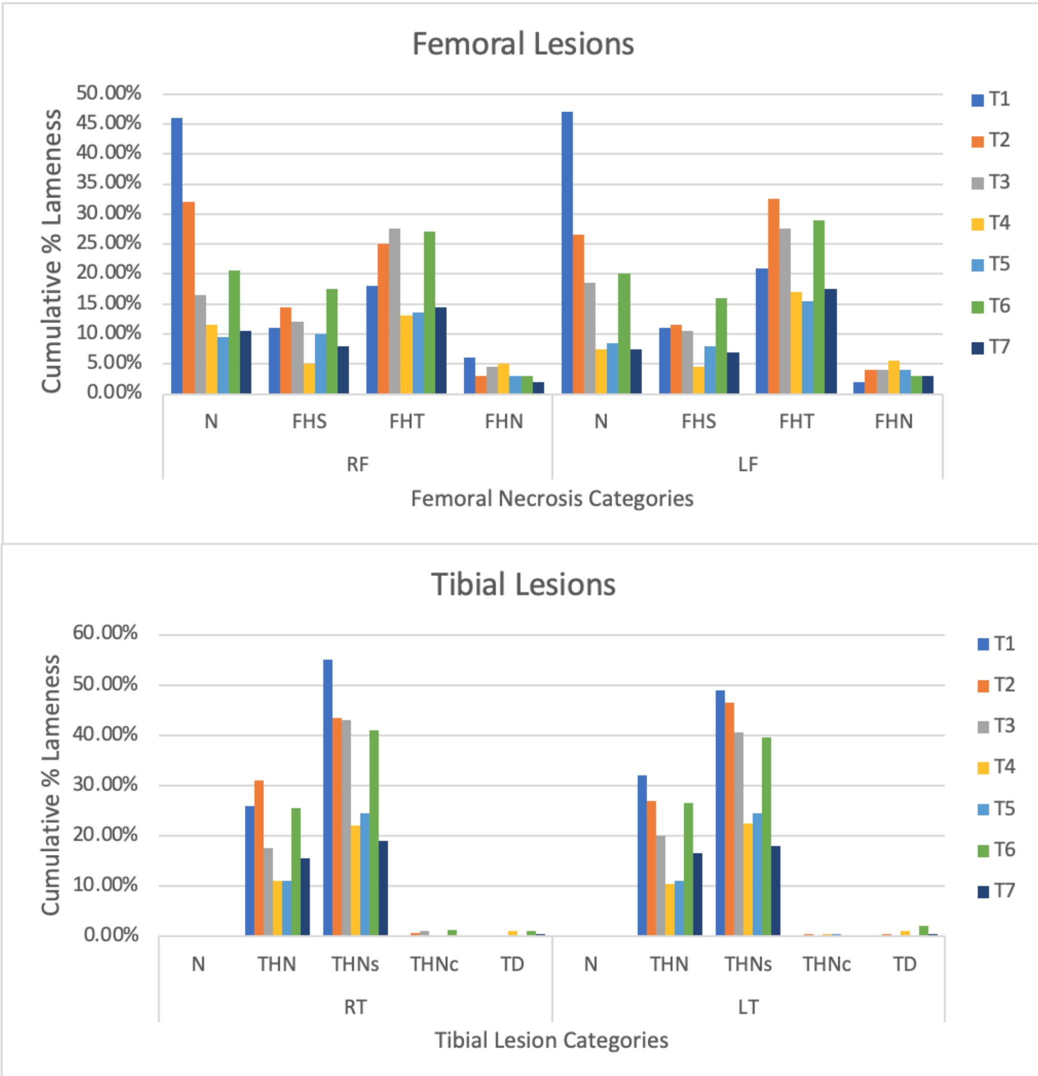

Figure 4. Tibial and Femoral lesion ratings for birds diagnosed as lame. The percentage of lame birds with the indicated BCO lesion(s) is plotted for each of the bones by treatment. RT- Right Tibia, LT- Left Tibia, RF- Right Femur, LF- Left Femur, N = Femur head, and proximal tibia appear entirely normal, FHS = Proximal Femoral Head Separation (epiphyscolysis), FHT = Proximal Femoral Head Transitional degeneration, FHN = Proximal Femoral Head Necrosis (bacterial chondronecrosis with osteomyelitis, BCO), THN = Proximal Tibial Head Necrosis , THNC = Proximal Tibial Head Necrosis Caseous , THNS = Proximal Tibial Head Necrosis Severe .

The percentage of healthy, dead, and lame incidences for each treatment is shown in **table 3**. The highest percentages of lameness were shown in treatments 1 and 2 at 81% and 74%, respectively. The lowest percentages of lameness were shown in treatments 4 and 7 at 34% and 35%, respectively. In reflection of this treatment 1 had the lowest percentage of healthy at 17% and treatment 4 had the highest percentage of healthy at 60%. Treatment 5 had the highest percentage of birds found either DOA or SDS with a combined 6%.

Percent	T1	T2	T3	T4	T5	T6	T7
DOA	0.0	0.5	0.0	0.5	1.0	0.0	0.0
SDS	1.0	1.5	1.0	1.0	5.0	2.0	1.5
KB	1.0	1.5	2.5	4.5	3.0	0.5	4.0
LAME	81.0	74.0	60.5	34.0	35.0	68.0	35.0
HEALTHY	17.0	22.5	36.0	60.0	56.0	29.5	59.5
Note: KB is one type of LAME.							

Table 3. Percentage of healthy, dead, and lame incidence for each treatment.

The percentages of lame birds with and without death per treatment is shown in **table 4**. The highest number of deaths occurred in treatments 4, 5, and 7 with treatment 5 having the most at 16 deaths. However, treatment 5 also had the lowest percent of lame birds without death at 28.5%.

Table 3. Lame birds with and without deaths							
	T1	T2	T3	T4	T5	T6	T7
% Lame birds with deaths	81.00	74.50	60.50	34.50	36.00	68.00	35.00
Total death	2	6	7	11	16	5	11
% Lame birds without deaths	79.00	71.50	57.00	29.50	28.50	65.50	31.00

Table 4. Lame birds with and without deaths

Table 5 shows variation in the number of lame birds per pen. This can be explained because the lameness originates from the wire flooring pens which occurred due to pathogenic infection from the environment. Lameness can vary with the first few infected birds, spreading the infection to the other birds in the pen.

Treatments	Lame per pen				Mortality per pen			
T1	39	42			1	1		
T2	39	35	36	39	1	2	2	1
T3	29	29	32	31	0	3	3	1
T4	15	18	22	14	4	0	3	4
T5	19	14	23	16	2	6	1	7
T6	29	35	36	36	1	1	1	2
T7	20	21	12	17	0	2	5	4

Table 5. Lame and mortality count for birds within treatments

At the end of the 56 days experiment we randomly selected five healthy birds from each pen and weighed them, this data is shown in **table 6**. Treatment 1 was shown to have the lowest average body weight of 4.28 kg and was significantly different from treatments 4, 6, and 7 (P>0.05). This is due to the high indices of lameness that the birds in treatment 6 endured, which compromised their growth. Treatment 6 was shown to have the highest average body weight of 4.94 kg, except it had no statistical difference from the other treatments (P>0.05), not including treatment 1.

Treatments	Weight (Kg)	Ave (Kg)	Treatments	Weight (Kg)	Ave (Kg)
T1	4.40	4.28[a]	T5	4.84	4.69
T1	4.02		T5	4.28	
T1	4.18		T5	4.46	
T1	4.74		T5	5.10	
T1	4.04		T5	4.76	
T2	4.42	4.53	T6	4.79	4.94[b]
T2	4.36		T6	4.68	
T2	4.70		T6	4.72	
T2	4.54		T6	5.48	
T2	4.62		T6	5.02	
T3	4.78	4.59	T7	4.82	4.82
T3	4.46		T7	4.56	
T3	4.28		T7	5.00	
T3	4.96		T7	4.56	
T3	4.46		T7	5.16	
T4	4.94	4.88	Note:		
T4	5.10		(a) means the lowest body weight data		
T4	4.90		(b) means the highest body weight data		
T4	4.62				
T4	4.84				

Table 6. Individual and average weight (Kg) of five birds from each treatment.

Discussion

From the data analysis that was conducted we can conclude that treatment 4 yielded the lowest number of lame chickens at the end of the trial with treatment 7 as a close second. Both treatments used diet 3 which had a Panbonis content of 100 g/ton. There was no significant difference in the percentage of lameness between treatments 4 and 7. There was a significant difference in the percent of lame chickens between treatments 6 and 7. Treatment 7 uses diet 3 for the first 28 days and then is switched to diet 1. The opposite is done in treatment 6 in which diet 1 is used for the first 28 days and then is switched to diet 3. This means that the diet has the most impact on the chicken's potentiality to develop lameness before it becomes fully grown. The statistical significance between the percent lame in treatment 4 and treatments 1 and 2 proves that it is the best choice at preventing BCO lameness in broiler chickens.

Chicken feed with a Panbonis supplement content of 100 g/ton is the optimum way of preventing the spread of BCO lameness in broiler chicken. This is not only because of the data we have found, but because it is most cost efficient and practical. As stated earlier, the poultry industry is continuously growing and there has never been a greater demand. This means that slowing down production by growing the chickens at a slower rate will hurt the industry more than it would help it, even if it does promote animal welfare. Also, as stated previously, antibiotics are no longer a viable way to stop bacterial diseases due to antibiotic resistance and the push to end the use of antibiotics in agriculture. The use of a supplement, such as Panbonis, allows the poultry industry to continue to grow chickens at the same rate, while also ensuring animal welfare without the use of antibiotics.

References

Alrubaye, A. A., Ekesi, N. S., Hasan, A., Elkins, E., Ojha, S., Zaki, S., Dridi, S., Wideman, R. F., Rebollo, M. A., & Rhoads, D. D. (2020). Chondronecrosis with osteomyelitis in broilers: further defining lameness-inducing models with wire or litter flooring to evaluate protection with organic trace minerals. Poultry Science, 99(11), 5422–5429.

Al-Rubaye, A. A., Ekesi, N. S., Zaki, S., Emami, N. K., Wideman, R. F., & Rhoads, D. D. (2017). Chondronecrosis with osteomyelitis in broilers: Further defining a bacterial challenge model using the wire flooring model. Poultry Science, 96(2), 332–340.

Al-Rubaye, A. A. K., Couger, M. B., Ojha, S., Pummill, J. F., Koon, J. A., Wideman, R. F., & Rhoads, D. D. (2015). Genome Analysis of Staphylococcus agnetis, an Agent of Lameness in Broiler Chickens. PLOS ONE, 10(11), e0143336.

Ando, T., Brown, R. F., Berg, R. D., & Dunn, A. J. (2000). Bacterial translocation can increase plasma corticosterone and brain catecholamine and indoleamine metabolism. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 279(6), R2164–R2172.

Bacterial Chondronecrosis with Osteomyelitis - Lameness. (2022). DSM. https://www.dsm.com/anh/challenges/supporting-animal-health/bcolameness.html

Bradshaw, R., Kirkden, R., & Broom, D. (2002). A Review of the Aetiology and Pathology of Leg Weakness in Broilers in Relation to Welfare. Avian and Poultry Biology Reviews, 13(2), 45–103.

Gaußmann, B., Hess, C., Grafl, B., Kovacs, M., Troxler, S., Stessl, B., Hess, M., &

Paudel, S. (2018). Escherichia coli isolates from femoral bone marrow of broilers exhibit diverse pheno- and genotypic characteristics that do not correlate with macroscopic lesions of bacterial chondronecrosis with osteomyelitis. Avian Pathology, 47(3), 271–280.

Granquist, E., Vasdal, G., de Jong, I., & Moe, R. (2019). Lameness and its relationship with health and production measures in broiler chickens. Animal, 13(10), 2365–2372.

Hancock, C. E., Bradford, G. D., Emmans, G. C., & Gous, R. M. (1995). The evaluation of the growth parameters of six strains of commercial broiler chickens. British Poultry Science, 36(2), 247–264. https://doi.org/10.1080/00071669508417773

Hartcher, K., & Lum, H. (2019). Genetic selection of broilers and welfare consequences: a review. World's Poultry Science Journal, 76(1), 154–167.

Jiang, S., Yan, F. F., Hu, J. Y., Mohammed, A., & Cheng, H. W. (2021). Bacillus subtilis-Based Probiotic Improves Skeletal Health and Immunity in Broiler Chickens Exposed to Heat Stress. Animals, 11(6), 1494.

Jiang, T., Mandal, R. K., Wideman, R. F., Khatiwara, A., Pevzner, I., & Min Kwon, Y. (2015). Molecular Survey of Bacterial Communities Associated with Bacterial Chondronecrosis with Osteomyelitis (BCO) in Broilers. PLOS ONE, 10(4), e0124403.

Knowles, T. G., Kestin, S. C., Haslam, S. M., Brown, S. N., Green, L. E., Butterworth, A., Pope, S. J., Pfeiffer, D., & Nicol, C. J. (2008). Leg Disorders in Broiler Chickens: Prevalence, Risk Factors and Prevention. PLoS ONE, 3(2), e1545.

Kpomasse, C. C., Oke, O. E., Houndonougbo, F. M., & Tona, K. (2021). Broiler production challenges in the tropics: A review. Veterinary Medicine and Science, 7(3), 831–842.

Lara, L., & Rostagno, M. (2013). Impact of Heat Stress on Poultry Production. Animals, 3(2), 356–369.

Lee, S. H. (2015). Intestinal Permeability Regulation by Tight Junction: Implication on Inflammatory Bowel Diseases. Intestinal Research, 13(1), 11.

Madhwal, A., Ghodasara, D., Joshi, B., Bhanderi, B., Dave, C., Jani, P., Chaudhari, S., & Choudhary, K. (2020). Etiopathology of femoral head necrosis with special reference to Escherichia coli in broilers. Indian Journal of Veterinary Pathology, 44(1), 29.

Mandal, R. K., Jiang, T., Al-Rubaye, A. A., Rhoads, D. D., Wideman, R. F., Zhao, J., Pevzner, I., & Kwon, Y. M. (2016). An investigation into blood microbiota and its potential association with Bacterial Chondronecrosis with Osteomyelitis (BCO) in Broilers. Scientific Reports, 6(1).

McCabe, L. R., & Parameswaran, N. (2018). Impact of Enteric Health and Mucosal Permeability on Skeletal Health and Lameness in Poultry. In Understanding the Gut-Bone Signaling Axis (Softcover reprint of the original 1st ed. 2017 ed., pp. 185–197). Springer.

McNamee, P. T., & Smyth, J. A. (2000). Bacterial chondronecrosis with osteomyelitis ('femoral head necrosis') of broiler chickens: A review. Avian Pathology, 29(5), 477–495.

Panbonis. (2022). Herbonis. https://herbonis.com/en/panbonis

Rostagno, M. H. (2020). Effects of heat stress on the gut health of poultry. Journal of

Animal Science, 98(4).

Szafraniec, G. M., Szeleszczuk, P., & Dolka, B. (2022). Review on skeletal disorders caused by Staphylococcus spp. in poultry. Veterinary Quarterly, 42(1), 21–40. Wideman. (2013). Bone circulatory disturbances in the development of spontaneous bacterial chondronecrosis with osteomyelitis: a translational model for the pathogenesis of femoral head necrosis. Frontiers in Endocrinology.

Wideman, R. F. (2016). Bacterial chondronecrosis with osteomyelitis and lameness in broilers: a review. Poultry Science, 95(2), 325–344.

Wideman, R., Hamal, K., Stark, J., Blankenship, J., Lester, H., Mitchell, K., Lorenzoni, G., & Pevzner, I. (2012). A wire-flooring model for inducing lameness in broilers: Evaluation of probiotics as a prophylactic treatment. Poultry Science, 91(4), 870–883.

Wijesurendra, D. S., Chamings, A. N., Bushell, R. N., Rourke, D. O., Stevenson, M., Marenda, M. S., Noormohammadi, A. H., & Stent, A. (2017). Pathological and microbiological investigations into cases of bacterial chondronecrosis and osteomyelitis in broiler poultry. Avian Pathology, 46(6), 683–694.

Appendix

Figure 1. Pen Setup for the Herbonis (Nuproxa) experiment

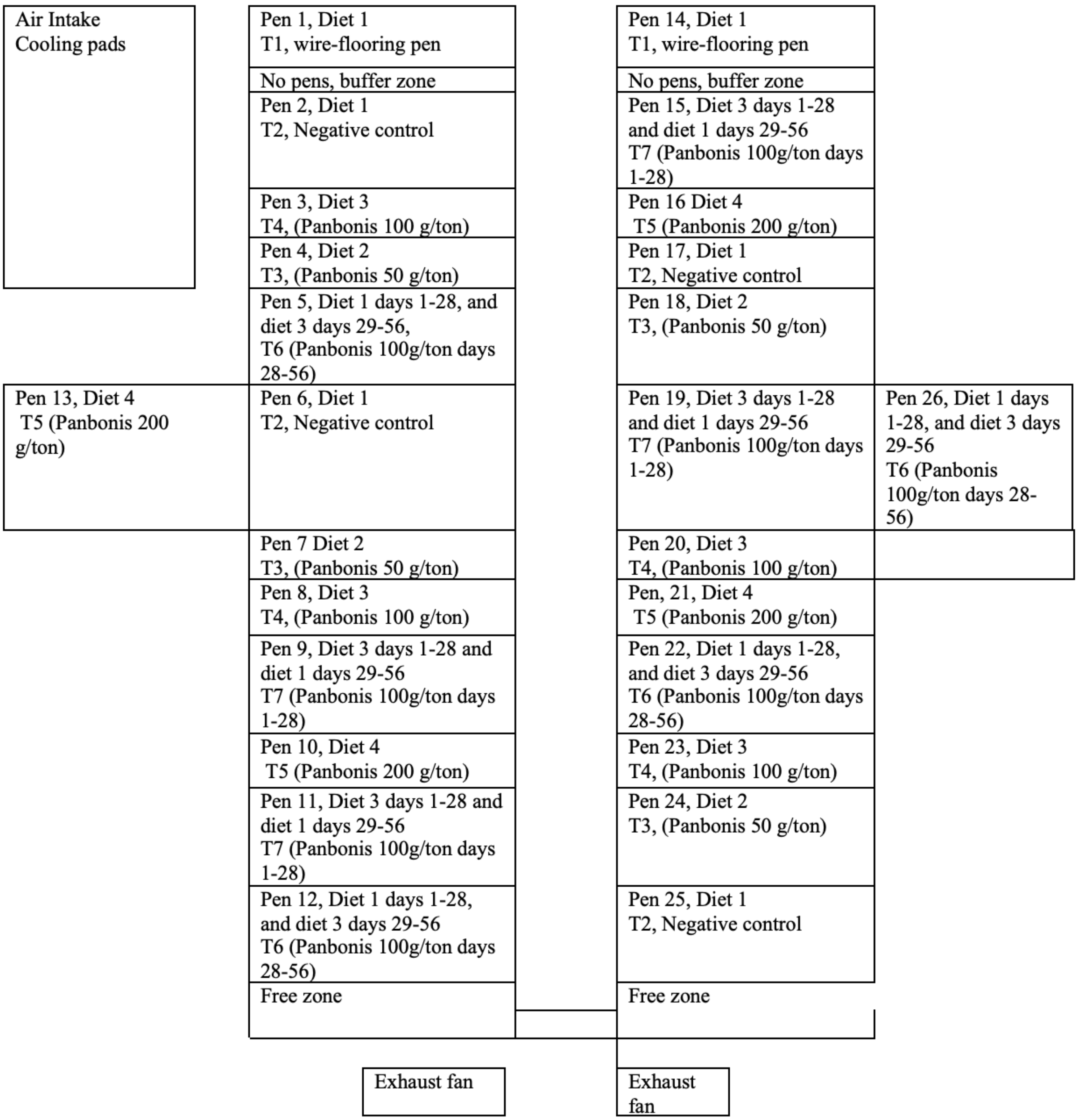

Table 1. Treatment descriptions

Treatment	Description
T1	Diet 1: Wire-flooring pens. Pen 1 & 14
T2	Diet 1: Negative control, Pen 2, 6, 17, 25
T3	Diet 2: Pen 4, 7, 18, 24 (Panbonis 50 g/ton)
T4	Diet 3: Pen 3, 8 ,20, 23 (Panbonis 100 g/ton)
T5	Diet 4: Pen 10, 13, 21, 16(Panbonis 200 g/ton)
T6	Diet 1 days 1-28, and diet 3 days 29-56, Pen 5, 12, 22, 26 (Panbonis 100g/ton days 29-56)
T7	Diet 3 days 1-28 and diet 1 days 29-56: Pen 9, 11, 15, 19 (Panbonis 100g/ton days 1-28)

Tables 2-5. Standard feed formulations

Adnan Herbonis Trial: Starter **Diet 1 - Control**			
Amount:	4500	**Form:**	Crumble
Ingredient Name	**%**	**Total, lb**	**Batch, lb**
Corn - Evonik	57.1208	2,570.4360	1,285.2180
SBM (48%) - Evonik	36.7594	1,654.1730	827.0865
Poultry Fat	2.5034	112.6530	56.3265
DL-methionine	0.3398	15.2910	7.6455
L-lysine HCl	0.1966	8.8470	4.4235
L-threonine	0.1131	5.0895	2.5448
Limestone	1.0885	48.9825	24.4913
Dicalcium phosphate	1.0445	47.0025	23.5013
Salt	0.4104	18.4680	9.2340
Sodium bicarbonate	0.0784	3.5280	1.7640
OptiPhos2000 (0.5lb/ton)	0.0250	1.1250	0.5625
Choline chloride (60%)	0.0500	2.2500	1.1250
Tyson 2x Broiler Vit	0.0550	2.4750	1.2375
BioCox60	0.0500	2.2500	1.1250
UofA TM (0.10%; Max = 0.12%)	**0.1000**	4.5000	2.2500
Inert Filler (cellulose or sand)	**0.0651**	2.9295	1.4648
Total	**100.0000**	**4,500.0000**	**2,250.0000**

Adnan Herbonis Trial: Starter **Diet 2**			
Amount:	1800	**Form:**	Crumble
Ingredient Name	**%**	**Total, lb**	**Batch, lb**
Corn - Evonik	57.1208	1,028.1744	514.0872
SBM (48%) - Evonik	36.7594	661.6692	330.8346
Poultry Fat	2.5034	45.0612	22.5306
DL-methionine	0.3398	6.1164	3.0582
L-lysine HCl	0.1966	3.5388	1.7694
L-threonine	0.1131	2.0358	1.0179
Limestone	1.0885	19.5930	9.7965
Dicalcium phosphate	1.0445	18.8010	9.4005
Salt	0.4104	7.3872	3.6936
Sodium bicarbonate	0.0784	1.4112	0.7056
OptiPhos2000 (0.5lb/ton)	0.0250	0.4500	0.2250
Choline chloride (60%)	0.0500	0.9000	0.4500
Tyson 2x Broiler Vit	0.0550	0.9900	0.4950
BioCox60	0.0500	0.9000	0.4500
UofA TM (0.10%; Max = 0.12%)	**0.1000**	1.8000	0.9000
Inert Filler (cellulose or sand)	**0.0651**	1.1718	0.5859
Total	**100.0000**	**1,800.0000**	**900.0000**
Panbonis		**41 gms**	**20.5 gms**

Adnan Herbonis Trial: Starter **Diet 3**			
Amount:	3600	**Form:**	Crumble
Ingredient Name	**%**	**Total, lb**	**Batch, lb**
Corn - Evonik	57.1208	2,056.3488	1,028.1744
SBM (48%) - Evonik	36.7594	1,323.3384	661.6692
Poultry Fat	2.5034	90.1224	45.0612
DL-methionine	0.3398	12.2328	6.1164
L-lysine HCl	0.1966	7.0776	3.5388
L-threonine	0.1131	4.0716	2.0358
Limestone	1.0885	39.1860	19.5930
Dicalcium phosphate	1.0445	37.6020	18.8010
Salt	0.4104	14.7744	7.3872
Sodium bicarbonate	0.0784	2.8224	1.4112
OptiPhos2000 (0.5lb/ton)	0.0250	0.9000	0.4500
Choline chloride (60%)	0.0500	1.8000	0.9000
Tyson 2x Broiler Vit	0.0550	1.9800	0.9900
BioCox60	0.0500	1.8000	0.9000
UofA TM (0.10%; Max = 0.12%)	**0.1000**	3.6000	1.8000
Inert Filler (cellulose or sand)	**0.0651**	2.3436	1.1718
Total	**100.0000**	**3,600.0000**	**1,800.0000**
Panbonis		164 gms	82 gms

Adnan Herbonis Trial: Starter **Diet 4**			
Amount:	1800	**Form:**	Crumble
Ingredient Name	**%**	**Total, lb**	**Batch, lb**
Corn - Evonik	57.1208	1,028.1744	514.0872
SBM (48%) - Evonik	36.7594	661.6692	330.8346
Poultry Fat	2.5034	45.0612	22.5306
DL-methionine	0.3398	6.1164	3.0582
L-lysine HCl	0.1966	3.5388	1.7694
L-threonine	0.1131	2.0358	1.0179
Limestone	1.0885	19.5930	9.7965
Dicalcium phosphate	1.0445	18.8010	9.4005
Salt	0.4104	7.3872	3.6936
Sodium bicarbonate	0.0784	1.4112	0.7056
OptiPhos2000 (0.5lb/ton)	0.0250	0.4500	0.2250
Choline chloride (60%)	0.0500	0.9000	0.4500
Tyson 2x Broiler Vit	0.0550	0.9900	0.4950
BioCox60	0.0500	0.9000	0.4500
UofA TM (0.10%; Max = 0.12%)	**0.1000**	1.8000	0.9000
Inert Filler (cellulose or sand)	**0.0651**	1.1718	0.5859
Total	**100.0000**	**1,800.0000**	**900.0000**
Panbonis		164 gams	82 gms

Table 6. Experiment Schedule

Age	Date	Day	Comments
1	04 -May	Wednesday	Place 60 chicks per pen. All pens on Starter crumbles. Temp at 90F
2	05- May	Thursday	
3	06- May	Friday	
4	07- May	Saturday	Reduce temp to 88 F
5	08- May	Sunday	
6	09- May	Monday	
7	10- May	Tuesday	Reduce temp to 85 F
8	11- May	Wednesday	
9	12- May	Thursday	
10	13- May	Friday	Reduce temp to 80 F
11	14- May	Saturday	
12	15- May	Sunday	
13	16- May	Monday	
14	17- May	Tuesday	-Cull to 50 birds per pen.
15	18- May	Wednesday	Reduce temp to 75 F. From this day forward, any reticent bird to walk is marked with paint, and then if it persists during the next inspection, it will be euthanized, necropsied, and cultured/sampled for histology.
16	19- May	Thursday	
17	20- May	Friday	
18	21- May	Saturday	Reduce temp to 70 F
19	22- May	Sunday	
20	23- May	Monday	
21	24- May	Tuesday	
22	25- May	Wednesday	Begin recording all deaths, lame and infirmed.
23	26- May	Thursday	
24	27- May	Friday	
25	28- May	Saturday	
26	29- May	Sunday	
27	30- May	Monday	
28	31- May	Tuesday	Switch diets in T6 & T7 only
29	01- Jun	Wednesday	
30	02- Jun	Thursday	
31	03- Jun	Friday	
32	04- Jun	Saturday	
33	05- Jun	Sunday	
34	06- Jun	Monday	
35	07- Jun	Tuesday	Switch all pens to finisher pellets
36	08- Jun	Wednesday	
37	09- Jun	Thursday	

38	10- Jun	Friday	
39	11- Jun	Saturday	
40	12- Jun	Sunday	
41	13- Jun	Monday	
42	14- Jun	Tuesday	
43	15- Jun	Wednesday	
44	16- Jun	Thursday	
45	17- Jun	Friday	
46	18- Jun	Saturday	
47	19- Jun	Sunday	
48	20- Jun	Monday	
49	21- Jun	Tuesday	
50	22- Jun	Wednesday	
51	23- Jun	Thursday	
52	24- Jun	Friday	
53	25- Jun	Saturday	
54	26- Jun	Sunday	
55	27- Jun	Monday	
56	28- Jun	Tuesday	
57	29- Jun	Wednesday	- 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