

Effects of dietary Vitamin D and 25-OH D₃ levels on 0-53 d broiler chicken performance,
processing, tibia ash, and serum Ca and 25-OH D₃ status

J.D. Striplin, R.A. Hirai, and K.G.S. Wamsley

Broiler chicken production is Mississippi's top agricultural commodity. Feed and feed manufacture represent ~70% of the costs required to rear poultry; therefore, any small change in diet formulation can have a dramatic impact on production cost, as well as broiler performance and health. Previous research has demonstrated improved breast yield and bone mineralization for broilers fed diets supplemented with 25-OH D₃. The objective of this study was to investigate the effects of dietary Vitamin D and 25-OH D₃ supplementation on day 0-53 broiler performance, processing yield, tibiae ash; as well as serum Ca and 25-OH D₃. The following inclusions were made to a common corn, soybean meal and DDGS based diet: treatment (Trt) 1-Low Vitamin (Vit) D₃ (165 IU/kg); Trt 2-Low Vit D₃ + 34.8 ppb Bio-D; Trt 3-High Vit D₃ (2756 IU/kg); Trt 4-High Vit D₃ + 34.8 ppb Bio-D; Trt 5-High Vit D₃ + 69.7 ppb Bio-D. On day 0, 1140 male chicks were equally placed in 60 pens; each Trt was randomly assigned to pen with 12 replications per trt. On day 0, 14, 28, 42, and 52 blood was collected from 3 birds/pen and analyzed for serum Ca and 25-OH D₃ levels. Also, on day 14 and 28 tibiae were extracted from 3 birds per pen and on day 53 from processed birds for bone ash measurements. Birds fed Trt 1 exhibited decreased performance, as well as lowest tibiae ash weight and serum Ca and 25-OH D₃ levels. Birds fed Trts 2-5 demonstrated improved performance as compared to Trt 1, along with serum 25-OH D₃ levels displaying a stepwise increase from treatment 1-5 ($P < 0.001$). Overall, birds fed a diet with added 25-OH D₃ and Low Vit D (165 IU/kg) levels had improved performance as compared to birds fed High Vit D (2756 IU/kg).

Joshua Striplin

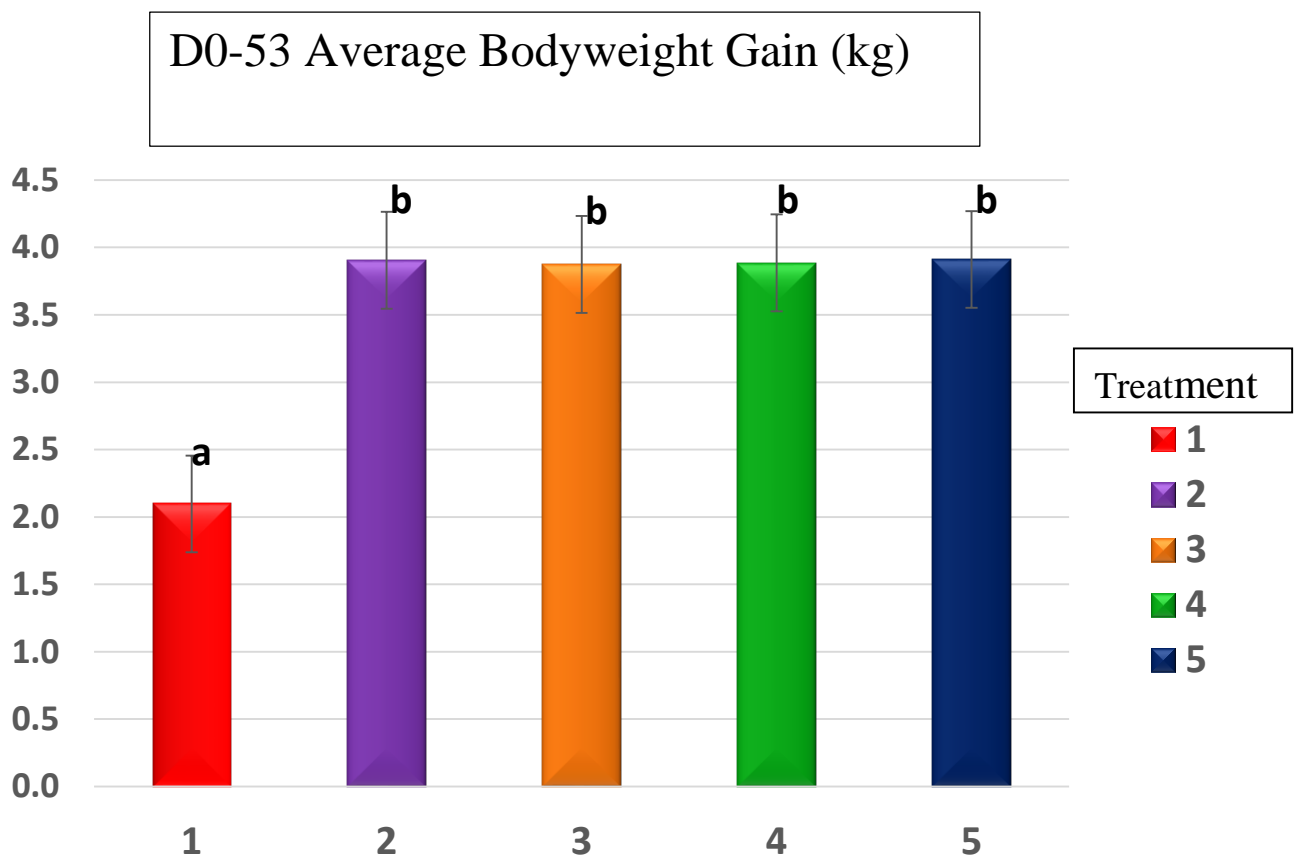
Dr. Kelley Wamsley

Undergraduate Research Experience

During the undergraduate research opportunity, one of the research projects that was conducted was testing the dietary effects of Vitamin D and 25-Hydroxyvitamin-D3 on the male Ross x Ross 708 birds throughout a grow-out period from day zero to day fifty-three. The factors that were tested for were broiler performance, processing yields, tibia ash content, and the serum levels of calcium in the blood. The project ran smoothly with no complications that occurred. Currently, the effects of adding supplementary Vitamin D and 25-Hydroxyvitamin-D3 have not been a heavily researched topic. In recent years, more research has been conducted on laying hens; however, not much research has been produced on the benefits of the vitamin supplements for broilers but that trend is starting to shift (Persia et al 2013). This aspect made the topic very interesting to be investigated to discover the results of adding these vitamin supplements to the feed. The hypotheses that were formulated were that birds that consumed a higher level of Vitamin D in the diet would show improved performance and tibia ash content (Garcia et al 2013). Also, birds eating a diet with the added 25-Hydroxyvitamin-D3 would demonstrate better performance, processing yields, and tibia ash content (Fritts and Waldroup 2003). The overall data that was achieved from the experiment led to incredibly interesting results.

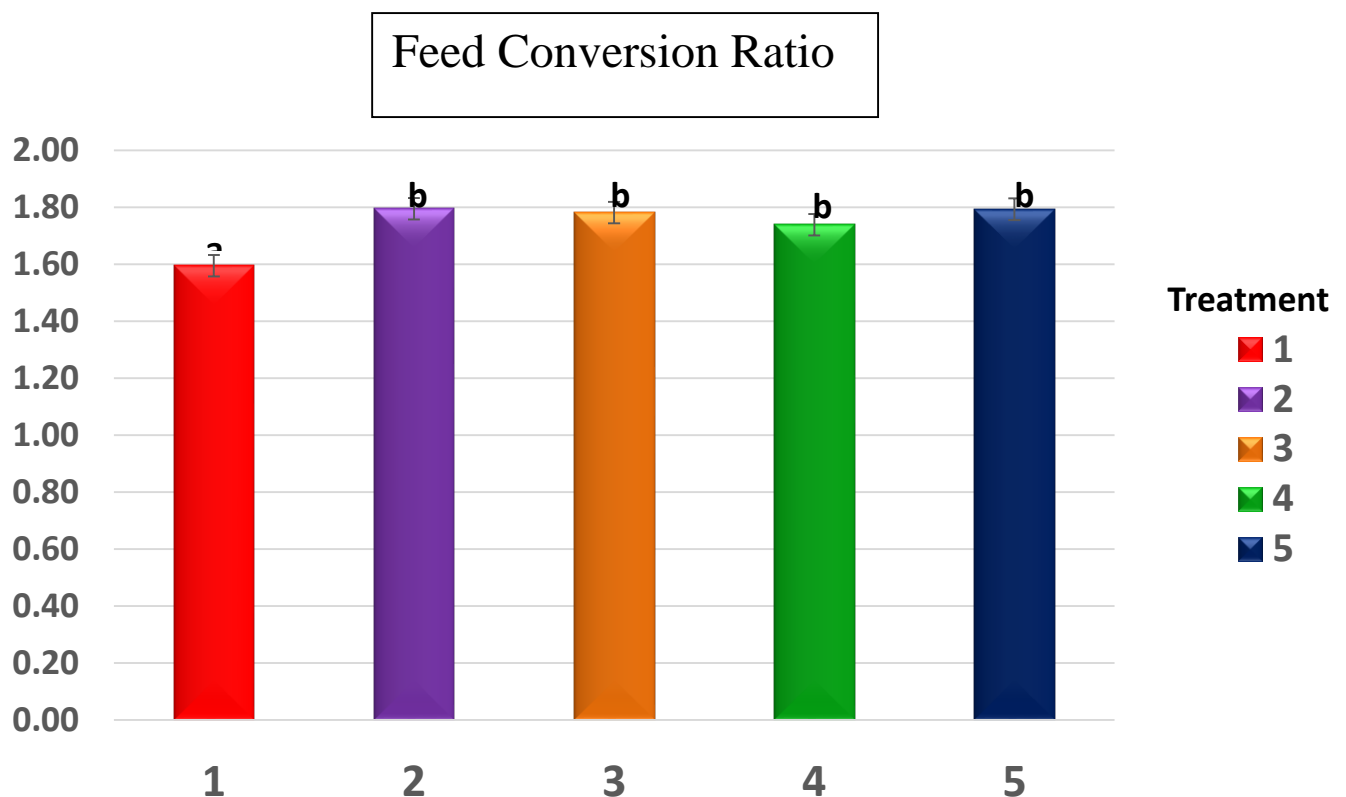
By taking body weight measurements on day zero, fourteen, twenty-eight, forty-two, and fifty-two, body weight gain was able to be measured. Having these measurements allowed us to calculate the bodyweight gain. The data achieved for bodyweight gain was quite enlightening. The birds fed from the treatment that had a low level of Vitamin D added to the diet and no supplementary dietary 25-Hydroxyvitamin-D3 showed the lowest performance as compared to

the other treatments. Treatments that had higher levels of Vitamin D and 25-Hydroxyvitamin-D3 added to the diet showed a significant increase in performance over the treatment with the low level of Vitamin D and no supplementary 25-Hydroxyvitamin-D3 added to the diet. One diet had higher levels of Vitamin D added to the diet as compared to the first diet, but it did not have the 25-Hydroxyvitamin-D3 added to the diet. This diet exhibited similar results as compared to the treatments that had the higher levels of Vitamin D and the 25-Hydroxyvitamin-D3 added to the diet. The data that was analyzed showed no significant difference between the diets that had higher levels of Vitamin D and that did or did not included the 25-Hydroxyvitamin-D3 supplement.



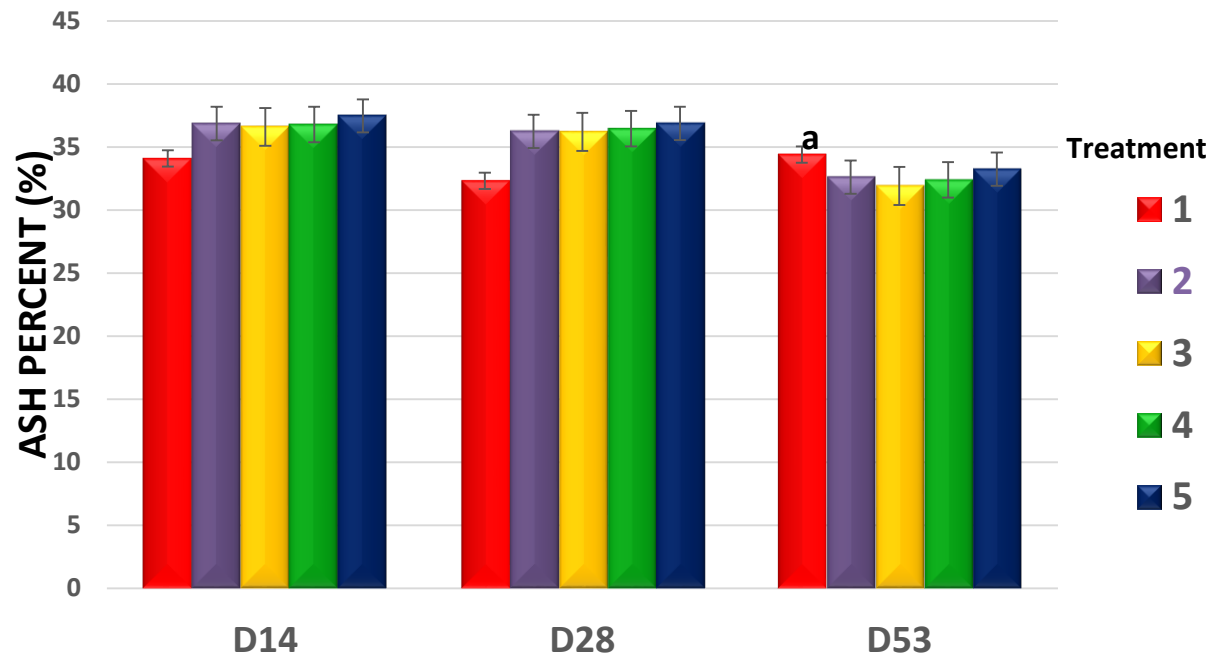
On days that the birds were weighed to attain average bodyweight gain, the feed was also weighed to determine the amount of feed consumed during those specific times which allowed

for the feed conversion ratio to be calculated. Having a low feed conversion ratio is a benefit when feeding birds due to the fact that the birds are converting the feed to muscle at a better rate as compared to other birds (Cowieson and Selle 2012). This will lead to birds being more efficient with the feed they consume which saves money throughout the grow-out process. The treatment that had the low level of Vitamin D and no 25-Hydroxyvitamin-D3 added to the diet exhibited the lowest feed conversion ratio when compared to the diets with higher levels of Vitamin D and whether the supplementary 25-Hydroxyvitamin-D3 was or was not added to the diet.

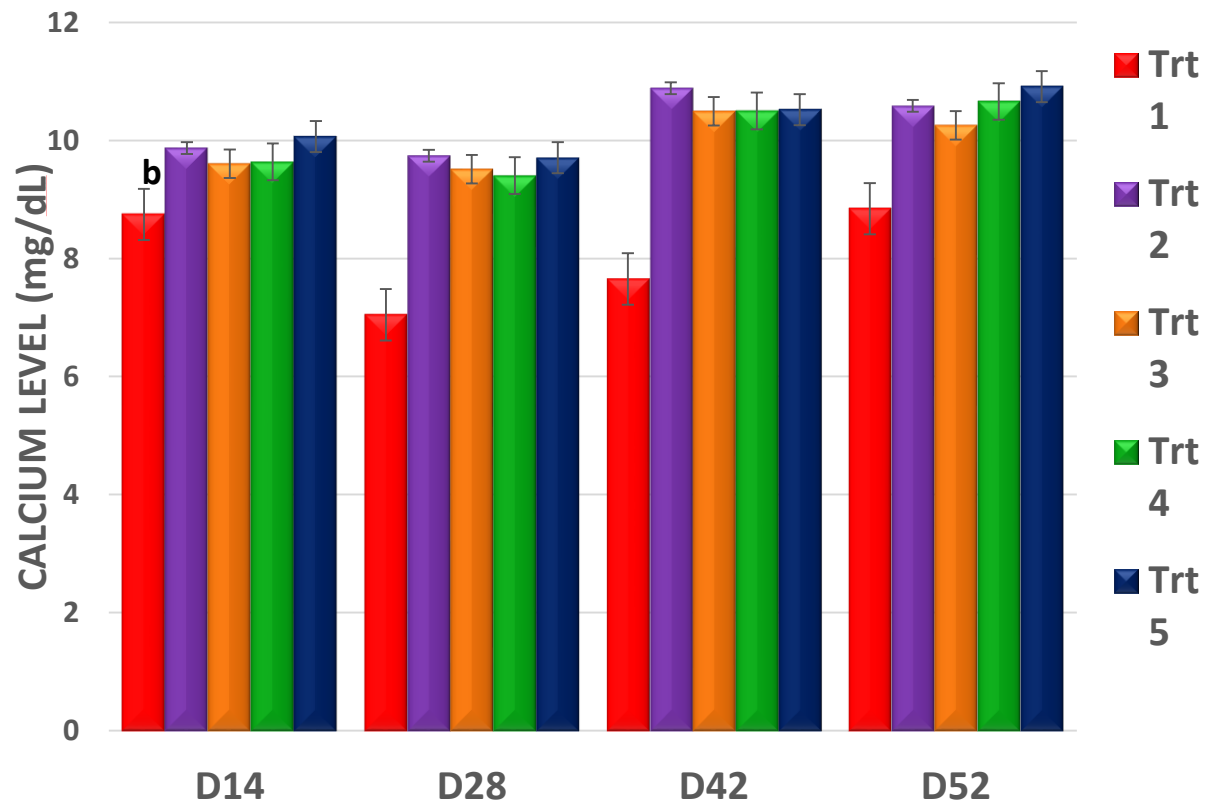


Another factor that was evaluated was tibia ash content. Tibias were collected from birds and sampled on days fourteen, twenty-eight, and fifty-three. The ash content was analyzed since bone ash content is an indicator of available phosphorous which makes it an easy variable to

measure (Hall et al 2003). The right tibia was used from all birds that were used, dried, and crushed to determine ash content. On days fourteen and twenty-eight, birds fed the diet containing the low amount of Vitamin D and no 25-Hydroxyvitamin-D3 had the lowest percentage of bone ash as compared to the other diets. The other diets showed no significant difference between the percentages of bone ash on these days. However, on day fifty-three there was a change in the findings of bone ash percentage between the treatments. The diet with the low level of Vitamin D and no added 25-Hydroxyvitamin-D3 had the highest percentage of bone ash as compared to the other diets. The diet with a high level of Vitamin D and the supplement 25-Hydroxyvitamin-D3 added to it showed the second highest percentage. Two diets each with differing levels of Vitamin D and the 25-Hydroxyvitamin-D3 added to the diet displayed similar results below the previous two diets while the diet containing just a high level of Vitamin D had the lowest percentage of bone ash.



The final factor that was tested was blood serum levels across each of the diets. Calcium is a key component of the diet in broilers due to it being pivotal in the homeostasis process in broilers (Proszkowiec-Weglarz and Angel 2013). When the diets were formulated, they were designed to contain low levels of calcium to determine the effects Vitamin D and 25-Hydroxyvitamin-D₃ have on calcium content in the serum. Blood was collected from birds on days fourteen, twenty-eight, forty-two, and fifty-two to be analyzed. The data showed fairly similar results across each of the days that the serum was collected and tested. The diet that had the just low level of Vitamin D added to the diet displayed the lowest level of calcium in the blood as compared to the other diets. The other diets showed no real significant difference between each other across the sampling days.



After the experiment ended, the data was fully analyzed and compared to the original hypotheses. The findings help confirm the hypotheses that were formulated and showed the important effects of Vitamin D in the diet. Not only was the level of Vitamin D in the diet important, adding 25-Hydroxyvitamin-D3 to the diet aided in yielding improved results (Garcia et al 2013). The way that this was proven was that one diet contained a low level of Vitamin D and had 25-Hydroxyvitamin-D3 added into the diet and displayed similar results to the diet with just a high level of Vitamin D and the diet with a high level of Vitamin D and the 25-Hydroxyvitamin-D3. Overall, the experiment was successful and provided a deeper insight to the positive effects of the inclusion level of Vitamin D and supplemental 25-Hydroxyvitamin-D3 in the diet on multiple aspects of broiler performance.

Throughout the entirety of the undergraduate research program, I learned a plethora of helpful traits and characteristics that will aid through the rest of my life and in the business world. Learning to ask for help from others was one of the key principles that I acquired. The research project was incredibly in-depth and required a ton of help. Many of the tasks that were necessary to achieve crucial data involved help from others. Weigh days were one of these aspects. The amount of personnel required to just weigh birds was more than one person could handle. Also, extracting tibias from birds was quite time consuming and if attempted alone would have lasted much longer without help from others. Not only that but on the day of processing, many hands were needed to record data and make sure all of the parts of processing ran smoothly. The amount of help that was vital to record and achieve proper data was not the only area in which I needed aid. Many times various questions would occur that I would not know the answer to and needed help to discover the answer. Without the assistance of my advising professor Doctor Kelley Wamsley, several graduate students, and coworkers the answers to these questions would have never been discovered. These essential resources were pivotal to the learning experience and helped me to attain success.

Another tool acquired during this process was learning how to coordinate schedules to have much needed aid in attaining all data and help to answer questions. Everyone who assisted me throughout the project each had their own schedules to work around which required planning for each process in advance. Coordinating with all parties who worked on this project was a challenge; however, weekly lab meetings helped everyone be informed on the coming week's activities so that all were informed. These meetings were a crucial part in reaching success throughout this project. One of the main parts of this project was the processing day at the end of project. The processing day possessed many crucial areas in which necessary attention was

mandatory to reach the desired result of acquiring data. The members of the immediate lab team were not enough to cover every area so coordinating with the schedules of additional parties was imperative. Organizing and assigning tasks to all workers ahead of the desired date made the job much more manageable and helped the project run smoothly. By learning how to organize and coordinate schedules, this has helped me prepare to gather aid in advance from multiple sources for future tasks that will be assigned later in life.

Every individual has their own unique personality and gaining the knowledge on how to work with similar and opposing personalities was another crucial component that was learned throughout this whole experience. By working with differing personality types, choosing one of the many ways on how to deal with each one was discovered. The same approach did not work for all personalities which revealed several different decisions on how to handle each situation. By listening to all opinions and views, a reasonable consensus was achieved from all parties which enabled an informed decision to be made. Methods for dealing with differing personalities which may not always agree with your own were able to be discerned from working with others with these types of personalities during this project.

The final lesson that was learned during this entire process was how deep and arduous a task it is to conduct research. One must give necessary attention to all the meticulous details that every research project has in store for the researcher. Small deviations from any project can lead to catastrophic results in the long term for the project. Proper checks and balances are critical whenever research is being conducted. These systems help maintain that the project continues to run smoothly without errors. Without these systems, minor errors early or later on in the research project can lead to detrimental outcomes with the project. Having others double check work that has been done or data that has been inputted is essential in acquiring proper and correct results.

Conducting a research experiment is not a one person job and having others aid in the process helps achieve the desired outcome. Not only are details and other's opinions a vital component of the process, the time commitment necessary for each project is not to be taken lightly. The time needed for each step must be properly allotted to ensure a successful conclusion to the experiment. One must give full dedication of time, energy, and heart to any research project to ensure a desired outcome. In conclusion, a great deal of respect must be given to anyone who has chosen to commit their life to conducting research due to the immense time and effort required to complete any experiment.


During the research symposium, I presented on the experiment of the effects of adding Vitamin D and supplementary 25-Hydroxyvitamin-D3 to the diet. Presenting on this project helped me realize the complete difference in presenting research as compared to any other presentation. Research is precise and gives definitive answers to questions that were formulated. The data is concrete and leaves little room for interpretation which is unlike presenting on other topics. Other topics allow for different interpretations to be formulated which lead to one's own opinion on the matter. This is unlike research since the results are unwavering in nature and what has been reported cannot change. Proper attention must be given to any research presentation to ensure that the audience is correctly informed on the findings. By discussing a research topic, I have been enlightened on how different the world of research is as compared to different presentations such as a sales one.

By partaking in the undergraduate research experience, a whole new side of the world has been revealed to me. Nutrition is a subject that I am greatly interested in and learning about how crucial Vitamin D is to broilers has been a superb experience. Also, seeing how adding a 25-Hydroxyvitamin-D3 supplement to broiler's diets can significantly increase results during the

grow-out period was incredibly insightful. By viewing the results of average body weight gain, tibia ash content, and blood serum levels after the experiment was finished helped put everything in perspective for what results the project had accomplished. Learning about one nutrition aspect of broiler's diets was not the knowledge I gained from the experience. Acquiring the experience in asking for help when you do not know the answer was very helpful. Planning and scheduling for certain tasks with multiple parties was an essential tool obtained during this process which will be much needed throughout the rest of my career and life. Dealing with various personality types and methods of reaching a similar outcome was another key aspect that was gained from this opportunity. Finally, by partaking in a research project and seeing it to its conclusion aided in revealing the true amount of work and dedication that is required to have a career in the field of research. It also divulged the full amount of effort given by all people who assist on any research experiment. Overall, the undergraduate research experience was an amazing opportunity and experience in which I was allowed to partake in and would recommend the opportunity to anyone who is will to dedicate to this superb learning program and endeavor.

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SPRING 2015 UNDERGRADUATE RESEARCH SYMPOSIUM

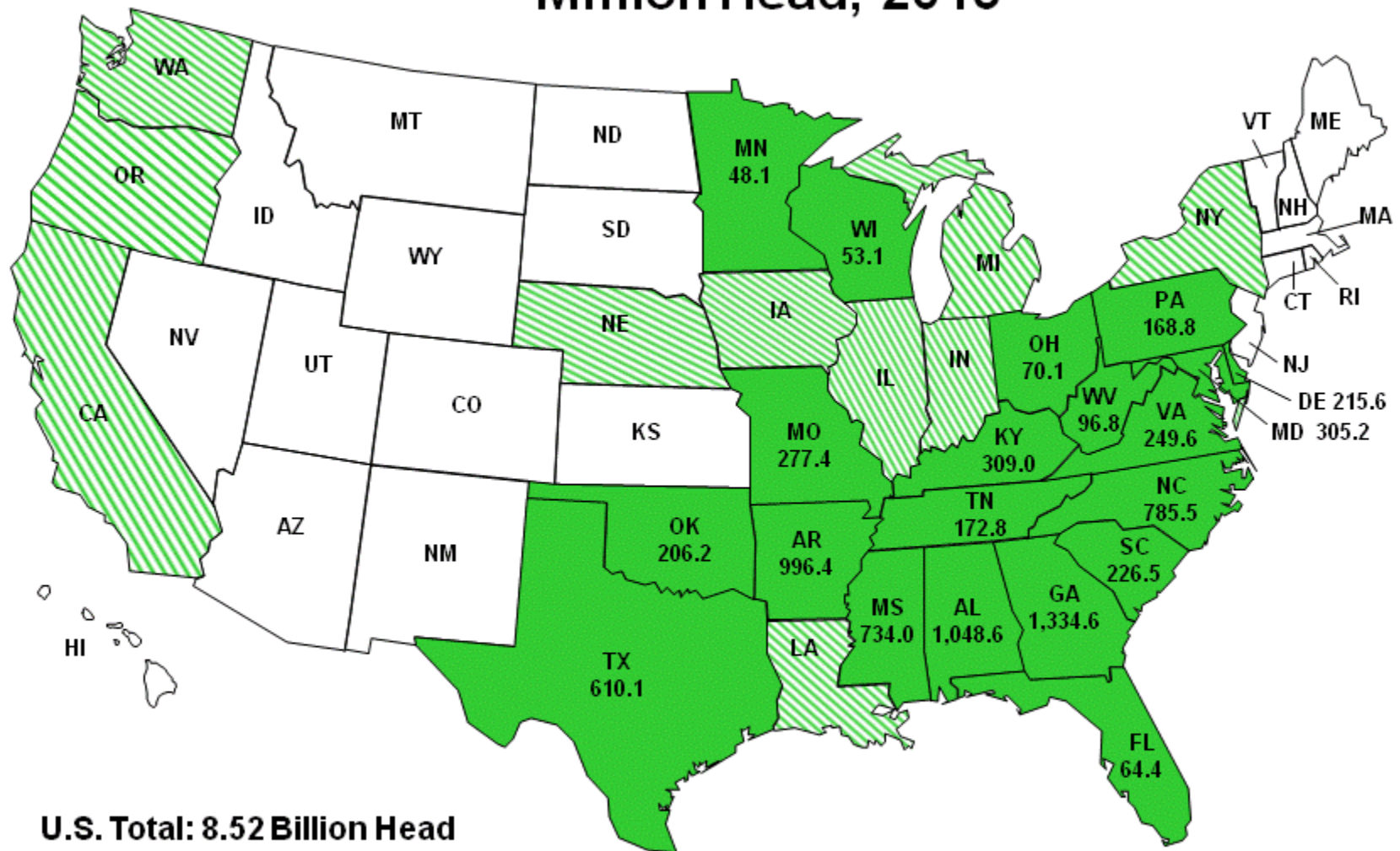
MISSISSIPPI STATE UNIVERSITY

Mississippi Ranking of Market Value of Ag Products Sold

| Item | Farms | Sales (\$1,000) | Rank by Sales | Percent of Total Sales |
|------------------|--------|-----------------|---------------|------------------------|
| Poultry and eggs | 2,742 | 2,744,048 | 1 | 42.6 |
| Aquaculture | 303 | 185,241 | 5 | 2.9 |
| Cattle | 13,178 | 332,491 | 4 | 5.2 |
| Hogs | 306 | 141,139 | 6 | 2.2 |
| Total sales | 38,076 | 6,441,025 | (x) | 100.0 |

Source: USDA, 2012

Broiler Production by State Million Head, 2013



U.S. Total: 8.52 Billion Head

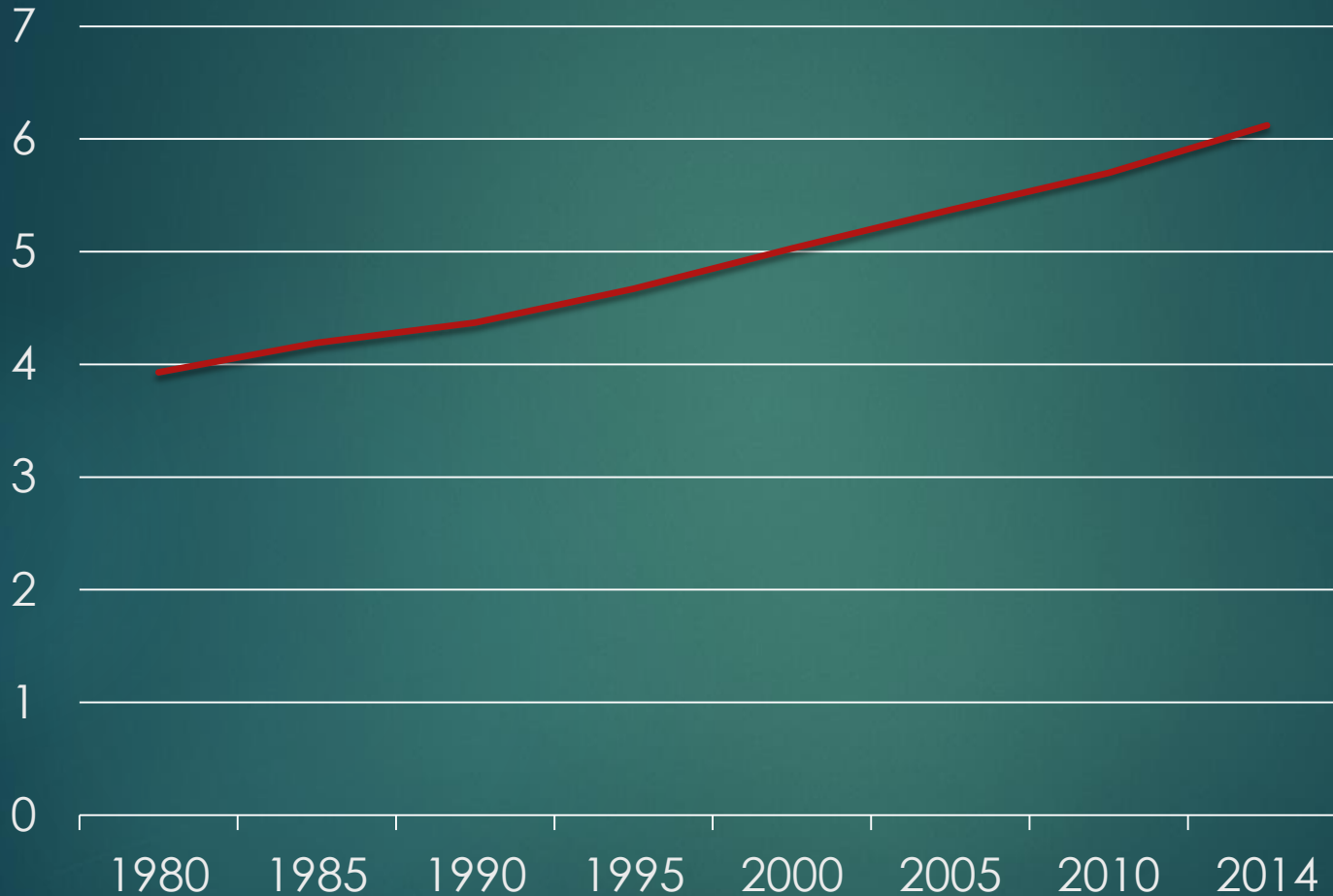
7.97 Billion Head, 93.5% of U.S. Total

 All Other Production States

USDA-NASS
04/29/14

U.S. Broiler Performance

Market Weight (lbs)



— Market Weight (lbs)

***estimated**
Source: National
Chicken Council
January 9, 2015

Introduction: Vitamin D Benefits

- ▶ Calcium and phosphorus metabolism in bone development and maintenance (Ward, 2014)
- ▶ Improved immune system (Reinhardt and Hustmyer, 1987)
- ▶ Improved growth performance (Yarger et al., 1995; Brito et al., 2010) and meat quality (Enright et al., 1999; Han et al., 2012)



Vitamin D3 - Cholecalciferol

- ▶ Vitamin D3 is one of the most important compound of the Vitamin D group
- ▶ After sunlight was established for its role in the prevention of rickets, it became known as the “sunshine vitamin” (Ward, 2014)



Deficiency of Vitamin D

- ▶ **Elevation in blood glucose levels of chicks**
(Hunt and Nielsen, 1987)
- ▶ **Skeletal and Beak Deformities**



25-Hydroxyvitamin D3

- ▶ 25-OH D3 is a vitamin D metabolite
- ▶ Provides a plethora of commercial advantages (Ward, 2014)
- ▶ Reduce Tibial Dyschondroplasia (Sunde, 1975) and increase bone ash (Applegate et al., 2003)
- ▶ Studies had been completed to evaluate 25-OH D3 on the live performance characteristics due to its potential improvements in broiler performance (Ward, 1995)

Objective

- ▶ To investigate the effects of Vitamin D3 level and 25-OH D3 source on 0-53 d broiler performance, processing yield, tibia ash, and blood serum measurements

Hypotheses

- ▶ Birds consuming a diet with a higher inclusion level of Vitamin D will exhibit improved performance and tibia ash measurements
- ▶ Inclusion of 25-OH D3 in diet with lower levels of Vitamin D will improve the performance, processing yields, and tibia ash measurements

Materials and Methods

- ▶ 109E House – South Farm, MSU
- ▶ 1140 male chicks Ross x Ross 708
- ▶ Experimental Period:
D0-D53
- ▶ Water and feed ad libitum



Materials and Methods

► Vaccination:

Live coccidiosis vaccine (5x manufacturer recommendations)



Experimental Design

- ▶ Randomized complete block:
 - 5 Treatments
 - 12 blocks
- ▶ Experimental Unit:
 - One pen with 19 male broilers Ross 708
- ▶ SAS (2013 Version):
 - ▶ Analysis of Variance (ANOVA)
 - ▶ Fisher's Least Significant Differences (LSD)
 - ▶ $P \leq 0.05$



Treatment outline

| Treatment | D3 premix | 25-OH D3 Inclusion (ppb) | 25-OH D3 Analyzed ³ (ppb) |
|-----------|-----------------------|--------------------------------|--------------------------------------------|
| 1 | NRC ¹ | - | 0.4 |
| 2 | NRC ¹ | 34.8 | 35.5 |
| 3 | Industry ² | - | 0.7 |
| 4 | Industry ² | 34.8 | 33.3 |
| 5 | Industry ² | 69.7 | 68.0 |

¹NRC D3 = 5 g/ton of feed = 165 IU/kg

²Industry = 83.3 g/ton of feed = 2756 IU/kg

³Samples from Grower phase feed

Basal Diet Formulation for Each Phase

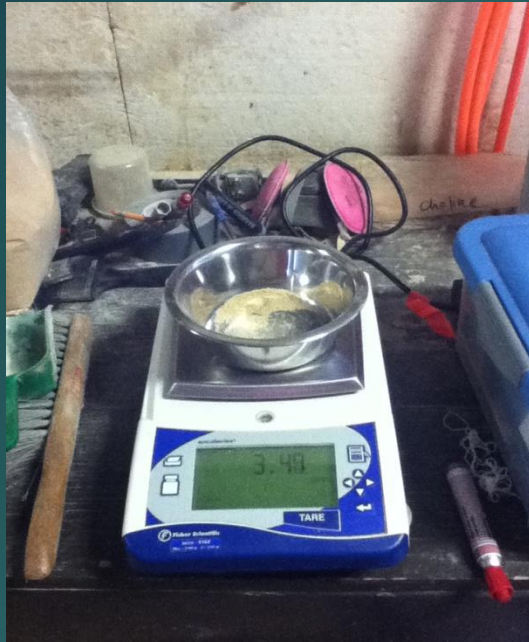
| Ingredient Name | Starter (D0-14) | Grower (D14-28) | Finisher 1 (D28-42) | Finisher 2 (D42-53) |
|----------------------|--------------------|--------------------|------------------------|------------------------|
| Corn | 53.7 | 58.8 | 61.6 | 63.4 |
| SBM (48% CP) | 35.2 | 28.6 | 24.8 | 23.5 |
| Corn DDGS | 4.0 | 7.0 | 8.0 | 8.0 |
| Poultry Fat | 3.5 | 2.1 | 2.3 | 2.0 |
| Dicalcium Phosphate | 1.1 | 1.1 | 0.95 | 0.90 |
| Limestone | 0.61 | 0.64 | 0.61 | 0.43 |
| Salt | 0.30 | 0.29 | 0.28 | 0.28 |
| Sodium Bicarbonate | 0.30 | 0.28 | 0.29 | 0.29 |
| HCl-Lysine | 0.23 | 0.22 | 0.23 | 0.24 |
| DL-Methionine | 0.32 | 0.27 | 0.24 | 0.21 |
| L-Threonine | 0.08 | 0.07 | 0.07 | 0.06 |
| Choline-Cl | 0.02 | 0.0002 | 0.01 | 0.01 |
| Selenium Premix | 0.02 | 0.02 | 0.02 | 0.02 |
| VIT/TM Premix | 0.50 | 0.50 | 0.50 | 0.50 |
| Phytase | 0.03 | 0.03 | 0.03 | 0.03 |
| Corn or 25OHD Premix | 0.10 | 0.10 | 0.10 | 0.10 |

Phytase (sparing 0.12% Ca and available P). Available P and Ca were reduced to 75% of breeder recommendations

Experimental Diet Manufacture

- ▶ Basal diets batched at Mississippi State University Poultry Research Unit
- ▶ Pellets were manufactured at USDA Poultry Research Unit (Starkville, MS)
Trt Order: 1, 2, 3, 4, 5





Measured Variables

- ▶ Live performance
- ▶ Blood collection
- ▶ Tibia collection and ash determination
- ▶ Processing and Debone characteristics

| Measured variable | D0 | D14 | D28 | D42 | D52 | D53 |
|-------------------|----|-----|-----|-----|-----|-----|
| Live performance | X | X | X | X | X | |
| Blood | X | X | X | X | X | |
| Tibia | | X | X | | | X |
| Processing | | | | | | X |

Live performance

| Measured variable | D0 | D14 | D28 | D42 | D52 | D53 |
|-------------------|----|-----|-----|-----|-----|-----|
| Live performance | X | X | X | X | X | |

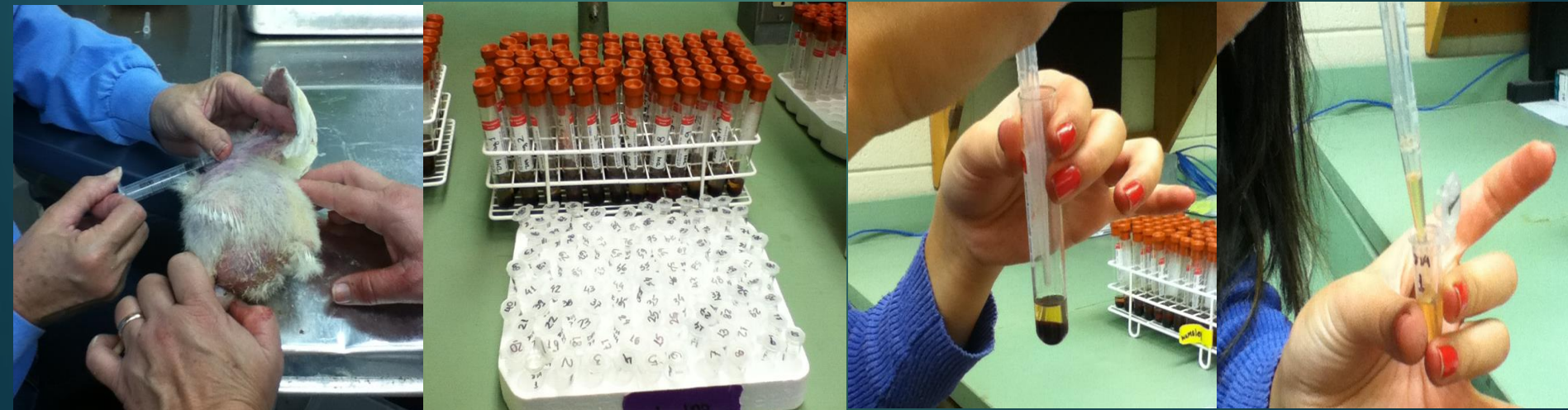
- Birds and feed were weighed by pen
- BW gain, feed consumption, and feed conversion ratio were calculated



Blood collection

| Measured variable | D0 | D14 | D28 | D42 | D52 | D53 |
|-------------------|----|-----|-----|-----|-----|-----|
| Blood Collection | X | X | X | X | X | |

- ▶ On D0, ten chicks were bleeding for serum analysis to obtain a baseline 25-OH D3 level
- ▶ Three birds from 10 replicate pens were used for serum analysis



Tibia collection and ash determination

| Measured variable | D0 | D14 | D28 | D42 | D52 | D53 |
|-------------------|----|-----|-----|-----|-----|-----|
| Tibia | | X | X | | | X |

- ▶ Three birds were used per pen
- ▶ On D 53, four birds per pen were chosen



Tibia collection and ash determination

► METHODOLOGY:

- Tibiae extracted from right leg of each bird
- Crucibles and tibiae were dried (dryer at 105°C, 12h)
- Muffle at 600°C, overnight
- All steps were weighed and recorded



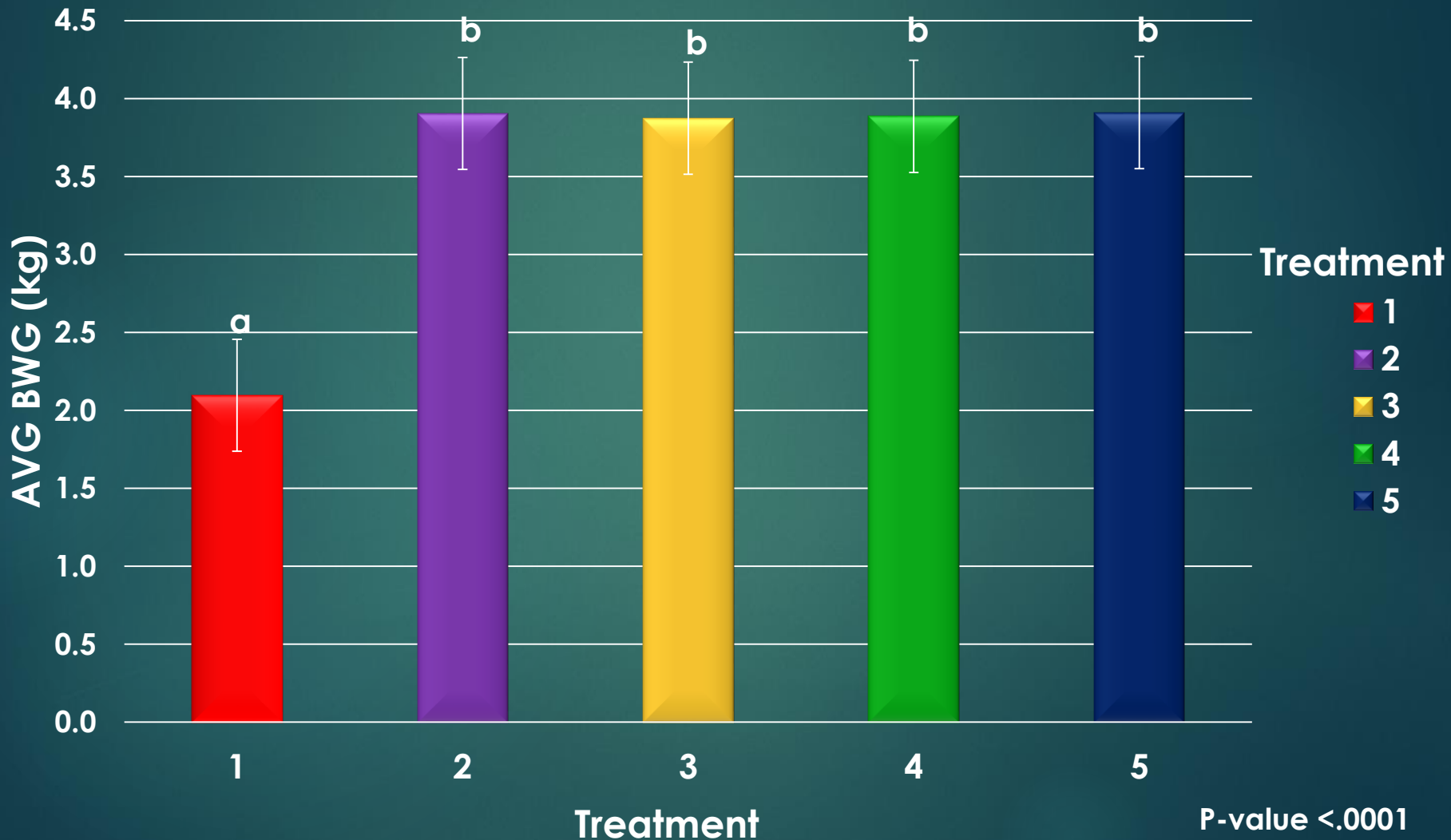
Processing

| Measured variable | D0 | D14 | D28 | D42 | D52 | D53 |
|-----------------------|----|-----|-----|-----|-----|-----|
| Processing and debone | | | | | | X |

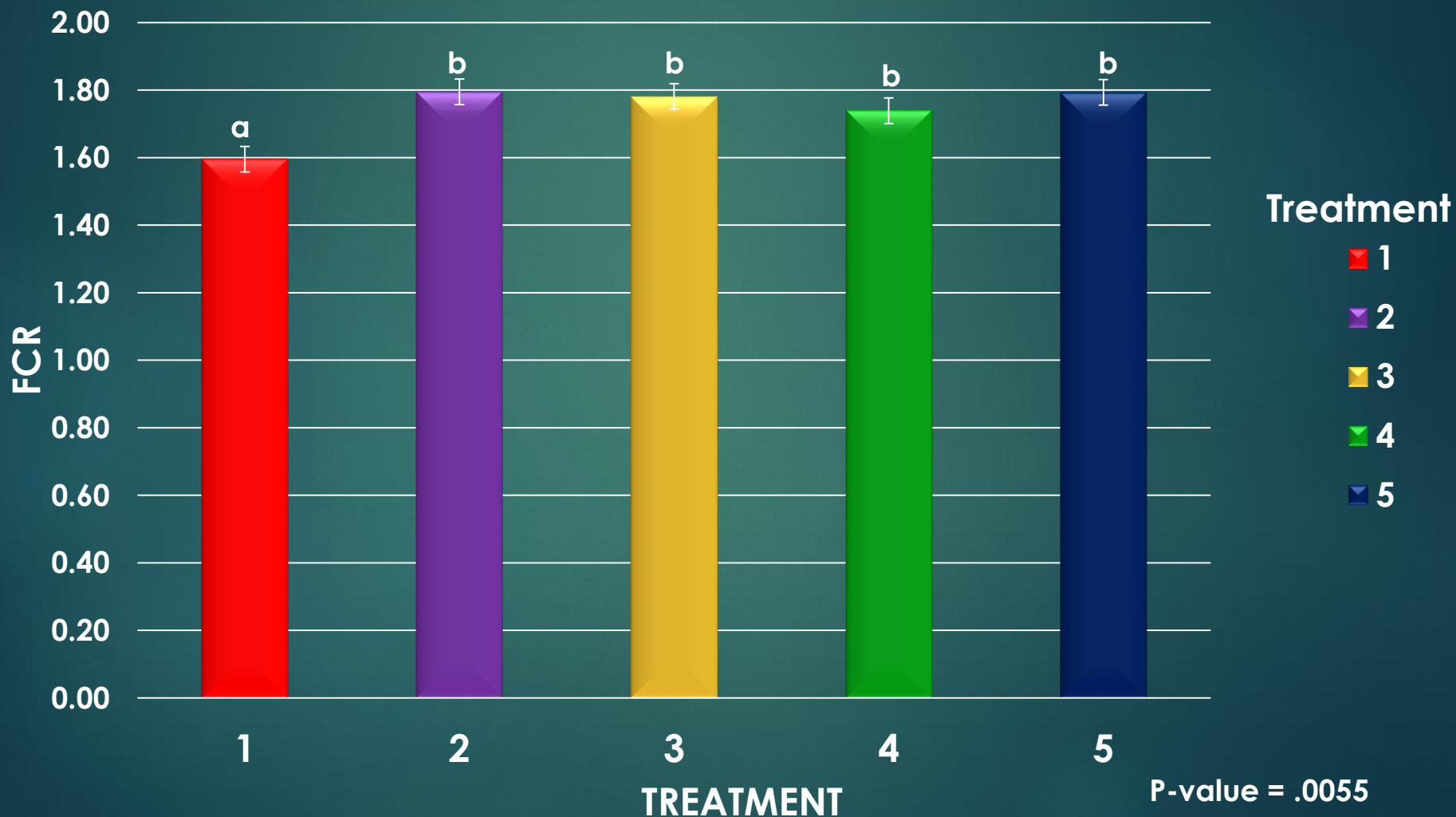
- ▶ On D53, four birds per pen were processed
- ▶ Determine carcass, abdominal fat pad, breast, tender, wing and leg weight/yield



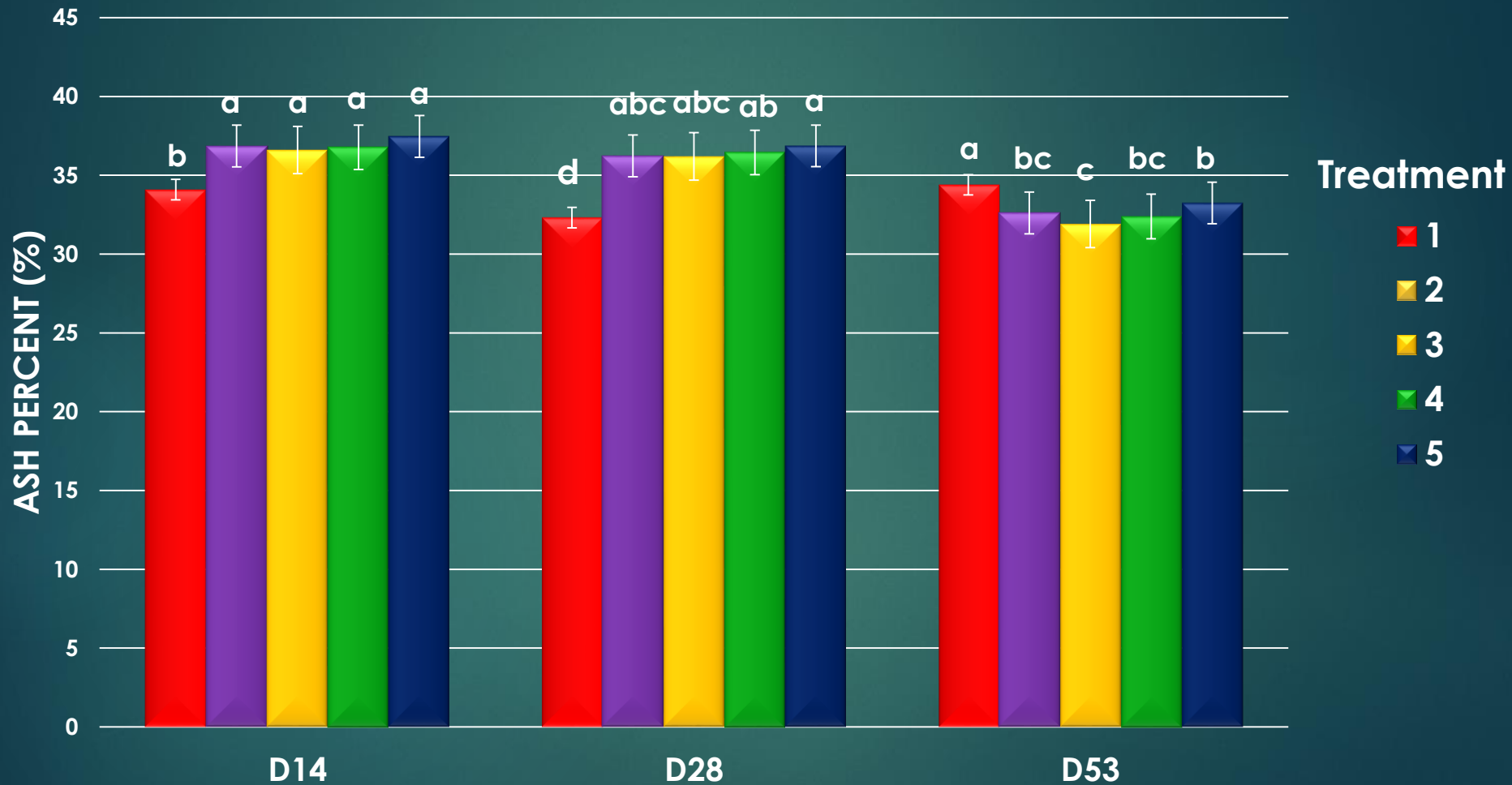
Results: D0-52 Avg. Body Weight Gain



Results: D0-52 Feed conversion ratio

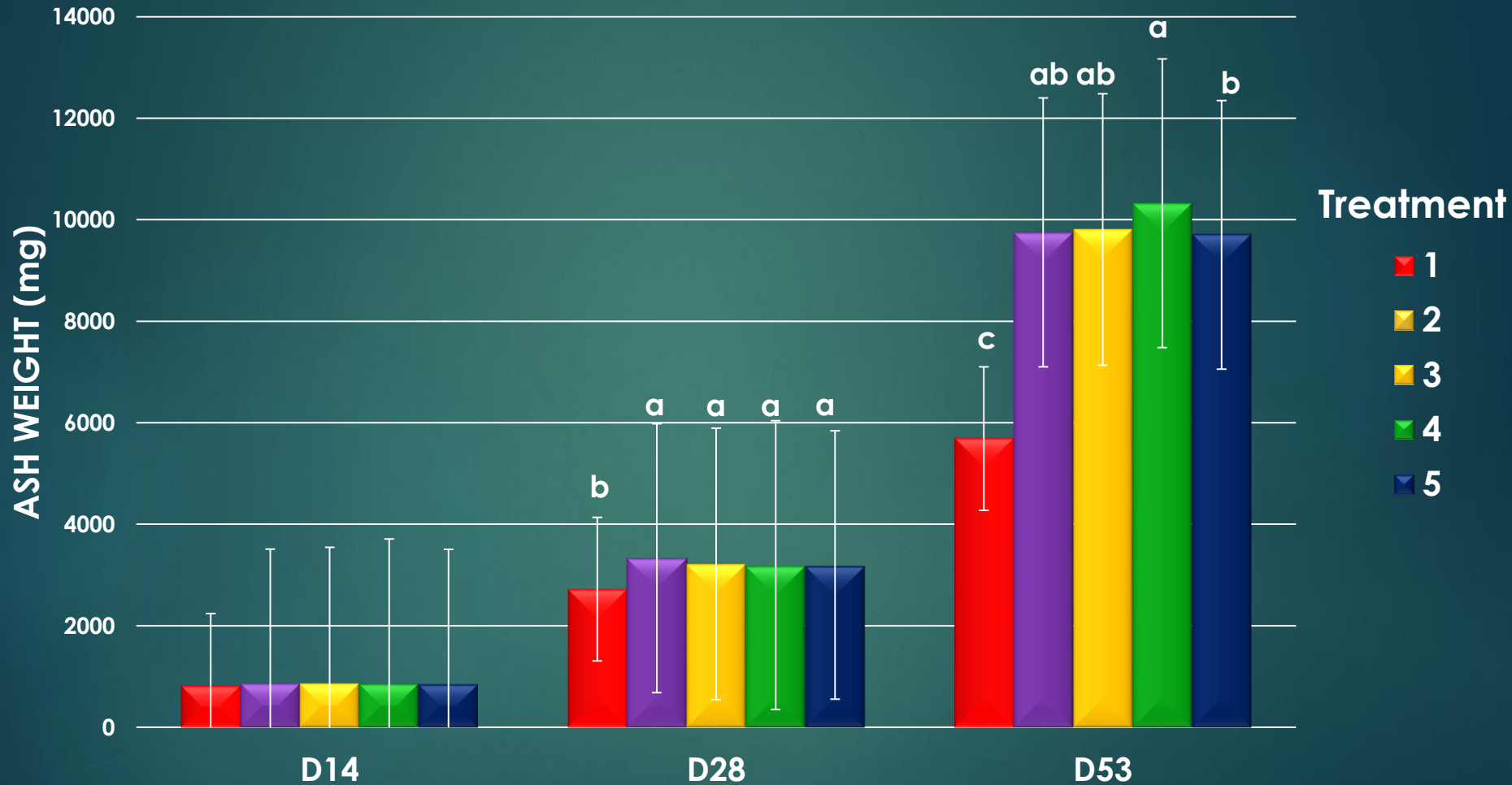


Results: Tibia Ash (%)



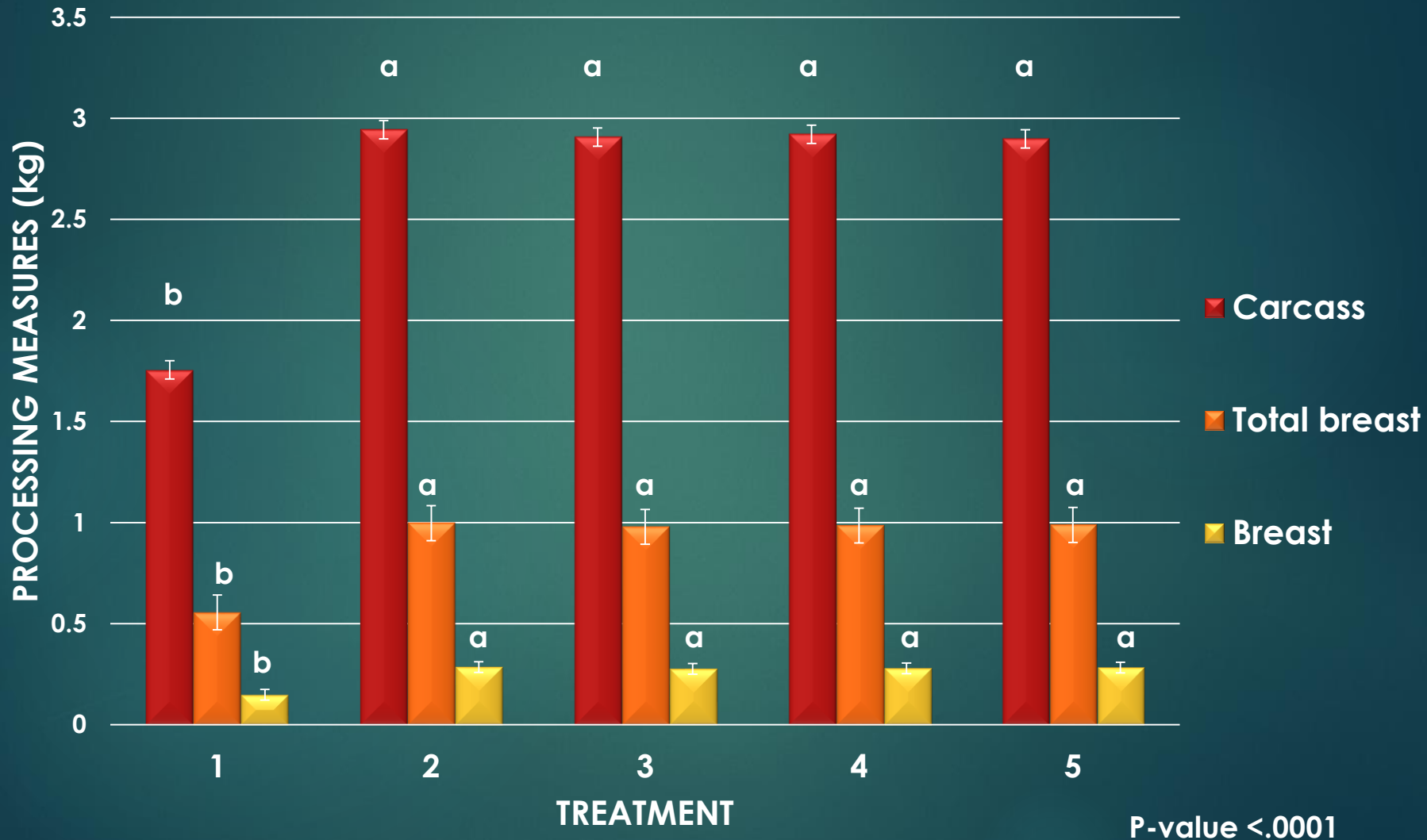
P-value <.0001

Results: Tibia weight (mg)

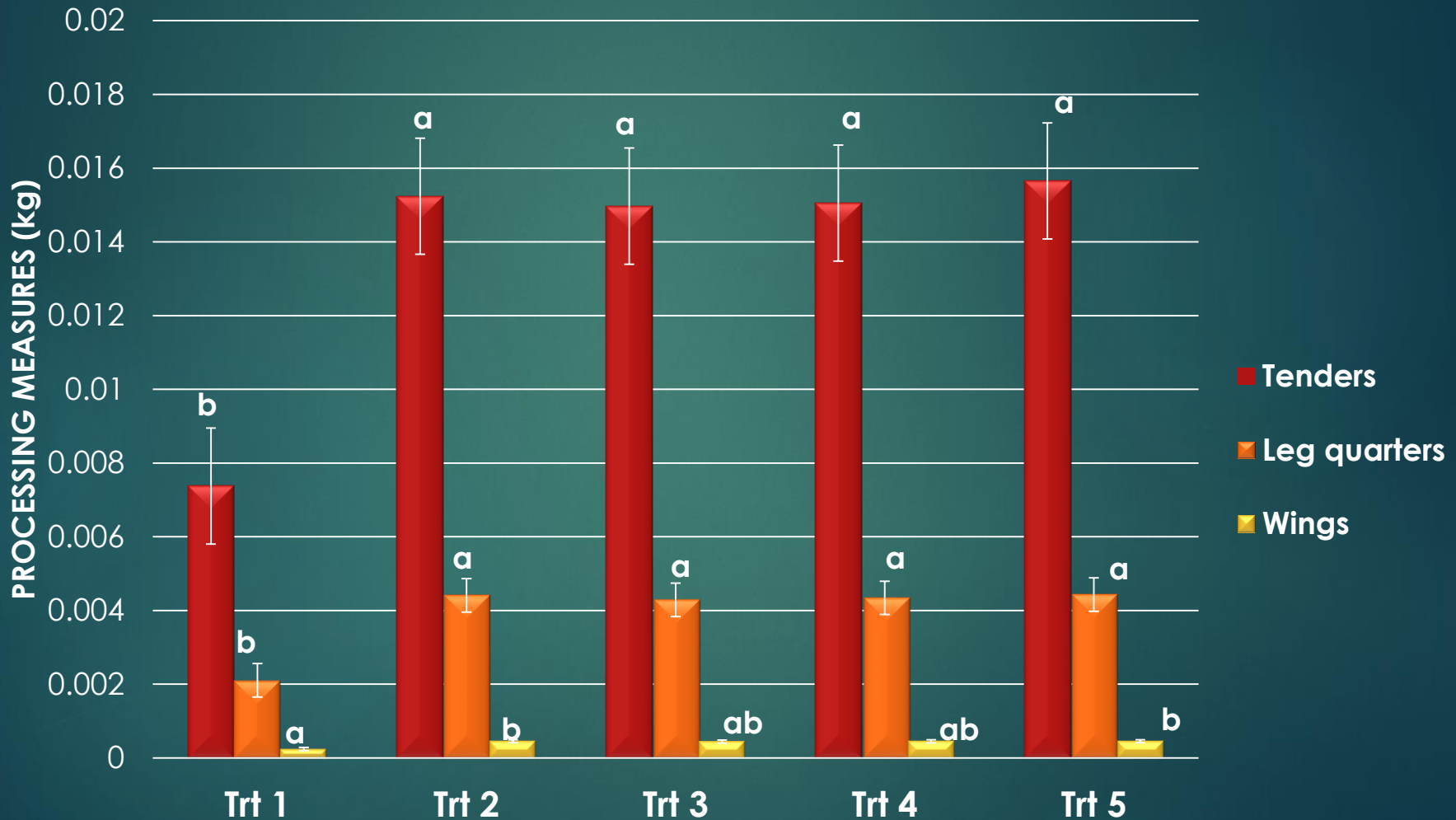


P-value <.0001

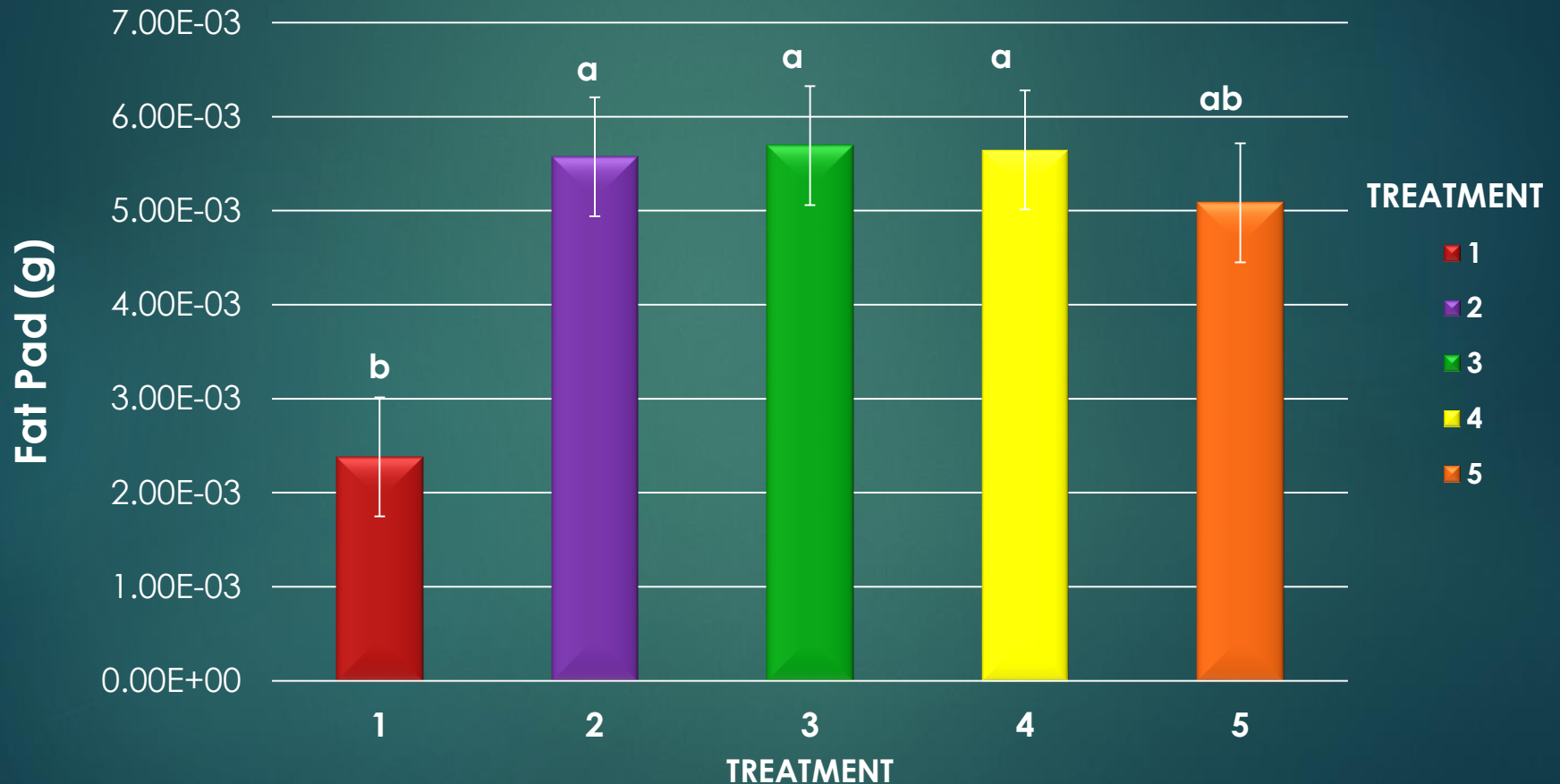
Results: Processing measures (kg)



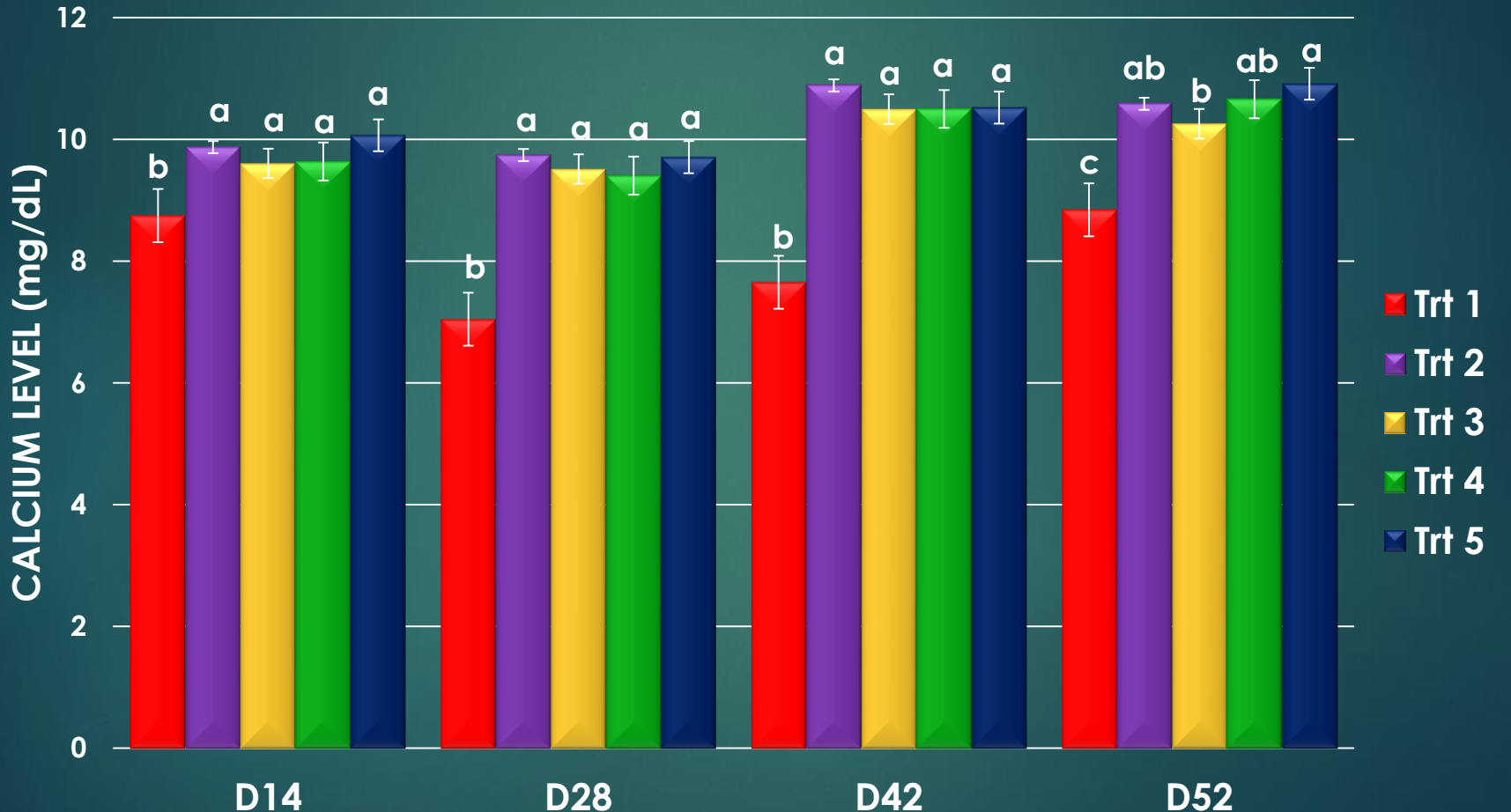
Results: Processing measures (kg)



Results: Processing measures (g)

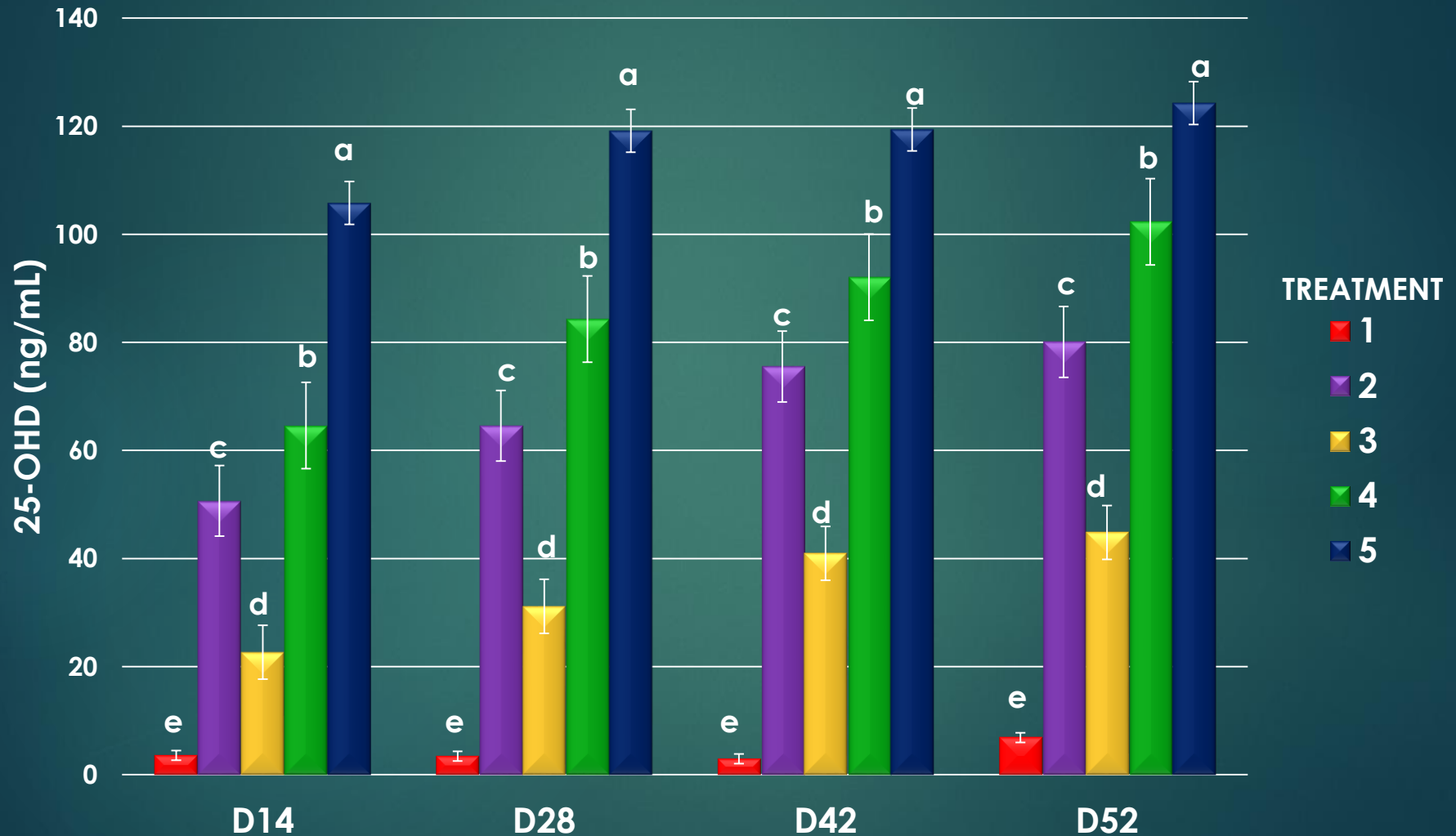


Results: Blood Serum Calcium



P-value <.0001

Results: Blood Serum 25-OH Vitamin D



P-value <.0001

Discussion

- ▶ Adding supplemental 25-OH D3 increased bone density
- ▶ No significant difference on growth performance
- ▶ Increased Phosphorus serum levels

Source: Hui et al 2013

Discussion

- ▶ Gurel et al (2013):
 - ▶ 2 different doses of 25-OH D3 commercial source without adding Vit D3 did not contribute on bone development
 - ▶ However, the supplementation of 25-OH D3 on the feeds which contain 2500-5000 IU/D3 effect the bone development and the strength

Take Home Message

- ▶ Birds fed Low inclusion of Vitamin D (165 IU/kg) exhibited the lowest performance results
- ▶ The remaining birds consuming different diets than birds fed low inclusion of Vitamin D displayed improved results
- ▶ Serum levels displayed an increase dependent on the inclusion level of 25-OH D3 source when used in conjunction with Vitamin D



Thank You