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Evaluation of Selected Nuproxa Feed Additives for protection against lameness and improving the wellbeing of broilers in a lameness challenge model

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Evaluation of Selected Nuproxa Feed Additives for protection against lameness and improving the wellbeing of broilers in a lameness challenge model

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Abstract

A common concern in commercial industries profiting from broiler chickens is their inability to yield a completely healthy flock. Bacterial Chondronecrosis with Osteomyelitis poses a threat to these companies by impairing the birds' proximal and distal tibial and femoral heads. In 2000, roughly 17.3% of broiler chickens were affected by Bacterial Chondronecrosis with Osteomyelitis which caused a substantial economic loss in the industry (McNammee et al., 2000). BCO cannot be tied to a particular bacterial species; this experiment focuses on evaluating the efficacy of certain commercial feed additives while determining the bacterial species causing BCO in this set of the flock by process of induction of the disease, manipulation, and maintenance of flock lifestyle, and eventual necropsy of the chickens. The farm setup where this experiment was conducted consisted of 26 pens and 1560 male chicks. Of the 26 pens, 2 of the pens were wire floored to create physical stress on the lower extremities, while the other 24 pens were litter floored. The pens were arranged in two rows of 13 and were 5*10 feet squared. The initial two pens of each row, pens 1 and 14, were the wire floored pens. Each pen began with 60 chicks and was eventually culled down to 50 chicks per pen on day 14. The pens received standard feed starter in crumbles from days 1-34 and then were switched to a finisher given through pellets from days 35-56. The six different treatment groups were distributed over the 26 pens and consisted of varying amounts of Nuproxa feed additives. Pens 1 and 14 were designated under treatment one and were the pens used to induce lameness through wire flooring and a standard diet without additives. A further detailed description of the treatments can be found in Table 1. Beginning on day 22, the birds were weighed and diagnosed as lameness began to appear. On day 56, the remaining birds underwent an extensive necropsy on the last day of the trial. They

were weighed, their tibia and femurs were evaluated, samples of their lesions on their tibia and femur were taken, and Inflammasome and FITC-D analysis was completed.

Introduction

Lameness in association with Bacterial chondronecrosis with osteomyelitis (BCO) can be considered one of the leading causes of illness in broiler chickens. With its initial recognition dating back to 1972, this illness alone has since causes detrimental monetary loss in commercial industries that substantially use broiler chickens (Alrubaye et al., 2020, Mandal et al., 2016). Worldwide, 66 billion broiler chickens are slaughtered and used for consumption annually (Hartcher et al. 2019). Aside from monetary loss, the use of birds infected with BCO could prove to cause health concerns for consumers (Gaußmann et al., 2017). When evaluated post-mortem, copious amounts of broiler chickens suffering from lameness were associated with abnormal color and odor, though a direct link could never be made (Granquist et al., 2019). The development of lameness and BCO invites industries to attempt to create solutions for this problem. Taking advantage of the rapid growth rates of the broiler chicken, industries have begun to decrease slaughter ages of the birds which directly impacts quality for the consumer. With a growing demand for meat as human population increases, poultry industries are in dire need for a solution. (Hartcher et al., 2019). The bacterial species causing BCO initially resides in the host's respiratory tract and gastrointestinal tract and ultimately makes its way towards the growth plates of the proximal femoral and tibial heads, thus promoting the initial steps towards lameness (Wideman Jr., 2012; Perpetua et al., 2000). After the bacteria translocate at the growth plates from the gastrointestinal system, the introduction of bacteria into the blood and bone leads to two outcomes within the femoral and tibial heads: damage to the cartilage cells of the growth plates continued by mass collections of osteochondrotic clefts (Wideman et al., 2013). In addition, the repeated amounts of stress on the lower extremities induce degeneration of the femoral and tibial

heads (Wideman Jr., 2016). Prior research shows that the most efficient method to incite repeated stress, both psychologically and physically, is through the addition of wire flooring in the pens where the Broiler chickens spend much of their gestation period.

It is rare that BCO is induced by a singular type of bacteria. To this day, numerous unidentified bacterial communities contribute to the development of BCO (Mendal et al., 2016). Because environments differ, each bacterial species is unequally represented deponing on the bone where the lesion resides and the type of lesion. (Jiang et al., 2015). From various studies, Staphylococcus aureus and Escherichia coli are the greatest causes of BCO. Following Staphylococcus aureus, coagulase- negative staphylococci and Enterococcus spp. are the most causing agents (McNamee et al., 2000; Gaußmann et al.2017). If let to run its course, these bacteria lead to Femoral and Tibial head necrosis. FHN and THN is the leading cause in lameness, particularly in our flock of interest, broiler chickens (Madhawl et al., 2020) An important method to determining the bacterial species causing BCO is through Polymerase Chain Reaction followed by gene resequencing. These tools serve to amplify the samples of DNA collected and compare the DNA to the species in question. Because this is such a pressing problem, species that serve as a potential risk already contain a pre-marked biomarker (Mandal et al., 2016). Identification of the bacterial species is crucial to combating lameness in broiler chickens. By identifying the leading bacterial species, commercial industries may develop protocols to ensure the health of their birds. Healthy birds will reduce the monetary loss in the industry and precent potential infection risks in consumers.

The introduction of wire pen flooring produces instability within natural gaited walk in the chickens leading to the growth of lesions within the already infected bone

(Wideman Jr., 2012). Because lesions are being created on the epiphyseal-physeal cartilage of the femur and tibia, this flooring method is crucial to transmitting the bacterial species into the bloodstream (Wideman, 2016; Al-rubaye et al., 2017). This step is crucial to inducing lameness because of the vulnerability that the flock possesses arising from their unnaturally quick growth rate. Growth rates of broiler chickens have increased by over 300% from the constant demand from consumers (Knowles et al., 2008). Additionally, growth rate is doubled in male broiler chickens than in female broiler chickens. Restricting feed during primal growth of the chickens is suggested to reduce the vulnerability to microfractures and osteochondritic clefts. (Bradshaw et al., 2002). This method allows the birds to reach their regular weight but significantly slows the rate at which they reach it. Though, in theory, feed restrictions will slow growth rate and reduce physical stress of birds, feed restrictions cause psychological stress to the birds. The inability for the birds to receive enough feed leaves the birds in constant states of hunger (Hartcher et al., 2019). It can be argued that psychological stress is the leading contributing factor for BCO. The addition of psychological stress activates the hypothalamo-pituitary-adrenocortical (HPA) axis in the brain which directly contributes to an immunocompromised gut. Stressors create an ideal environment to promote translocation from the gut to, not just the bones but various parts of the host (Ando et al., 2000). Although different factors affect overall lameness in Broiler chickens, it is proposed that improving overall immune health is the most efficient method to reduce the incidence rate within the chickens. More importantly, focusing on gastrointestinal health may be a predominant aspect of improving immune systems in Broiler chickens. Gastrointestinal health promotes increased osteoblast and osteoclast activity, which

indirectly impacts the slowing degeneration of the femoral and tibial head (McCabe et al., 2017). Essentially, to create an optimal experimental setting, both psychological and physical stress must be managed. By reducing the physical stress causing microfractures, minimal bacteria is translocated because of a healthy gut. Consequently, the minimal bacteria that is translocated to the bones would have no place to reside thus terminating the formation of a lesion. One main reason that BCO is still a prevalent issue within the poultry industry is its low incidence rate. Though it causes a substantial loss within the market, the prevenance is still low enough to where it becomes hard to study (Granquist et al. 2019). This experiment can generate beneficial data by inducing lameness. While controlling the infection and lifestyle management of the chickens, feed additives that could aid gut health were used to determine a reduction in lameness. In this trial, two Nuproxa products were used, separately and in conjunction, to determine if BCO can be diminished within an environment where BCO is expansively present. BCO was generated through a control group that was placed entirely on wire flooring and housed in the same facility as other chickens placed on litter. The type of research setup used in this experiment allows the bacterial species to travel via respiratory infection. This can be used to determine which type of feed additive combination is most effective in combating the generation of lameness within Broiler chickens. BCO proves to be a limiting factor in the commercial industry primarily because it can lead to premature death resulting in noticeable economic loss (Perpetua et al., 2000). Research with feed additives, specifically Nuproxa in this research trial, may provide supplemental information when considering best practices in poultry farming (Al-Rubaye et al., 2015). The research also allowed for a further in-depth look at which specific microorganisms were associated

with this specific case of BCO. Once the trail concluded, additional microbiological work was conducted. Identification of bacterial species is important in this context because of how bacterial species colonize differently depending on the bone location (Gaußmann et al., 2017). By taking samples of the tibial and femoral head of each necropsied bird, species can be eventually separated into pure cultures. In our research lab, identification begins through the reculturing of the bacterial species to obtain pure cultures. Once pure cultures have been obtained, DNA extraction of the pure cultures are separated and organized. To receive an accurate identification, polymerase chain reaction is run on the samples to amplify the extracted DNA. Lastly, gene sequencing allows for eventual identification of the bacterial species. Being able to recognize the bacterial species allows industries to potentially neutralize the deleterious effects of the bacteria. Ultimately, the goal is to be able to take steps to utilize the data to determine how to best promote gut health.

Literature Review

Bacterial Chondronecrosis with Osteomyelitis leads the commercial industry in overall lameness with broiler chickens. Approximately $80-$120 million are lost in the commercial broiler industry from lameness. About 12.3% of lameness is due to lesions caused by BCO (*Bacterial Chondronecrosis with Osteomyelitis – Lameness*, 2022). BCO is a disease that is not associated with any microbial species but can be introduced into the host through various species (Jiang et al., 2021). Species most associated with BCO include Staphylococcus spp., Enterococcus spp., E. coli., and Mycobacterium spp. These species of bacterium make their way and remain in the gastrointestinal system for the duration of the infection (DSM, Szafraniec, et al., 2022). The experiment conducted rapid

induction of Bacterial Chondronecrosis with Osteomyelitis in the flock of broiler chickens. Many different factors can manipulate gastrointestinal health. Arguably the most crucial aspect of gastrointestinal health in this research has to do with the intestinal permeability of the flock (Jiang et al., 2015). Because the translation of bacteria causes BCO from the gut to the joints, the decreased intestinal permeability creates a more efficient pathway for the bacteria and an expedition of the production of clefts and lesions. Intestinal permeability decreases when the flock is introduced into high-stress environments (Jiang et al., 2021). The problem leading to this experiment combines both the impending question of which bacteria caused BCO in the flock of broiler chickens and which forms of additives targeted towards improving gut health correlate with a decrease in BCO.

Stress is directly proportional to intestinal permeability, meaning that if the host, in this case, a broiler chicken, is highly exposed to stress, their intestinal permeability increases (Jiang et al., 2015). Intestinal permeability works by allowing fluid exchange between the lumen of the intestine and the tissues in the body. Usually, the tight epithelial junctions that control permeability are keen to allow the passage of ions, nutrients, and water and prevent the passage of dangerous toxins and pathogens, meaning that an increased permeability gives rise to a higher chance of bacterial translocation (Lee, 2015). Neural connections create a prominent link between our brain and digestive systems. The brain-gut axis is a bidirectional network that creates constant communication, which allows considerable influence in both top-down and bottom-up processes between these two systems. In turn, Gastrointestinal health is susceptible to stressors because of this connection. Heat stress is a particularly prominent psychological

stress corresponding with broiler chickens. Higher temperatures increase the need for thermoregulation and activate the hypothalamic-pituitary-adrenal (HPA) axis. Since the HPA axis helps make up the brain-gut axis, activation of the HPA axis directly affects how the gastrointestinal system will react to stress. (Lara et al.. 2013, Rostagno, 2020). Broiler chickens optimally grow in zones of 64 degrees Fahrenheit to 75 degrees Fahrenheit (Kpomasse et al., 2021). In the experiment, the heat lamps above each pen left the chicks to grow in temperatures upwards of 90 degrees Fahrenheit. These temperatures mimic temperatures seen during transportation and commercial farming plants, leading to immense heat stress within the developing flock.

Psychological and physical stress work simultaneously to create the optimal environment for BCO. Bacteria associated with BCO capitalize from the leaky epithelial tight junctions within the gastrointestinal epithelium caused by psychological stress. Broiler chickens are a specific flock that can grow substantially quickly compared to other types of aviation. This alacrity in growth causes poorly mineralized bones, makings them more porous and prone to fractures and injury when placed under significant strain (Singh et al., 2021, Szafraniec et al., 2022, Wideman et al., 2013). Once the bacteria have passed through the tight junctions and spread to the head of the joints, they implant into the microfractures at the growth plates caused by physical stress (Jiang 201,5, Hancock et al., 1995). It is rare for blood circulation to penetrate the entirety of the growth plate. The development of microfractures and clefts allows blood to circulate within the growth plate and allows the bacteria to colonize.

Furthermore, wire floor models aid in microfracture and cleft production in the bones. Continuous footing instability from the wire flooring during development keeps a

resurgence of fractures on the growth plates. Ultimately, since the microfractures are constantly being formed on the proximal tibial and femoral heads of the flock from the strain, the translocated bacterium from the gut can exploit the openings to begin the process of lesions and eventual lameness (Wideman et al., 2013).

A multitude of different bacterial species can cause BCO. Identification of the bacterial species is crucial to developing a plan for reducing lameness in the flock. By exposing the flock to a wire floor pen model, incidences of BCO can be seen at high rates without having to expose the flock to toxins or pathogens directly. This also allows for insight into which bacteria species are most likely to infect the flock since no singular bacterium is being introduced into the environment (Jiang et al., 2015, Wideman et al., 2013).

Materials and Methods

The experiment took place in A365W and required 60 male chicks supplied from Cobb. The chicks were distributed within 26 5*10 feet squared floor pens. Pens 1 and 14 had floors made of wire, while Pens 2-13 and 15-26 had litter-covered floors. Each pen consisted of 2 tube-type feeders at the east end of each pen and a row of nipples that served as a tap water dispenser on the west end. Pens 1 and 14 contained feeders adapted for gravity feed water from 20L carboys. The temperature was controlled through thermoregulation in A365W. The temperatures were set for 90 °F for days 1-3, 88 °F for days 4-6, 85 °F for days 7-10, 80 °F for days 11-14, 75 °F for days 14-17, and 70 °F for the remainder of the experiment. A3655W was set up to allow 23 hours of light and one hour of dark for the duration of the experiment. All pens received standard feed (crumbles) from days 1-34 and were then switched to finisher (pellets) from days 35-56.

The treatments were separated into 6 (Table 1) and were randomized in a block design (Figure 1). On day 14, 10 chicks were culled from all pens so that each one now contained 50 chicks. Starting on day 21, the birds were "walked" daily by being prompted by a broomstick. Pens 1 and 14 served as the infection source via air transmission and were placed at the westmost end of A356W. The wire floor and air transmission simulated infection seen in the poultry industry. Ventilation fans placed at the eastmost end of A356 pulled air from the westmost end to the eastmost end. On day 22, recording and diagnosis of cumulative lameness began. On day 56, select birds were weighed and necropsied for lameness lesions, were given a gross evaluation of the tibia and proximal femur, and were given an inflammasome and FITC-D analysis. 2 birds from each pen were given a microbial sampling of either the proximal tibia or femur for a total of 104 samples. Every time a bird died or developed, lameness was recorded by date, pen number, and treatment, then necropsied.

Assessment of Clinical Lameness: Starting on day 21, the birds were "walked." Those who could not do so were diagnosed as "clinically lame" and consequently humanely euthanized. They were recorded by date, pen number, and treatment. They were necropsied, and their femoral and tibial heads were assigned under one of the categories shown in figures 1 and 2:

N = Femur head and proximal tibia appear entirely normal

Cull = Runts and individuals that failed to thrive or appeared to be clinically ill

U = Unknown cause of death

NE = Necrotic Enteritis

SDS = Sudden Death Syndrome (Flipover, Heart Attacks)

PHS = Pulmonary Hypertension Syndrome, Ascites

KB = Kinky Back (Spondylolisthesis)

TW = Twisted Leg or Slipped Tendon (perosis)

TD = Tibial Dyschondroplasia

Lame-UNK = Lameness for undetermined reasons

FHS = Proximal Femoral Head Separation (epiphyseolysis)

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

Total Lame = FHS + FHT + FHN + THN

Figure 1: Femoral head BCO lesions progression. Lesion severity begins from regular progress from left to right and downward.

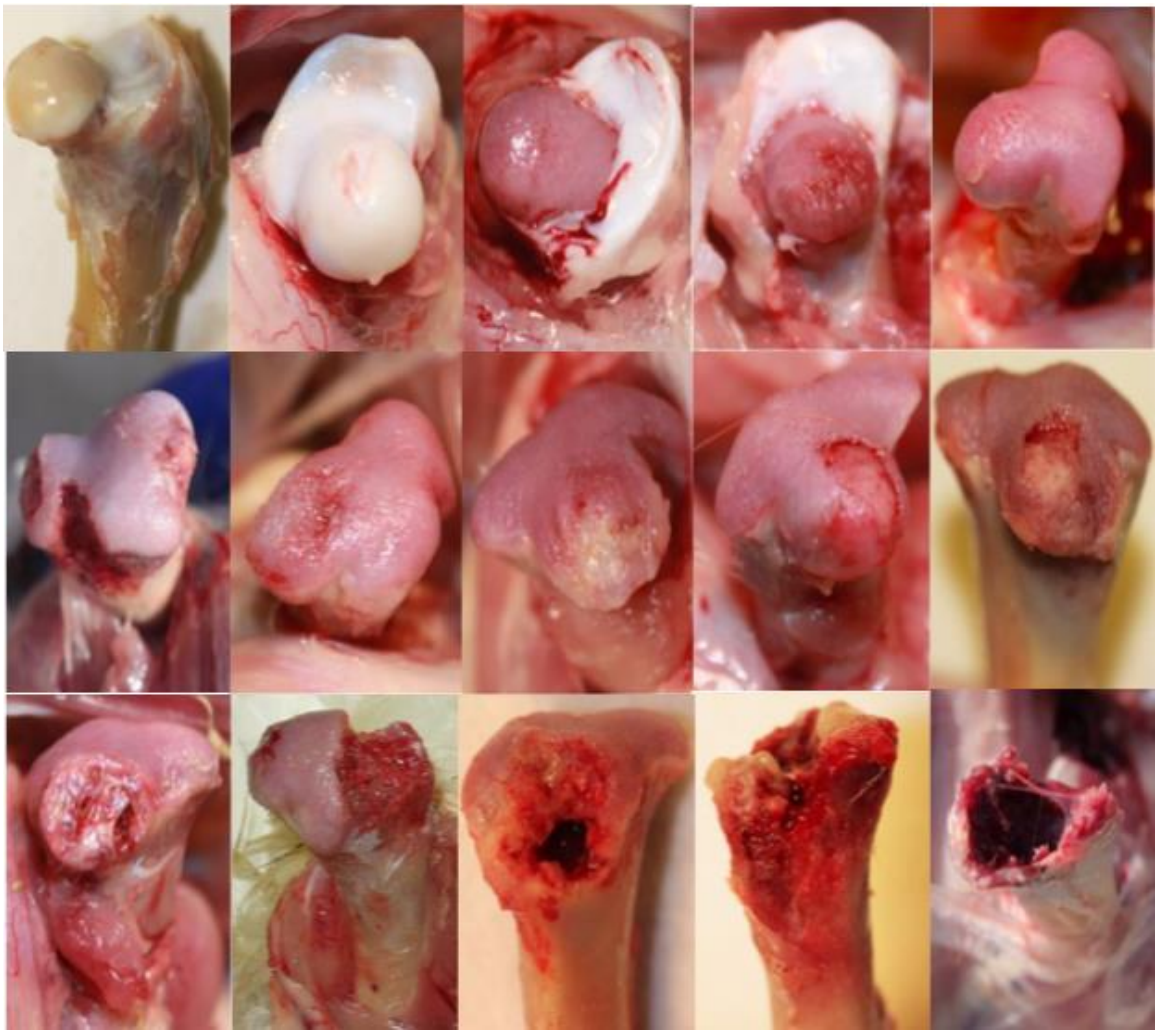
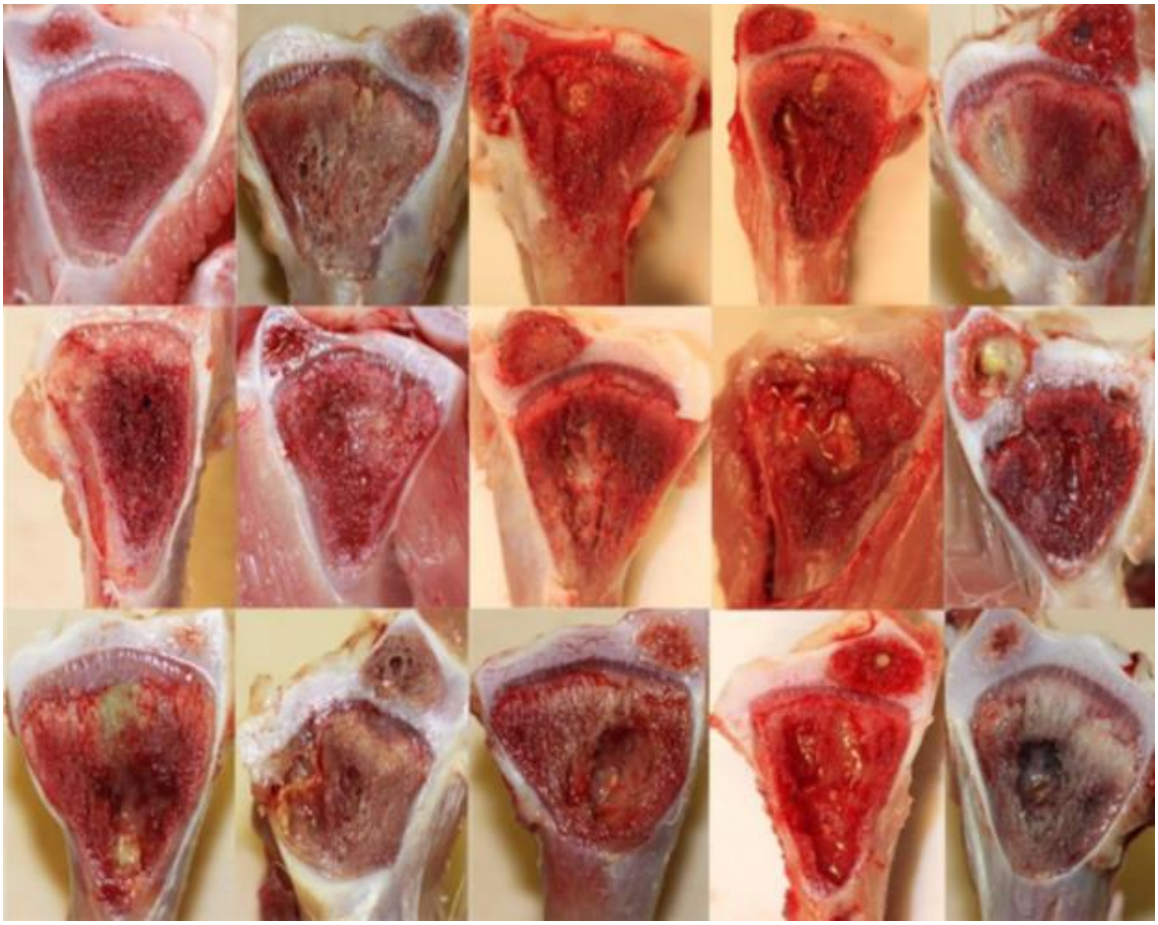


Figure 2: Tibial head BCO lesions progression. Lesion severity begins from regular progress from left to right and downward.



On day 56, a selected number of birds from each treatment are necropsied for lameness through gross anatomical inspection of the head of the tibia and proximal femur, culture samples from two gross lesions at either proximal tibia or femur from 2 birds from each pen for a total of 104 samples. Following, DNA extraction, Polymerase Chain Reaction (PCR), and sequencing will be conducted on all bacterial samples for 156 samples.

Results

Though this experiment was set for 56 days, inclement weather extended the trial time by four days for 60 days. Through Figure 1, cumulative lameness through percentages within each treatment can be analyzed. As expected, the pens under Treatment 1, a standard diet, and wire flooring have the highest incidence of cumulative lameness. Treatment 2, a standard diet on litter flooring, had a similar incidence though slightly lower than treatment 1. The following treatments, treatments 3-7, are statistically significant when determining whether they reduce the incidence of lameness. In comparison to treatment 2, each treatment (T3-T7) had a statistically significant P-value ranging between 1.5E-10to <2.0E-16, as shown in Table 1. Treatment 5 (diet 4) seems to have created the highest incidence among treatments 3-7.

Figure 1: Cumulative Lameness for the seven treatment groups.

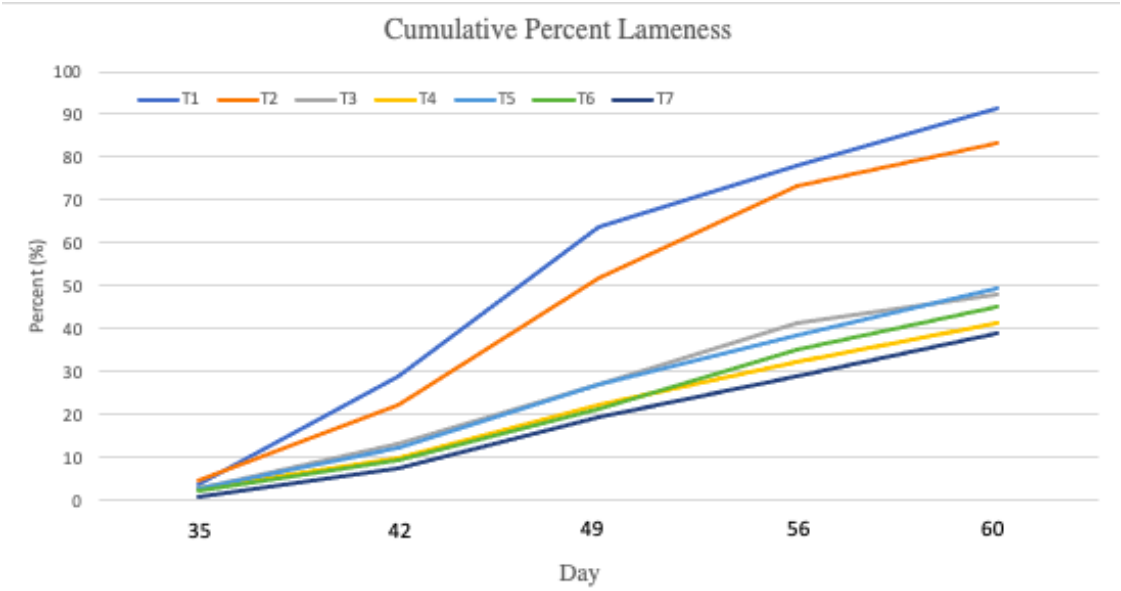
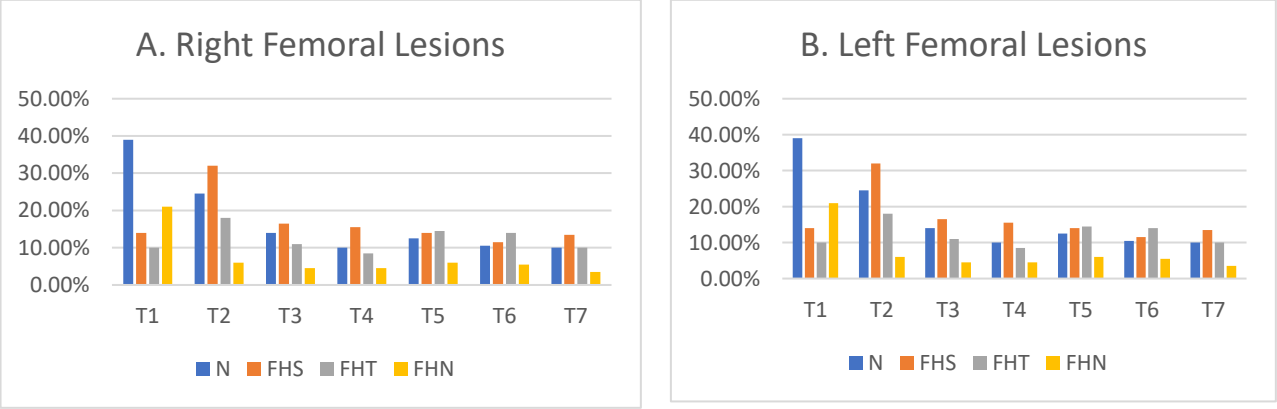


Table 1: P values from the GLM analysis for treatments 1-7 on day 60.

GLM Analysis	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
	Negative control	Panbois high	Panbois low	Panbois low + NatuFeat	Panbois Low + Animunim	Panbois + Thyme oil
P-Value	T2	T3	T4	T5	T6	T7
T1	0.64	2.7E-07	1.2E-11	4.5E-08	1.2E-09	2.9E-07
T2		1.5E-10	<2.0E-16	1.5E-11	3.1E-14	3.4E-10
T3			0.033	0.78	0.21	0.41
T4				0.05	0.33	0.29
T5					0.32	0.61
T6						0.76

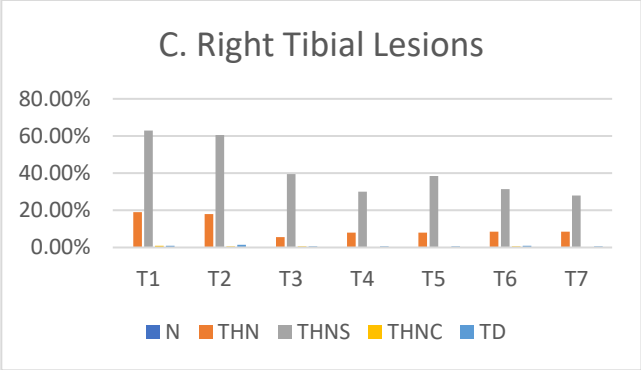
During the gross anatomical analysis, the conditions of the proximal femoral heads were recorded and can be seen in figure 2. Within the femoral heads of treatments 3-7, femoral head separation (FHS) was the most prominent within the treatment groups, followed by femoral head transitional degradation (FHT). No clear pattern was determined based on the findings.

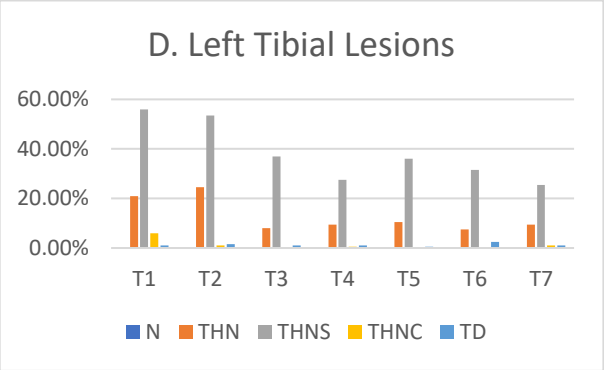
Figure 2: Femoral Lesions for treatments T1- T7 at day 60. N- normal; FHS- femoral head separation; FHT- femoral head transitional degeneration; FHN- femoral head necrosis



During the gross anatomical analysis, the conditions of the proximal tibial heads were recorded and can be seen in figure 3. Across all the treatments, Total head necrosis severe (THNS) was the most prevalent. Total head necrosis (THN) was the next most apparent within tibial heads. Again, there was no clear pattern that was determined through these findings.

Figure 3: Tibial Lesions for treatments T-T7 at day 60. N- normal; THN- Tibial head necrosis; THNS- Tibial head necrosis severe; THNC- Tibial head necrosis casein; TD- Tibial death.





Cumulative percent lameness at days 35, 41, 49, 56, and 60 were measured in Table 2. Treatment 1 and 2 conjured the greatest percent lameness by 40%. Following treatments 1 and 2, Treatment 7 contained the least number of lame birds.

Table 2: Cumulative percent lameness for specific days

Day	T1	T2	T3	T4	T5	T6	T7
35	4.0	4.5	3.0	3.0	3.0	2.5	1.0
42	29.0	22.5	13.5	10.0	12.5	9.5	7.5
49	64.0	52.0	27.0	22.5	27.0	21.5	19.5
56	78.0	73.5	41.5	32.5	38.5	35.0	29.0
60	91.3	83.4	47.9	41.6	49.7	45.1	38.9

Consequently, birds who become lame eventually suffer mortality. Table 3 summarizes the number of lame birds per treatment along with the mortality per treatment.

Table 3: Lame and mortality count for birds within treatments

Trt	Lame per pen				Mortality per pen			
T1	45	39			5	3		
T2	43	42	38	38	0	0	3	4
T3	23	19	22	28	3	0	2	3
T4	24	20	18	15	0	8	4	3
T5	24	26	20	24	4	1	1	5
T6	17	17	20	19	1	8	1	6
T7	18	22	16	18	2	0	2	6

Like Table 3, Table 4 includes the percentage of lame birds and the number of total deaths. Additionally, it includes the percentage of birds that went lame without any deaths.

Table 4: Lame birds with and without death

	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**
% Lame	84.00	80.50	46.00	38.50	47.00	41.50	37.00
Total Deaths	8	7	8	15	11	16	10
% Lame w/o Deaths	91.30	83.42	47.92	41.62	49.74	45.11	38.95

Discussion

We compared the P-values of the different treatment groups and the cumulative lameness to how high of an effect the Nuproxa additives were at combating lameness. A P-value below .05 indicates that the treatments were likely to reduce lameness and thus reduce the incidence of BCO. The additive, Panbois, serves as a probiotic, which was determined to aid gastrointestinal health (Wideman et al., 2012). Treatment groups 3 (diet 2), 4 (diet 3), 5 (diet 4), 6 (diet 7), and 7 (diet6) all presented P values lower than $P < .05$ when compared to treatment 2, which served as the negative control in the flock. Treatment 7 (diet 6), which consisted of Panbois and Thyme oil, seemed to be the additive that caused the most significant decrease in lameness by the end of the trial and was the most statistically significant during the GLM analysis. Though the additives may have reduced the incidence of lameness within the chickens, the severity of lesions across all treatments are similar.

The additives were shown to decrease the lameness when compared to treatments 1 and 2, but some additives functioned better than others. Table 2 shows that treatments 4, 6, and 7 had the lowest number of lame birds over the course of the experiment. Table 3 compared the number of lame birds per pen with the mortality per pen. The pens that share treatments have variation in the number of lame and the mortality per pen. This can be explained by the random block design within the farm. The way that the air flowed and by the uneven spread of the first birds that were infected. Table 2 explains this phenomenon by showing that the cumulative percentage of those lame are relatively similar at day 35. As the infection can spread more regularly, the effects of the feeds are seen by the great contrast of cumulative lameness by the end of the trail on day 60. Table 4, like table 3, shows the percentage of lame birds with and without deaths. This table

shows that the percentage of birds that were lame is the highest for treatment 3 (diet 2) and the lowest were treatments were treatments 4 (diet 3) and 7 (diet 6). Treatment 3 was the only treatment that contained Panbonis high (1000g/ton) whereas all the other treatments contained Panbonis low (100g/ton). This concludes that although all the treatments with additives reduced lameness in the chickens, Panbonis high protected against lameness less than the diets with Panbonis low did. Consequently, the information gathered throughout this experiment would conclude that the feed additives including Panbonis low would be the most effective additive to reduce lameness in Broiler chickens.

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Appendix

Figure 1: Map of pen setup in A365W

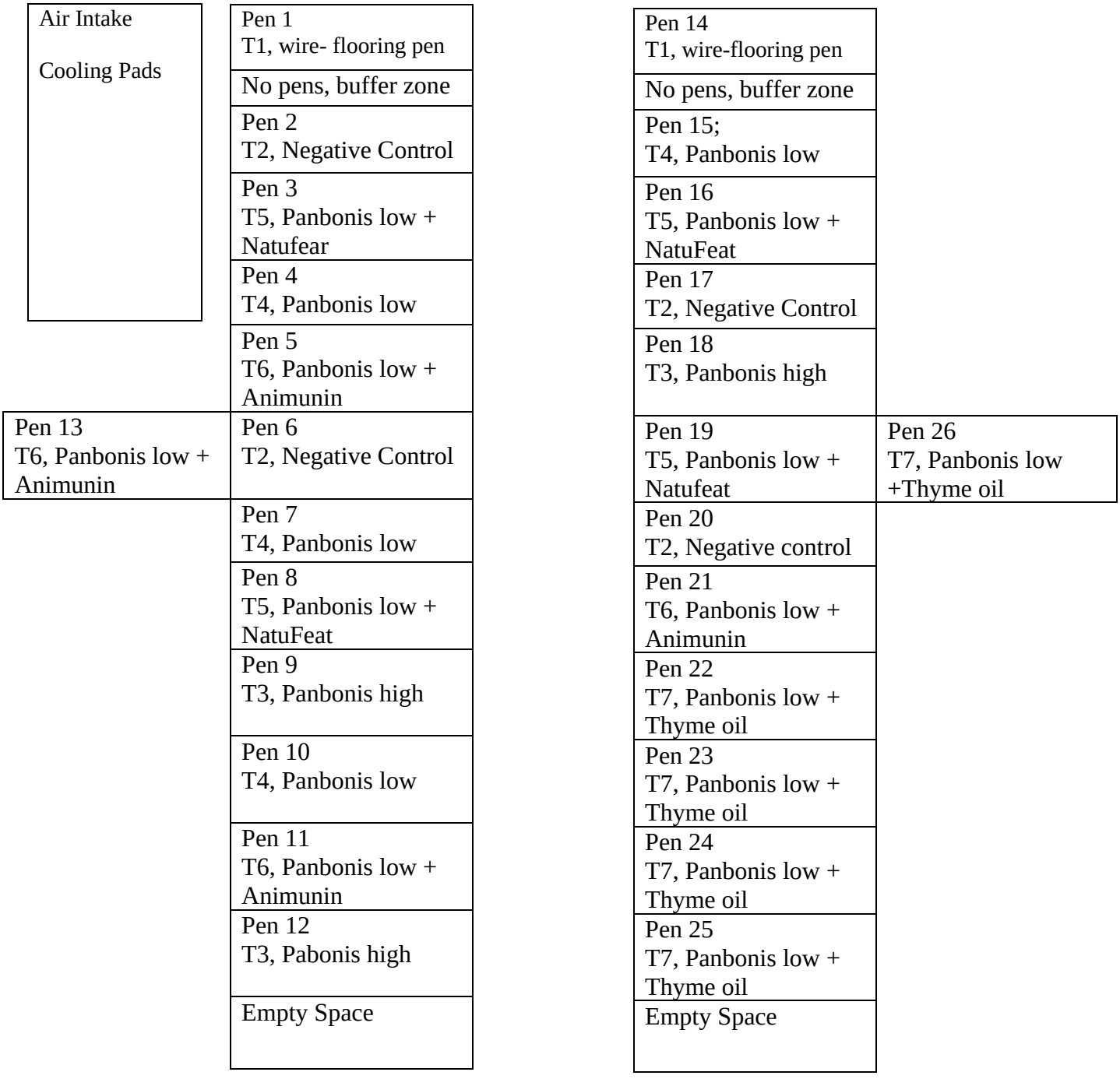

Table 1: Treatment descriptions

Treatment	Pens	Description
T1	1 & 14	Diet 1
T2	2, 6, 17, & 20	Diet 1: Negative Control
T3	9, 12, 18, 24	Diet 2: Panbonis high (1000 g/ton)
T4	4, 7, 10, & 15	Diet 3: Panbonis low (100 g/ton)
T5	3, 8, 16, & 19	Diet 4: Panbonis low (100g/ton) + NatuFeat (500 g/ton)
T6	5, 11, 13, & 21	Diet 5: Panobis low (100 g/ton) + Animunin (750 g/ton)
T7	22, 21, 25, & 26	Diet 6: Panobis low (100 g/ton) + Thyme oil (500 g/ton)

Table 2: Standard Feed Formulation Diet 1

Ingredient Name	%
Corn- Evonik	57.1208
SBM (48%)- Evonik	36.7594
Panbonis	0
Poultry Fat	2.5034
DL- methionine	0.3398
L-lysine HCl	0.1966
L- threonine	0.1131
Limestone	1.0885
Dicalcium phosphate	1.0445
Salt	0.4104
Sodium bicarbonate	0.0784
OptiPhos2000 (0.5lb/ton)	0.025
Choline Chloride (60%)	0.05
Tyson 2x Broiler Vit	0.055
Bio Cox60	0.05
UofA TM (0.10%; Max = 0.12 %)	0.10
NatuFeat	0
Animunin	0
Thyme Oil	0
Inert Filler (cellulose or sand)	0.0651
Total	100.0

Table 3: Standard Feed Formulation Diet 2

Ingredient Name	%
Corn- Evonik	57.1208
SBM (48%)- Evonik	36.7594
Panbonis	0.1
Poultry Fat	2.5034
DL- methionine	0.3398
L-lysine HCl	0.1966
L- threonine	0.1131
Limestone	1.0885
Dicalcium phosphate	1.0445
Salt	0.4104
Sodium bicarbonate	0.0784
OptiPhos2000 (0.5lb/ton)	0.025
Choline Chloride (60%)	0.05
Tyson 2x Broiler Vit	0.055
Bio Cox60	0.05
UofA TM (0.10%; Max = 0.12 %)	0.10
NatuFeat	0
Animunin	0
Thyme Oil	0
Inert Filler (cellulose or sand)	0.0651
Total	100.10

Table 4: Standard Feed Formulation Diet 3

Ingredient Name	%
Corn- Evonik	57.1208
SBM (48%)- Evonik	36.7594
Panbonis	0.1
Poultry Fat	2.5034
DL- methionine	0.3398
L-lysine HCl	0.1966
L- threonine	0.1131
Limestone	1.0885
Dicalcium phosphate	1.0445
Salt	0.4104
Sodium bicarbonate	0.0784
OptiPhos2000 (0.5lb/ton)	0.025
Choline Chloride (60%)	0.05

Tyson 2x Broiler Vit	0.055
Bio Cox60	0.05
UofA TM (0.10%; Max = 0.12 %)	0.10
NatuFeat	0
Animunin	0
Thyme Oil	0
Inert Filler (cellulose or sand)	0.0651
Total	100.1

Table 5: Standard Feed Formulation Diet 4

Ingredient Name	%
Corn- Evonik	57.1208
SBM (48%)- Evonik	36.7594
Panbonis	0.01
Poultry Fat	2.5034
DL- methionine	0.3398
L-lysine HCl	0.1966
L- threonine	0.1131
Limestone	1.0885
Dicalcium phosphate	1.0445
Salt	0.4104
Sodium bicarbonate	0.0784
OptiPhos2000 (0.5lb/ton)	0.025
Choline Chloride (60%)	0.05
Tyson 2x Broiler Vit	0.055
Bio Cox60	0.05
UofA TM (0.10%; Max = 0.12 %)	0.10
NatuFeat	0.05
Animunin	0
Thyme Oil	0
Inert Filler (cellulose or sand)	0.0651
Total	100.06

Table 6: Standard Feed Formulation Diet 5

Ingredient Name	%
Corn- Evonik	57.1208
SBM (48%)- Evonik	36.7594
Panbonis	0.01
Poultry Fat	2.5034

DL- methionine	0.3398
L-lysine HCl	0.1966
L- threonine	0.1131
Limestone	1.0885
Dicalcium phosphate	1.0445
Salt	0.4104
Sodium bicarbonate	0.0784
OptiPhos2000 (0.5lb/ton)	0.025
Choline Chloride (60%)	0.05
Tyson 2x Broiler Vit	0.055
Bio Cox60	0.05
UofA TM (0.10%; Max = 0.12 %)	0.10
NatuFeat	0
Animunin	0.075
Thyme Oil	0
Inert Filler (cellulose or sand)	0.0651
Total	100.085

Table 7: Standard Feed Formulation Diet 6

Ingredient Name	%
Corn- Evonik	57.1208
SBM (48%)- Evonik	36.7594
Panbonis	0.01
Poultry Fat	2.5034
DL- methionine	0.3398
L-lysine HCl	0.1966
L- threonine	0.1131
Limestone	1.0885
Dicalcium phosphate	1.0445
Salt	0.4104
Sodium bicarbonate	0.0784
OptiPhos2000 (0.5lb/ton)	0.025
Choline Chloride (60%)	0.05
Tyson 2x Broiler Vit	0.055
Bio Cox60	0.05
UofA TM (0.10%; Max = 0.12 %)	0.10
NatuFeat	0
Animunin	0
Thyme Oil	0.05
Inert Filler (cellulose or sand)	0.0651
Total	100.06

Table 8: Experimental Time Line

Age	Date	Day	Comments
1	08- Dec	Wednesday	Place 60 chicks per pen. All pens on Starter crumbles. Temp at 90F
2	09- Dec	Thursday	
3	10- Dec	Friday	
4	11- Dec	Saturday	Reduce temp to 88F
5	12- Dec	Sunday	
6	13- Dec	Monday	
7	14- Dec	Tuesday	Reduce temp to 85F
8	15- Dec	Wednesday	
9	16- Dec	Thursday	
10	17- Dec	Friday	Reduce temp to 80F
11	18- Dec	Saturday	
12	19- Dec	Sunday	
13	20- Dec	Monday	
14	21- Dec	Tuesday	Cull to 50 per pen
15	22- Dec	Wednesday	Reduce temp to 75F. 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	23- Dec	Thursday	
17	24- Dec	Friday	
18	25- Dec	Saturday	Reduce temp to 70F
19	26- Dec	Sunday	
20	27- Dec	Monday	
21	28- Dec	Tuesday	
22	29- Dec	Wednesday	Begin recording all deaths, lame, and infirmed.
23	30- Dec	Thursday	
24	31- Dec	Friday	
25	01-Jan	Saturday	
26	02- Jan	Sunday	
27	03- Jan	Monday	
28	04- Jan	Tuesday	
29	05- Jan	Wednesday	
30	06- Jan	Thursday	
31	07- Jan	Friday	
32	08- Jan	Saturday	
33	09- Jan	Sunday	
34	10- Jan	Monday	
35	11- Jan	Tuesday	

36	12- Jan	Wednesday	Switch all pens to finisher pellets, FITC-D analysis
37	13- Jan	Thursday	
38	14- Jan	Friday	
39	15- Jan	Saturday	
40	16- Jan	Sunday	
41	17- Jan	Monday	
42	18- Jan	Tuesday	
43	19- Jan	Wednesday	
44	20- Jan	Thursday	
45	21- Jan	Friday	
46	22- Jan	Saturday	
47	23- Jan	Sunday	
48	24- Jan	Monday	
49	25- Jan	Tuesday	
50	26- Jan	Wednesday	
51	27- Jan	Thursday	
52	28- Jan	Friday	
53	29- Jan	Saturday	
54	30- Jan	Sunday	
55	31- Jan	Monday	
56	01-Feb	Tuesday	
57	02- Feb	Wednesday	
58	03- Feb	Thursday	
60	04- Feb	Friday	
61	05- Feb	Saturday	- 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