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Growth and Development of Two Broiler Strains with Low Protein and Crystalline Amino Acid Supplemented Diets

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GROWTH AND DEVELOPMENT OF TWO BROILER STRAINS WITH LOW PROTEIN
AND CRYSTALLINE AMINO ACID SUPPLEMENTED DIETS

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science in Animal Science

in

The School of Animal Sciences

by

Chaoyang Li

B.S., Hunan Agricultural University in Changsha, Hunan, China, 2014
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TABLE OF CONTENTS

ACKNOWLEDGEMENTS	ii
LIST OF TABLES	v
LIST OF FIGURES	vi
ABSTRACT.....	ix
1. INTRODUCTION	1
2. LITERATURE REVIEW	3
2.1 Low Protein Diets for Livestock.....	3
2.2 Low Protein Diets for Poultry	6
3. METHODS AND MATERIALS	14
3.1 Data Collection	17
3.2 Statistical Analysis	19
4. RESULTS.....	20
4.1 The Absolute Growth Performance	20
4.2 The Relative Growth Performance	50
5. DISCUSSION	82
6. CONCLUSIONS	95
REFERENCES	96
VITA	105

LIST OF TABLES

Table 1. Composition of Broilers Starter, Grower, and Finisher Diets with Different Nutrients Density	16
Table 2. The Effect of Diets (positive control, low protein and low protein+ CAA) and Breed (Cobb broilers and Ross broilers) on the Growth of Organ, Muscle and Bones ²	21
Table 3. The Effect of Diets (positive control, low protein and low protein+CAA) and Time (7 weeks) on the Growth ¹ of Organ, Muscle and Bone ¹	24
Table 4. The Effect of Breeds (Ross broilers, Cobb broilers) and Time (7 weeks) on the Growth of Organ, Muscle and Bone ¹	25
Table 5. Effect of Diet (Control, Low Protein and Low Protein + CAA) and Breeds (Ross broilers, Cobb broilers) on the Growth of Organ, Muscle and Bone over seven 7 weeks ¹	31
Table 6. The Effect of Diets (positive control, low protein and low protein+ CAA2) and Breed (Ross broilers and Cobb broilers) on the Relative Growth ¹ of Organ, Muscle and Bone ²	52
Table 7. The Effect of Diets (positive control, low protein and low protein+ CAA2) and Time on the Relative Growth ¹ of Organ, Muscle and Bone ²	52
Table 8. The Effect of Breed (Ross broilers and Cobb broilers) and Time on the Relative Growth ¹ of Organ, Muscle and Bone ²	53
Table 9. The Effect of Diets (positive control, low protein and low protein+ CAA2), Breed (Ross broilers and Cobb broilers) and Time on the Relative Growth ¹ of Organ, Muscle and Bone ²	54

LIST OF FIGURES

Figure 1. Effect of Diet and Breed on Body Weight.....	21
Figure 2. The Effect of Breeds on Body Weight over Weeks	22
Figure 3. The Effect of Different Diets On Body Weight over Weeks	22
Figure 4. The Effect of Diets, Breeds, and Time on Body Weight	23
Figure 5. Effect of Diet and Breed on Heart Weight.....	26
Figure 6. The Effect of Diet and Time on Heart Weight.....	27
Figure 7. The Effect of Breed and Time On Heart Weight	27
Figure 8. Effect of Diet, Breed and Time on Heart Weight	28
Figure 9. Effect of Diet and Breed on Liver Weight.....	29
Figure 10. Effect of Breed and Time on Liver Weight	29
Figure 11. The Effect of Diets and Time on Liver Weight.....	30
Figure 12. Effect of Diet, Breed and Time on Liver Weight.....	30
Figure 13. Effect of Diet and Breed On Pectoralis Weight.....	35
Figure 14. The Effect of Breed and Time on Pectoralis Weight	36
Figure 15. Effect of Diet and Time on Pectoralis Weight.....	36
Figure 16. Effect of Diet, Breed and Time On Pectoralis	37
Figure 17. Effect of Diet and Breed on Peroneus Weight.....	38
Figure 18. Effect of Breed and Time on Peroneus Weight.....	38
Figure 19. Effect of Diet and Time on Peroneus Weight	39
Figure 20. Effect of Diet, Breed, and Time on Peroneus Weight.....	39
Figure 21. Effect of Diet and Breed On Iliotibialis Weight	40
Figure 22. Effect of Breed and Time on Iliotibialis Weight.....	41

Figure 23. Effect of Diet and Time on Iliotibialis Weight	41
Figure 24. Effect of Diet, Breed and Time on Iliotibialis Weight.....	42
Figure 25. Effect of Diet and Breed on Femur Weight	43
Figure 26. Effect of Breed and Time on Femur Weight.....	44
Figure 27. Effect of Diet and Time on Femur Weight	44
Figure 28. Effect of Diet, Breed, and Time on Femur Weight.....	45
Figure 29. Effect of Diet and Breed on Tibia Weight	46
Figure 30. Effect of Breed and Week on Tibia Weight	46
Figure 31. Effect of Diet and Time on Tibia Weight.....	47
Figure 32. Effect of Diet, Breed, and Time on Tibia Weight	47
Figure 33. Effect of Diet and Breed on Radius Weight	48
Figure 34. Effect of Breed and Time on Radius Weight.....	49
Figure 35. Effect of Diet and Time on Radius Weight.....	49
Figure 36. Effect of Diet, Breed, and Time on Radius Weight	50
Figure 37. Effect of Diet and Breed on Heart to Body Weight Ratio	57
Figure 38. Effect of Diet and Time on Heart to Body Weight Ratio	58
Figure 39. Effect of Breed and Time on Heart to Body Weight Ratio.....	58
Figure 40. Effect of Diet, Breed and Time on Heart to Body Weight Ratio	59
Figure 41. Effect of Diet and Breed on Liver to Body Weight Ratio	60
Figure 42. Effect of Diet and Time on Liver to Body Weight Ratio.....	61
Figure 43. Effect of Breed and Time on Liver to Body Weight Ratio	61
Figure 44. Effect of Diet, Breed and Time on Liver to Body Weight Ratio	62
Figure 45. Effect of Diet and Breed on Pectoralis to Body Weight Ratio	63

Figure 46. Effect of Diet and Time on Pectoralis to Body Weight Ratio.....	64
Figure 47. Effect of Breed and Time on Pectoralis to Body Weight Ratio.....	64
Figure 48. Effect of Diet, Breed and Time on Pectoralis to Body Weight Ratio.....	65
Figure 49. Effect of Diet and Breed on Peroneus to Body Weight Ratio.....	66
Figure 50. Effect of Diet and Time on Peroneus to Body Weight Ratio.....	67
Figure 51. Effect of Diet Breed and Time on Peroneus to Body Weight Ratio.....	67
Figure 52. Effect of Diet, Breed and Time on Peroneus to Body Weight Ratio.....	68
Figure 53. Effect of Diet and Breed on Iliotibialis to Body Weight Ratio.....	69
Figure 54. Effect of Diet and Time on Iliotibialis to Body Weight Ratio.....	70
Figure 55. Effect of Breed and Time on Iliotibialis to Body Weight Ratio.....	70
Figure 56. Effect of Diet, Breed and Time on Iliotibialis to Body Weight Ratio.....	71
Figure 57. Effect of Diet and Breed on Femur to Body Weight Ratio.....	73
Figure 58. Effect of Diet and Time on Femur to Body Weight Ratio.....	73
Figure 59. Effect of Breed and Time on Femur to Body Weight Ratio.....	74
Figure 60. Effect of Diet, Breed and Time on Femur to Body Weight Ratio.....	74
Figure 61. Effect of Diet and Breed on Tibia to Body Weight Ratio.....	76
Figure 62. Effect of Diet and Time on Tibia to Body Weight Ratio.....	77
Figure 63. Effect of Breed and Time on Tibia to Body Weight Ratio.....	77
Figure 64. Effect of Diet, Breed and Time on Tibia to Body Weight Ratio.....	78
Figure 65. Effect of Breed and Diet on Radius to Body Weight Ratio.....	79
Figure 66. Effect of Diet and Time on Radius to Body Weight Ratio.....	80
Figure 67. Effect of Breed and Time on Radius to Body Weight Ratio.....	80
Figure 68. Effect of Diet, Breed, and Time on Radius to Body Weight Ratio.....	81

ABSTRACT

The objective of this research was to compare the growth performance of broilers from two commercial breeds with control, low protein and low protein supplemented with crystalline amino acids diets. This was a randomized block design, and identical experiments were conducted on successively in two years. In each experiment, day-old chicks, Ross 708 broilers and Cobb 405 broilers, were randomly assigned into three dietary treatments: 1) positive control, 2) low crude protein (LP), and 3) LP + crystalline amino acids (CAA). A three phase feeding program was used. Feed and water were provided *ad-libitum*. On d 12, 19, 26, 33, 40, 47, and 54, two birds per pen were randomly selected, weighed, and euthanized by carbon dioxide asphyxiation for further dissection. Three muscles (*M. peroneus longus*, *M. iliotibialis*, and *M. pectoralis thoracica*), and three bones (tibia, femur, and radius), and organs were collected. Abdominal fat was only collected at the end of the experiment in the first year.

The results showed that dietary protein restriction by 6% units had a retarding influence on the growth and development of visceral organs, muscle tissues and bone mass. The supporting effect of CAA helped compensate the negative effect of low protein diet on the body weight, organs, muscles and bones growth, but only during the early growing stages. Cobb broilers had a significantly heavier body weight with both low protein and low protein with CAA diets. However, Ross broilers produced significant heavier pectoralis, and had more pectoralis yield than Cobb broilers by feeding the control and low protein with CAA diets. The relative growth of pectoralis in both breeds was significantly inhibited by feeding low protein diet, and the decrease of pectoralis proportion even showed a week earlier, compared to the absolute pectoralis growth. The CAA supplementation enabled both breeds to produce of close pectoralis proportion compared to those on control diet, and this supportive effect of CAA on Ross broiler lasted a

week longer than on Cobb broilers.

Key words: low crude protein, crystalline amino acids, broilers, organ weights, body composition, pectoralis, absolute growth, relative growth.

1. INTRODUCTION

The total value produced by broilers in 2015 was about 29 billion dollars (USDA, 2016). The development of the broiler industry resulted from the transformation of locally fragmented businesses into vertically integrated companies (MacDonald, 2009), which heavily accelerated the production and enhanced the growth efficiency. Expanded broiler production provides affordable and healthful meat and other useful products as fertilizer or additives. Chickens are believed to be the most efficient livestock to convert corn-soy diets into meat and poultry nutritionists found that high energy and grain-based diets will improve the feeding conversion efficiency. Accordingly, the current boiler breeds are more and more quickly reaching market weight than the past in order to meet the ever-increasing market demand (Kleyn and Chrystal, 2008). On the other side, the concentrated feeding of large numbers of poultry leads to environmental issues including air, water and soil pollution, which have received close attention by the public. Environmental pollution results from the poisonous or deleterious materials and will affect human beings, animal production and other organisms (Williams, 1995). Moreover, poultry production inevitably results in waste necessary for disposal. Chicken or turkey farms bring about more harmful materials such as antibiotics, heavy metals, cysts, and larvae than hog or dairy operations, even though these produce more manure than poultry operations (Allison, 1998).

Reducing nitrogen (N) excretion by manipulating animal diets is feasible and feeding animals with low protein diets is one strategy effective in controlling N waste and ammonia (NH₃) emission (Ferguson, 1998). Chickens fed low protein diets would excrete significantly less N than those fed high-protein diets (Bregendahl et al., 2002). The reduction of dietary protein will lead to higher profitability in poultry production if efficiencies remain the same or similar

because feed is the greatest cost of production. However, lowering the content of dietary protein in feeds will impair the chicken growth (Bregendahl et al., 2002). Low crude protein (CP) diets will also lead to decreased meat yields and increased fat deposition (Pinchasov et al., 1990; Bartov, 1996; Ferguson et al., 1998; Aletor et al., 2000). The impairment of growth performance and carcass composition of chickens fed low protein led to examination of whether low protein diets supplemented with essential amino acids (EAA) would restore the normal growth and development (Summers, 1985; Nakajima 1985). Feeding broilers with 14% CP supplemented with methionine and lysine resulted in no differences in feed efficiency from broilers fed 18.1% CP (Bornstein & Lipstein, 1975). Optimal body weight and broiler gain was obtained by feeding 16% CP fortified with methionine and lysine, but adding EAA did not increase feeding efficiency to the level obtained by feeding 20% CP (Uzu, 1983). Fancher (1989) stated that optimal body weight and feed efficiency is inconsistent with low CP diets supplemented with EAA.

Influences of low CP on relative development of muscles, bones and organs in broilers have not been identified while information is available on turkey growth and development (Summers et al., 1989; Sell et al., 1994). The objective of this experiment was to explore growth performance and body development of broilers from two genetic lines fed low CP diets fortified with crystalline amino acids.

2. LITERATURE REVIEW

2.1 Low Protein Diets for Livestock

Low protein diets have been widely studied among livestock including dairy and swine, with the purpose of decreasing input of nitrogen, reducing nitrogen excretion from manure and enhancing nitrogen utilization, which may also assist in reproduction efficiency. Dairy producers can gain more profit by balancing the risk of losing milk production and reducing feed costs. Reduction in CP content can be harmful to cattle if the requirements cannot be met for a long term. Metabolizable protein is what a dairy cow requires and is composed of protein synthesized by rumen microorganisms and feed protein passed through the rumen, but absorbed in the small intestine (NRC, 2001). The supplementation of rumen-protected amino acid was proven to be a successful strategy for the maintenance of milk production (Davidson et al., 2008; Benefield et al., 2009). Histidine has been considered as a limiting amino acid in dairy cattle diets and the addition of histidine to 13% CP diets improved the Dry Matter Intake (DMI), which further triggered the increase in milk yield (Lee et al., 2012). The question is how low can the CP level be without significant decreases in production. Aschemann et al. (2012) reported that dietary CP level can be reduced as low as 12% without significant adverse influence on milk yield, but depressed digestibility of nutrients and synthesis of microbial protein. Olmos Colmenero and Broderick (2006) fed dairy cattle with low CP (13.3%) and reported a relatively reduced DMI and milk production, the discrepancy could be from the productivity variations among the dairies that were studied. The former study was conducted with relatively lower productive dairy cows with lower feed intake which means that these dairy cows were restricted with feed intake and the effect of protein level on feed efficiency may be less significant. Studies with high-productivity dairy cows on low CP demonstrated an adverse effect on DMI accompanied with a

decline in milk production, but milk production was not affected when DMI was maintained (Lee et al., 2012; Giallongo et al., 2014).

Protein is also an extremely important nutrient in swine production. Studies have shown that feeding swine with sufficient protein during the whole growing period would lead to superior growth performance, including feed efficiency and lean output, compared to the lower CP diet. The reduction of excess amino acids in swine production can also result in decreased feed cost and relieve environmental concerns without compromising growth performance. Ideal protein serves as a standard tool to lowering protein level in swine production. Ideal protein means all the non-essential amino acids required for animal growth can be obtained when the requirement for one amino acid is established (Lenis et al., 1993). An increase in N efficiency has been demonstrated in feeding swine low-protein diets supplemented with crystalline EAA on the basis of the ideal protein concept (Kerr and Easter, 1995). On the contrary, feeding crystalline amino acids was reported to lower protein deposition in body (muscle) compared to the amino acid coming from intact protein. Moreover, crystalline amino acids appeared much more slowly in the portal blood, which slowed down their utilization for protein synthesis more than intact peptides (Rerat et al., 1992), and may result from the competition of different amino acids for transport into the blood stream. Feeding pigs three times per day or *ad libitum* is likely to reverse this situation. Low protein diets also showed a negative effect on the FE during the finishing phase, but had no significant influence in the growing phase (Tuitoeck et al., 1997), which suggested the requirement for protein or amino acid fluctuated with the increase in body weight (Hahn and Baker, 1995). The suppression of low CP on FE could be alleviated when ideal amino acids ratio was obtained by providing non-EAA source because certain non-EAAs are required for favorable N utilization. A better FE was achieved when adding glutamate, glycine, and

proline to low CP diet instead of only using glutamate (Chung and Baker, 1992).

Another main reason for reduction in protein level is to decrease the malodor caused by swine production. Odor has been determined as a problem related to animal production, which is highly associated with N excretion that is due to CP digestion. The primary volatile compounds causing the unfavorable odor are derived from excess protein degradation and certain carbohydrate fermentation in the manure. The incomplete anaerobic degradation of protein and carbohydrate results in the production and accumulation of volatile compounds including alcohol, phenols, carbonyls and organic acids. The manipulation of feed has been proven to change the composition of swine manure, especially in reducing N content. Lowering the CP level by 8.4% with addition of amino acids helped reduce N deposition in manure ranging from 3.2 to 62%, with the variation due to pig size (Kerr and Easter, 1995). Aarnink et al. (1993) also reported that dietary CP reduced by 10g/kg caused 9% reduction in ammonia. Reducing CP in growing and finishing diets by 3% and fortifying with synthetic amino acids like lysine, methionine and threonine reduced the emission of ammonia by 28% (Sutton et al., 1996). The ammonia and total excretion of N can be reduced as much as 43% and 56%, respectively, which requires an almost 10% reduction in dietary CP supplemented with amino acid (Sutton et al., 1996). Moreover, supplementation with amino acids in low CP diets showed a decreasing trend in pH reduction, which helped curtail the emission of NH_3 .

The dietary manipulation is also favorable to meat quality in pork. The loss of blood during slaughtering switches aerobic into anaerobic metabolism and excessive amounts of glycogen rapidly decrease pH below 5.5 by accumulation of lactic acid. Coupled with high body temperature, muscle proteins denature and pale, soft, exudative (PSE) meat could form. Manipulating glycogen content in muscle pre-slaughter plays a key role in forming high water

holding capacity and fresh pork color. Research on dietary adjustment has demonstrated that glycogen concentration could be reduced by increasing fat, protein and lower-digestible carbohydrates (Bee et al., 2006). Stressors are widely acknowledged to influence glycogen reserves and pig responses to stress and aggression can be modified by supplying amino acid tryptophan to increase serotonin (Guzik et al., 2006). Intramuscular fat (IMF) content or marbling is another factor affecting meat palatability and consumers' perceptions, with 2.5 to 3 % IMF needed to ensure palatability (DeVol et al., 1988). Much effort has been to increase IMF so reducing the CP and lysine in growing and finishing diet is an effective strategy. IMF content was reported to elevate by 13.7 to 17.6% with decreased FE. Chronic exposure to low CP results in lower growth performance, but no negative impact was detected when feeding a reduced CP diet during the last 5 or 6 weeks of finishing phase (Cisneros et al., 1996).

2.2 Low Protein Diets for Poultry

Dietary protein is extremely important for poultry regarding various functions, especially muscle deposition, because of its biologically active role in the body. Satisfying the protein requirement is more complicated than meeting other nutrient requirements. High protein content is always required by poultry for rapid growth and is essential for feed efficiency (FE) and other performance characteristics. Protein supply in the early stage of chicken is important for the proliferation and development of muscle cells and digestive tracts (Lemme, 2003). Thus, dietary protein plays a key role in regulating the early age growth and development and some carry-over effects on the overall growing period would also be highly associated with the early growth (Firman and Boling, 1998). However, excessive protein intake would be detrimental to poultry themselves because the inevitable liberation of ammonia through protein metabolism is toxic to cells. Excess protein excretion mainly results from less efficient digestion and metabolism. Extra

amino acids could be regulated by being converted into uric acid in poultry. Manure, composed of feces and urine, contains undigested nutrients including protein. Excessive nitrogen from animals will deposit in manure or urine and atmospheric ammonia is one of the pathways of nitrogen loss from concentration of animal feeding operations (Ndegwa et al, 2008). Nitrogen is one of the major pollutants to which much concern has been given. Nitrogen will contribute to pollution of air as atmospheric ammonia and of water as nitrate in groundwater. Atmospheric ammonia creates a series of problems like malodor, a hazard to human health and vegetation. Chronic exposure to high concentrations of fine particulate aerosols is the reason for some respiratory diseases. Nitrates also exert health hazards on humans by polluting groundwater and leading to the eutrophication of waterways. Another major concern related to the excessive excretion of nitrogen is climate change and global warming, which is highly correlated with the increased atmosphere content of nitrous oxide. Therefore, these critical environmental issues derived from animal production are more likely to lead the livestock and poultry industry into a situation with public opposition. Corresponding problems could arise and further obstruct future development. Numerous approaches have been conducted to alleviate the risk of nitrogen pollution and the manipulation of dietary nutrition to increase feed efficiency (FE) is one of current methods. The reduction in excretion of nitrogen is achieved by lowering the CP content and supplementing with synthetic essential animal acids to increase the usage of nitrogen. It was reported that every 1% decrease in CP would reduce N excretion by 7% (Ferguson et al., 1998). The reduction of CP is attributed to lowering soybean meal content and can help decrease the cost of feed, which is the most expensive part of poultry production. Replacing the CP with synthetic amino acid demonstrated a reduction of 10 or 27% N content in broiler manure (Blair et al., 1999). It is of essential importance to keep poultry production more sustainable and less

controversial. Sustainable agriculture is also built upon economical viability and making concessions to restore the environment by reducing profitability is not wise. If the growth performance can be maintained equal to feeding normal protein diets without compromising carcass composition and meat quality, there will be more incentives for industry to use low protein feed for profitability.

Protein sources can be generally divided into two groups, plant and animal products. The obtaining of essential amino acids (EAA) from the protein source is the major way to evaluate its usefulness as a feedstuff for poultry. Non-carnivorous animal's dietary proteins are mainly gained from plants, and soybean is the widely used protein source. Soybean has a good balance of amino acid ratios, but a deficiency in certain EAA and limiting amino acids, which may require supplementation with animal protein (Akhter et al., 2008). Animal proteins tend to be more expensive and contain anti-nutritional factors, which limit their use in animal production. The protein requirement would also be highly associated with environmental concerns and growth performance can be impaired if the requirement wasn't met under certain environment.

Temperature plays a critical role in chicken growth. Heat is one of the stressors in poultry production and affects both protein synthesis and breakdown, with the former being more influenced. An increase in dietary protein content would not reverse the trend and restore protein synthesis. Lowering the protein level was reported to increase the heat production during the early growing stage (Buyse et al., 1992) and increase the requirement for metabolic energy (Nieto et al., 1997). This would lead to poorer performance such as lower FE and higher fat deposition since chickens would eat more to meet their protein requirement (Buyse et al., 1992). The relatively lower feed efficiency would lead to a decrease in profitability. It is urgent to improve the feed efficiency as the cost of feed continues to increase together with environmental

concerns. The advent of synthetic amino acid facilitated the formulation of ideal amino acid profile and resulted in the increase in lean output (Sleman et al., 2015). The supplementation of crystalline amino acids can help alleviate the susceptibility to heat stress (Baker et al., 1998). Therefore, more interest is placed on incorporating certain levels of CP with synthetic amino acids supplying the required amino acids.

Feeding only lowered protein diets could inhibit the growth performance by lowering FE and body weight gain (BWG), although other nutrient requirements are satisfied (Waldroup, 2000). The synthesis of Non Essential Amino Acid (NEAA) in poultry can be maintained with a specific amount of EAA and enough nitrogen instead of the whole CP (NRC, 1994). It seems to be other factors accompanied with reduction of CP level that mainly affect performance. Further, it has been documented that the major factors affecting performance rely on the ratio of macronutrients (Nieto et al., 1997; Collin et al., 2003). It could be speculated that the deleterious effect of reducing CP may include the decrease in nitrogen pool for NEAA synthesis, which might be one of the factors causing the failure in performance. The lack of capacity for NEAA synthesis has also been reported to be a possible problem and the fortification of low protein diets with L-glutamic acid or the addition of EAA including arginine (Arg), threonine (Thr), isoleucine (Ile), and tryptophan (Trp) had a positive effect on FE, but didn't impact BWG (Fancher, 1989a; 1989b). Moreover, the potassium concentration and dietary electrolyte balance (DEB) were assumed to be another factor.

The DEB is of great importance to maintain the acid-base equilibrium. Dietary cation anion difference (DCAD) is used to indicate DEB. The adjustment in dietary nutrients could easily lead to the change in dietary anions or cations levels and the reduction in soybean meal which enriches in potassium may result in a negative DCAD or acidic conditions. Further, the addition

of amino acids tends to increase the acidosis and interfere the acid-base equilibrium. Murakami et al. (2003) adjusted the DEB on low protein diets by increasing Na and K concentration and reported a linear effect on the FE and body weight. But some opposite consequences over the DEB still exist. Si et al. (2004) reported no significant influence of fortifying DEB to a standard level to relieve the negative effect of low protein diets. The inability to fully restore the growth performance through dietary manipulation of these factors led to the proposal that different ratios of various amino acids like tryptophan to other large neutral amino acid and cysteine to methionine might be a factor (Waldroup, 2000). A low CP diet (20%) supplemented with EAA and glutamine completely sustained equal growth performance of both fast or slow growing broiler species compared with 23% protein diet (Han et al., 1992). CP level can also be reduced as low as 16% and the growth performance including FE can still be substantiated with the addition of EAA and glycine (Dean et al., 2006). Glycine is considered as the semi-essential amino acid for chicken, which restricts the synthesis of other amino acids and not only serves as a building block for protein itself, but is also needed for the formation of DNA, RNA and substrates for creatine (Ngo et al., 1997). Additional glycine in corn-soybean meal diet contributes to the improvement in chicken growth (Ngo et al., 1997; Dean et al., 2006). Opposite results were reported by Waldroup et al. (2005). Even though the body weight increased with the addition of 0.2 or 0.4% glycine, it was not fully restored and there was no significant effect on FE (Waldroup et al. 2005). Some other studies reported that crystalline amino acid had an inhibition effect on feed intake (Carew et al., 1997; Si et al., 2004) and Peng and Harper (1970) also found a negative correlation between the high level of amino acids plus their metabolites in plasma and appetite-controlling mechanism. It is still likely that there is more capacity of chicken fed with lower protein to show equal or similar performance as chicken fed with normal

diets if there is a well-balanced combination of several amino acids. To minimize the undesirable effects of low protein diets, the growth and developing trend of broiler with low protein content and supplementation with crystalline EAA should be taken into consideration.

Together with FE and body weight gain (BWG), body composition is another parameter that must be considered regarding growth performance. Costumers' demand for the whole chicken has shifted to specific cuts like chicken breast and thigh. Increased demand also has led the poultry industry to increase muscle output. Another side effect of low protein diets on poultry is the decrease in carcass value by undesirable increases in fat deposition (Si et al., 2001). It is well-documented that the muscle mass is highly affected by the intrinsic factors insulin-like growth factors (IGF-1 and IGF-2), and IGF is the key regulator of muscle development, regeneration and hypertrophy. The transcription of IGF-2 in skeletal myogenesis can be regulated by nutritional status, especially since the activation of IGF transcription requires sufficient amino acids (Erbay et al., 2003). IGF-1 is produced in liver and some local tissues and its expression is associated with the abundance of peptide in skeletal muscle (Adams and Haddad, 1985).

Lowering the CP content may necessitate the use of alternate feedstuffs to meet other nutritional needs. A decrease in soybean meal in the feed leads to the increased amount of corn to satisfy the requirement for certain amount of metabolic energy (ME), which inevitably increases the ratio of ME to protein. It is more efficient for dietary energy to be converted into fat than for protein disposition (Blaxter, 1989). Therefore, the increase in ME to CP ratio may cause the promotion of fat retention by either increasing the lipogenesis or poor growth performance. Research had been conducted to identify if the change in ME to CP content would reverse these trends.

Kamran et al. (2008) reported that low protein levels fortified with EAA and constant ME to protein ratios adversely influenced the body composition and performance of broilers. Feed

intake increased with reduction of ME and protein level, which could be driven by the nature of the birds and they would consume enough to be satiated (Leeson et al., 1993). However, increases in feed intake didn't lead to high FE. The carcass yield and abnormal fat were not significantly affected by reducing ME and protein level if adequate amount of EAA were met, including Lys and Met, both of which are regarded exclusively for protein accretion (Baker et al., 2002). This study is similar to the feed restriction which had been initially acknowledged for its role in reducing fat deposition and improving the FE at the expense of body weight loss (Fisher, 1984). Research on feed restriction by Plavnik and Hurwitz (1985) showed that there was a compensatory growth after a short severe feed restriction. Catch-up growth is characterized as an increased growing rate compared to the normal rate at the same age in the same breed. Catch-up or compensatory growth may also result in the excessive growth of certain parts of the body in compensation of the loss of other parts. One side-effect of adding crystalline AA to low protein diets is the increase in fat deposition, especially in the abdominal cavity (Namroud et al., 2008). Therefore, results only including FE and body weight may not sufficiently substantiate the positive effect of supplementation of EAA in restoring the growth performance. The 'catch-up weight' might be the result of the increase in fat deposition. It is of interest if the negative effect of feeding lowering protein level can be alleviated or even reversed by re-feeding or supplementation of crystalline amino acid. The timing, severity of protein level, and the duration of restricted protein level could be significant factors on the capacity of an animal recovering from a protein deficit (Yu & Robinson, 1992). However, it is still unclear how low protein diets and the supplementation of crystalline amino acid will influence growth patterns and different breeds may also possess various responses to protein level or crystalline EAA level.

Growth is defined as the increase in size with the accretion of tissues and is generally

accomplished by hypertrophy and hyperplasia (Gerrard and Grant, 2003). In general, tissues start growing at a slow rate with the order of organ, bone and muscle. After organs reach nearly maximum size and the completion of bone deposition, muscle reaches its maximal growing rate. Fat develops last after 90% of final muscle deposition. Thus, by measuring the body weight, organ size, bone and muscle weight, the growth pattern affected by low protein and the addition of crystalline can be monitored.

This experiment aimed at identifying the influence of low protein diets and low protein diets fortified with crystalline EAA on the growth and development of broiler chicken by monitoring the weight of organs, bones, and muscle. In addition, the responses of two different broiler breeds to the dietary treatments were compared.

3. METHODS AND MATERIALS

This experiment was conducted with broilers of two genotypes, Ross×Ross broilers 708 and Cobb×Cobb broilers 405, to evaluate the effect of low protein diet fortified with crystalline AA for broiler growth performance and changes in specific body components with time. One-day-old male and female broilers were obtained from a local poultry company (Raeford Farm) and transported to the LSU Ag Center Central Research Station Poultry Farm. Chicks were weighed and then randomly assigned by breed to labeled pen floors (2.85 x 7.1m) with 30 birds per pen to guarantee 0.7 m² per chicken at the beginning and 0.13 m² per chicken at the ending of study. The experimental design was the same as Ross two years except each treatment combination (2x3) was replicated with 10 pens per treatment combination (60 pens total) in the first year, and replicated with 5 pens per treatment combination (30 pens total) in the second year. Each pen had wood shavings and was equipped with a hanging feeder and six nipple drinkers. Feed in mash form and water were provided *ad libitum* throughout the whole experiment. Light was maintained constantly at 24h light, 20h light, 18h light and 20h light for 0-3 days, 3 to 11 days, 11 to 28 days, and 28 days to end, respectively.

The mortality was monitored throughout the whole experiment. Daily room temperature ranged from 24.22°C to 30.9°C with an average of 27.2°C and the floor temperature ranged from 25.8°C to 36.4°C with average of 29.7°C. The daily humidity was recorded, ranging from 0% to 40% RH, with the average range of 22% to 29%.

This was a two-year experiment and structured as random block design, which was blocked by year. In each year, experiment was a 2 by 3 by 7 factorial design including 2 breeds, 3 diets and 7 weeks. All the physical dissection samples were taken every week starting with second week, for 7 weeks in total. The whole experiment period each year was split into three

phases, starter (0-14 d), grower (14-25 d) and finisher phase (25-56 d). Feeds for each specific phase were formulated according to the recommendation from Broiler Nutrition Specification (BNS; 2007) for Ross 708 broilers (Table 1). Restrictions were placed on certain nutrients to formulate the low protein diets including the amino acids, but calcium and metabolism energy (ME) were still maintained to meet the basic requirement for Ross 708 broilers. For all three dietary treatments, soybean meal and corn were used as base ingredients. For the positive control diets, all the nutrients were at a minimum 100% of BNS recommendation, which generally contained 25%, 22%, and 20% CP for the three phases, respectively. The low protein diet (low lysine) was reduced by 6% units of CP of the original basis (Dean et al., 2006). The third diet was the low CP diet supplemented with crystalline amino acids and level of lysine was used to adjust to ideal amino acid ratios (Baker, 1997). In addition to the lysine level being maintained at 1.43%, 1.24% and 1.09% for three different phases, methionine, threonine, arginine, valine, isoleucine, glycine, histidine, tryptophan and phenylalanine were also added. The calcium levels in low protein diets were lower than the requirement so monocalcium phosphate was added so that calcium was maintained at 1.09%, 0.9% and 0.85%, during the three phases, respectively. The three modified diets contained 3025 kcal, 3150 kcal, and 3200 kcal metabolic energy in the different periods, respectively (Table 1).

Table 1. Composition of Broilers Starter, Grower, and Finisher Diets with Different Nutrients Density

	Starter Phase			Grower Phase			Finisher phase		
Composition%	Control	Low CP	Low CP+CA A	Control	Low CP+CA A	Low CP+CA A	Control	Low CP	Low CP+CA A
Corn	45.77	63.61	64.13	52.59	70.48	71.62	58.75	76.68	78.68
Soybean Meal	45.68	30.27	27.62	38.49	23.01	19.66	32.75	17.21	13.09
Poultry Fat	4.02	1.73	1.03	4.84	2.53	1.76	4.68	2.35	1.43
Limestone	1.34	1.4	1.42	1.11	1.17	1.19	1.09	1.15	1.16
Mono-calcium	1.68	1.79	1.81	1.5	1.61	1.64	1.4	1.51	1.54
Salt	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
LSU mineral mix ¹	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
NutraBlend vitamins ²	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.31		0.45	0.27		0.4	0.23		0.38
Biolys			0.94			0.98			1.02
L-Threonine			0.27			0.27			0.34
L-Arginine HCl			0.38			0.43			0.48
L-Valine			0.27			0.27			0.27
L-Isoleucine			0.21			0.23			0.25
Glycine			0.21			0.22			0.04
L-Histidine			0.05			0.06			0.07
L-Tryptophan			0.01			0.03			0.05
L-Phenylalanine	0.05	0.05	0.05			0.04			0.1
Choline chloride	0.05	0.05	0.05	0.05	0.05	0.05	0	0	0
Ethoxyquin	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05

Table Cont'd

	Starter Phase			Grower Phase			Finisher phase		
BMD	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
	100	100	100	0.05	0.05	0.05	0	0	0
				100	100	100	100	100	100
ME (kcal/kg)	3025	3025	3025	3150	3150	3150	3200	3200	3200
CP	25.19	19.19	20.36	22.33	16.33	17.34	20.1	14.1	14.8
Calcium	1.05	1.05	1.05	0.9	0.9	0.9	0.85	0.85	0.85
Phosphate	0.8	0.76	0.75	0.73	0.69	0.68	0.68	0.65	0.64
Available P	0.5	0.5	0.5	0.45	0.45	0.45	0.42	0.42	0.42
Lysine	1.43	1.03	1.43	1.24	0.84	1.24	1.09	0.69	1.09
Methionine	0.68	0.3	0.73	0.6	0.26	0.63	0.53	0.23	0.59
Met+Cys	1.07	0.61	1.03	0.95	0.61	0.89	0.86	0.48	0.82
Threonine	0.97	0.74	0.96	0.86	0.54	0.83	0.77	0.53	0.8
Tryptophan	0.32	0.23	0.23	0.28	0.19	0.2	0.24	0.16	0.19
Valine	1.17	0.89	1.1	1.04	0.76	0.95	0.93	0.65	0.84
Phe+Tyr	2.17	1.65	1.55	1.92	1.4	1.3	1.72	1.2	1.14
Leucine	2.05	1.67	1.59	1.86	1.48	1.38	1.71	1.33	1.21
Isoleucine	1.08	0.8	0.96	0.95	0.67	0.83	0.84	0.56	0.73
Glycine	1.05	0.8	0.96	0.93	0.68	0.83	0.83	0.58	0.55
Glycine+Serine	2.3	1.76	1.85	2.04	1.49	1.57	1.83	1.28	1.15
Arginine	1.73	1.27	1.5	1.51	1.05	1.3	1.34	0.88	1.14
Histidine	0.67	0.52	0.53	0.59	0.44	0.46	0.54	0.38	0.4

¹Ingredients per kg of diets: Cu (copper sulfate), 15 mg; I (calcium iodate), 1.25 mg; Fe (ferrous sulfate H₂O), 50 mg; manganese (manganese sulfate), 100 mg; Se (sodium selenite), 0.30 mg; Zn (zinc sulfate), 100 mg.

²Ingredients per kg of diets: vitamin A, 8,002.78 IU; vitamin D₃, 3003. 8 IU; vitamin E, 25 IU; vitamin K, 1.5 mg; vitamin B12, 0.02 mg; biotin, 0.1 mg; folic acid, 1 mg; niacin, 50 mg; panthothenic acid, 15 mg; pyridoxine, 4 mg; riboflavin, 10 mg; thiamin, 3 mg.

3.1 Data Collection

The trials lasted 56 days in total and data were collected starting with the second week. Two birds were selected from each pen with body weight close to the pen average every week, avoiding birds with visual signs of abnormalities or excessive or deficient growth. The two birds selected from each pen were euthanized by suffocating in a sealed container with carbon dioxide gas, identified by pen number with tag around the foot at the farm, and then transported to the

Louisiana State University Agricultural Center Meat Laboratory for further dissection.

Laboratory personnel and students were trained to weigh each bird before dissecting and in the desired removal and trimming of the heart, liver, three muscles (*M. peroneus longus*, *M. iliotibialis*, and *M. pectoralis thoracica*), and three bones (tibia, femur, and radius). Muscles and bones were identified using diagrams (Jacob and Pescatore, 2013a, b) and pictures of a broiler in sequential stages of muscle and bone removal. The first dissected bird from each pen was identified as bird one and the second bird from each pen as the second duplicate animal. Birds were generally placed on a cutting board, breast upward, to allow cutting of the skin parallel to the clavicle (breast bone) and down the thigh and leg using scalpels on the starter and grower chicks and meat boning knives on broilers in the finisher phase. The *M. peroneus longus*, *M. iliotibialis*, and *M. pectoralis thoracica* were removed, trimmed of excess connective tissue and fat, and identified by pen number before weighing. The chest cavity was opened by separating the clavicle and ribs from the back vertebrae to remove the heart and liver. The gall bladder was trimmed from the liver. Bones (femur, tibia, and radius) were removed by cutting through cartilage connections and any remaining loose connective tissue and muscle residue were removed before weighing. The abdominal fat pad was removed and weighed on the birds dissected on d 56 only in the first year experiment. The dissections were performed on alternating weeks by student workers or by undergraduate students in the Growth and Development class, School of Animal Sciences, Louisiana State University. All dissection procedures were closely supervised by laboratory managers, graduate students, and class instructors. Students used the data to prepare laboratory reports on the growth and relative part development of broiler chickens.

The growth performance of broilers on different diets were evaluated by the absolute and

relative growth. The relative growth of each muscle, bone and organ was calculated by dividing the specific body part weight by the body weight and times 100%.

3.2 Statistical Analysis

All the data were analyzed by three-way ANOVA using the R program and using the model for random block design as $y = \text{Diet} * \text{Breed} * \text{Week} + \text{Year}$ (y is responsive value). Each pen was considered as an experimental unit. Tukey's HSD was chosen as post hoc test method to compare the means. Significance was detected when type 1 error was less than 0.05. Interaction effects were dropped whenever there was not significance. All the results were presented as least square means by using LSMEAN package. Line graphs and bar charts were constructed in R program by using the GGPlot2 package.

4. RESULTS

4.1 The Absolute Growth Performance

Table 2 shows the effect of diets with different protein level and breeds on the average development of organ, muscle and bone weights over weeks and years. The average body weight over the weeks significantly decreased with the lower CP level. The interaction between breed and diet had significant effect on the body weight which mainly resulted from higher resistance of Cobb broilers to lower CP diets. When feeding normal diets, Ross broilers were not heavier than Cobb broilers. Adding CAA in low protein diet increased the body weight of both strains more than low protein diets without CAA ($P < 0.05$), but not to the weights of birds fed control diet. The supplementation of CAA seemed to restore more body weight of Cobb broilers and be less effective on Ross broilers, which could result from the significance in the interaction breed and week (Figure 1). The two strains didn't show significant differences in weight until the last week when Cobb broilers demonstrated a faster growing rate than Ross broilers, resulting in a heavier final body weight (Figure 2, Figure 4).

The difference exerted by diet on body weight began to show at 2nd week and protein scarcity inhibited body weight gain. CAA addition was only effective until the 3rd week (Figure 3, Table 2). The interaction of diet, breed and time significantly affected the body weight, and significant differences did not present in the first three weeks, and then both strains fed the control diet grew more than the broilers on the two other diets (Figure 1, Table 5). Cobb broilers fed the low protein diet tended to grow faster compared to Ross broilers after the third week, and almost reached the same growth level as Ross birds fed low proteins diet with CAA. The administration of CAA to Ross broilers had a positive impact on body weight during the starter and grower phase. The effect of CAA on Cobb broilers was consistently positive, and both low

protein diets had less effect on Cobb broilers when compared to Ross broilers.

Table 2. The Effect of Diets (positive control, low protein and low protein+ CAA) and Breed (Cobb broilers and Ross broilers) on the Growth of Organ, Muscle and Bones²

	Positive Control		Low Protein		Low Protein+ CAA		SEM
Weight/g	Ross Broilers	Cobb Broilers	Ross Broilers	Cobb Broilers	Ross Broilers	Cobb Broilers	
Body Weight	1879.45d	1838.79d	1223.32a	1345.61b	1329.02b	1432.07c	27.583
Heart	9.01a	9.29b	7.02a	7.25a	7.05a	7.47a	0.167
Liver	50.17d	48.99d	37.76a	42.77c	38.21ab	41.24bc	0.671
Pectorallis	151.9d	138.90c	63.69a	65.16a	89.17b	90.61b	2.43
Peronaeus	30.40b	32.11b	20.41a	21.48a	23.28a	23.84a	0.605
Iliotibialis	41.37c	41.78c	26.15a	27.75ab	28.71ab	30.83b	0.767
Tibia	16.165d	15.84d	11.91a	12.54ab	13.13bc	13.99c	0.258
Femur	11.66c	11.34c	9.66b	9.83c	8.84a	9.25ab	0.179
Radius	1.52b	1.54b	1.26a	1.25a	1.19a	1.16a	0.027
Abdominal fat	49.49ab	53.90ab	67.06b	53.9ab	37.65a	48.62ab	5.06

¹The results were presented in weight as least square mean and averaged over the levels of weeks and years. Confidence level used: 0.95.

²Least Square mean in the same row with different letters are different ($P < 0.05$)

³The total experimental period lasted 8 weeks and all the samples were initially taken from 2nd week but was labeled as Week 1

⁴ Abdominal fat was only collected at the final week

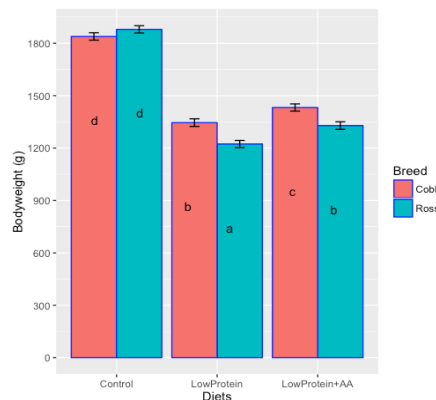


Figure 1. Effect of Diet and Breed on Body Weight

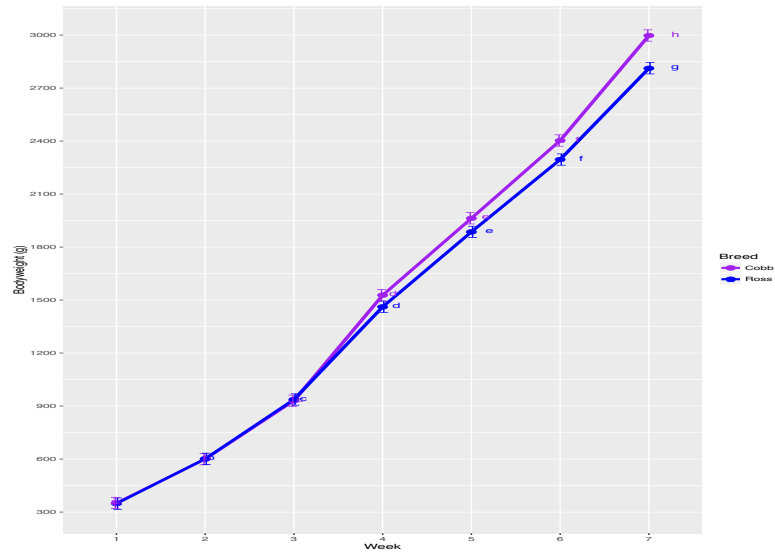


Figure 2. The Effect of Breeds on Body Weight over Weeks

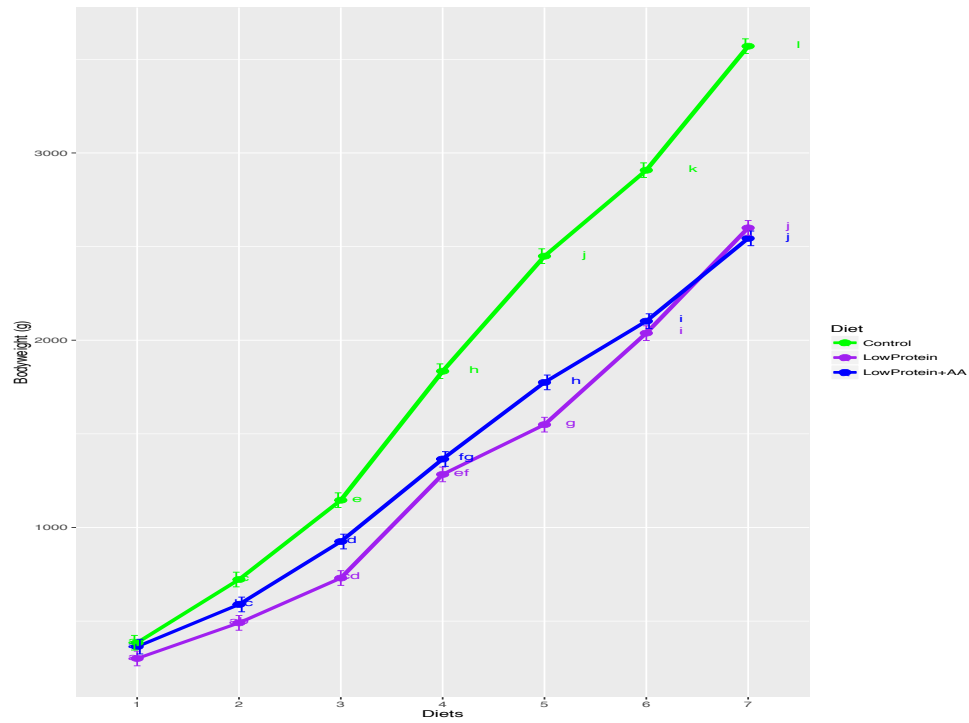


Figure 3. The Effect of Different Diets On Body Weight over Weeks

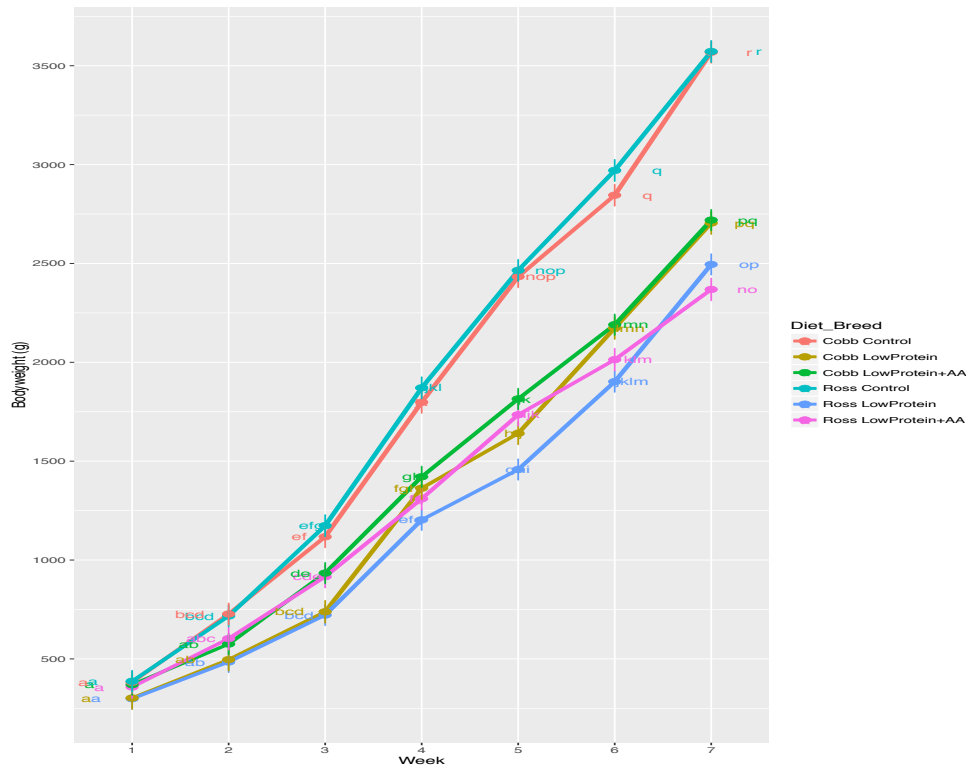


Figure 4. The Effect of Diets, Breeds, and Time on Body Weight

The overall heart weight was not affected by breed or diet with the exception of Cobb broilers; these broilers had heavier heart weights when fed with control diet (Table 2, Figure 5) when the time effect is ignored. Regardless of breed, feeding low protein diet with CAA didn't increase heart weight. The impact of protein level on heart weight was not significant until the 4th week when low protein diets decreased heart weight compared to birds on control diet (Figure 6, Table 3). Then variation among the three groups disappeared until the final week, and the control diets resulted in the heaviest heart weights. There was no significant effect of breed ($P > 0.05$) on heart weight gain over the first six weeks, but Cobb broilers gained more heart weight in the final week than the Ross broilers ($P < 0.05$, Figure 7, Table 4). The combined effects of diet, breed, and time were not obvious until the 6th week, and only the Cobb broilers fed the two low protein diets displayed a decline in the heart weights when compared to the other treatments (Figure 8, Table 5).

Table 3. The Effect of Diets (positive control, low protein and low protein+CAA) and Time (7 weeks) on the Growth1 of Organ, Muscle and Bone¹

	Week 1			Week 2		
Weight/g	Positive Control	Low protein	Low Protein + CAA	Positive Control	Low protein	Low Protein + CAA
Body weight	384.57a	300.48a	363.8a	722.09c	490.49ab	588.93bc
Heart	2.32ab	2.09a	2.39ab	3.69abcd	3.08abc	3.21abc
Liver	14.69ab	13.17a	14.36ab	26.62cd	21.65bc	23.90cd
Pectoralles	21.59ab	13.13a	20.05a	41.68bc	21.57ab	33.78ab
Peroneaus	5.24a	3.878a	4.73a	10.78abc	5.75ab	8.14ab
Iliotibialis	6.1ab	4.95a	6.38ab	13.59abc	7.63ab	11.14ab
Tibia	3.58a	2.78a	3.52a	6.44bc	4.32ab	5.45ab
Femur	2.80ab	2.26a	2.69ab	4.81cde	3.43abc	3.99bcd
Radius	0.66ab	0.51a	0.68abc	0.69abc	0.49a	0.58a
	Week 3			Week 4		
Weight/g	Positive Control	Low protein	Low Protein + CAA	Positive Control	Low protein	Low Protein + CAA
Body weight	1145.74e	730.01cd	924.96d	1834.44h	1283.37ef	1365.57fg
Heart	6.10de	4.88bcde	5.15cde	10.18ghi	7.54efg	7.07ef
Liver	35.29ef	25.66cd	29.37de	50.39hi	44.50gh	41.62fg
Pectoralies	72.25de	34.47ab	60.66cd	132.31hi	61.75cd	84.05ef
Peroneaus	10.77abc	10.32abc	14.10bcd	31.70ghi	20.75def	23.18efg
Iliotibialis	22.15cd	12.71abc	15.84bc	41.26fg	27.80de	27.75de
Tibia	9.91d	6.87bc	8.36cd	16.53fg	12.63e	13.08e
Femur	7.70fg	5.32de	6.42ef	11.83h	8.85g	9.23g
Radius	1.14cde	0.78abc	0.89abcd	1.49efg	1.14cde	1.12bcde
	Week 5			Week 6		
Weight/g	Positive Control	Low protein	Low Protein + CAA	Positive Control	Low protein	Low Protein + CAA
Body weight	2448.92j	1549.15g	1774.76h	2907.87k	2037.94i	2101.84i
Heart	11.37hijk	9.02fgh	9.52fgh	12.61ijk	10.41hijk	10.34hij
Liver	63.05jk	45.65gh	48.96gh	72.43l	61.71j	57.18ij
Pectoralies	186.25j	74.81de	111.55gh	239.47k	102.89fg	137.69i
Peroneaus	43.11jkl	27.54fgh	30.78ghi	51.25lm	35.63hij	37.856ijk
Iliotibialis	56.77h	35.24ef	39.46fg	68.48i	41.92fg	45.92f

Table Cont'd

	Week 5			Week 6		
Weight/g	Positive Control	Low protein	Low Protein + CAA	Positive Control	Low protein	Low Protein + CAA
Tibia	21.09i	14.93ef	18.22gh	25.62k	20.78hi	22.02ij
Femur	15.18i	11.19h	12.80h	17.78j	14.97i	15.12i
Radius	1.93ghi	1.35def	1.68fgh	2.27ij	1.89ghi	1.82fghi
	Week 7			SEM		
Weight/g	Positive Control	Low protein	Low Protein + CAA			
Body weight	3570.19l	2599.83j	2543.93j		39.15	
Heart	17.77l	12.96jk	13.13k		0.54	
Liver	84.61m	69.49kl	62.69jk		1.51	
Pectoralles	324.17l	142.35i	181.44j		4.28	
Peroneaus	59.11m	42.72jkl	46.16kl		4.28	
Iliotibialis	82.62j	58.39h	61.87hi		1.95	
Tibia	28.77l	23.30ijk	24.28jk		0.53	
Femur	20.40k	17.16j	18.14j		0.34	
Radius	2.53j	2.04hi	2.05hi		0.09	

¹The results were presented in weight as least square mean and averaged over the levels of weeks and years. Confidence level used: 0.95. Least Square mean in the same row with different letters are different ($P < 0.05$)

Table 4. The Effect of Breeds (Ross broilers, Cobb broilers) and Time (7 weeks) on the Growth of Organ, Muscle and Bone¹

	Week 1		Week 2		Week 3	
Weight/g	Cobb Broilers	Ross Broilers	Cobb Broilers	Ross Broilers	Cobb Broilers	Ross Broilers
Bodyweight	351.45a	347.79a	599.61b	601.40b	929.98c	937.16c
Heart	2.30a	2.23a	3.32ab	3.33ab	5.28bc	5.47c
Liver	14.22a	13.92a	24.59bc	23.53b	30.49d	29.72cd
Pectoralles	17.72a	18.79a	30.94a	33.75a	52.45b	59.14b
Peroneaus	4.66a	4.57a	8.21ab	8.24ab	13.81b	14.22b
Iliotibialis	6.00a	5.66a	10.45ab	11.11ab	17.38b	16.42b
Tibia	3.27a	3.32ab	5.33bc	5.47c	8.29d	8.47d
Femur	2.60a	2.57a	3.92b	4.23b	6.49c	6.48c
Radius	0.58a	0.65ab	0.57a	0.6ab	0.95bc	0.93abc

Table Cont'd

	Week 4		Week 5		Week 6	
Weight/g	Cobb Broilers	Ross Broilers	Cobb Broilers	Ross Broilers	Cobb Broilers	Ross Broilers
Bodyweight	1527.53d	1461.40d	1962.74e	1885.81e	2403.06f	2295.36f
Heart	8.48de	8.05d	9.74def	10.19ef	11.23f	11.01f
Liver	47.38ef	43.62e	53.35g	51.76fg	65.98hi	61.57h
Pectoralles	89.63c	95.78c	120.57d	127.83d	158.59e	161.44e
Peronaeus	25.99c	24.44c	34.68d	32.94d	43.73ef	39.42de
Iliotibialis	32.23c	32.32c	45.47de	42.18d	53.48f	50.73ef
Tibia	14.49e	13.67e	18.53f	17.64f	22.82g	22.79g
Femur	10.07d	9.87d	12.97e	13.15e	16.09f	15.82f
Radius	1.29cd	1.22cd	1.57de	1.73ef	1.96fg	2.03fg
	Week 7					
Weight/g	Cobb Broilers	Ross Broilers	SEM			
Bodyweight	2997.39h	2811.91g	32.04			
Heart	15.68h	13.56g	0.44			
Liver	74.32j	70.21ij	1.24			
Pectoralles	214.31f	217.66f	3.5			
Peronaeus	49.60f	49.06f	1.43			
Iliotibialis	69.15g	66.11g	1.59			
Tibia	26.16h	24.73gh	0.43			
Femur	18.88g	18.25g	0.28			
Radius	2.29g	2.12g	0.07			

The results were presented in weight as least square mean and averaged over the levels of weeks and years. Confidence level used: 0.95. Least Square mean in the same row with different letters are different ($P < 0.05$).

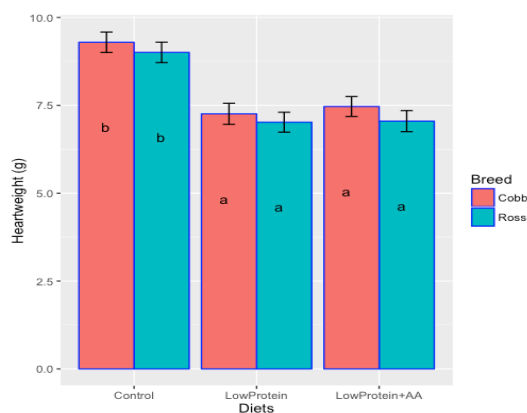


Figure 5. Effect of Diet and Breed on Heart Weight

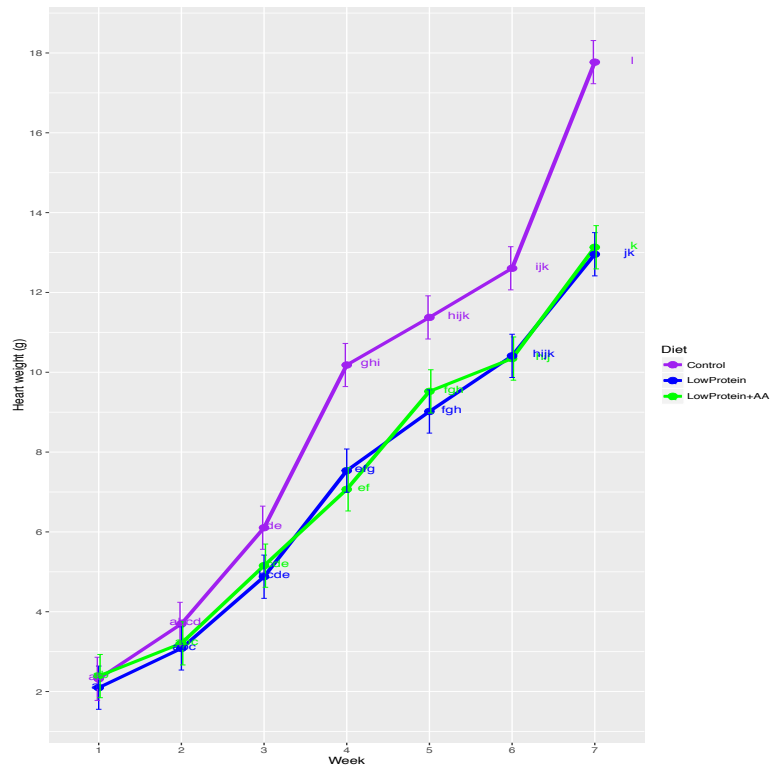


Figure 6. The Effect of Diet and Time on Heart Weight

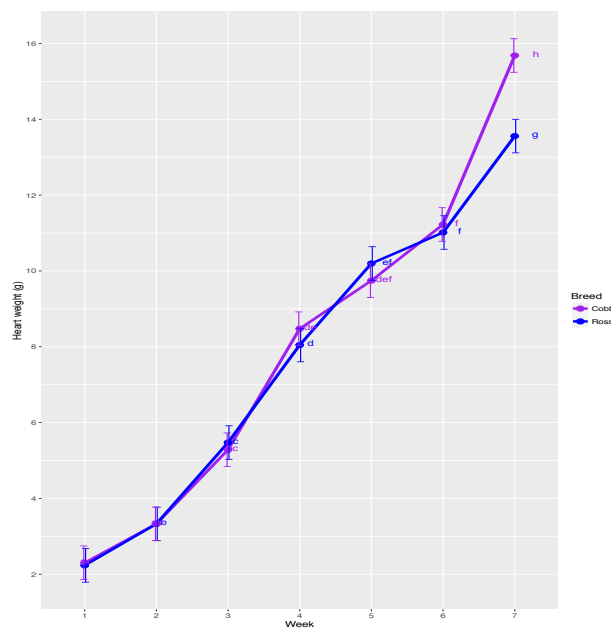


Figure 7. The Effect of Breed and Time On Heart Weight

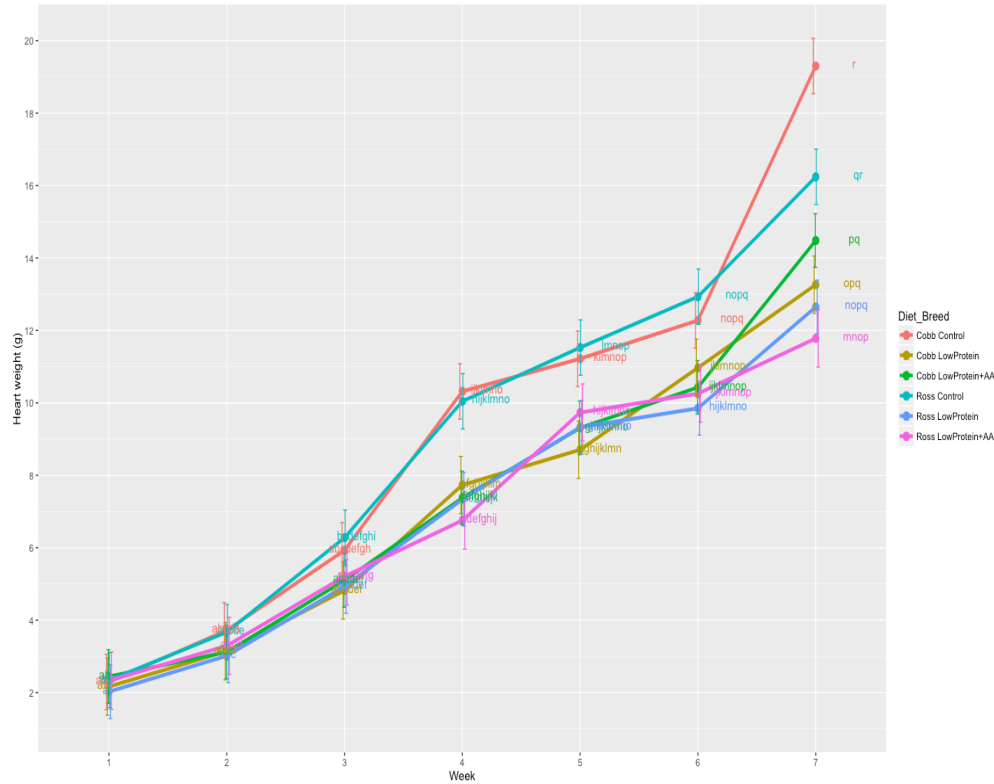


Figure 8. Effect of Diet, Breed and Time on Heart Weight

The liver growth was highly affected by the breed and diet ($P < 0.01$). Cobb broilers had heavier livers than Ross broilers on low CP diets (Table 2). The two breeds fed three diets did not show any difference in liver growth until the final week; however, Cobb broilers fed the control diet gained more liver weight when compared to the birds that were fed low protein and low protein + CAA diets (Figure 9, Table 4). Lowering protein level had a negative influence on liver weight and administration of CAA did not help restore the liver growth. There was no interaction effect between breeds and time on the liver growth over time ($P > 0.05$, Figure 10, Table 4), but the interaction effect of time and diet was significant. In the first 3 weeks, adding CAA maintained the liver weight to that of control diets; the effect of CAA on low protein diets started to decline after that (Figure 11). Birds fed control diets began showing heavier liver weights than those on the other two diets, but there were no differences between the two low protein diets.

Liver weights on the different diets for the two strains demonstrated no differences until the fourth week when Ross broilers fed control diets grew heavier livers than the Cobb broilers on low protein diets supplemented with CAA (Figure 12). There was a significant difference between the control diet and the two low protein diets after the fifth week (Table 5). During the finisher phase, Cobb broilers fed low protein diets gained more liver weight than the birds on other diets or the Ross broilers fed the two diets. The final liver weight of Cobb broilers on the low protein diets was not significant from control group, while Cobb broilers on CAA fortified the low protein diet produced significantly lighter liver weights than birds on control diet ($P < 0.05$).

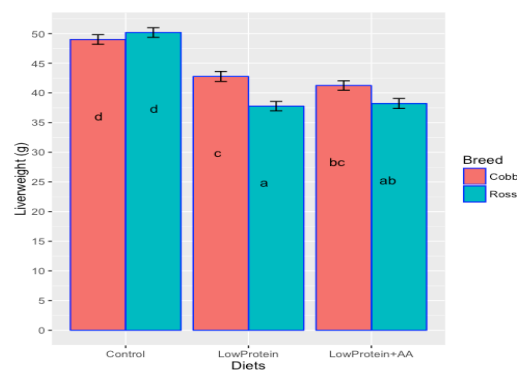


Figure 9. Effect of Diet and Breed on Liver Weight

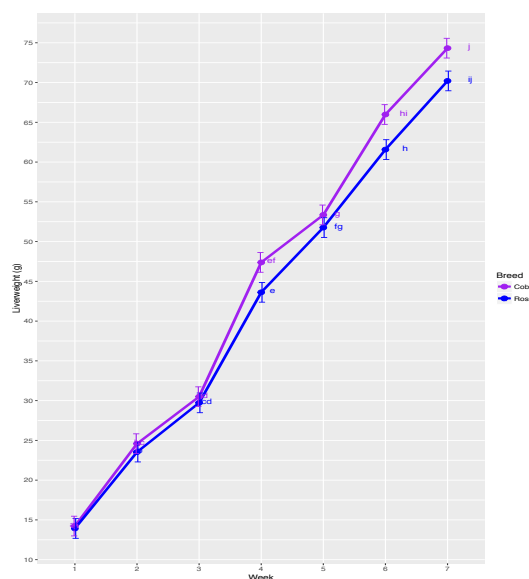


Figure 10. Effect of Breed and Time on Liver Weight

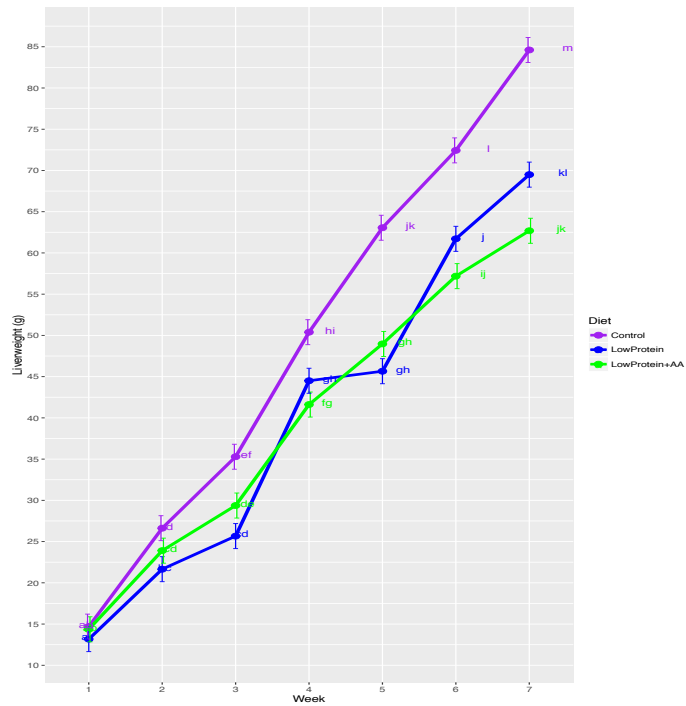


Figure 11. The Effect of Diets and Time on Liver Weight

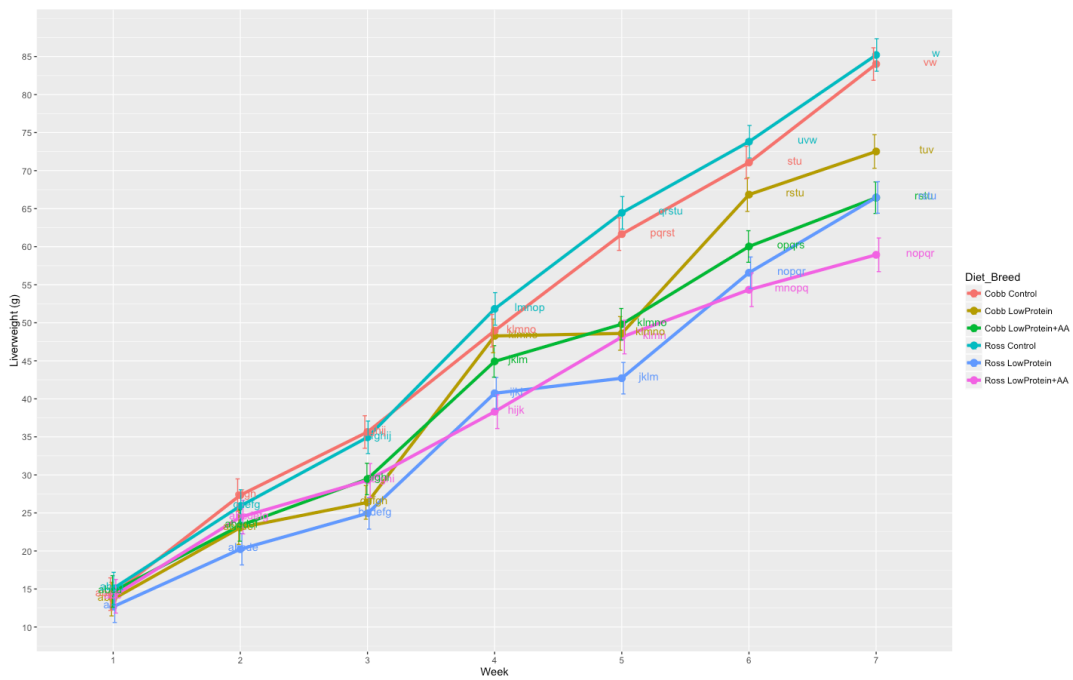


Figure 12. Effect of Diet, Breed and Time on Liver Weight

Table 5. Effect of Diet (Control, Low Protein and Low Protein + CAA) and Breeds (Ross broilers, Cobb broilers) on the Growth of Organ, Muscle and Bone over seven 7 weeks¹

	Week 1						Week 2					
	Low Protein		Low Protein + CAA		Positive Control		Low Protein		Low Protein + CAA		Positive Control	
Weight/ g	Ross Broli ers	Cobb Broli ers	Ross Broliers	Cobb Brolier s	Ross Brolier s	Cobb Brolie rs	Ross Broliers	Cobb Broliers	Ross Broliers	Cobb Broliers	Ross Broliers	Cobb Broliers
Bodywe ight	298.6 9a	302.2 8a	357.79a	369.82 a	386.91 a	382.2 4a	484.51a b	496.48a b	602.69ab c	575.18a b	717.01b cd	727.16b cd
Heart	2.03 a	2.17 ab	2.33 ab	2.45 ab	2.34 ab	2.29 ab	3.02 abc	3.14 abcd	3.29 abcd	3.12 abc	3.67 abcde	3.72 abcde
Liver	12.67 a	13.67 ab	14.05 abc	14.68 abcd	15.06 abcd	14.33 abc	20.23 abcde	23.07 abcdef	24.45 abcdefg	23.35 abcdef	25.91cd efg	27.34efg h
Pectoral les	13.76 a	12.5 a	19.71 a	20.38 a	22.91 a	20.27 a	21.62 a	21.54 a	36.35 abc	31.22 ab	43.29 abcde	40.07 abcd
Peronae us	3.64 a	4.12 ab	4.5 ab	4.96 ab	5.58 ab	4.9 ab	5.71 ab	5.8 ab	8.94 abcde	7.33 abc	10.07 abcdef	11.48 abcdef
Iliotibia lis	4.43 a	5.49 a	6.77 ab	5.98 a	5.79 a	7.59 abc	7.67 ab	7.59 abc	12.38 abcde	9.89 abc	13.29 abcdef	13.89 abcdef
Tibia	2.89 ab	2.67 a	3.43 ab	3.61 ab	3.65 ab	3.53 ab	4.31 abc	4.34 abcd	5.72 abcde	5.2 abcd	6.4 abcdef	6.47 abcdef
Femur	2.2 a	2.33 ab	2.62 abc	2.76 abcd	2.89 abcd	2.72 abcd	3.59 abcd	3.26 abcd	4.21 abcdef	3.78 abcde	4.89 bcdefg	4.73 abcdef
Radius	0.59 abc	0.44 a	0.73 abcdef	0.62 abcd	0.63 abcd	0.7 abcde	0.5 ab	0.5 ab	0.61 abcd	0.54 ab	0.7 abcde	0.69 abcde

Table Cont'd

	Week 3						Week 4					
	Low Protein		Low Protein + CAA		Positive Control		Low Protein		Low Protein + CAA		Positive Control	
Weight/g	Ross Broilers	Cobb Broilers	Ross Broilers	Cobb Broilers	Ross Broilers	Cobb Broilers	Ross Broilers	Cobb Broilers	Ross Broilers	Cobb Broilers	Ross Broilers	Cobb Broilers
Body weight	721.76bcd	738.25bcd	916.01cde	933.91de	1173.72efg	1117.77ef	1203.24efg	1363.50fgh	1309.99fg	1421.16gh	1870.96jkl	1797.93jk
Heart	4.93abcdef	4.82abcdef	5.21abcdefg	5.1abcdef	6.28bcdefghi	5.93bcdefgh	7.34defghijk	7.73efghijklm	6.76cdefghij	7.38defghijkl	10.04hijklmno	10.32ijklmno
Liver	24.94bcdefg	26.38defgh	29.28efghi	29.46efghi	34.94fghij	35.64ghij	40.73ijkl	48.27klmno	38.31hijk	44.92jklm	51.82lmnop	48.96klmno
Pectoral	35.43abc	33.51abc	63.62bcdef	57.7bcdef	78.37fghi	66.13cdefg	60.46bcdef	63.04bcdef	86.35fghi	81.76fghi	140.55klmn	124.07jklm
Peroneus	9.14abcd	11.51abcdef	15.59abcdegh	12.61abcdegh	17.93bcdeghi	17.31bcdeghi	20.26cdefghij	21.25defghijk	22.98efghijk	23.39fghijk	30.07ijklm	33.33jklmn
Iliotibialis	11.62abcd	13.8abcdef	14.93abcdegh	16.77abcdegh	22.71cdefgh	21.59bcdegh	25.77defghi	29.83ghijk	27.78efghij	27.73fghi	43.41klmn	39.12ijklm
Tibia	6.77abcdef	6.98bcdef	8.57defgh	8.17cdefg	10.07fghi	9.74efghi	11.5ghij	13.75ijkl	12.34hijk	13.81ijkl	17.17lmno	15.9klm
Femur	5.33defgh	5.32cdefgh	6.57fghij	6.28efghi	7.54ghijk	7.86hijk	8.49ijk	9.22jkl	8.91ijkl	9.55kl	12.21mn	11.44lm
Radius	0.87abcdefg	0.7abcde	0.9abcdegh	0.9abcdegh	1.02abcdeghi	1.27cdefghijk	1.13abcdeghijk	1.14abcdeghijk	1.06abcdeghijk	1.19bcdeghijk	1.46fghijklmno	1.53ghijklmno

Table Cont'd

	Week 5						Week 6					
	Low Protein		Low Protein + CAA		Positive Control		Low Protein		Low Protein + CAA		Positive Control	
Weight/g	Ross Broilers	Cobb Broilers	Ross Broilers	Cobb Broilers	Ross Broilers	Cobb Broilers	Ross Broilers	Cobb Broilers	Ross Broilers	Cobb Broilers	Ross Broilers	Cobb Broilers
Body weight	1457.70 ghi	1640.60	1734.64ijk	1814.88 jk	2465.07nop	2432.76nop	1902.23jklm	2173.65lmn	2013.42klm	2190.26mn	2970.44q	2845.28q
Heart	9.33ghijklmno	8.71 fghijklmn	9.73 hijklmno	9.31 ghijklmno	11.53lmnop	11.22 klmnop	9.85 hijklmno	10.97 jklmnop	10.26 ijklmnop	10.43 ijklmnop	12.93 nopq	12.28 nopq
Liver	42.71 jklm	48.6 klmno	48.12 klmn	49.81 klmno	64.46 qrst	61.65 pqrst	56.58n opqr	66.85rst u	54.34m nopq	60.03op qrs	73.8u vw	71.07 stu
Pectoral	75.84 efgh	73.77 defgh	112.11 ijklm	110.98ij kl	195.5 4pq	176.96 op	97.93g hij	107.86 hijk	131.6 klm	143.78 mn	254.7 9r	224.1 5qr
Peroneus	25.67ghi jkl	29.41hij klm	32.65 jklmn	28.9hijk lm	40.5 mno	45.72 nop	33.12jk lmn	38.14 lmno	34.22kl mn	41.49 mno	50.94 opq	51.56 opq
Iliotibial	34.31hij kl	36.19 hijkl	39.19 ijklm	39.75 ijklm	53.06 mnop	60.49o pq	40.03 ijklm	43.81 klmn	43.34 jklmn	48.51 lmno	68.83 qr	68.12 pqr
Tibia	14.86 jkl	15 jkl	17 lm	19.45 mnop	21.05 nopqr	21.13 nopqr	20.17 nopq	21.39 opqr	21.38 opqr	22.65pq r	26.83s tu	24.42 rst
Femur	11.1 lm	11.3 lm	13.11 mno	12.49 mn	15.25 opqr	15.11 opqr	14.43 nop	15.8 opqrs	14.66 nopq	15.28 opqr	18.4 stu	17.17 qrst
Radius	1.38 efghijklmn	1.33 defghijklm	1.74 ijklmnop	1.62 hijklmno	2.07 nopq	1.78 jklmnop	1.9 mnop	1.89 lmnop	1.82 klmnop	1.81 klmnop	2.35 pq	2.18 opq

Table Cont'd

	Week 7					
	Low Protein		Low Protein + CAA		Positive Control	
Weight/g	Ross Broilers	Cobb Broilers	Ross Broilers	Cobb Broilers	Ross Broilers	Cobb Broilers
Bodyweight	2495.14op	2704.54pq	2368.56no	2719.29pq	3572.03r	3568.37r
Heart	12.65 nopq	13.26 opq	11.78 mnop	14.48 pq	16.24 qr	19.3 r
Liver	66.47rstu	72.52tuv	58.93 nopqr	66.44rstu	85.21w	84.01vw
Pectoralles	140.81klm	143.89lmno	174.47nop	188.42p	327.66s	320.67s
Peronaeus	45.31 nop	40.13mno	44.11nop	48.2 opq	57.74 pq	60.48q
Iliotibialis	59.23 opq	57.56 nopq	56.56 nopq	67.19 pq	82.54 r	82.71 r
Tibia	22.87pqrs	23.46pqrs	23.46pqrs	25.1rst	27.88 tu	29.66u
Femur	16.77 pqrst	17.55 rst	17.55 rst	18.73 tu	20.44 u	20.36 u
Radius	1.96 mnopq	2.13 opq	1.96 mnopq	2.14 opq	2.42 pq	2.63 q

¹The results were presented in weight as least square mean and averaged over the levels of weeks and years. Confidence level used: 0.95. Least Square mean in the same row with different letters are different ($P < 0.05$)

Muscle growth was highly affected by the protein level in diets. Pectoralis was correlated with diet, and no breed effect were found except when incorporating with diet effect ($P < 0.05$). Lowering protein level significantly decreased pectoralis weight in both strains. Although CAA helped regain some weight ($P < 0.05$), pectoralis from the birds fed that diet was still lighter than those from control groups. There was no difference in pectoralis weight between the two breeds fed with the two low protein diets. When comparing the two strains fed control diet, Ross broilers had significantly heavier pectoralis than Cobb broilers ($P < 0.05$, Figure 13). There was no difference in pectoralis caused by breed over the whole experimental period (Figure 14). When incorporating the time factor into diet and breed model, no variation among each group was found until the third week (Figure 15) when Ross broilers fed the control diet started showing significant differences from the low protein fed diet ($P < 0.05$). Difference between control and low protein showed 50% inhibition on pectoralis growth in Cobb broilers. Significance between control and the two low protein diets was shown since the 4th week, and administration of CAA helped pectoralis grow, but the influence was not detectable ($P > 0.05$, Figure 16). In the final week, almost 57% and 55% decline in pectoralis weight were discovered from Ross broilers and Cobb broilers on low protein diet ($P < 0.05$), respectively. There was about 20% and 23% increase in pectoralis from Ross broilers and Cobb broilers, respectively, with the fortification of CAA in the low protein diet ($P > 0.05$).

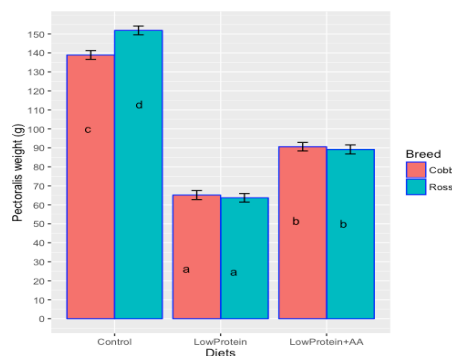


Figure 13. Effect of Diet and Breed On Pectoralis Weight

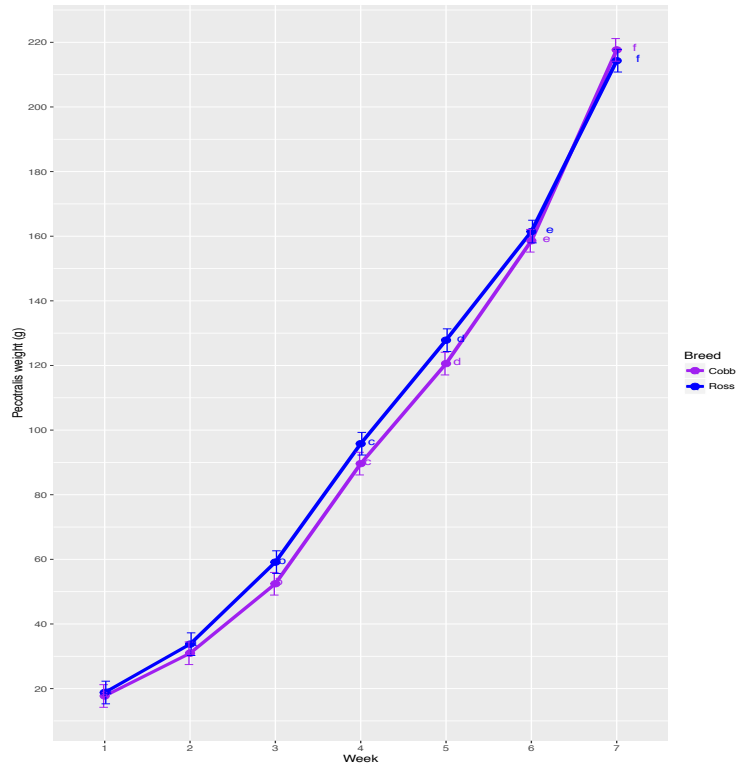


Figure 14. The Effect of Breed and Time on Pectoralis Weight

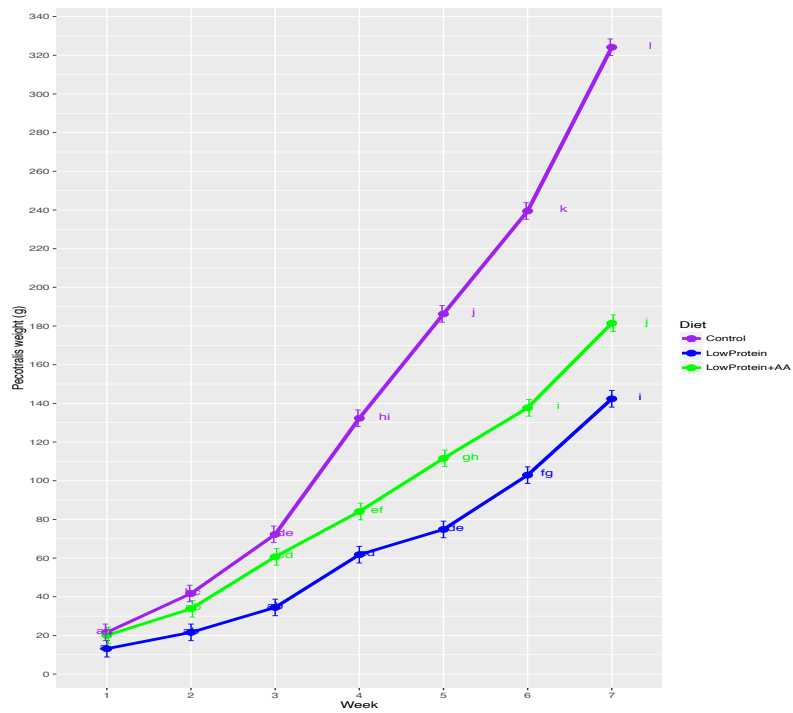


Figure 15. Effect of Diet and Time on Pectoralis Weight

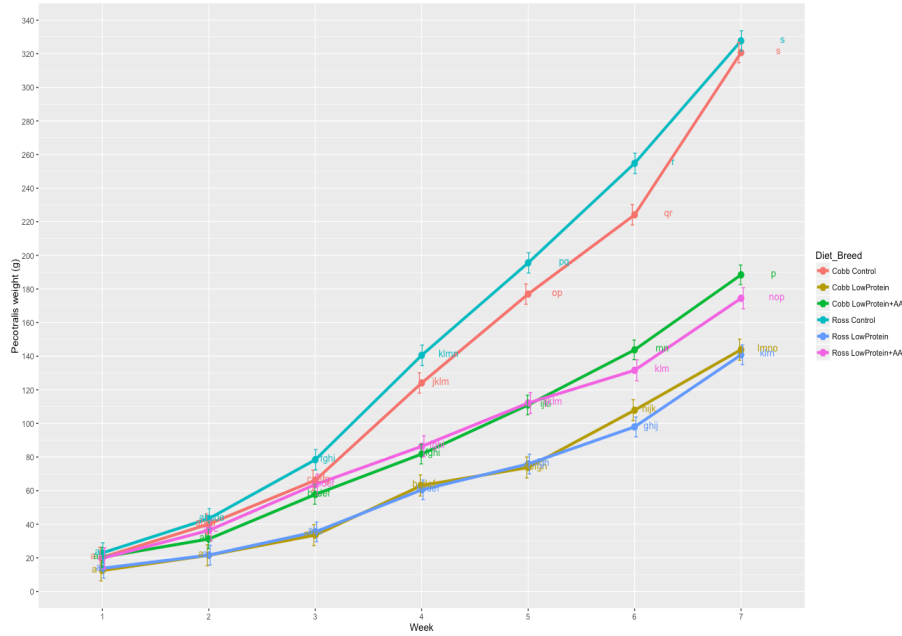


Figure 16. Effect of Diet, Breed and Time On Pectoralis

Peroneus weights, taken from hind leg, were significantly influenced by protein level and time. The breed didn't exert an effect on changing peroneus weight, but the interaction effect of diet and breed on peroneus weight was significant. Peroneus weight decreased when protein level declined ($P < 0.05$), and Cobb broilers showed a marginally heavier, but not significant, peroneus growth than Ross broilers with each of the three diets (Figure 17). No detectable difference was found among the two breeds over 7 weeks. The peroneus from Cobb broilers was inclined to gain more weight between the 4th and 6th week (Figure 18, Table 4). Results of peroneus with diet over 7 weeks were similar to those for the pectoralis, and no differences in weights were generated among the three diets until 4th week. Then peroneus weight from birds fed control diet exceeded that from low protein diets, and CAA was still able to maintain the requirement for peroneus growth. Difference of peroneus weight was significantly shown in 5th week between control and two low protein diet and CAA was no longer able to contribute to peroneus growth at the same rate (Figure 19). Roughly, 23% decrease in peroneus weight resulted from a 6% units decreased protein content in the final

week (Table 3). The joint influence of diet, breed and diet (Table 5, Figure 20) indicated that no compelling differences were detected until 5th week and only Ross broilers fed low protein initiatives showed low peroneus weight compared to both strains fed the control diet. This continued until the last week where significance no longer existed in weights among the three diets for Ross birds. On the contrary, Cobb chickens fed low protein diet declined about 33% in peroneus weight in the final week. The inclusion of CAA in low protein diet effectively maintained peroneus growth in each breed, except for the 6th week for Ross birds.

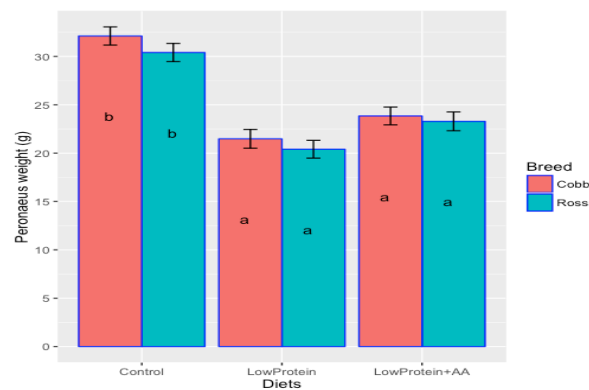


Figure 17. Effect of Diet and Breed on Peroneus Weight

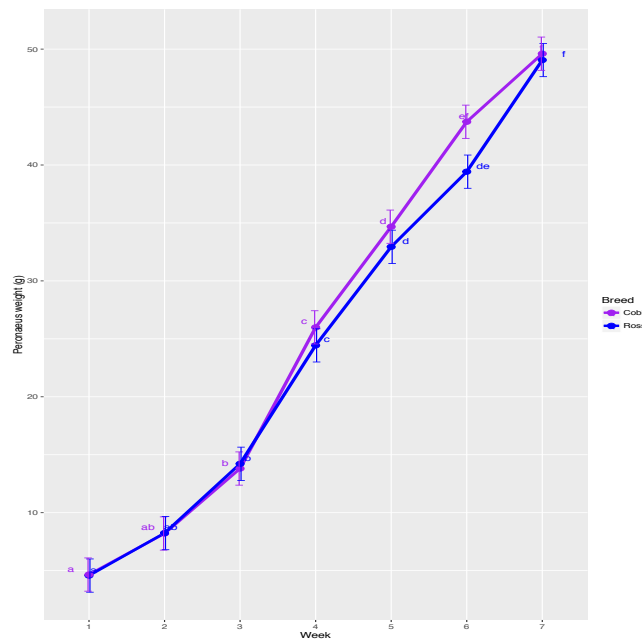


Figure 18. Effect of Breed and Time on Peroneus Weight

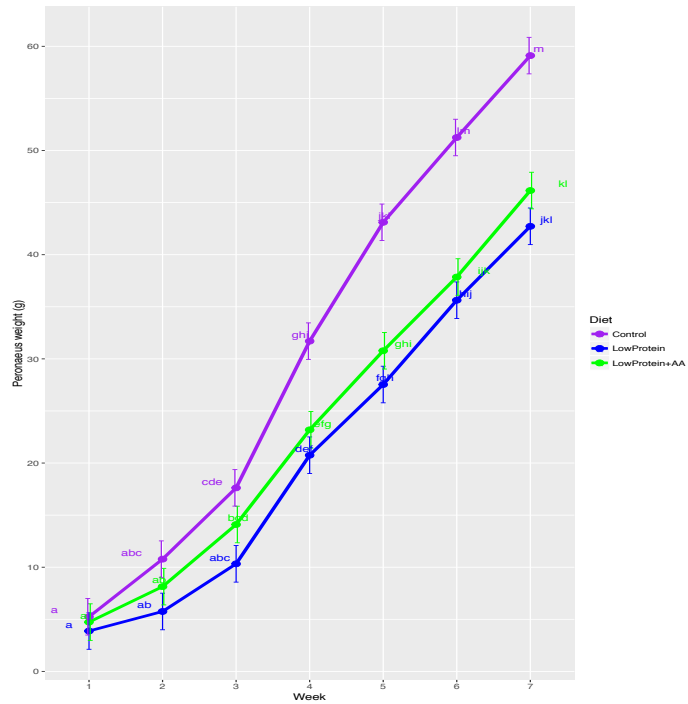


Figure 19. Effect of Diet and Time on Peroneus Weight

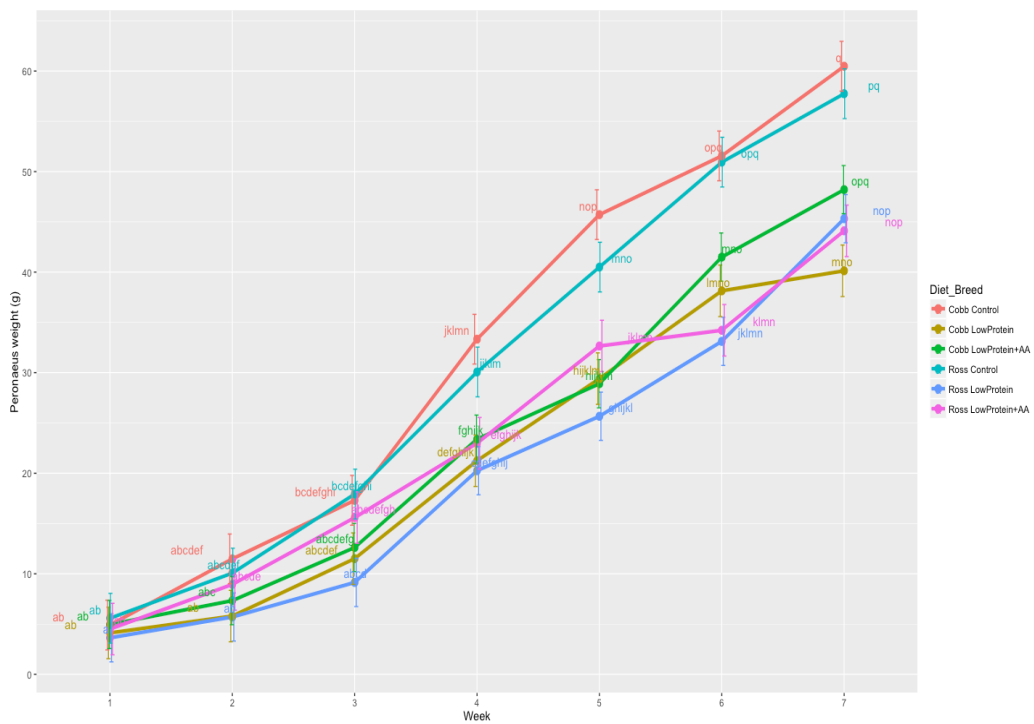


Figure 20. Effect of Diet, Breed, and Time on Peroneus Weight

Iliotibialis was removed from thigh and the weight was also greatly associated with protein level, time ($P < 0.05$) and breed ($P = 0.07$). In general, decreasing protein level rendered

about 10% less weight of iliotibialis compared to normal diet treatment and no change was exerted by adding CAA for both strains. However, even though CAA had more beneficial effect on restoring iliotibialis weights in Cobb broilers than Ross broilers, there was no statistically significant difference between the two breeds (Table 2, Figure 21). Breed by time did not play a role in changing iliotibialis weight (Figure 22, Table 4). The response of iliotibialis growth to the associative influences of diet and time (Figure 23) was similar to the other two muscles with no noticeable differences found with the three diets during first three weeks. Unlike the peroneus growth, CAA did not express anticipated effect of weight restoration on iliotibialis during 4th week. Iliotibialis weight was impaired about 29% with low protein levels at the end of the trial (Table 3). When incorporating breed effect into diet and time interaction, significant differences were found only between control and low protein diets (Figure 24). After the 5th week, CAA was no longer adequate in assisting iliotibialis growth in both breeds. Birds on control diet produced significant heavier iliotibialis than low protein diet at 3rd week, but the dietary effect showed up a week later when the breed effect was taken into consideration. Therefore, breed might play a compensatory effect on the iliotibialis weight.

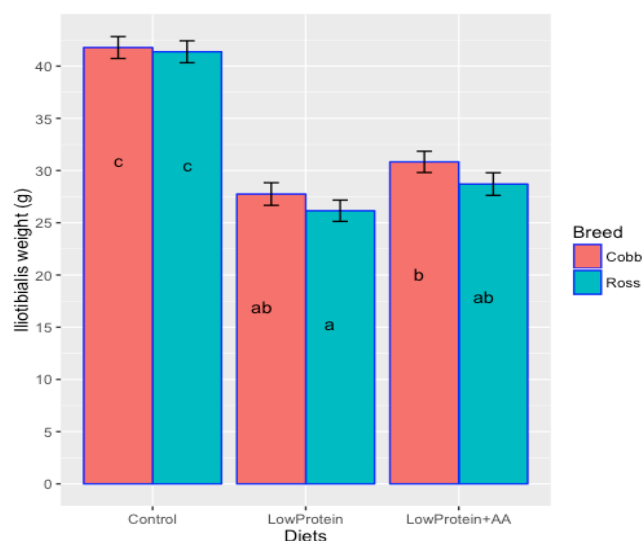


Figure 21. Effect of Diet and Breed On Iliotibialis Weight

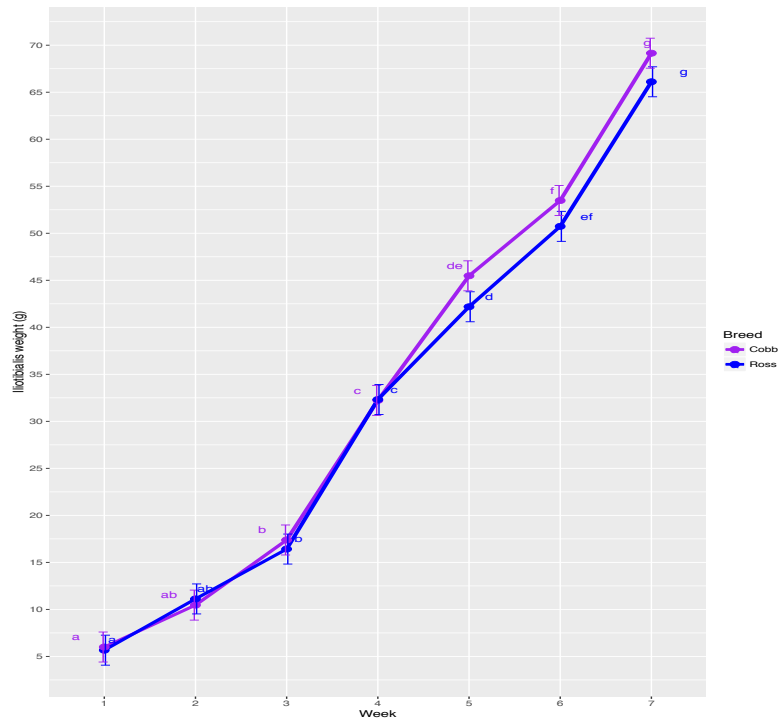


Figure 22. Effect of Breed and Time on Iliotibialis Weight

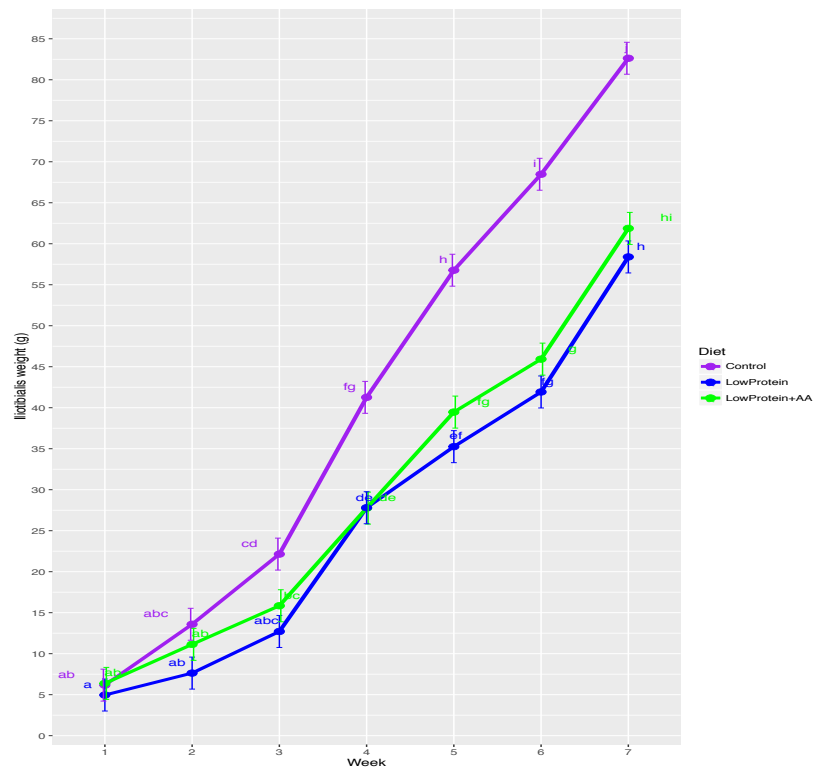


Figure 23. Effect of Diet and Time on Iliotibialis Weight

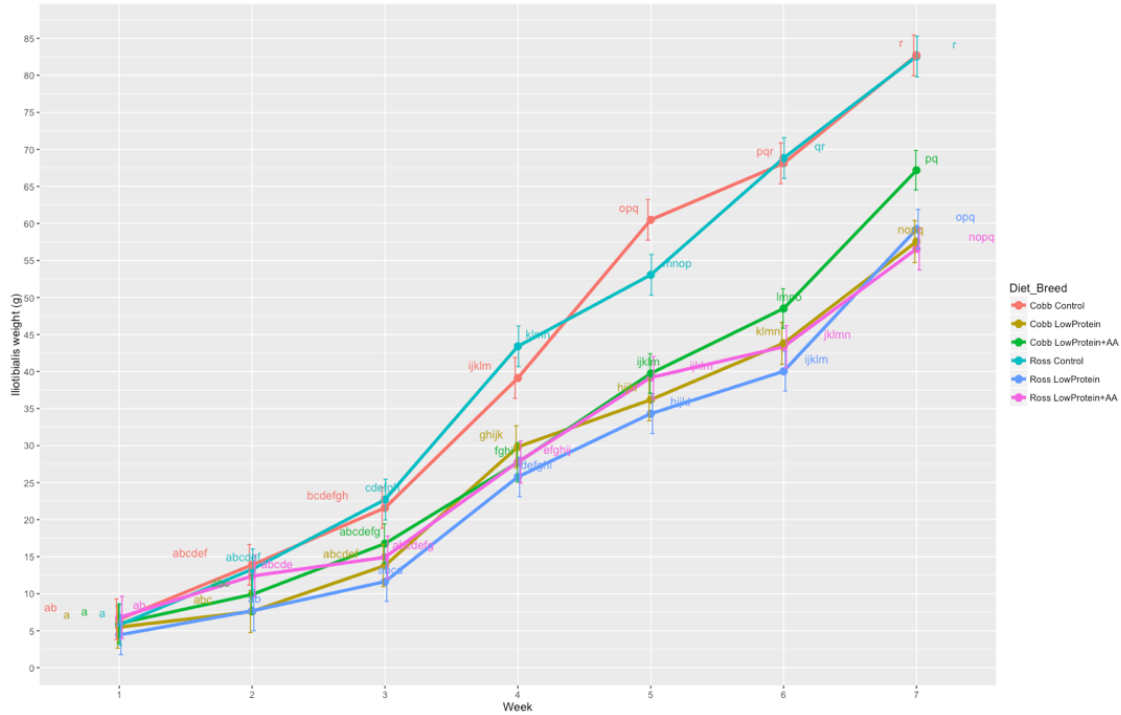


Figure 24. Effect of Diet, Breed and Time on Iliotibialis Weight

The bone grows earlier than muscle. Femur, taken from the thigh, was significantly influenced by the restriction of dietary protein level (Table 2). The addition of CAA significantly increase the femur weight only on Ross broilers, compared to those fed the low protein diet (Figure 25). However, the supplementation of CAA could not completely restore the femur growth for both breeds. In general, Ross broilers fed with control diet exhibited relatively heavier femur than Cobb broilers ($P > 0.05$), while Cobb broilers had more resistance towards low protein diets. No significant effect exerted by the breed on femur weight was found over 7 weeks (Figure 26, Table 3). Femur weight started showing significant variation among three diets at the 3rd week, when control diets for the two breeds produced heavier femurs than in birds with low protein diet. The CAA addition was still able to maintain a similar femur growth of birds fed the low protein diet as those fed the control diet until the 3rd week (Figure 27) ($P > 0.05$). However, birds under control diet developed heavier femur than the low protein diets, even with the addition of the CAA. By combining the effect of diet, breed and time (Figure 28, Table 5), there

was no significant difference on the femur development until the 4th week. However, there was a difference noticed between the Ross broilers that were fed the control diets versus those fed the low protein diets. No variation was found among the other treatments. By the 5th week, only the Ross broilers fed the low protein diet fortified with CAA could obtain similar femur weight with those broilers fed the control diet, but the influence of CAA on Ross broilers became significant during the last two weeks. The Cobb broilers on the low protein with CAA diet demonstrated opposite results compared to the Ross broilers during the 5th week ($P < 0.05$). There was also a significant difference in the femur between the control and low protein with CAA diets, but that difference disappeared within the last two weeks ($P > 0.05$).

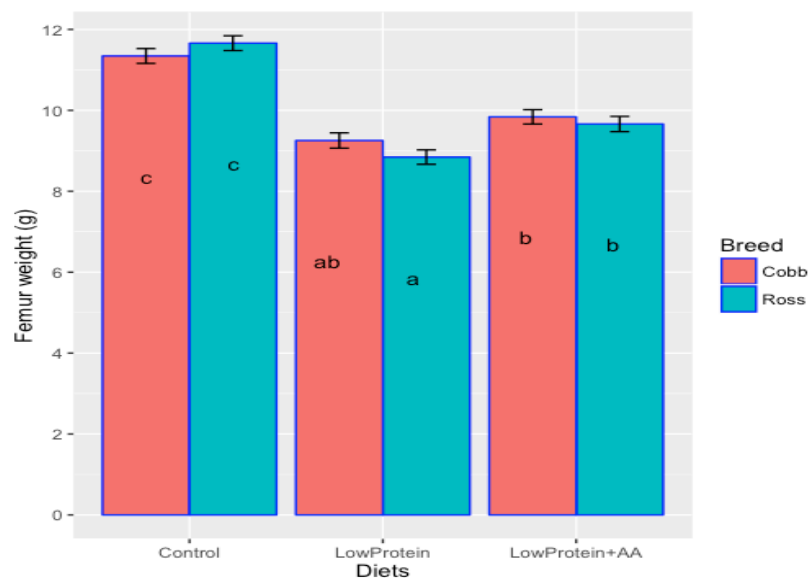


Figure 25. Effect of Diet and Breed on Femur Weight

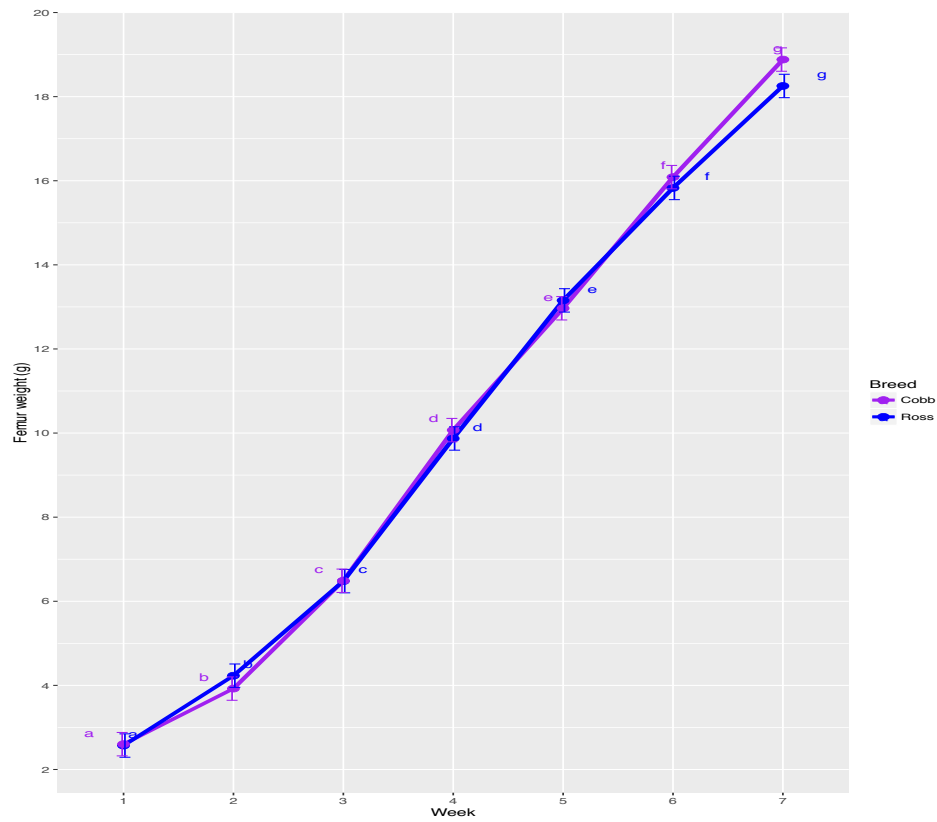


Figure 26. Effect of Breed and Time on Femur Weight

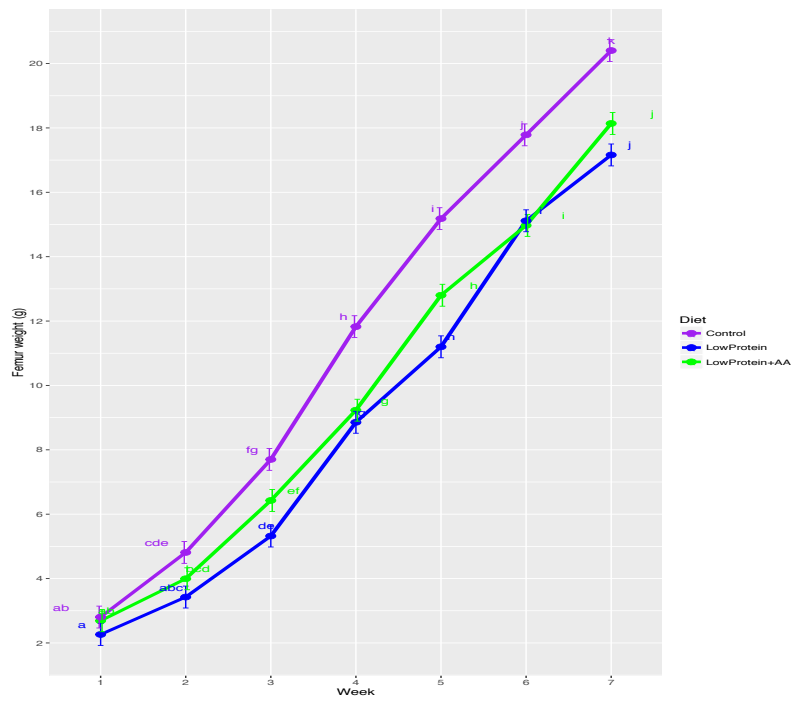


Figure 27. Effect of Diet and Time on Femur Weight

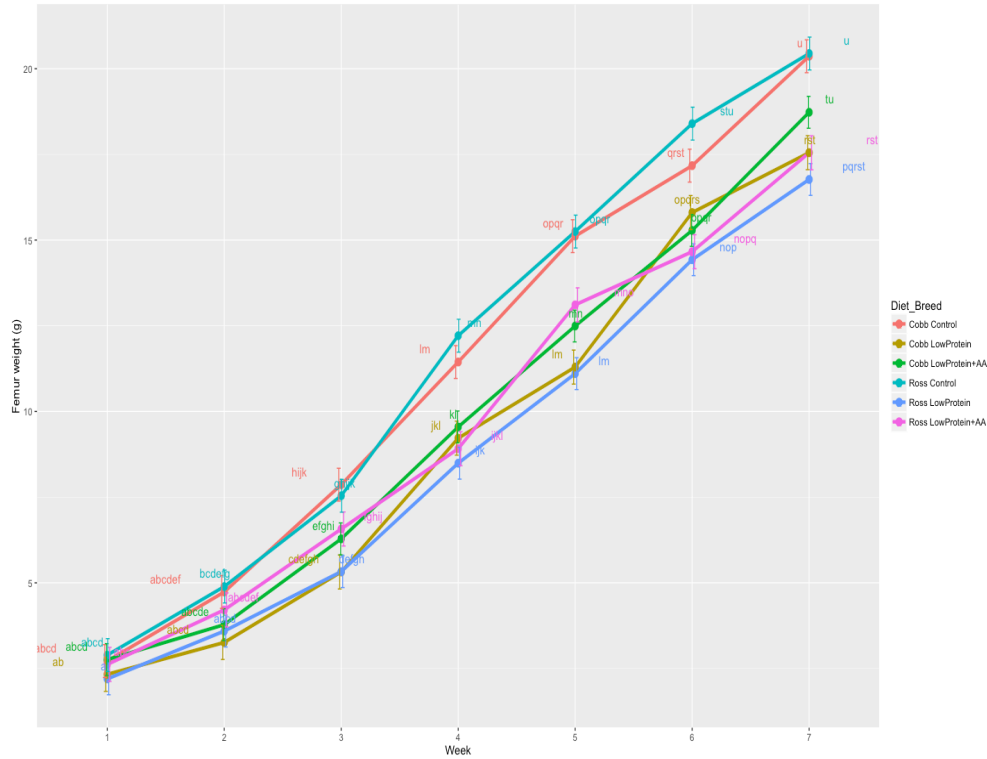


Figure 28. Effect of Diet, Breed, and Time on Femur Weight

When both breeds were given a reduced protein diet, congruent losses in tibia and femur weight were exhibited among both breeds. Both breeds that were fed a CAA added diet displayed positive femur and tibia development when compared to those fed the low protein diet (Figure 29, Table 2). During the 3rd week, there was a negative effect on tibia weight for broilers fed the low protein diet, when compared to those fed the control diet (Table 5, Figure 31). The restorative effect of the CAA on maintaining tibia weight lasted until the 3rd week; however, there was no differences in weight between the two low protein diets throughout the experimental period. This was excluding the 5th week when the birds on the low protein diet with CAA produced heavier tibias than those on diets without CAA. Ross broilers fed the low protein diet, first revealed significant decrease in the tibia weight on the 4th week. The beneficial effect of the CAA lasted two weeks less on Ross broilers than on Cobb broilers (Figure 32, Table 5). The two breeds reacted differently in the last two weeks. Ross broilers on the control diet

produced significantly heavier tibias when compared to the other low protein diets. Cobb broilers did not show any variation in tibia weight among the three diets during the 6th week. However, there was an abrupt increase in the tibia weight only in the Cobb broilers fed the control diet, which contributed to the significant difference of the tibia weight compared to those fed the low protein with CAA at the 7th week. Ross broilers on the control diet produced significantly heavier tibia weights compared to the Ross broilers on the two low protein diets, and this variation lasted from the 6th week to the final week.

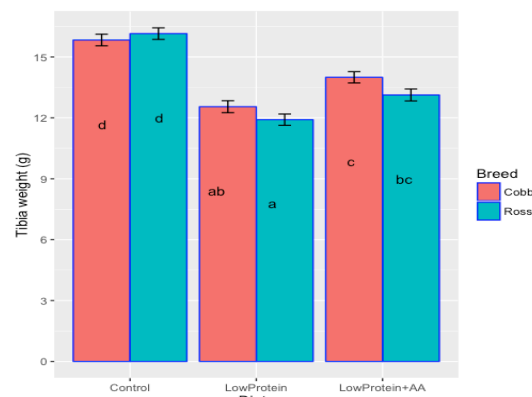


Figure 29. Effect of Diet and Breed on Tibia Weight

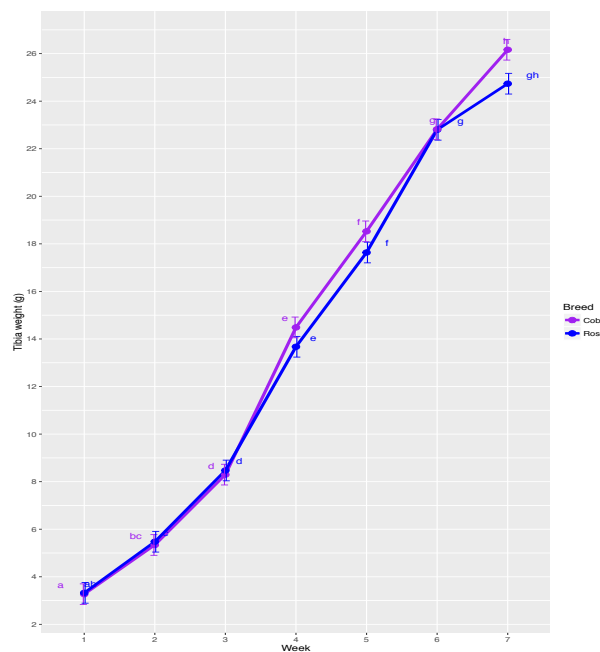


Figure 30. Effect of Breed and Week on Tibia Weight

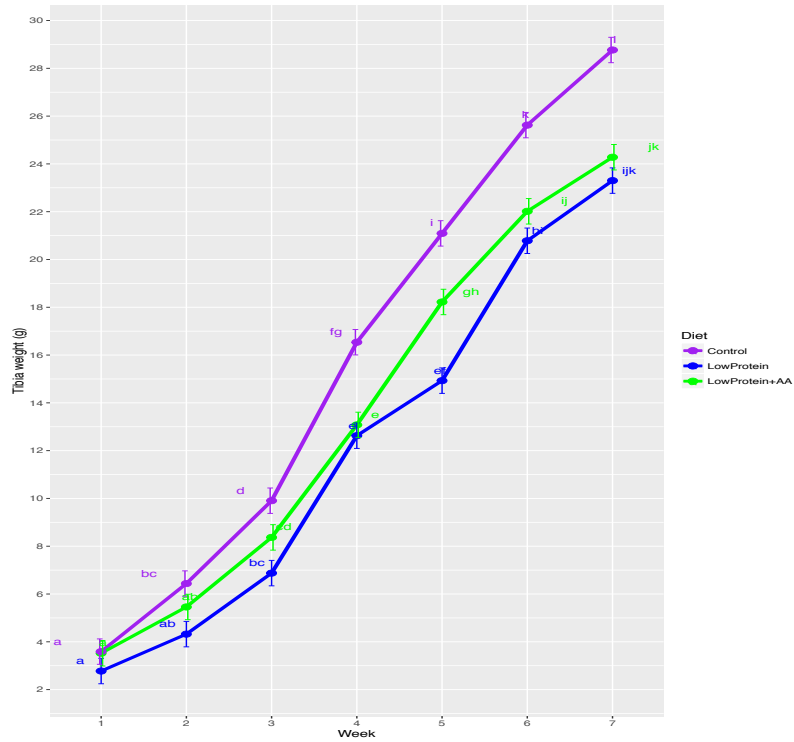


Figure 31. Effect of Diet and Time on Tibia Weight

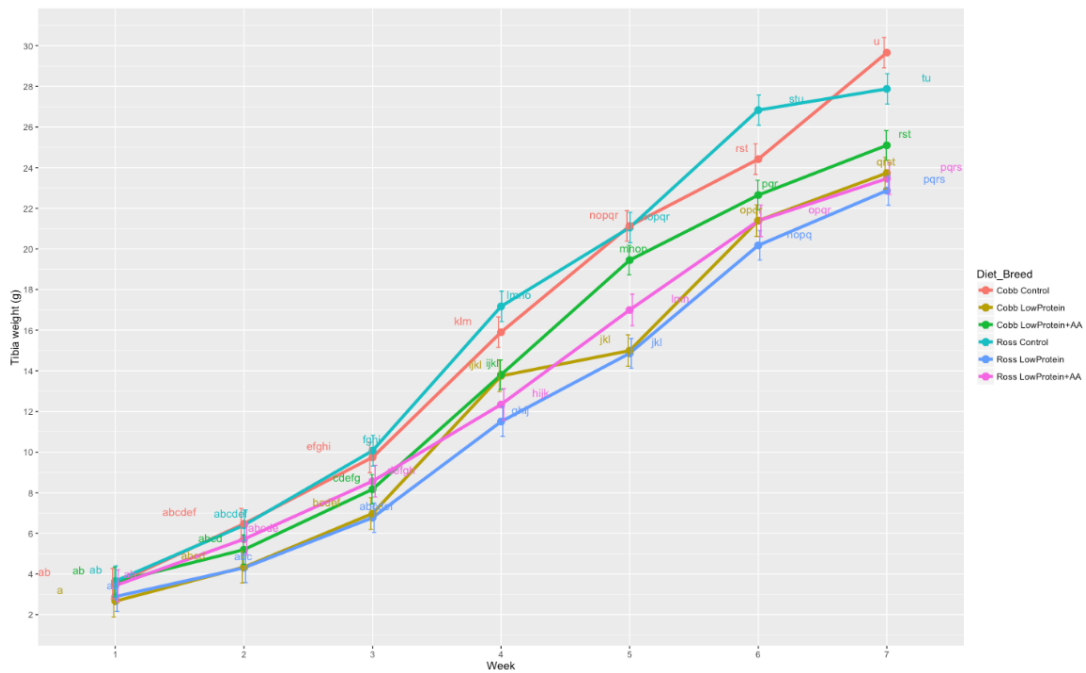


Figure 32. Effect of Diet, Breed, and Time on Tibia Weight

The radius taken from the wing was only significantly affected by time and dietary protein level. The overall radius weights declined in the both breeds that were fed low protein

diets compared to those on control diet ($P < 0.05$) (Figure 33, Table 2). When the birds were fed the low protein diet with CAA, ($P > 0.05$) the radius weights were increase to a certain degree, but not statistically significant. The radius growth was similar between the two breeds over the whole experimental period, but Cobb broilers showed a more consistent growing trend than Ross broilers (Figure 34). The slower growth of radius from the low protein diet was not noticeable until the 4th week, and reduction of 6% units of crude protein in diet caused about 31.5% deceased radius weight (Figure 35, Table 4). The effectiveness of CAA on maintaining radius weight was also negligible during the whole experimental period. Moreover, birds on the control diet tended to have a more uniform radius growth trend than those on the other two diets. The two breeds fed three different diets had an imperceptible influence on the radius development over the whole experimental period (Table 5, Figure 36).

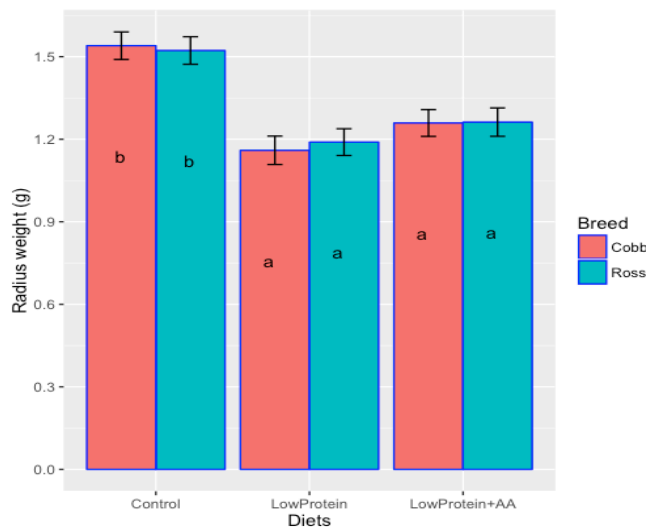


Figure 33. Effect of Diet and Breed on Radius Weight

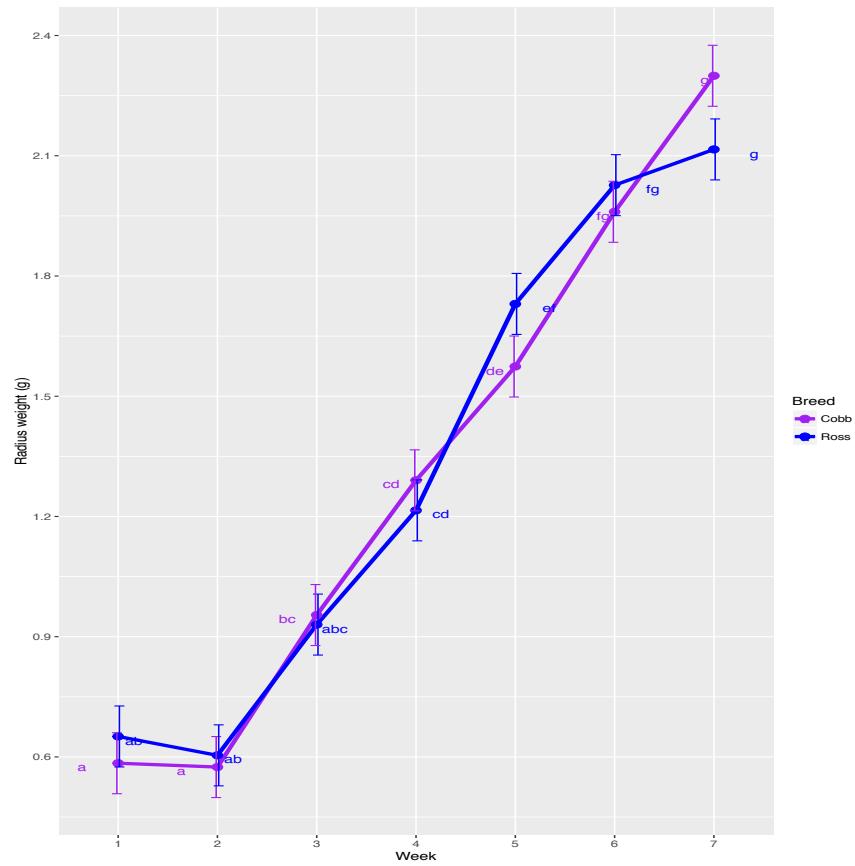


Figure 34. Effect of Breed and Time on Radius Weight

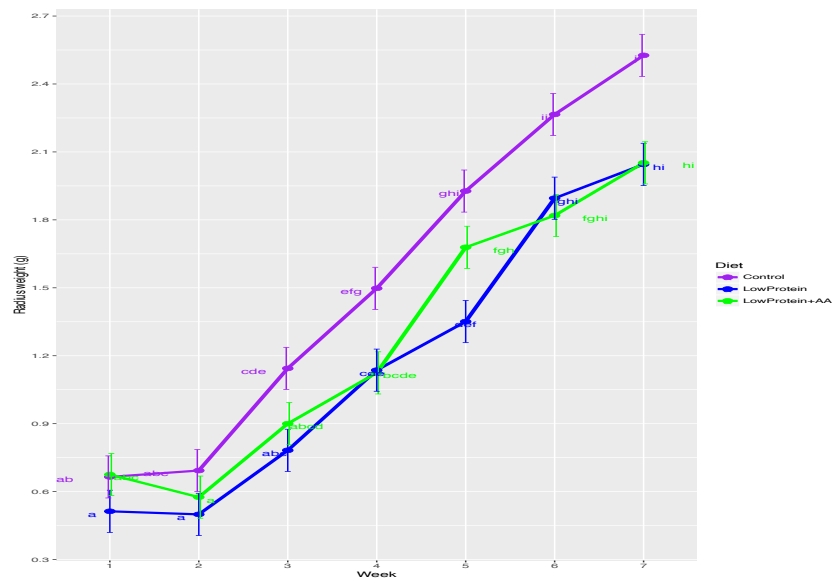


Figure 35. Effect of Diet and Time on Radius Weight

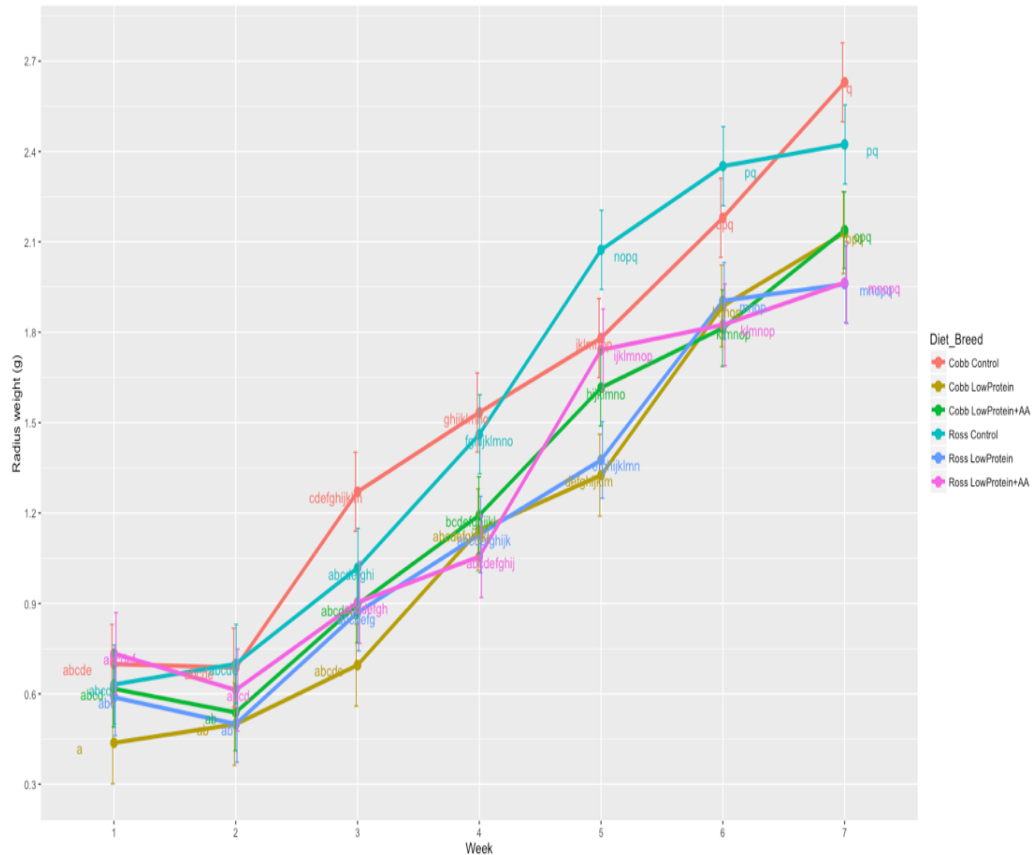


Figure 36. Effect of Diet, Breed, and Time on Radius Weight

The abdominal fat was collected at the last week of the experiment. Neither the dietary protein content nor the breed exerted any effect on the fat deposition; however, Cobb broilers showed relative heavier abdominal fat than Ross broilers in both low protein with CAA and the control diets. The significant difference in abdominal fat was only found between low protein and CAA fortified diets fed to Ross broilers. The CAA resulted in a 47% decrease in abdominal fat deposition compared to the birds on the low protein diet (Table 4).

4.2 The Relative Growth Performance

The relative growth is another important parameter that can strengthen the understanding of changes in body development over different protein levels, species, and time. Heart to body weight ratio (HBR) was significantly higher in both breeds fed the two low protein diets (Table 6). In addition to this, Ross broilers featured a significantly higher HBR with the low protein diet

than with the CAA fortified diets. There was no statistical difference between the two breeds on the same diet, but Cobb broilers seemed to have a higher heart proportion than the Ross broilers on the control diet; however, Ross broilers displayed a higher heart ratio when fed the two low protein diets (Figure 37). The birds on CAA added diets demonstrated an overall declining trend in HBR (Figure 38). There was a short and mild increase in HBR after the 2nd week for birds on all three diets, and the relative increase trend lasted a week longer for the birds on the control diet. The low protein diet only had significant higher HBR than those on the control diet during the 2nd week ($P < 0.05$) (Table 7). Also, no differences were detected between the two breeds over time, and HBR generally showed a declining trend for both breeds (Figure 39, Table 8), except for an erratic increase in HBR during the last week for Cobb broilers, even though the change was not statistically significant. The collective effect of the protein level, breed and time was not significant on the relative heart development ($P > 0.05$), and HBR of both breeds on three different diets tended to fluctuate over the weeks (Table 9). Both breeds on the low protein diet had the highest HBR, followed by CAA fortified low protein diets, and then the control diet (Figure 40). There was an increased in HBR during grower and finisher phases for the two breeds on three different diets, excluding Cobb broilers. When feeding low protein with CAA diets, Cobb broilers had a relatively smooth declining trend of HBR, with a sudden increase during the final week. The two breeds fed control diets demonstrated a larger variation over the weeks. Cobb broilers had a 23% increase in HBR during the 6th and 7th week. Moreover, Ross broilers expressed more inconsistency in the relative development of heart compared to the Cobb broilers on the two low protein groups (Figure 40).

Table 6. The Effect of Diets (positive control, low protein and low protein+ CAA2) and Breed (Ross broilers and Cobb broilers) on the Relative Growth¹ of Organ, Muscle and Bone²

	Positive Control		Low Protein		Low Protein + CAA		SEM
	Ross Boilers	Cobb Boilers	Ross Boilers	Cobb Boilers	Ross Boilers	Cobb Boilers	
Heart percent	0.53 a	0.55 a	0.65 c	0.62 bc	0.59 ab	0.58 ab	0.014
Liver percent	3.04 a	3.06 a	3.53 c	3.71 d	3.27 b	3.29 b	0.04
Pectoralis percent	7.26 e	6.71 d	4.71 a	4.39 a	6.21 c	5.84 b	0.087
Peronaeus percent	1.73 a	1.84 a	1.73 a	1.68 a	1.84 a	1.77 a	0.044
Iliotibialis percent	2.25 a	2.36 a	2.22 a	2.18 a	2.32 a	2.27 a	0.05
Femur percent	0.65 a	0.65 a	0.74 c	0.7 b	0.73 bc	0.7 b	0.009
Tibia percent	0.89 a	0.89 a	0.99 b	0.95 ab	1 b	0.99 b	0.15
Radius percent	0.1 a	0.1 a	0.12 a	0.1 a	0.11 a	0.1 a	0.049
Abdominal fat percent	1.45a	1.6a	2.58b	2.59b	1.71a	1.96ab	0.175

¹The relative growth of organ, muscle and bone were calculated by dividing the specific body parts by body weight and times 100%.

² The results were presented in the percentage as least square mean, and averaged over the levels of weeks and years. Confidence level used: 0.95. Least Square mean in the same row with different letters are different (P < 0.05).

Table 7. The Effect of Diets (positive control, low protein and low protein+ CAA2) and Time on the Relative Growth¹ of Organ, Muscle and Bone²

		Heart Percent	Liver Percent	Pectoralis Percent	Peronaeuse Percent	Femur Percent	Tibia Percent	Radius Percent
Positive Control	Week 1	0.66 defgh	4.1 jk	5.25 cde	1.81 ab	0.75 ef	0.97 bcd	0.19 b
	Week 2	0.55 abcd	3.85 ij	5.38 de	1.73 ab	0.67 bcde	0.91 abc	0.1 a
	Week 3	0.56 abcde	3.15 efg	6.21 fg	1.69 ab	0.67 bcde	0.88 ab	0.1 a
	Week 4	0.58 bcdef	2.74 abcd	7.12 hi	1.87 b	0.64 abcd	0.91 abc	0.08 a
	Week 5	0.47 ab	2.61 abc	7.59 ij	1.84 ab	0.62 abc	0.88 ab	0.08 a
	Week 6	0.44 a	2.52 ab	8.18 j	1.84 ab	0.61 ab	0.88 ab	0.08 a
	Week 7	0.51 abc	2.4 a	9.16 k	1.7 ab	0.57 a	0.81 a	0.07 a

Table Cont'd

		Heart Percent	Liver Percent	Pectoralis Percent	Peronaeuse Percent	Femur Percent	Tibia Percent	Radius Percent
Low Protein	Week 1	0.79 h	4.79 m	3.7 a	1.75 ab	0.77 f	0.99 bcd	0.18 b
	Week 2	0.68 efgh	4.66 lm	3.91 ab	1.42 a	0.7 cdef	0.91 abc	0.11 a
	Week 3	0.71 fgh	3.69 hi	4.47 abc	1.63 ab	0.74 ef	0.95 abcd	0.11 a
	Week 4	0.61cdefg	3.51 ghi	4.65 bcd	1.77 ab	0.69 bcdef	1 bcd	0.09 a
	Week 5	0.6 bcdefg	2.98 cdef	4.77 cd	1.87 b	0.74 ef	0.99 bcd	0.09 a
	Week 6	0.52 abc	3.03 def	4.91 cd	1.78 ab	0.75 ef	1.04 cd	0.09 a
	Week 7	0.51 abc	2.67 abcd	5.43 def	1.72 ab	0.67 bcde	0.92 abcd	0.08 a
Low Protein + CAA	Week 1	0.73 gh	4.23 jk	5.13 cde	1.77 ab	0.76 ef	1.04 cd	0.2 b
	Week 2	0.6 bcdefg	4.3 kl	5.23 cde	1.62 ab	0.69 bcdef	0.96 bcd	0.11 a
	Week 3	0.61 cdefg	3.31 fgh	6.21 fg	1.69 ab	0.71 def	0.93 abcd	0.1 a
	Week 4	0.55 abcde	3.0 def	5.86 efg	1.91 b	0.68 bcde	0.97 bcd	0.08 a
	Week 5	0.55 abcde	2.83 bcde	6.22 fg	1.84 ab	0.72 def	1.05 cd	0.09 a
	Week 6	0.51 abc	2.74 abcd	6.45 gh	1.93 b	0.72 def	1.07 d	0.09 a
	Week 7	0.53 abcd	2.47 ab	7.05 hi	1.89 b	0.72 def	0.97 bcd	0.08 a

¹The relative growth of organ, muscle and bone were calculated by dividing the specific body parts by body weight and times 100%.

² The results were presented in the percentage as least square mean, and averaged over the levels of weeks and years. Confidence level used: 0.95. Least Square mean in the same row with different letters are different ($P < 0.05$).

Table 8. The Effect of Breed (Ross broilers and Cobb broilers) and Time on the Relative Growth¹ of Organ, Muscle and Bone²

		Heart Percent	Liver Percent	Pectoralis Percent	Peronaeuse Percent	Femur Percent	Radius Percent	Tibia Percent
Ross Broilers	Week 1	0.72 e	4.35 f	4.56 a	1.77 a	0.75 c	0.2 b	0.75 c
	Week 2	0.61 cd	4.13 f	4.6 a	1.59 a	0.72 bc	0.11 a	0.72 bc
	Week 3	0.64 de	3.33 de	5.33 bcd	1.67 a	0.71 bc	0.1 a	0.71 bc
	Week 4	0.59 bcd	3.05 cd	5.63 cde	1.86 a	0.68 ab	0.09 a	0.68 ab
	Week 5	0.57abcd	2.82 bc	5.91 def	1.86 a	0.72 bc	0.09 a	0.72 bc
	Week 6	0.5 ab	2.76 abc	6.41 fg	1.79 a	0.7 abc	0.09 a	0.7 abc
	Week 7	0.49 ab	2.52 ab	7.09 hi	1.84 a	0.67 ab	0.08 a	0.67 ab

Table Cont'd

		Heart Percent	Liver Percent	Pectoralis Percent	Peronaeuse Percent	Femur Percent	Radius Percent	Tibia Percent
Cobb Broilers	Week 1	0.73 e	4.39 f	4.83 ab	1.79 a	0.76 c	0.18 b	0.76 c
	Week 2	0.61 cd	4.41 f	5.08 abc	1.59 a	0.66 ab	0.1 a	0.66 ab
	Week 3	0.61 cd	3.44 e	5.93 def	1.67 a	0.7 abc	0.1 a	0.7 abc
	Week 4	0.57 abcd	3.16 de	6.12 efg	1.85 a	0.66 ab	0.08 a	0.66 ab
	Week 5	0.51 abc	2.79 abc	6.48 fgh	1.85 a	0.67 ab	0.08 a	0.67 ab
	Week 6	0.48 a	2.77 abc	6.62 gh	1.91 a	0.68 ab	0.08 a	0.68 ab
	Week 7	0.54 abcd	2.51 a	7.34 i	1.7 a	0.64 a	0.08 a	0.64 a

¹The relative growth of organ, muscle and bone were calculated by dividing the specific body parts by body weight and times 100%.

² The results were presented in the percentage as least square mean, and averaged over the levels of weeks and years. Confidence level used: 0.95. Least Square mean in the same row with different letters are different ($P < 0.05$).

Table 9. The Effect of Diets (positive control, low protein and low protein+ CAA2), Breed (Ross broilers and Cobb broilers) and Time on the Relative Growth¹ of Organ, Muscle and Bone²

			Heart	Liver	Pectoralis	Peronaeus	Iliotibialis	Femur	Tibia	Radius
Positive Control	Ross Broilers	W1 ²	0.67 defghi j	4.16 jklm	5.5 efghijklm n	1.88 a	2.05 abc	0.76 ghi	0.76 ghi	0.18 cd
		W2	0.54 abcde fgh	3.75 hijkl	5.67 fghijklmn op	1.58 a	2.13 abc	0.69 abcde fghi	0.69 abcd efghi	0.1 ab
		W3	0.55 abcde fgh	3.04 bcdef g	6.65 nopqrs	1.68 a	2.11 abc	0.64 abcde fgh	0.64 abcd efgh	0.09 ab
		W4	0.58 abcde fghi	2.76 abcd	7.4 rst	1.74 a	2.45 abc	0.65 abcde fgh	0.65 abcd efgh	0.08 a
		W5	0.48 abcde	2.64 abc	7.89 stu	1.75 a	2.28 abc	0.62 abcde	0.62 abcd e	0.08 a
		W6	0.44 ab	2.53 ab	8.5 tuv	1.79 a	2.39 abc	0.61 abcd	0.61 abcd	0.08 a
		W7	0.45 abc	2.41 a	9.21 v	1.66 a	2.37 abc	0.57 a	0.57 a	0.07 a

Table Cont'd

Positive Control	Cobb Broilers		Heart	Liver	Pectoralis	Peronaeus	Iliotibialis	Femur	Tibia	Radius
		W1	0.66 cdefg hij	4.03 ijkl	5 bcdefghij	1.74 a	2.28 abc	0.73 cdefg hi	0.73 cdef ghi	0.2 d
		W2	0.55 abcde fgh	3.96 ijkl	5.09 bcdefghij k	1.88 a	2.29 abc	0.65 abcde fgh	0.65 abcd efgh	0.1 ab
		W3	0.56 abcde fgh	3.26 defgh	5.77 ghijklmn op	1.71 a	2.12 abc	0.7 abcde fghi	0.7 abcd efghi	0.11 abc
		W4	0.59 abcde fghij	2.72 abcd	6.85 opqrs	2.00 a	2.32 abc	0.64 abcde fg	0.64 abcd efg	0.09 ab
		W5	0.47 abcd	2.58 abc	7.29 rst	1.93 a	2.6 bc	0.62 abcde f	0.62 abcd ef	0.07 a
		W6	0.44 a	2.51 ab	7.86 stu	1.88 a	2.5 abc	0.6 abc	0.6 abc	0.08 a
		W7	0.57 abcde fgh	2.39 a	9.12 uv	1.74 a	2.4 abc	0.58 ab	0.58 ab	0.07 a
Low Protein	Ross Broilers	W1	0.78 ij	4.67 mno	3.83 ab	1.73 a	2.18 abc	0.75 efghi	0.75 efghi	0.21 d
		W2	0.68 efghij	4.35 lmno	3.99 abcd	1.46 a	1.97 abc	0.75 efghi	0.75 efghi	0.11 ab
		W3	0.73 ghij	3.62 ghijk	4.66 abcdefg	1.54 a	1.87 a	0.76 ghi	0.76 ghi	0.12 abc
		W4	0.64 abcde fghij	3.44 efghi	4.77 abcdefgh	1.86 a	2.35 abc	0.71 cdefg hi	0.71 cdef ghi	0.1 ab
		W5	0.65 bcdef ghij	2.96 abcde f	5.12 defghijk	1.87 a	2.52 abc	0.78 hi	0.78 hi	0.1 ab
		W6	0.52 abcde f	2.99 abcde f	4.98 bcdefghij	1.75 a	2.12 abc	0.76 ghi	0.76 ghi	0.1 ab
		W7	0.52 abcde f	2.65 abc	5.58 fghijklmn	1.9 a	2.5 abc	0.69 abcde fghi	0.69 abcd efghi	0.08 a

Table Cont'd

Low Protein	Cobb Broilers		Heart	Liver	Pectoralis	Peronaeus	Iliotibialis	Femur	Tibia	Radius
		W1	0.8 j	4.9 no	3.57 a	1.77 a	2.36 abc	0.79 i	0.79 i	0.16 bcd
		W2	0.68 defghi j	4.96 o	3.82 abc	1.38 a	1.85 ab	0.65 abcde fghi	0.65 abcd efghi	0.1 ab
		W3	0.7 fghij	3.75 hijkl	4.28 abcde	1.71 a	2.07 abc	0.73 cdefg hi	0.73 cdef ghi	0.1 ab
		W4	0.59 abcde fghij	3.58 fghij	4.53 abcdefg	1.68 a	2.36 abc	0.68 abcde fghi	0.68 abcd efghi	0.08 ab
		W5	0.55 abcde fgh	3 abcde f	4.42 abcdef	1.87 a	2.32 abc	0.7 abcde fghi	0.7 abcd efghi	0.08 a
		W6	0.51 abcde f	3.08 bcdef g	4.83 abcdefghi	1.8 a	2.1 abc	0.73 cdefg hi	0.73 cdef ghi	0.09 ab
		W7	0.5 abcde f	2.7 abcd	5.28 efghijklm	1.54 a	2.19 abc	0.65 abcde fgh	0.65 abcd efgh	0.08 a
Low Protein+CAA	Ross Broilers	W1	0.72 fghij	4.23 klm	5.14 cdefghijk l	1.69 a	2.4 abc	0.74 cdefg hi	0.74 cdef ghi	0.21 d
		W2	0.6 abcde fghij	4.28 lmn	5.58 efghijklm no	1.72 a	2.4 abc	0.71 bcdef ghi	0.71 bcde fghi	0.11 ab
		W3	0.63 abcde fghij	3.31 defgh	6.48 mnopqr	1.78 a	1.82 a	0.74 cdefg hi	0.74 cdef ghi	0.1 ab
		W4	0.55 abcde fgh	2.94 abcde f	6.2 jklmnopq r	1.97 a	2.36 abc	0.68 abcde fghi	0.68 abcd efghi	0.08 a
		W5	0.58 abcde fghi	2.86 abcde	6.43 lmnopqr	1.94 a	2.41 abc	0.76 fghi	0.76 fghi	0.1 ab
		W6	0.53 abcde fg	2.76 abcd	6.37 klmnopqr	1.83 a	2.29 abc	0.74 cdefg hi	0.74 cdef ghi	0.09 ab
		W7	0.51 abcde f	2.49 ab	7.24 qrst	1.95 a	2.55 abc	0.75 defghi	0.75 defg hi	0.08 a

Table Cont'd

Low Protein+CAA	Cobb Broilers		Heart	Liver	Pectoralis	Peronaeus	Iliotibialis	Femur	Tibia	Radius
		W1	0.74 hij	4.24 lm	5.11 defghijk	1.85 a	2.31 abc	0.77 ghi	0.77 ghi	0.19 d
		W2	0.6 abcde fghij	4.31 lmn	4.88 bcdefghi	1.52 a	2.06 abc	0.67 abcde fghi	0.67 abcd efghi	0.1 ab
		W3	0.59 abcde fghi	3.31 defgh	5.95 hijklmno p	1.59 a	2.05 abc	0.68 abcde fghi	0.68 abcd efghi	0.1 ab
		W4	0.54 abcde fgh	3.18 cdefg h	5.52 efghijklm n	1.86 a	2.16 abc	0.67 abcde fghi	0.67 abcd efghi	0.09 a
		W5	0.53 abcde fg	2.8 abcd	6.01 ijklmnop q	1.74 a	2.38 abc	0.69 abcde fghi	0.69 abcd efghi	0.09 ab
		W6	0.49 abcde	2.72 abcd	6.53 mnopqr	2.03 a	2.36 abc	0.71 bcdef ghi	0.71 bcde fghi	0.08 a
		W7	0.55 abcde fgh	2.45 ab	6.86 pqr	1.82 a	2.6 c	0.69 abcde fghi	0.69 abcd efghi	0.08 a

¹The relative growth of organ, muscle and bone were calculated by dividing the specific body parts by body weight and times 100%.

² The results were presented in the percentage as least square mean, and averaged over the levels of weeks and years. Confidence level used: 0.95. Least Square mean in the same row with different letters are different ($P < 0.05$).

² W1 represents week 1, and it applies to the entire column.

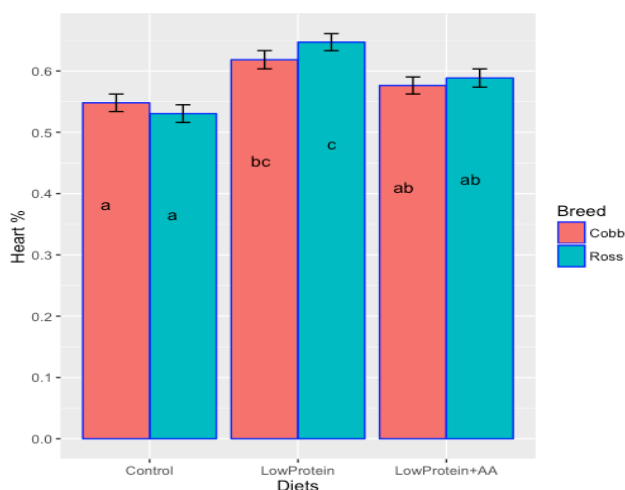


Figure 37. Effect of Diet and Breed on Heart to Body Weight Ratio

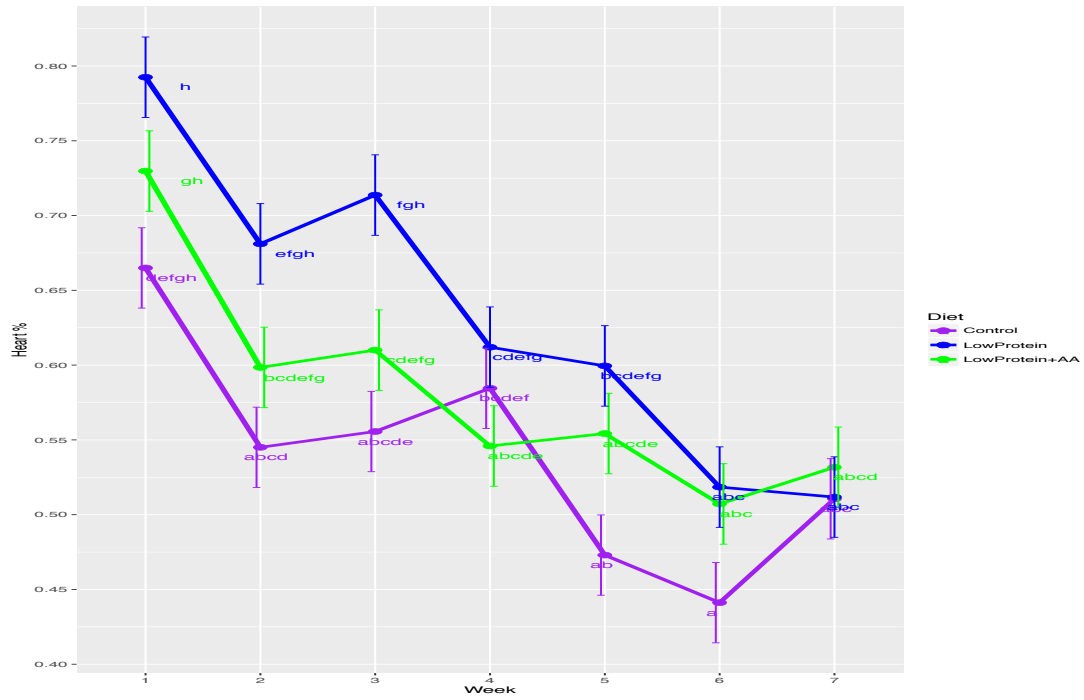


Figure 38. Effect of Diet and Time on Heart to Body Weight Ratio

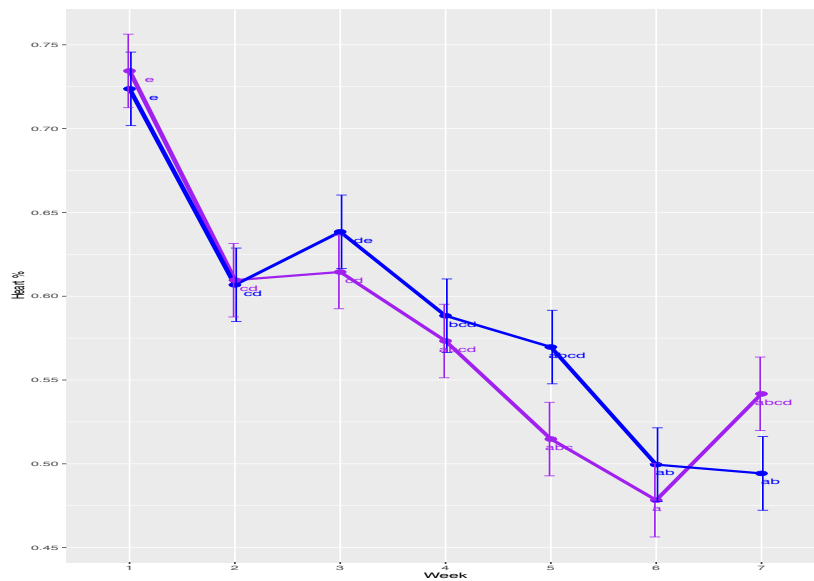


Figure 39. Effect of Breed and Time on Heart to Body Weight Ratio

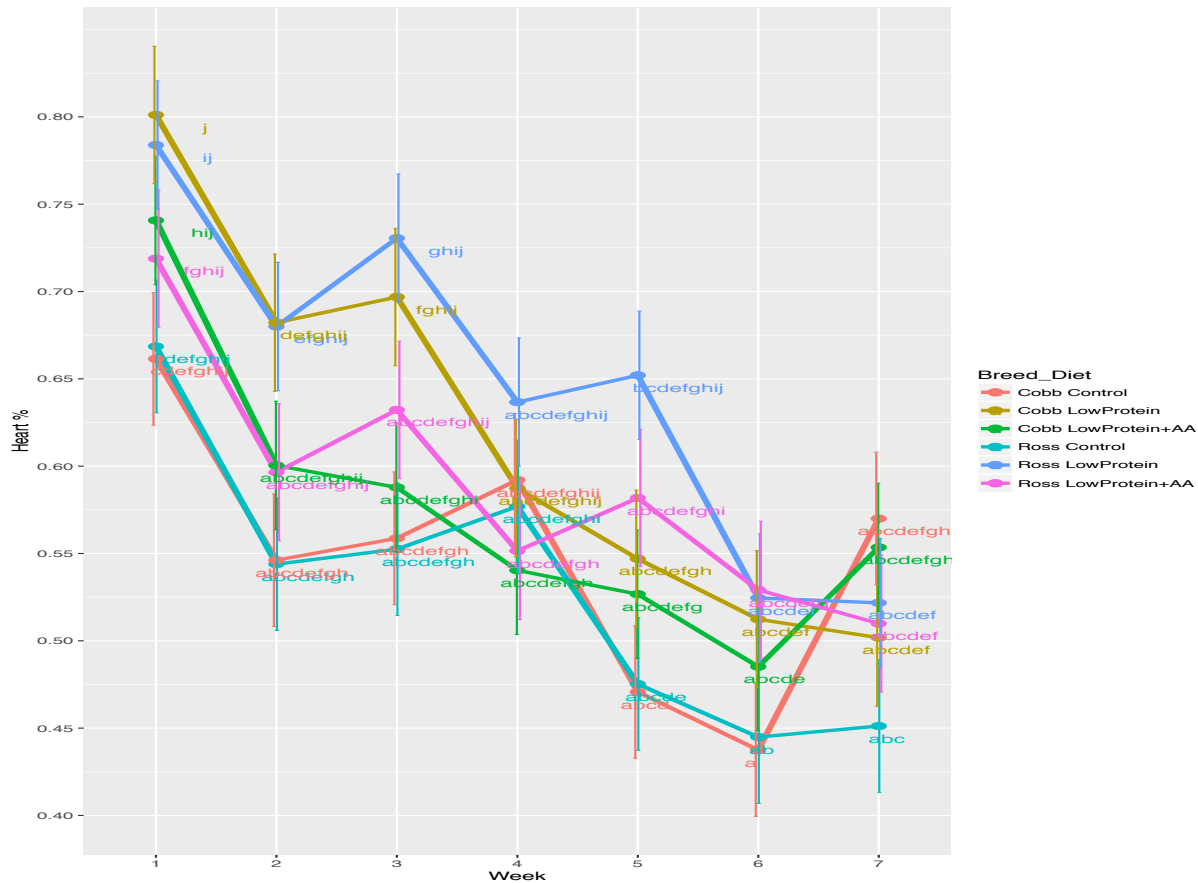


Figure 40. Effect of Diet, Breed and Time on Heart to Body Weight Ratio

The liver to body weight ratio (LBR) was significantly affected by the protein content in the diets and breed. The effect of the protein level on the LBR was similar to that of HBR, and the reduction in protein content increased the liver proportion in the two breeds, especially on the Cobb broilers (Table 5). The CAA addition helped reduce the liver proportion to a significantly lower level compared to that of birds on the low protein diet. However, the general LBR was still significantly higher than birds on the control diet ($P < 0.05$) (Figure 41). The LBR had a generally declining trend through the all 7 weeks, and the significant variation in LBR showed during the first week between the control and the two low protein diets (Figure 42). The birds fed the low protein diet had higher LBR than those on control diet until the 4th week, and the birds on CAA added diet maintained the LBR at a similar level to those on control diet ($P > 0.05$)

(Table 7). However, the birds on CAA diet had a slight increase in LBR during the 2nd week, resulting in the significant variation from those on control diet (Figure 42). Birds on the all three diets had a dramatic decline in the LBR during the 3rd week, and the control diet fed to birds resulted in reaching a constant LBR a week earlier compared to those on the other diets. Afterwards, birds on all three diets achieved a constant LBR after the 5th week. The breed influence on the relative liver development was less detectable (Table 8). However, the overall liver development curve for Ross broilers was less varied than Cobb broilers (Figure 43). The low protein diets resulted in a significant increase in the LBR, but only for Cobb birds before the 3rd week. While LBR from both breeds on low protein diets became significantly higher than for the birds on the control diet, when it came to the 4th week ($P < 0.01$), the supplementation of CAA adjusted the relative growth of liver, and erased the difference between the birds feed control and the low protein diets. In addition to this, both breeds fed low protein diets and Cobb broilers on the CAA added diet did not achieve the constant LBR until a week later than the other treatments (Figure 44).

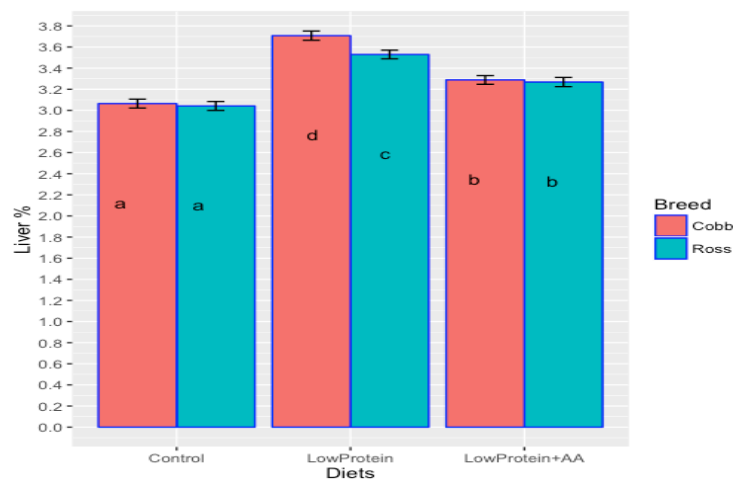


Figure 41. Effect of Diet and Breed on Liver to Body Weight Ratio

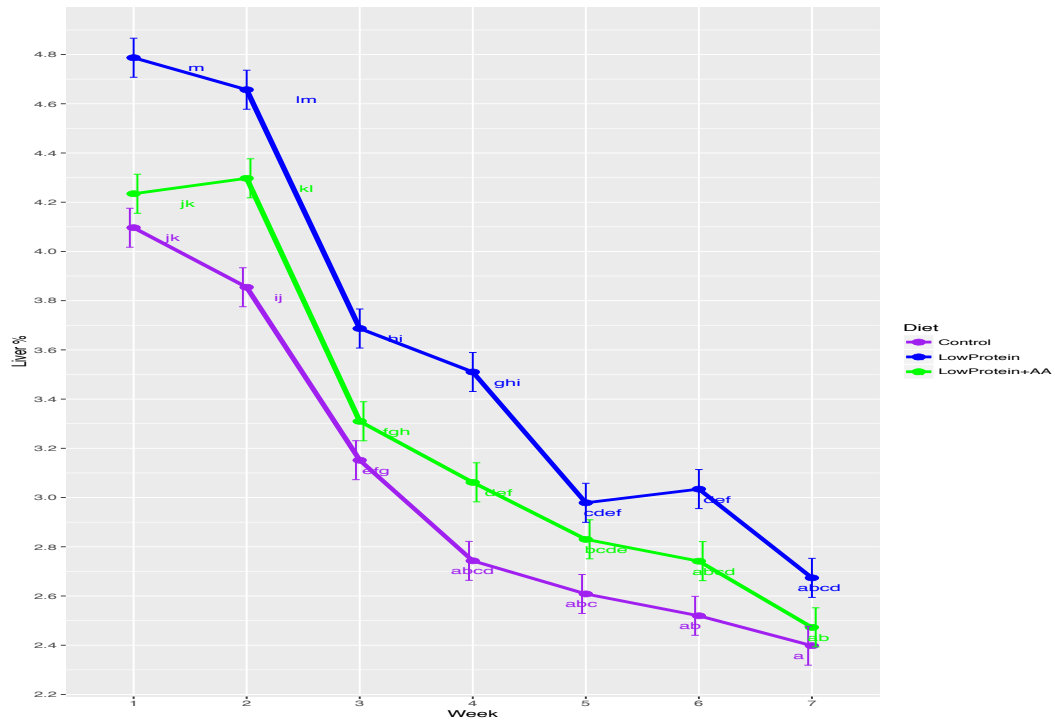


Figure 42. Effect of Diet and Time on Liver to Body Weight Ratio

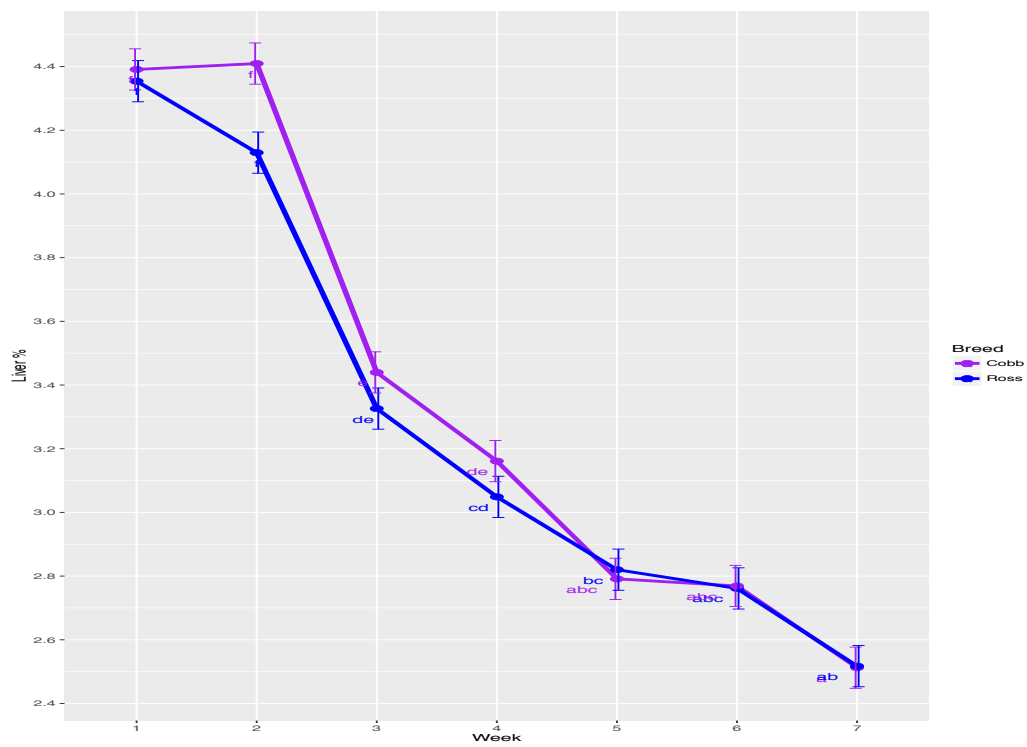


Figure 43. Effect of Breed and Time on Liver to Body Weight Ratio

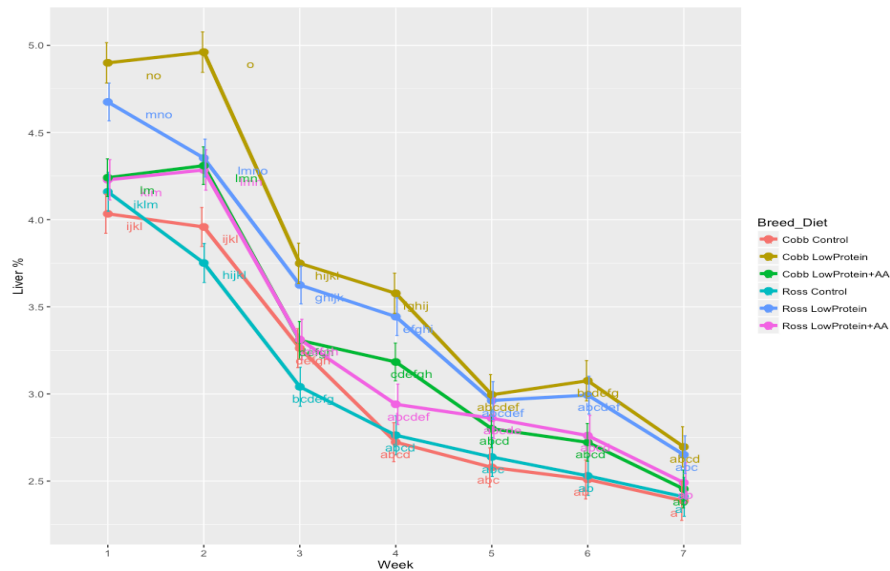


Figure 44. Effect of Diet, Breed and Time on Liver to Body Weight Ratio

The effect of protein level and breed on the relative pectoralis growth is in Figure 45. Both breeds experienced a negative decrease in pectoralis proportion when they were fed the restricted protein content. Ross broilers produced significantly higher pectoralis proportion (PBR) than Cobb broilers on either control or the low protein with CAA diets ($P < 0.05$) (Table 6). The CAA added diet restored the relative pectoralis growth in both breeds, but was unable to completely compensate the restrictive effect of low protein diets. The PBR among the birds on the three different diets showed a generally increasing trend, but birds on the low protein diets had the lowest PBR through the overall experimental period. Birds on low protein diets did not show significant gains in PBR until the 4th week, and then the PBR kept increasing at a relatively slow rate, without significant variation between each week (Figure 46, Table 9). When just considering the diet and time effect, it took three weeks for birds on low proteins diets to reach to the similar level of PBR that was achieved on the the other two diets within the 1st week. The addition of CAA could decrease the adverse effect of low protein diet on the PBR, but could only sustain the relative growth of pectoralis for the first three weeks. The birds fed control diets were characterized by significant increases at 3rd, 4th and 7th week, and there was a 9.2 % increase in

PBR during the final week (Table 7). The two breeds had similar increasing trends for pectoralis proportion, starting with relatively slow increases during the first two weeks and a dramatic improvement especially during the 2nd and 7th weeks (Figure 47,). However, there were no significant differences between two breeds until the last week (Table 8), even though Ross broilers expressed a relatively higher PBR than Cobb broilers throughout the time period. Figure 48 showed the combined effect of diets, breeds and time on the relative pectoralis development, and both breeds had similar PBR with the protein restriction. The Ross broilers and Cobb broilers had a slightly decrease in PBR, during 4th and 5th week, respectively. The relative growth curves for both breeds on CAA added diet were not steadily increasing. Those two breeds all had declining periods that were exhibited at the 2nd and 4th week for Cobb broilers, and at the 3rd and 5th for Ross broilers (Figure 48). Either breed on low protein diet could maintain the PBR with those from control diet, as long as the CAA was supplemented. These supportive effects of CAA on PBR did not fade until the 3rd week, after which the differences first showed up on Cobb broilers. The sustaining effect of CAA on Ross broilers lasted a week longer than on Cobb broilers, but finally disappeared at the 6th week. No difference was found between two breeds on control diets through the whole time, but Ross broilers had higher but not statistically significant ($P > 0.05$) PBR than Cobb broilers in all three diet throughout the time,

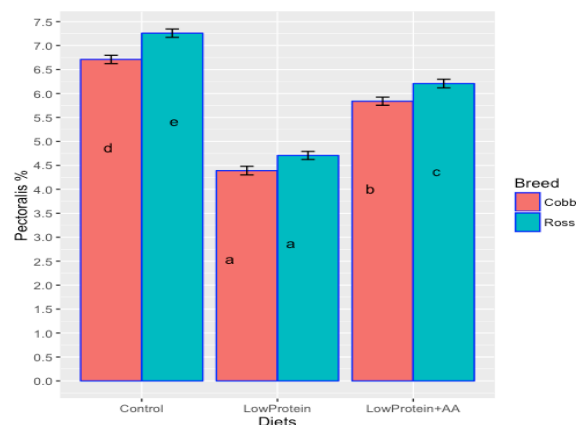


Figure 45. Effect of Diet and Breed on Pectoralis to Body Weight Ratio

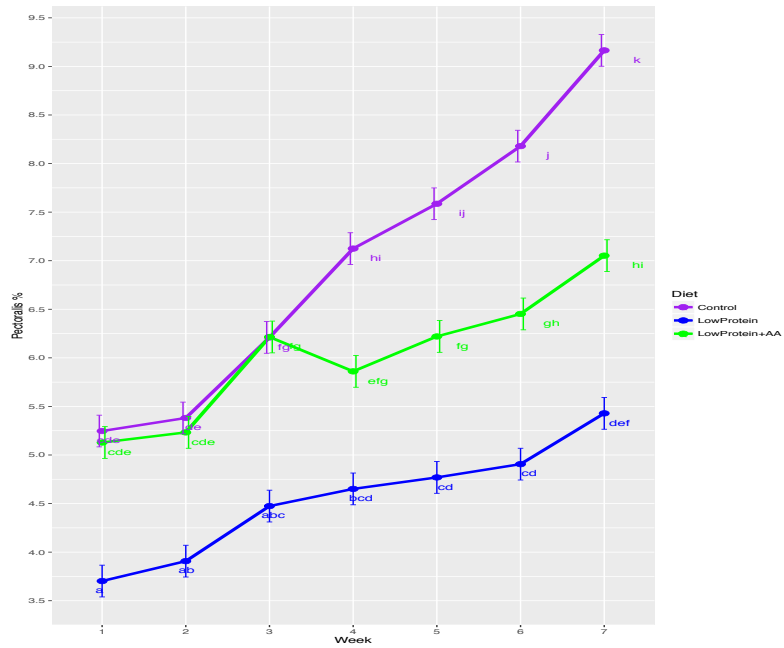


Figure 46. Effect of Diet and Time on Pectoralis to Body Weight Ratio

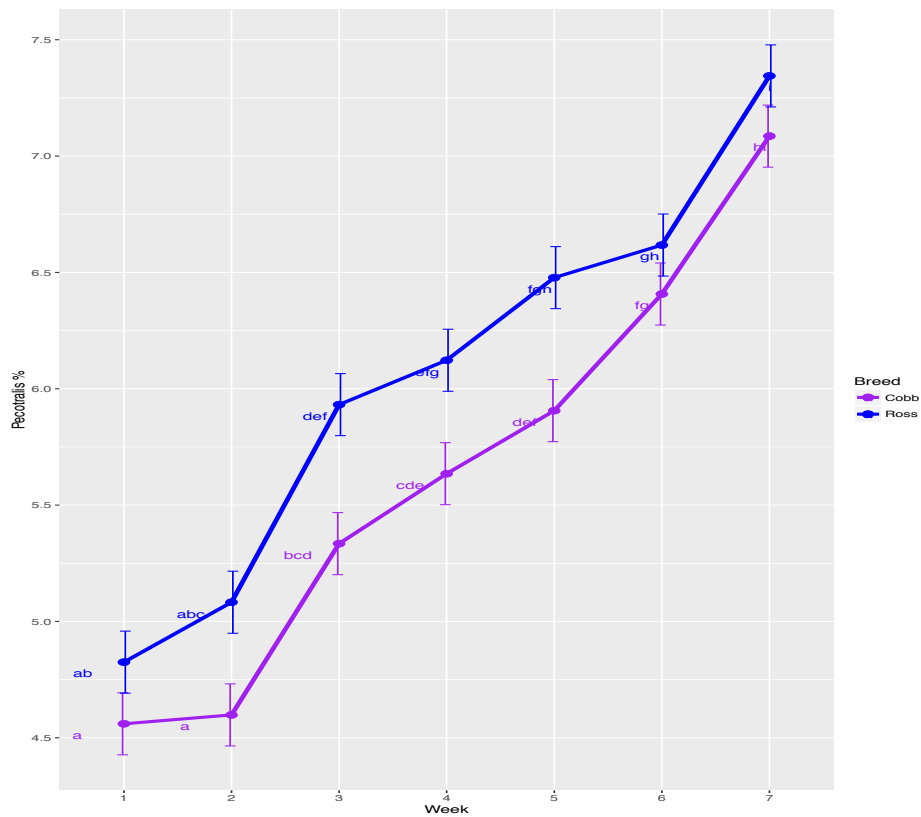


Figure 47. Effect of Breed and Time on Pectoralis to Body Weight Ratio

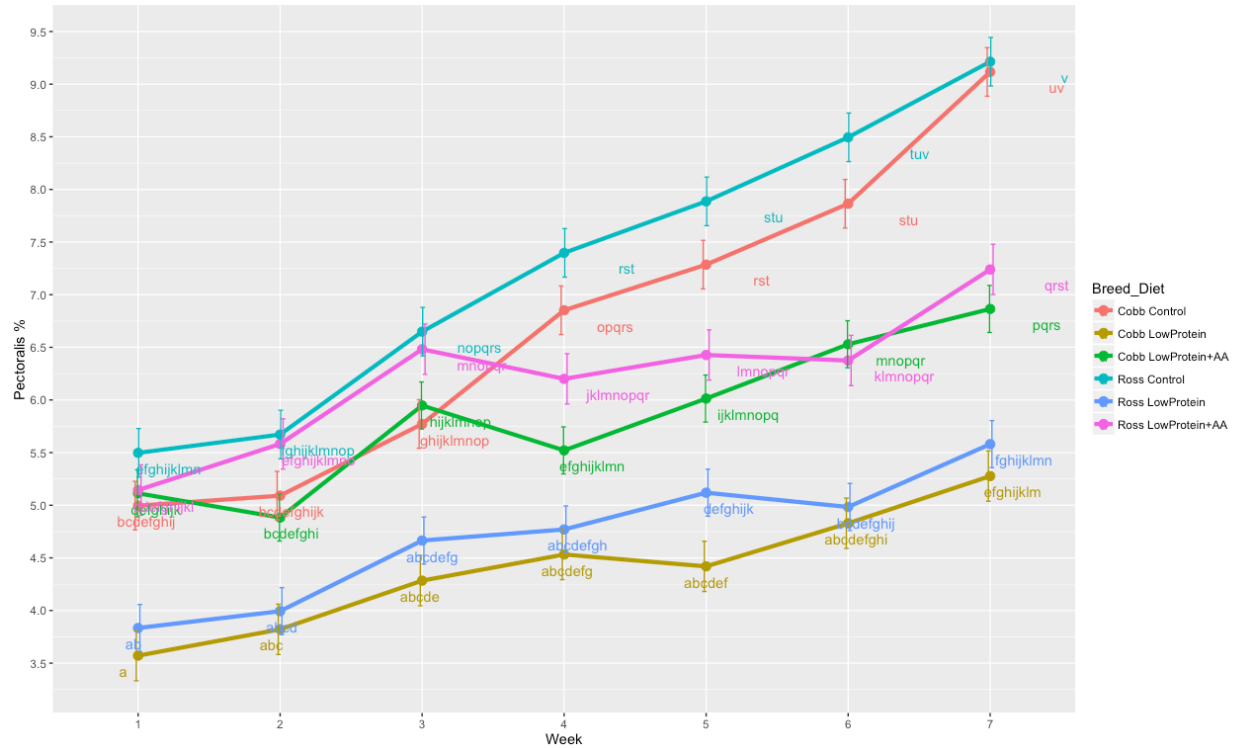


Figure 48. Effect of Diet, Breed and Time on Pectoralis to Body Weight Ratio

The diminished protein level and the breed of the birds had little influence on the relative peroneus growth (Table 6). Either breed on different diets failed to show any variation in peroneus to body weight ratio (PEBR), and two breeds on the same diet had really close PEBR (Figure 49). Regardless the breed effect, the birds fed all three diets had an erratic relative peroneus growth trend over time (Figure 50). PEBR of birds on different diets unexpectedly decreased after the 1st week, and the birds on the control diet has a week longer decreasing period than those on the other two diets. Moreover, PEBR of birds fed control diets tended to decrease after the 4th week. For the birds on low protein diet, there was a relative increase in PRBR during the grower phase, followed by a steady decrease during the last two weeks. Still, there was no statistical significance in those changes. Adding the CAA exerted more impact on PEBR, and there were three relative decreasing periods, 2nd, 5th and 7th week ($P > 0.05$). The PEBR between each week was not statistically significantly different, regardless of the diet

(Table 7). The PEBR was proportionately higher in birds fed CAA fortified low protein diets at the final week than those on the other two diets. The breed effect during the overall time was not significant on PEBR (Table 8), and neither breed showed significantly changes between each week. The two breeds presented similar growing pattern except during the last two weeks (Figure 51). The overall effect of diet, breed and time on peronaeus proportion was not significant ($P > 0.05$) (Table 9). Any variations among all 6 treatments were unable to be detected through the whole experimental period. Almost all of the groups reached the highest PEBR at 4th week, excluding birds of the two breeds fed low protein and Cobb broilers on CAA added diet (Figure 52). Ross broilers on the three diets had numerically higher PEBR than Cobb broilers ($P > 0.05$). The CAA added to LP diet fed to Ross broilers resulted in the relative highest PEBR.

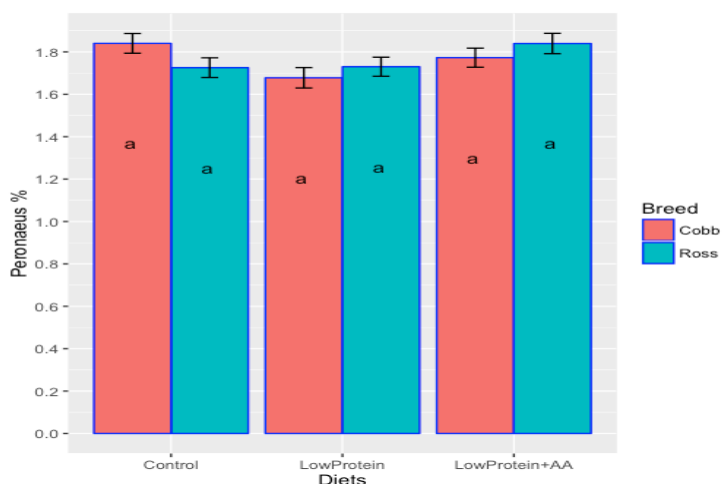


Figure 49. Effect of Diet and Breed on Peronaeus to Body Weight Ratio

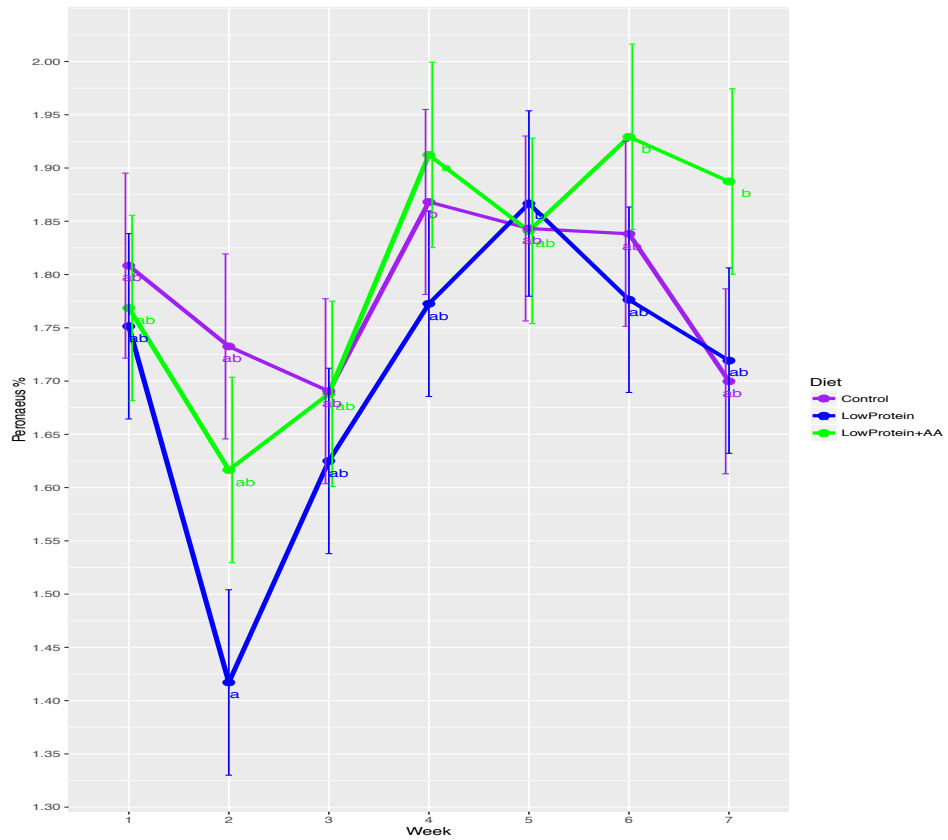


Figure 50. Effect of Diet and Time on Peronaeus to Body Weight Ratio

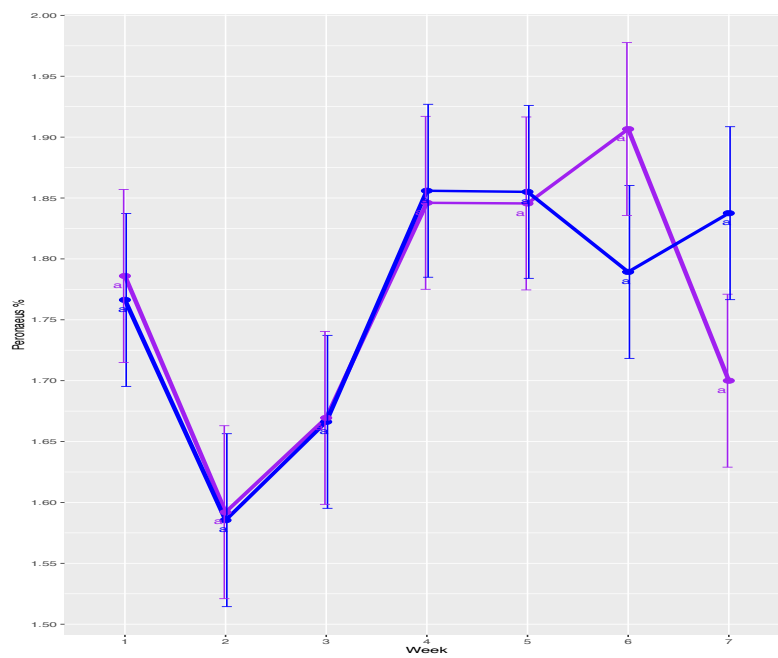


Figure 51. Effect of Diet Breed and Time on Peronaeus to Body Weight Ratio

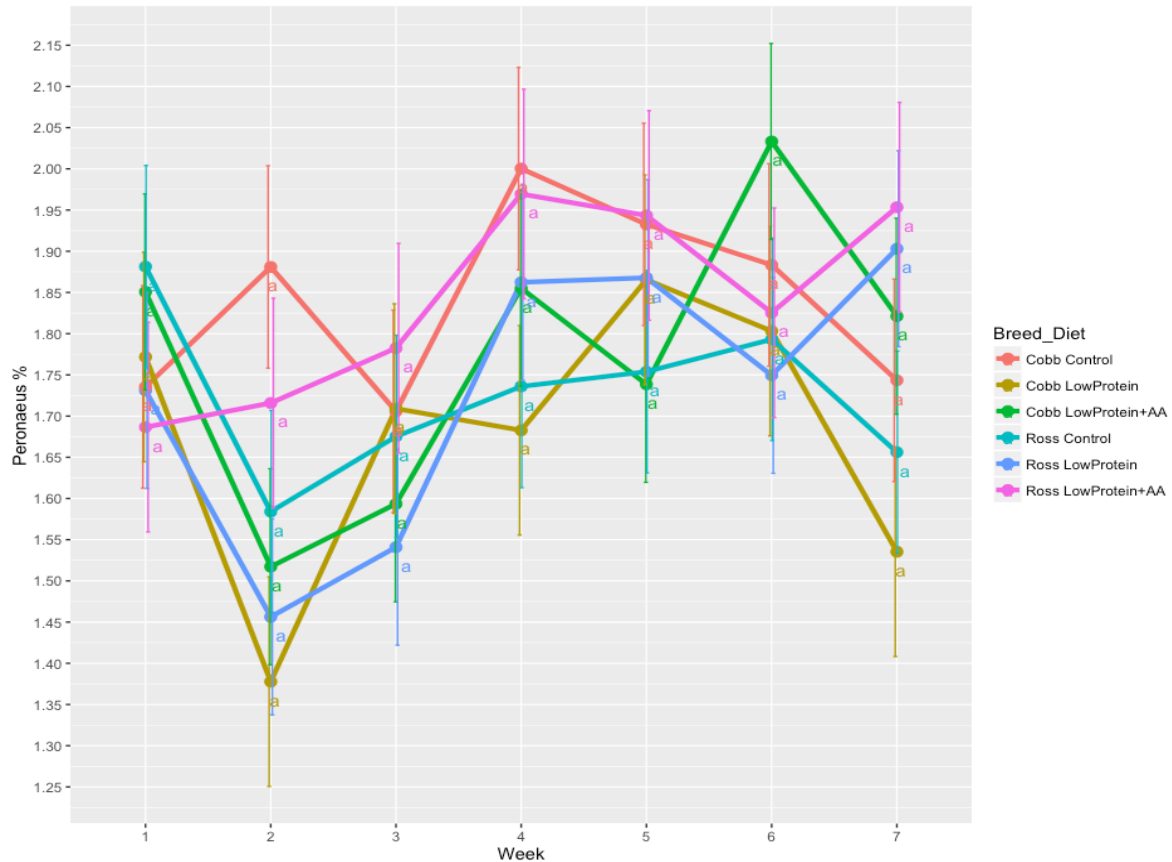


Figure 52. Effect of Diet, Breed and Time on Peroneus to Body Weight Ratio

Analogous consequences were found for the relative iliotibialis growth with the influence of breed and protein level. The breed and diet had no significant impact on changing the overall iliotibialis to body weight ratio (IBR) (Figure 53, Table 6). Even though there was no statistical difference among birds on the different diets fed over different weeks, the results still demonstrated how this type of muscle was affected by the dietary protein content throughout the time period. All three diets had an unfavorable effect on IBR, and decreased the IBR during the grower phase. The birds on control diets had the steadiest growing trend compared to those on the other two diets (Figure 54, Table 7). The restriction in protein content resulted in a sudden reduction in IBR during the 2nd and the 6th weeks, and resulting in the relatively lowest IBR ($P > 0.05$). The CAA supplemented diet featured a relative higher IBR at the beginning, but then

experienced a decline in IBR during different weeks. The relative growth of iliotibialis over the 7 weeks on each diet were not significant, but the birds on CAA supplemented diet displayed a significance increase between the 3rd and 7th week. The effect of breeds on IBR over weeks was shown in Figure 55. No significant effect on IBR was caused by breed during the overall experimental period. Both breeds experienced a trend for decreased IBR after the 1st week, and the relative growth ability was restored afterwards. However, Cobb broilers restored the growth ability in IBR a week earlier than Ross broilers. Ross broilers demonstrated a more changeable growing pattern than Cobb broilers, which largely fluctuated over weeks. Ross broilers started with a comparably lower, but ended up with a higher, IBR.

Both breeds exhibited no difference in IBR when fed the different diets over weeks ($P > 0.05$, Table 8), however, each breed on each of the certain diets showed erratic in relative iliotibialis growth patterns (Figure 56). Ross broilers on control diets started with the lowest IBR, but ended up with the highest IBR at the 4th week. Cobb broilers fed control diets reached the relative highest IBR a week later (5th week). Both breeds on the two low protein diets did not showed any significant changes over time, even with the CAA supplementation. There was an abrupt drop of IBR in Ross broilers on the CAA added diet, but soon 1st week. The two breeds on CAA added diets had the relatively highest IBR at the end of the experiment ($P > 0.05$) (Table 9).

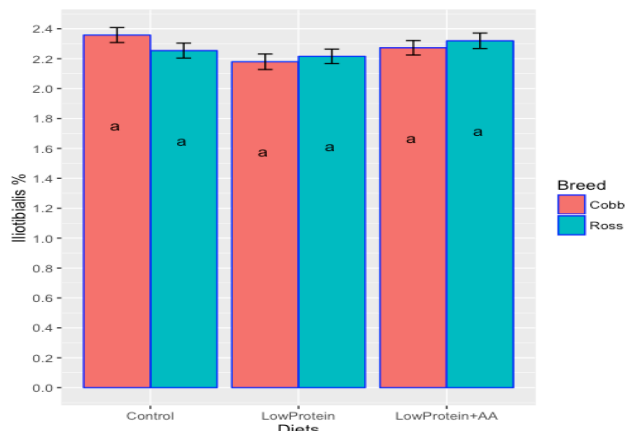


Figure 53. Effect of Diet and Breed on Iliotibialis to Body Weight Ratio

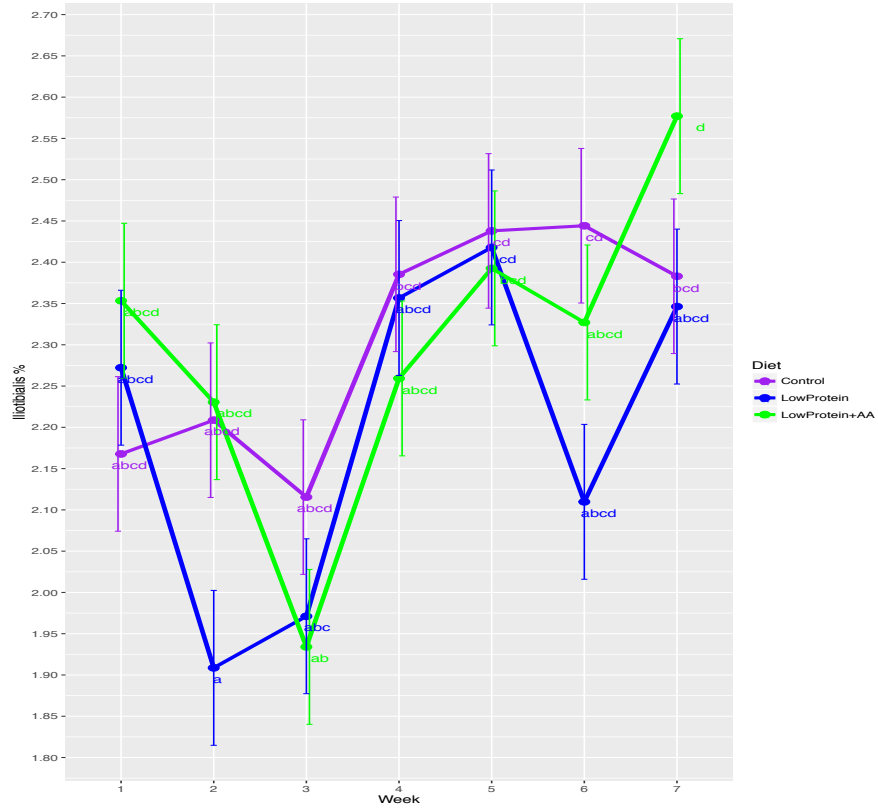


Figure 54. Effect of Diet and Time on Iliotibialis to Body Weight Ratio

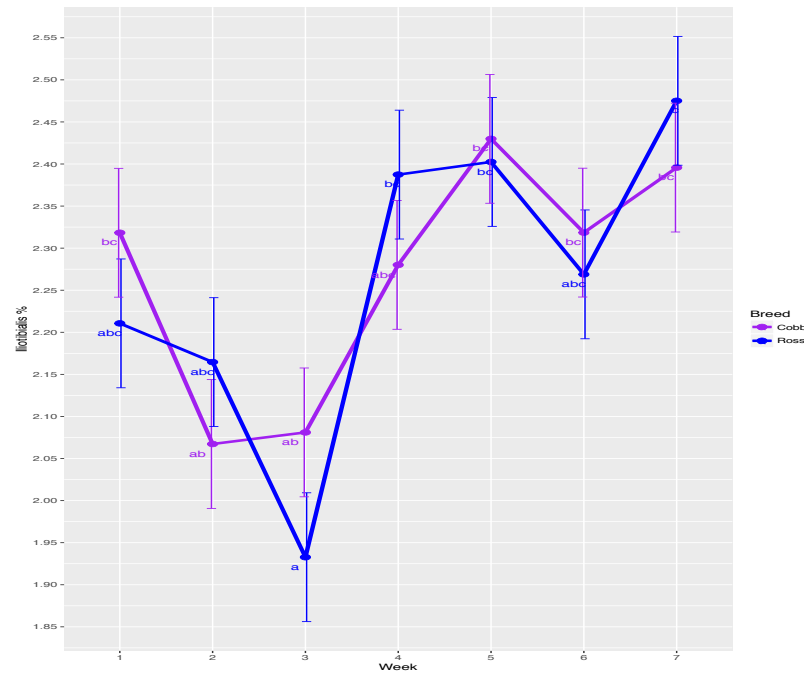


Figure 55. Effect of Breed and Time on Iliotibialis to Body Weight Ratio

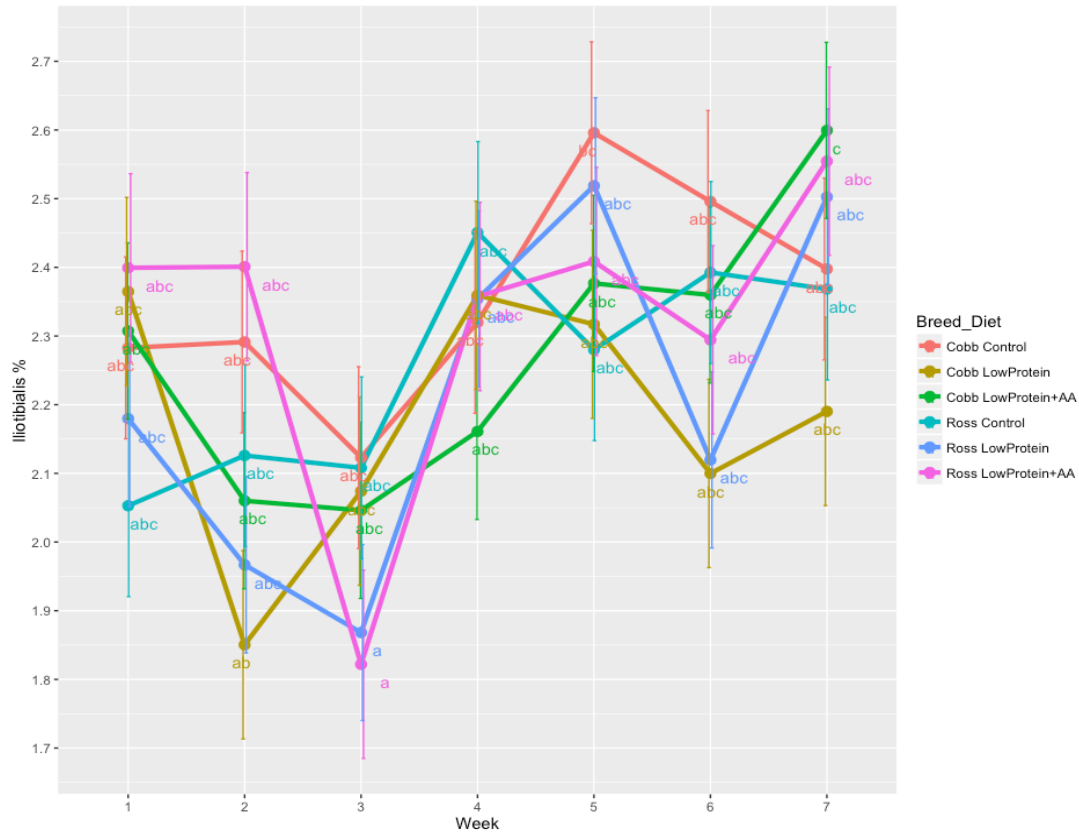


Figure 56. Effect of Diet, Breed and Time on Iliotibialis to Body Weight Ratio

The relative growth of all three bones with the influence of breed and diet were opposite to the organ or muscle growth pattern. Femur to body weight ratio (FBR) was significantly higher on low protein diet ($P < 0.05$), and there were no differences between the two breeds either on CAA added or control diets ($P > 0.05$) (Figure 57, Table 6). There was a significant difference in FBR between the two breeds, and Ross broilers had much higher FBR than Cobb broilers when both were fed low protein diets. Birds fed control diets had a constant decreasing trend in FBR over weeks except a flattening period during the 2nd week. The significant dietary effect was first found during the 5th week when birds fed control diet had a significantly lower FBR than those on the two low protein diets ($P < 0.05$) (Table 7). On the other hand, the effect of limited protein content and the CAA supplementation changed over time, and birds on both low protein diets shared with similar pattern for the most of the time. The birds on CAA added diet

had no significant changes in FBR through the whole experiment (Figure 58). However, the significant difference was found on the birds fed low protein between the 1st and final weeks. The breed affected the FBR less on each week than the diet, and Ross broilers had a relative consistent decreasing trend during all 7 weeks (Figure 59, Table 8). An abrupt decrease in FBR was first found at the 2nd week on Cobb broilers, and led to significant differences in FBR between these two weeks. However, differences from initial FBR in Ross broilers was not found until two weeks later (during the 4th week).

When looking at the collective effect of diet, breed and time on femur proportion development (Figure 60), both breeds especially Ross broilers on the control diet, had a relative constant decreasing trend through the weeks than any other treatments (Table 9). It took 4 weeks for Ross broilers on control diets to show significant decreases in FBR ($P < 0.05$). The significant time effect also showed with control diets fed to Cobb broilers, but the significant decrease in FBR from 1st did not appear until the final week (Table 9). Although each diet by breed combination exhibited highly various growth patterns in the femur proportion, there were only a few differences between each treatment among the weeks. The major differences were derived from the three diets fed to Ross broilers. The dietary effect on Ross broilers began to be determined at the 5th week when both breeds on low protein diets had significantly increased FBR ($P < 0.05$). Ross broilers on low protein diets produced significantly higher FBR than those on control diets, and these differences were detected during the 6th and 7th weeks. All three diets fed Cobb broilers resulted in a relative inclining trend in FBR, right after an abrupt decline between the 2nd and 3rd weeks. The Cobb broilers on CAA supplemented diets mitigated the sudden increase in FBR, compared with those on the other two diets. In spite of the rapid change given by the three diets in Cobb broilers, there was still no statistically significant variation

among them. Finally, Cobb broilers finished up with commensurably lowest FBR when on the control diet (Table 9).

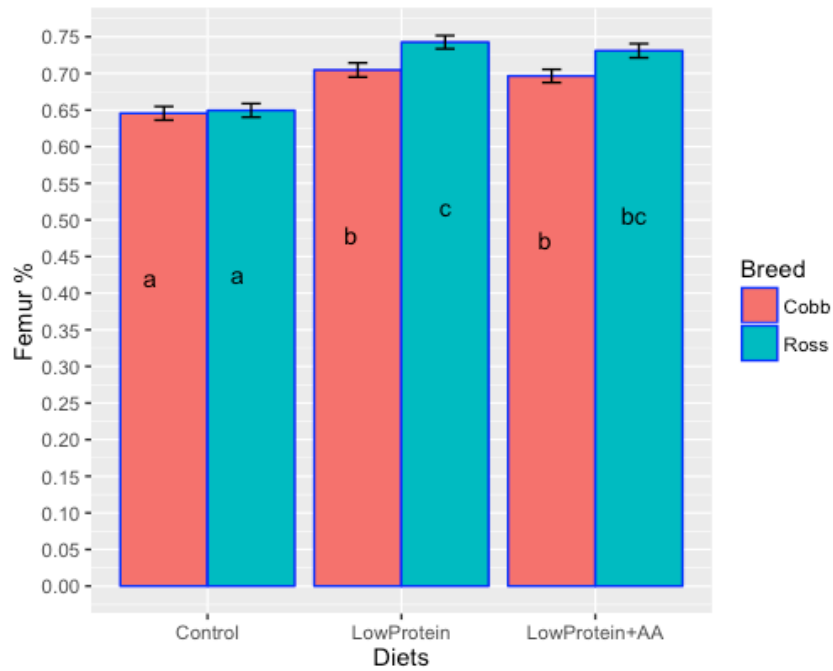


Figure 57. Effect of Diet and Breed on Femur to Body Weight Ratio

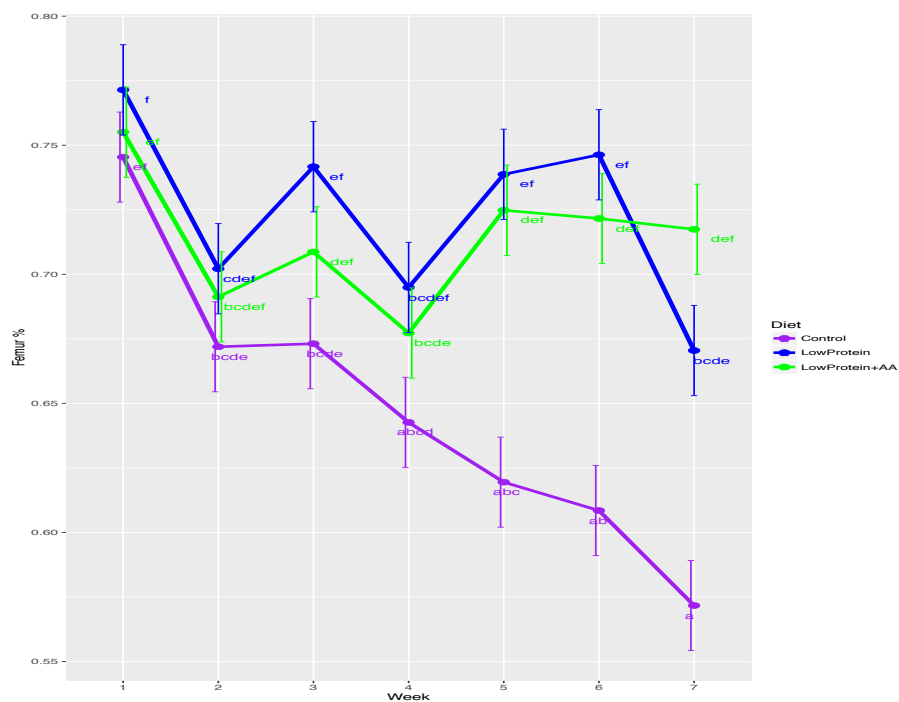


Figure 58. Effect of Diet and Time on Femur to Body Weight Ratio

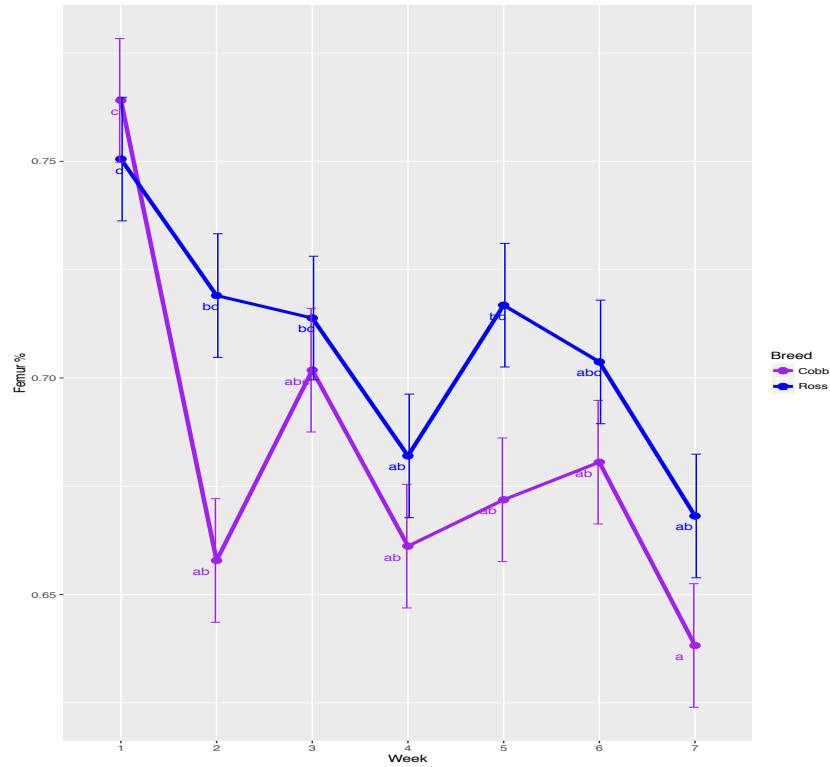


Figure 59. Effect of Breed and Time on Femur to Body Weight Ratio

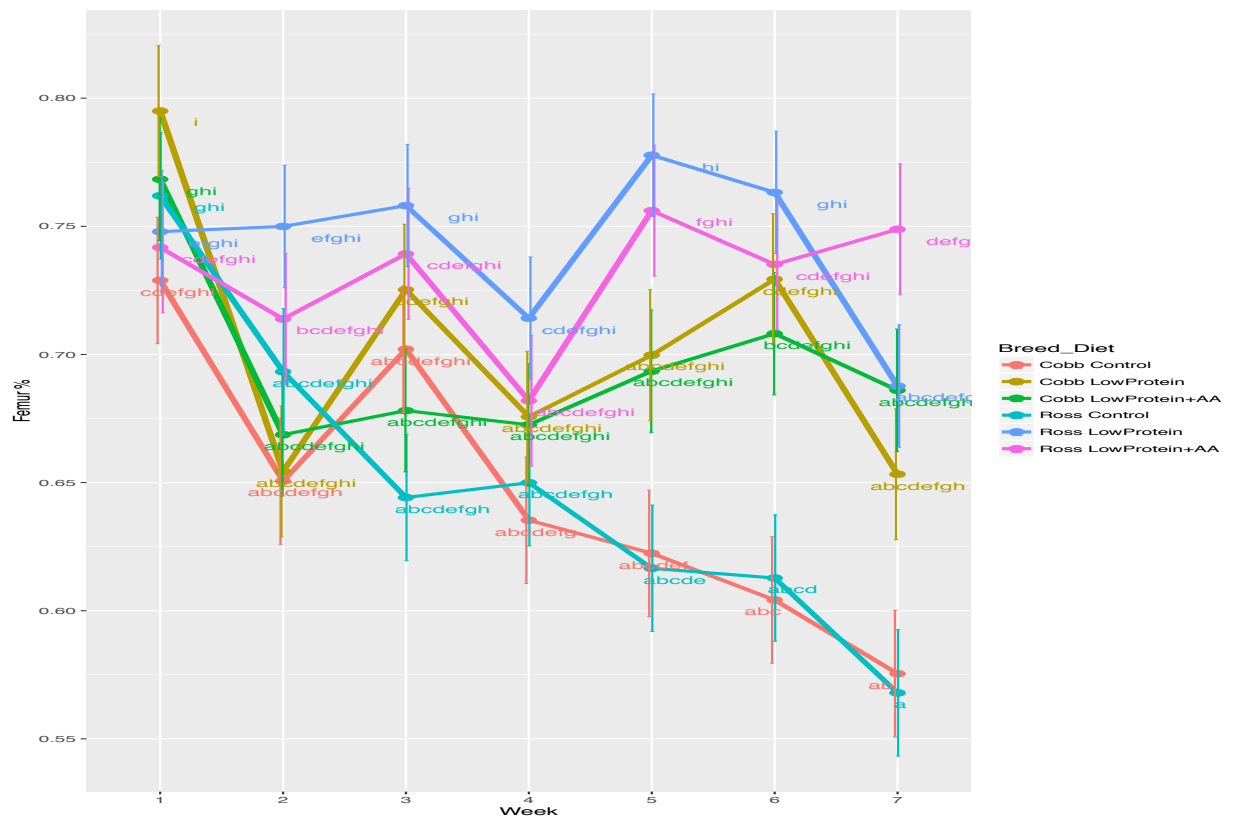


Figure 60. Effect of Diet, Breed and Time on Femur to Body Weight Ratio

The effect of breed by diet on relative tibia growth was shown in Figure 61. The restriction of protein fed Ross broilers possessed a significant increase in the tibia proportion compared to those on control diet. On the other hand, CAA supplementation significantly increased the tibia proportion in the two breeds compared to birds on control diet (Table 6). All the growth curves for corresponding diets experienced fluctuation during the whole experimental time, but the birds on the control diets seemed to have more stable decreased trends than the other two groups (Figure 62). The time effect on TBR was only detected in birds on the control diet, but the difference in tibia proportion was only found between the 1st and 7th weeks. No significant effect exerted by time was discovered on either the low protein or CAA supplemented diets. The decreasing trend in TBR from birds on low protein diets lasted only one week, and then the tibia proportion tended to increase until the 6th week. Similar growth patterns were also found on birds on CAA added diet, with the exception that the period of decrease was a week longer compared to birds on low protein diets. The difference in TBR among different diets was first detected during the 5th week, which was mainly derived between the CAA added and control diets. This variation lasted until the last week. Birds on low protein diets showed a difference in FBR from control diets during the 6th week, but the difference disappeared due to the relative decline during the last week (Figure 62). There were no differences in TBR between the two breeds throughout the study (Table 4). Both breeds possessed similar relative tibia development (Figure 63) after the decreases during the first two weeks. Ross broilers expressed a longer and faster increasing trend compared to Cobb broilers. The two breeds had a sharp decrease in FBR during the last week and ended up with a 9% and 10% losses in TBR compared to the initial values, respectively. Among the cumulative impact of the protein level, breed and time, the diet affected tibia proportion the most. All the growth patterns for tibia proportion can be generalized

by diets. The two breeds on the same diets shared analogous trends during each week (Figure 64). Control diet fed Cobb broilers had the most stable decreasing trend throughout the whole time among these 6 treatments. The birds in all treatments had a decrease in TBR during the first two weeks, except those on low protein diet which increased right after the 2nd week. The added CAA sustained the decreasing trend on both breeds until the 2nd week. Cobb broilers had similar two-week sharp incline when feeding low protein diets, while Ross broilers also displayed a longer increased growth. There was no statistically significant difference among each treatment on each week, with the exception that Ross broilers on CAA added diets produced significantly higher TBR ($P < 0.05$) than those on the control diet during the final week.

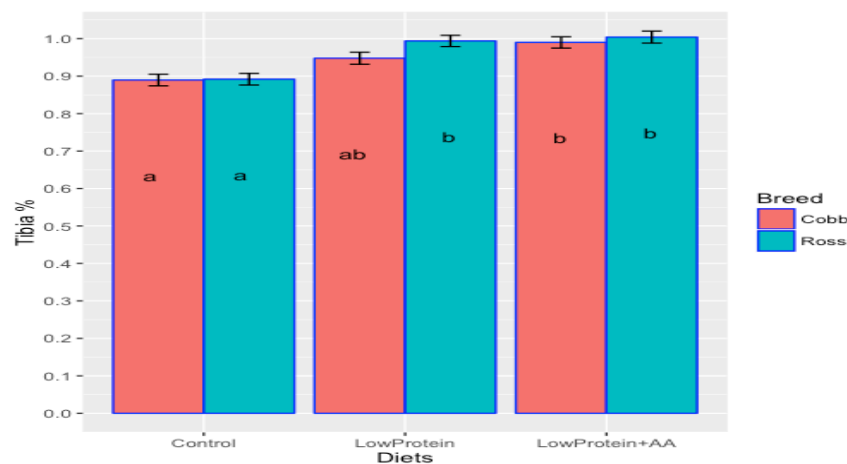


Figure 61. Effect of Diet and Breed on Tibia to Body Weight Ratio

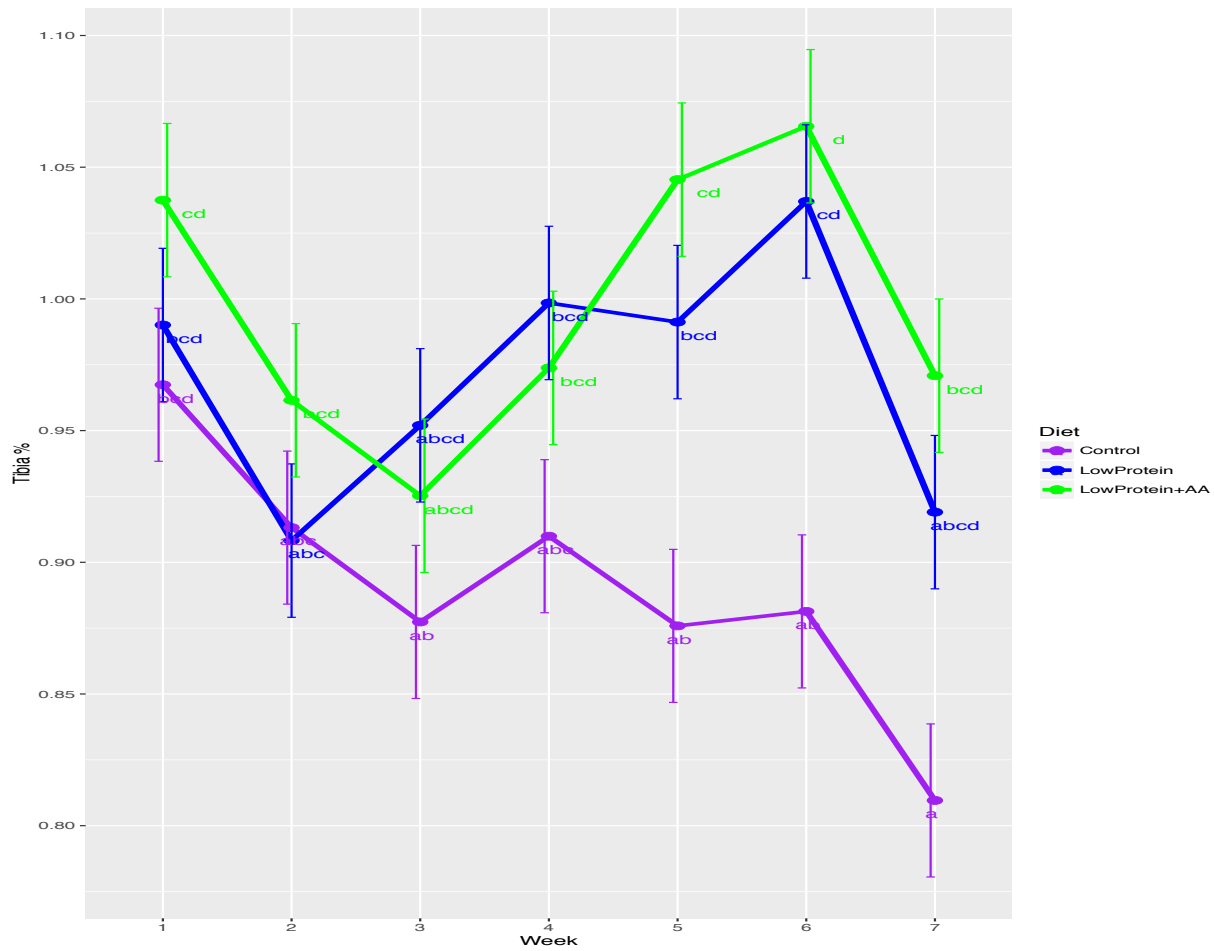


Figure 62. Effect of Diet and Time on Tibia to Body Weight Ratio

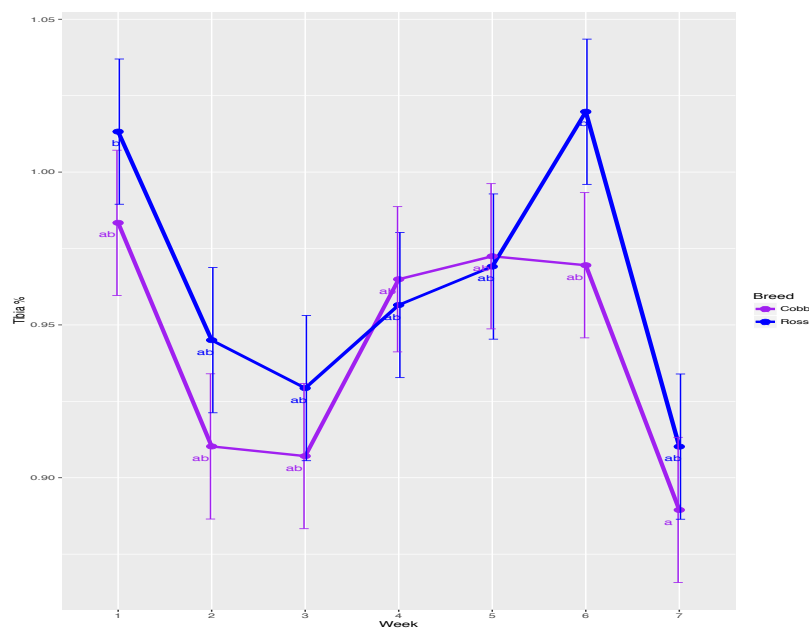


Figure 63. Effect of Breed and Time on Tibia to Body Weight Ratio

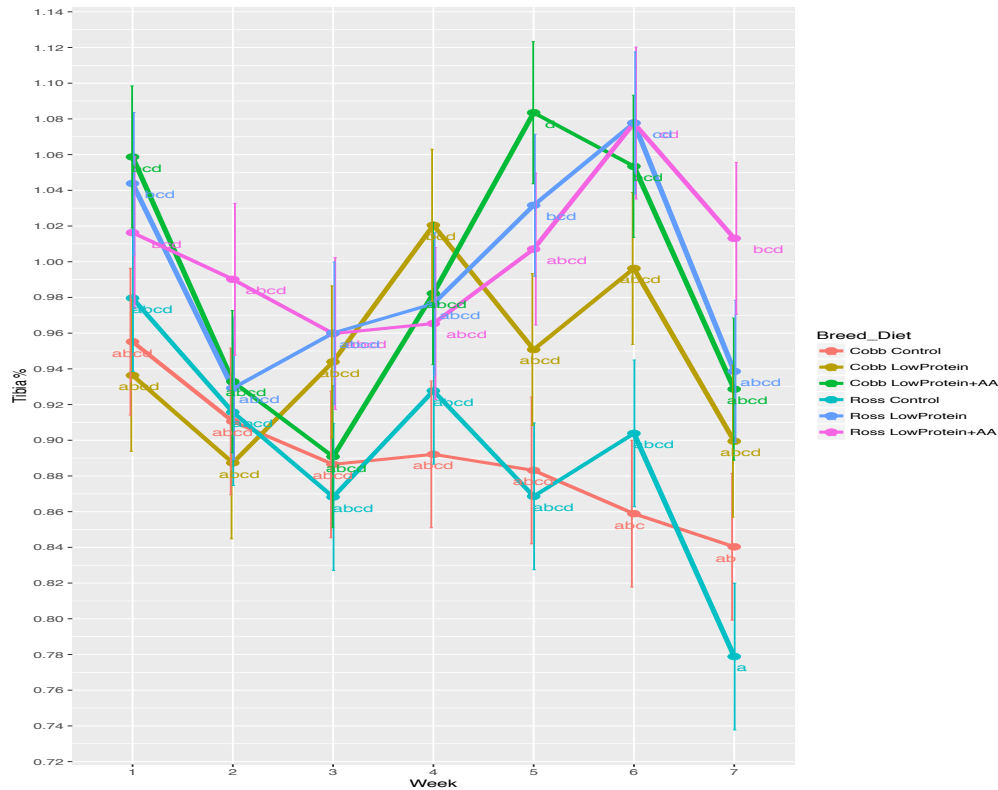


Figure 64. Effect of Diet, Breed and Time on Tibia to Body Weight Ratio

Relative radius growth was less influenced by either the protein level or the breed. No differences in radius to body weight ratio (RBR) were shown across all 6 treatments (Table 6). However, Figure 65 showed that Ross broilers had a relatively higher radius proportion by feeding both protein limited and CAA supplementation diets. Cobb broilers, on the contrary, possessed a relatively higher RBR than Ross broilers, when they were on the control diet. The relative radius growth trend with different dietary protein contents were uniform over weeks. After a sudden drop after the 1st week, RBR began to maintain similar growth among the different diets (Table 7). However, although no statistical significance was caused by diets on RBR on each week, the birds in the control group had a continuing tendency to decrease in RBR through the whole time compared to those on the other two diets. Both low protein and CAA added diets contributed a mild increase in radius proportion during the 5th week and 6th week,

respectively, which finally ended with relatively higher RBR than the birds on control diets (Figure 66). The relative growth of radius with the dietary effect enjoyed less fluctuation during the growing period, compared to the other two bones (Figure 66). Identical growth trends were found for the two breeds over weeks. Both breeds experienced a rapid decline after the 1st week, and then maintained similar RBR in the rest of weeks (Figure 67). Furthermore, the two breeds did not exhibit any difference in RBR between weeks (Table 8), but Ross broilers had increased RBR during the 4th week. The different diets for the two breeds resulted in similarly rapid drops right after the 1st week, and all the treatments started showing significantly lower RBR at the 2nd week. However, Cobb broilers fed low protein started with the relatively lowest RBR, and did not show a difference until the 5th week (Figure 68). The low protein diet fed to Ross broilers gave a relative unstable growth tendency including a sudden inclining at the 2nd week. The control diet fed to Ross broilers contributed the most constant decreasing trend through the whole study with only a mild increase at the 4th week. All treatments reached a constant RBR after the 2nd week.

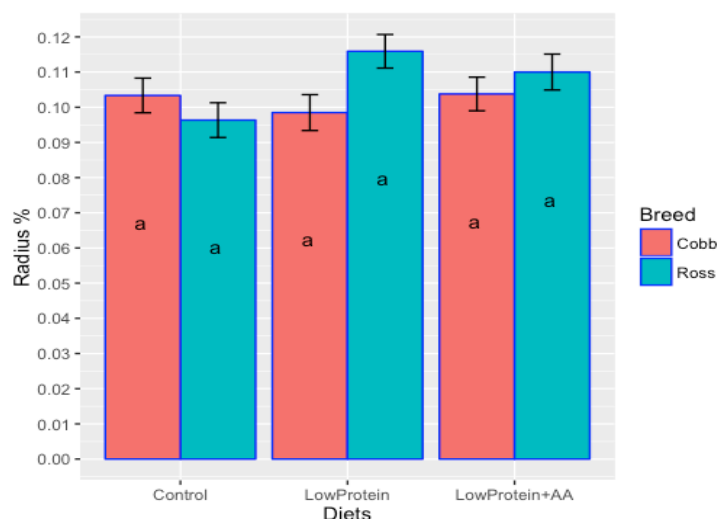


Figure 65. Effect of Breed and Diet on Radius to Body Weight Ratio

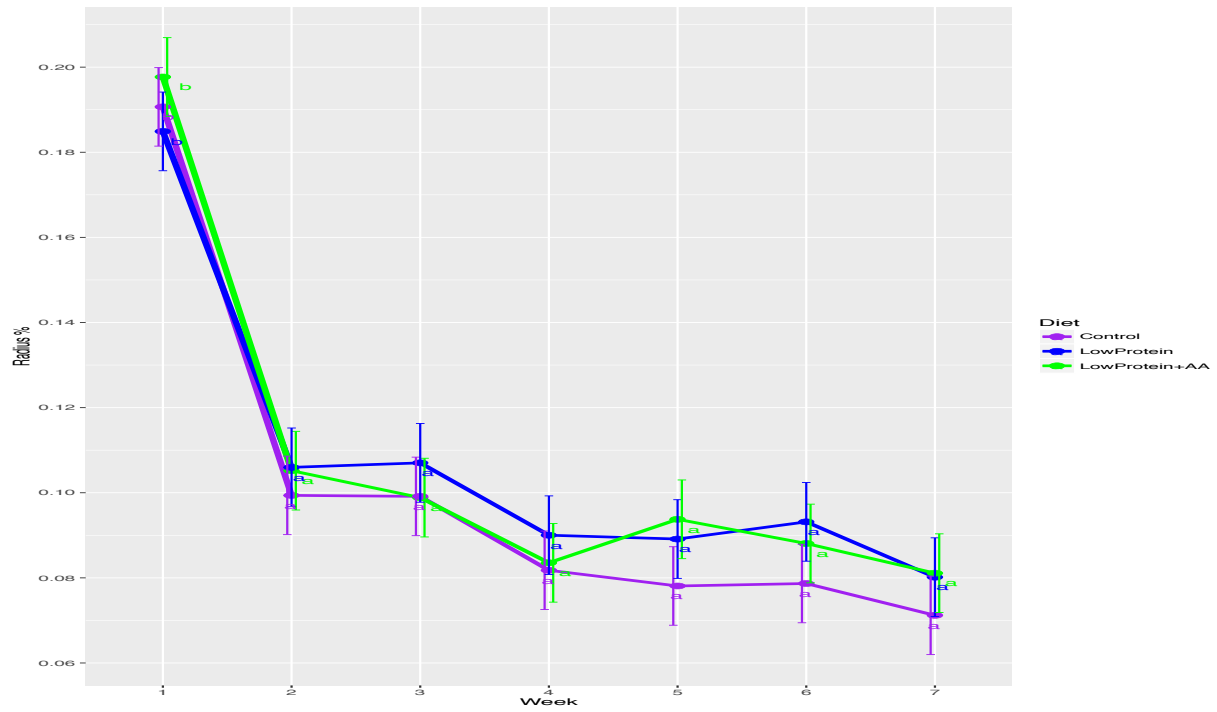


Figure 66. Effect of Diet and Time on Radius to Body Weight Ratio

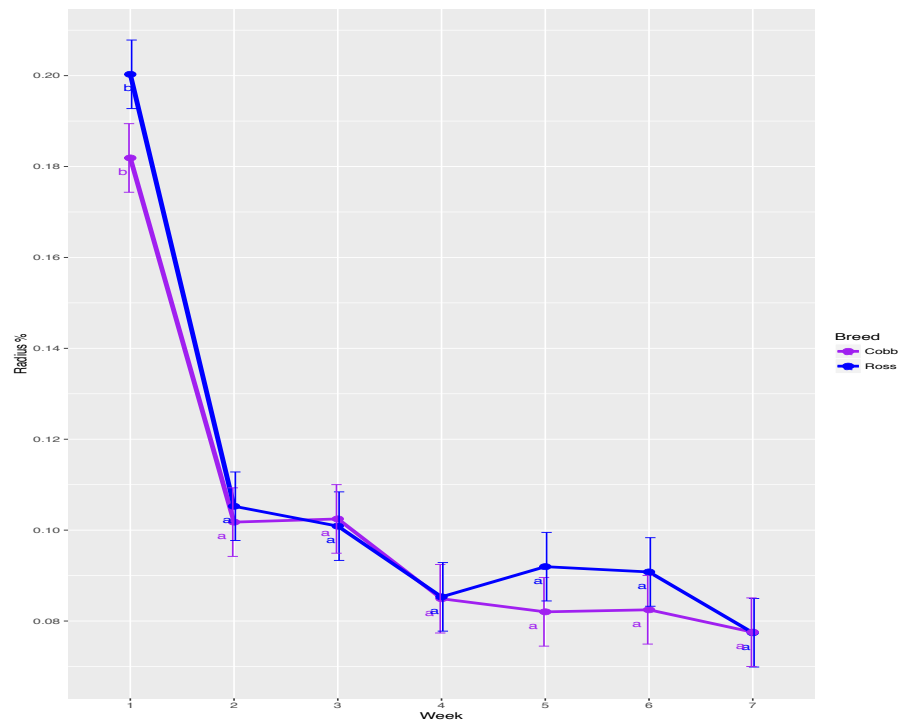


Figure 67. Effect of Breed and Time on Radius to Body Weight Ratio

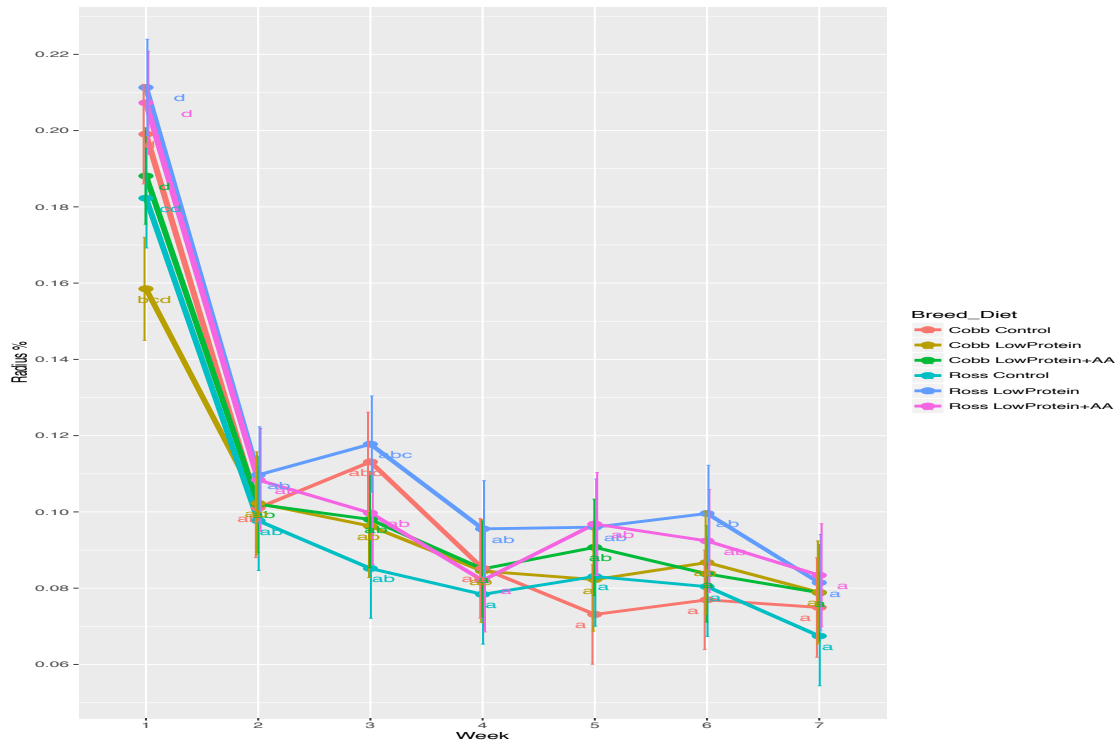


Figure 68. Effect of Diet, Breed, and Time on Radius to Body Weight Ratio

The relative abdominal fat deposition was significantly affected by the diets, with protein restriction increasing the fat proportion in both breeds. But the supplementation of CAA resulted in a fat percentage that resembled that of the birds on control diets. The Cobb broilers on all three diets had a relative higher abdominal fat percentage than Ross broilers, but there was no statistical significance ($P > 0.05$).

5. DISCUSSION

The reduction of crude protein level by nearly 6% units in both breeds' diet generally rendered a retarding effect on the general growth even with the compensation of CAA. The results, to a certain degree, were in agreement with previous studies on the negative effect of protein scarcity on body weight gain, and crystalline amino acid was not effective enough to replace the crude protein in the diet (Pinchasov *et al*, 1990; Colnago *et al*, 1991, Han *et al.*, 1992, Yamazaki *et al.*, 2006, Namroud *et al.*, 2008). But when looking into the process of how limited protein content and CAA influence the body weight, visceral organ, muscle, and bones over time, the results tended to be different from the former studies. It is not surprising that the reduction of crude protein level inevitably resulted in lowered body weight gain because of the deficiency in dietary essential amino acid especially lysine, methionine, and threonine, which are respectively the first, second and third limiting amino acid (Edmonds *et al*, 1985). Our results indicated that body weight could only be maintained in the low protein diet with the addition of CAA until the 3rd week. This results are in accordance to Schutte (1987) who reported that young chick's growth could be fortified by feeding at the low protein level of 16% if same CAA level as in 20% CP was supplemented in feeds. There is disagreement among studies over the effectiveness role of adding certain amino acids in inhibiting abdominal or hepatic fat deposition. Our results implied that low protein diets tended to increase more abdominal fat deposition, but no significant differences in actual fat weight was found among the broilers of both breeds fed the three diets. This contradiction could be due to the limited amount of abdominal fat samples. However, the difference in abdominal fat proportion did exist among broilers of the two breeds fed the three diets. Coupled with the lower body weight in low protein diets, the abdominal fat was more favorably deposited under protein restriction conditions. Our results also indicated that

the supplementation with CAA significantly improved the growth performance of birds on low protein diets, but was unable to contribute equal EAA levels as the control diets. The addition of CAA did decrease the proportion of abdominal fat, which indirectly assumed its certain effectiveness on restoring body weight gain without in compensation with abdominal fat. This observation partially agrees with the previous studies (Bunchasak et al., 1996, Leclercq et al., 1994) that proved the role of Met+Cys and arginine in decreasing abdominal fat deposition. On the contrary, Namroud (2008) implied the less usefulness of adding Lys, Trp, Thr, and Arg on reduction of abdominal fat, even though a level in excess of the normal requirement was added. Also, both dietary composition and the ratio of macronutrients play a key role on chicken growth performance (Collin et al., 2003), and the higher energy to protein ratio generally promotes the fat retention, which would explain the relatively higher fat deposition in the birds on low protein diet in the preset study. The potential mechanism of reducing fat retention in birds fed high energy to protein ratio could be associated with the decreasing triglyceride content in liver caused by the supplementation of glycine and glutamate to low protein diet (Bunchasak et al., 1998). On the other hand, the surplus protein or amino acids can also impair poultry growth performance by accelerating the heat increment. The body has rid of extra amino groups by the transamination, and finally turn the amino acids into uric acid (Namroud, 2008). This process requires the use of energy. Cobb broilers had a heavier body weight than Ross broilers at the final week, and this could be the reason for the relatively heavier fat amount collected from the Cobb broilers on all three diets. Together with the higher fat proportion found on Cobb broilers, the results indicated this breed has more potential for abdominal fat deposition, and may have less value in selling of carcass parts.

The body weight decreased in the birds on low protein diets, and was also accompanied

with decreases in the heart and liver weight. Our results indicated that the absolute heart and liver growth was regulated by the protein content, and the administration of CAA had no obvious effect on maintaining either weight. Table 6 implied that the relative growth of both organs was significantly increased, which means that protein restriction resulted in the higher proportion of these two organs. There were two reasonable mechanisms behind the increase in heart proportion of birds on low protein diets, especially during the early stages of the birds' growth. Results from Table 7 showed that the heart proportion became higher in birds on low protein than those on control diet at 2nd week, and then the variation was not detected within the next two weeks. It is assumed that the higher heart proportion may be caused by abnormal heat production. It is known that heart is an important component of the circulation system, and pumps the blood throughout the whole body to deliver nutrients and oxygen, and the removes the metabolic waste like CO₂ from the tissues. The circulation system will help regulate the body temperature to adapt to different environmental conditions, as the ambient temperature is crucial for the chicken growth performance. Higher sensitivity to temperature has been instilled into broilers along with the successive selection for rapid growth rate (Cahaner et al. 1995). The two breeds used in our research are widely used in the many poultry industry, and may indicate their need for more heat dissipation than other breeds. Our experiment was conducted with the average ambient temperature 29.7°C, which is considered as relatively higher than the optimal growth temperature for broilers. High ambient temperature was reported to depress body weight gain (Cahaner and Leenstra, 1992; Cahaner *et al*, 1993). Protein is considered as a high caloric nutrient. Thus, the reduction of protein content should have decreased the heat production of the birds and result in the reduction of heart proportions. Previous studies pinpointed that the lack of certain amino acid like Lys, Arg, Met, and Trp (Carew et al., 1997) would cause increased

concentrations of plasma triiodothyronine (T3), which is highly associated with heat production. The other mechanism of increasing heart proportion of birds on low protein diets involves the growing priority of different parts of the body. Different body parts have various priorities of development, and lowered protein level might restrain the developing process of the birds. Those birds on the low protein diet might still undergo development of the heart even after three weeks, while birds on the control diets had already entered the next development step. This mechanism could also explain that the broilers on all three diets finally reached a constant heart proportion in the late of the experiment.

The liver weight was expected to increase similarly to the the elevation in abdominal fat deposition caused by the lower ratio of protein to metabolic energy, according to the more active *de novo* lipogenesis in the liver of bird on low protein diet (Rosebrough and Steele, 1985; Swennen et al., 2006). Our results didn't quite agree, but liver weights decreased with lower protein levels. The supplementation of CAA was only able to sustain the absolute liver growth until the 4th week, and was unable to reverse the retarding effect of protein restriction in the remainder of the trial. The reason for this contradiction might be caused by the excessive limited protein level in this study, which rendered an inhibiting effect on the liver growth. When taking the breed effect into consideration (Table 5), the variation found on the 4th week in liver weight between CAA added and control diets was mainly in the Ross broilers, which was inconsistent with the results on the absolute heart growth. Heart weight of the two breeds on the control diet was significantly heavier than with the other two diets by the 4th week. These results implied that the potentially different abilities of the breeds in regulating liver and heart growth on different protein contents. The two breeds also had relatively variations in the utilization or adaptation of CAA, even though neither the absolute liver weight or percentage were different between two

breeds. However, the liver percentage weight increased with two low protein diets even with the addition of CAA, which is in agreement with the Namroud (2008). Also, similar consequences were found in our studies about the effect of supplemental CAA in reducing liver proportions compared to birds on low protein diet (Table 6). The mechanism involved in the increase in liver proportion by feeding the lower protein level could be associated liver function. Liver is one of the most important organ responsible for protein metabolism. Protein or amino acids will be broken down, absorbed in intestine, and then be transported to the liver where they are further distributed to other tissues and organs via the circulation system. The surplus amino acids in liver, if they are not used for the synthesis of proteins, would be catabolized into alpha-keto acids and ammonia. A similar consequence would also be found when the limiting amino acids are deficient and the rest of amino acids would be oxidized for energy instead of being used for structural or regulatory purposes. Low ratios of protein to energy can also lead to the lower use of protein in the diet, as the extra carbon skeleton provided by carbohydrates cannot get sufficient alpha-amino group from amino acid through transamination. All of these situations could result in the increased serum ammonia levels. High serum ammonia levels are toxic to birds and so increased metabolism in the liver is required to keep it below the toxic level. Higher ammonia levels in blood activate mechanisms in the liver, which may cause increased relative liver weight in order to adapt to the inflated ammonia level. The higher ammonia level can also explain the decrease in body weight, as the more active enzymes are used for uric acid production, the more inhibition of growth in the animal (Schimke, 1963). Namroud (2008) reported an increase in blood ammonia level for the birds fed CAA fortified and low protein diets, and concluded the correlation of CAA addition and higher ammonia level was based on a higher absorption rate of CAA than of intact protein. However, our results indicated that CAA

addition may not increase ammonia level in blood as the liver proportion of birds fed the low protein and CAA was decreased. This discrepancy might derive from the amount and kinds of CAA added in the diet. Namroud (2008) supplemented with excessive amount of CAA in low protein diet, and this was even higher than the level of EAA in the control diet in our study. Further research is necessary to determine if the protein restriction or which specific amino acids would inhibit or accelerate the activity of liver enzymes for uric acid production.

. The growth pattern of muscle is different from the visceral organs, bones, and fat. Generally, muscular tissue starts developing prenatally with continuous weight increase. Then the fattening phase takes growth priority after the muscle achieves its growth plateau, decreasing the muscular tissue percentage. Normally, animal development is affected by the internal factors like genotype, and external factors including nutrition. Three muscles were removed from the birds in our studies, pectoralis, pernaeus and iliotibialis, which represented the most popular cuts of chicken. The pectoralis (breast) is considered as the most valuable cut of the chicken, and had the most culinary value in poultry (Abeni et al., 2001). A slight decrease in breast yield might significantly impacted poultry market. The deleterious effect of protein restriction on pectoralis development were shown in Table 4.2 and Figure 4.15 indicated that birds started growing differently under the three nutrition plane at the 3rd week. It was also the time when the promoting effect of CAA ceased so birds were no longer able to maintain pectoralis growth fed low protein diet. The variations mainly came from the differences between Ross broilers fed the control and low protein diets. The variation in pectoralis appeared one week earlier than for the other two muscle tissues, which might be a result of their difference in biochemical and histological properties. Pectoralis is mainly composed of white muscle fibers, and is characterized with large diameters and larger quantities of glycogen because of the different

metabolic function from red muscle fibers. Marcu et al. (2011) and Suchy et al. (2002) reported that the much higher protein content was found in white fibers, almost 20% more than in red fibers. The development of larger diameter fibers inevitably requires higher density of proteins. Thus, the development of pectoralis required higher density of crude protein or addition of more amino acids, compared to the other two muscle tissues that are comprised of higher level of red muscle fibers. This assumption is in accordance to Marcu et al. (2013) who reported higher protein to energy level increased the muscle fiber size and the cross sectional area. Also, Roy et al. (2006) proposed the role of high density of protein in promoting the hypertrophy of white muscle fiber in animals. Moreover, Tang et al. (2007) studied the influence of metabolic energy and lysine supplementation on breast yield, and found that the addition of lysine in a lower protein diet (19%) increased the pectoralis weight. Similar results were also reported by Temim et al. (2000), and a lysine deficiency retarded the carcass performance of 50 day-old chicken. However, our studies found the balance of lysine content equal to control diets plus the beneficial effect of other various CAA didn't lasted more than three weeks. The discrepancy might be attributed by the extent of lowering crude protein level, which was almost 3% units lower in our studies than the other reports. Our study further found that the adverse effect of excessive restricted protein content cannot be reversed, even with the CAA addition. Moreover, the birds in the early growing period may have less demand for high protein diets, as they had not entered the major muscle development stage.

The other two muscle tissues from thigh and drumstick, presented a relatively different growing pattern from the pectoralis. These two types of muscle are generally composed of red muscle fibers, which have lower protein content and higher lipid content. However, they were affected by different factors according to our study. First, peroneus was not affected by the

dietary protein content until the 4th week, and difference in peroneus was mainly caused by the protein restriction. Our results indicated that the two genotypes did not grow differently in both skeletal muscles, but the Cobb broilers had relatively heavier weights in these two muscles than Ross broilers on the low protein diet. This could be attributed to the significantly heavier body weight of Cobb broilers than Ross broilers in response to the protein scarcity and the utilization of CAA. Although several studies investigated the effect of low protein concentration with isoenergetic diets on broiler carcass characteristics, most of those compared the boneless thigh weight or proportion (Kidd & Kerr, 1996, Widyaratne & Drew, 2011, Liu et al., 2014) various protein concentrations. There was no study that measured specific muscles except the pectoralis, unlike our study. Thus, there are no relative results that can be used to compare with results of our study and provide more specific information on growth of different muscles with different dietary protein conditions.

The distribution of the muscle tissue in different part of carcass is equivalent or more important than the gross carcass composition. The alteration of nutrition or genotype may possibly increase proportion of high-valued cuts compared with lower cost cuts. Wholesale or retail cuts may be more affected by the muscle or fat distribution. Namroud et al (2008) reported that limiting protein levels to low as 17% without CAA supplementation did not diminish the relative growth of pectoralis or the thigh. Our results showed the retarding effect of protein limitation on the pectoralis proportion, which was evident as soon as the first week of sampling. Our results also confirmed the role of CAA in maintaining the relative growth of pectoralis during the early growth. Widyaratne & Drew (2011) reported the reduction in white muscle yield percentage by feeding lower protein diet (19%) until the 35 days. In our observation, all breed by diet combinations had a relative more constant increasing trends of relative pectoralis except

with Ross broilers on the CAA added diet. Both breeds fed with CAA added diet had a decrease at the 3rd week, after which CAA addition was no longer able to maintain similar pectoralis proportions to the control diet. In opposition to the absolute growth, Ross broilers had a relatively higher level of pectoralis distribution than Cobb broilers, especially when fed the control and CAA added diets ($P < 0.05$). Liu et al. (2015) reported a different observation on the pectoralis proportion which did not show any decline in 84-day-old Wuji chickens (indigenous breed in China) on the low protein diet, although lower protein diets did reduce the absolute weights of pectoralis. This divergence result from the different strains used in the experiments, and this indigenous breed from China has longer growing period, and relatively slower growing rate than the two strains used in our study. Regardless of genotype variation, muscle samples were taken late in the 84th day when birds had already finished the muscle growth, and entered the fattening stage. The adverse effect of low protein diets on pectoralis proportion showed up at very early stage according to our results, while the other experiment started with 46-day-old chicken. Chicken were on normal diet before being put into low protein diet, and these birds would definitely have more developed musculature, and less carry-over effect would be exerted on the later relative pectoralis growth. Our results also showed that even though heavier muscle weight was obtained from Cobb broilers on low protein diet ($P > 0.05$), Ross broilers demonstrated more potential of pectoralis yield percentage. The protein limitation retarded more body weight but less muscle yield on Ross broilers than Cobb broilers. Unlike the growing pattern of pectoralis, relative iliotibialis and peroneus were not influenced either by genotype or protein content. Kidd & Kerr (1996) reported no alternation caused by lowering protein content on relative boneless thigh or drumstick at the 42 and 56 days, and added that neither CAA or sole Thr improved the distribution of both muscle groups. Gong et al. (2005) recorded the changes in

the percentage of thigh over three feeding phases, but a 2% drop of protein level from the normal diet didn't obviously inhibit thigh proportion. Dairo et al. (2010) investigated the effect of different metabolic energy to protein ratios on specific cut growth, and found that low protein level did caused significant change in both thigh and drumstick proportions compared to those on the control diet. On the contrary, higher protein levels than in our control diet significantly increased the thigh percentage. Liu et al. (2014) also concluded that 8% decrease in the crude protein content did not result in decreased in the thigh proportions. Even though these results can partially support our conclusion, they were still not instructive enough to corroborate our findings because either thighs and drumsticks are composed of multiple muscles, which might contribute to varying growing rates. The fluctuating relative growth curves could result from the difficulty of identifying these two specific muscle tissue for some of the students involved to cause the variations in the muscle samples.

The pressure of selecting fast-growing genotype is always associated with the evolution of certain skeletal diseases which may cause bone the weakness. Thus, bone-associated health problems are discussed and cited regarding to quality issues and animal welfare (Applegate & Lilburn, 2002). It was acknowledged that the reduction of the growing rate may improve the gait score, and further maintain a healthy chicken condition (Brickett et al., 2007). Moreover, the protein content has both detrimental and beneficial effect on the development of healthy bones, relying on several factors including protein sources, calcium metabolism, and others. (Heaney & Layman, 2008). Each growing bone has physiologically distinct regions which may contribute to the distinctive growth and development patterns. Therefore, several studies have investigated the growth of the tibia, femur, and humerus under restrictive feed (Bruno et al., 2000), protein, and calcium conditions (Skinner et al., 1991). Our results indicated that protein restriction

significantly inhibited all three bones in the both relative and absolute growth. Ross broilers produced comparatively less heavy bones on control diets, but had more tolerance to protein restriction than Cobb broilers. Applegate & Lilburn (2002) reported that the tibia weight grew differently after 28 days, but only in female birds, which partially matched our observations on the time effect. The interaction effect of diets by breeds by time revealed that the lower growth efficiency for the three bones was found in both breed strains fed low protein diets. Also, the femur bone from birds of the two breeds fed the different diets had similar growing patterns to those of the tibia. This observation indicated that the lack of certain amino acid would affect the bone growth, as the supplementation CAA maintained weight of the two bones similar to bones in birds on control diet. However, the radius weight presented a relatively different growing pattern from the femur and tibia. The influence of dietary protein content or the CAA on radius development was less than on the other two bones. The effect of protein content only showed up at the final week regardless of genotype, which implied some variations between two breeds even though there were no statistical differences. On the contrary, the relative growth of the radius was more constant than the other two bones, and presented steadily decreasing for all six treatments. The distribution of radius from all six treatments reached a constant value after 2nd week. However, the relative growing pattern of other two bones, femur and tibia, were highly affected by the crude protein level over the time. Our results collectively indicated that more alteration in the growth of femur and tibia were caused by change of protein content or the amount of amino acids than for the radius. The bone mass had a continual growth trend during the whole experimental time as the bone maturing periods were not detected in our study. Protein restriction even with CAA fortification evidently delayed on maturing of the three bones, especially the radius.

Protein intake is essential to animals, especially poultry which have higher requirements than mammals because of the relative higher body temperature and rapid passage in the digestive system. About one-third of the bone mass and 50% of bone volume were made of protein (Heaney, 2006). In general, the amount of protein intake would influence the bone development by influencing the IGF level, calcium absorption, and the formation of bone matrix (Heaney & Layman, 2008). In our study, the bone weight kept accumulating throughout the experimental time. The bone mass accumulation was inhibited when the protein level was reduced, around 3rd or 4th week in our experiment. The three muscle tissues also demonstrated the differences during these periods, which indicated that similar factors that affected muscle mass also influenced the bone mass. Sufficient daily protein supply is essential to rebuild the bone matrix as the proteolysis of protein from collagen fragments is less re-utilizable, and the modification of certain amino acids including lysine and proline by hydroxylation also assists in the cross-linking of collagen in bone. Moreover, the amount of the CAA supplementation in our study was determined by fixing the lysine level equal to the control diet. Soybean meal is enriched in calcium, and the reduction in soybean meal content inevitably led to the calcium deficiency. We compensated the calcium level in both low protein diets, but bone mass was still unable to be maintained equal to the control group. Although the protein seemingly plays a direct role in the bone development, the dietary composition alteration resulting the acid-base imbalance might have a further complicated results. Therefore, the mechanism behind this growth patterns may result from the low calcium absorption. The calcium utilization can also be changed by either addition of amino acids or changing in protein level, both of which might change the calcium homeostasis. In fact, the excessive protein intake has been cited as a factor in increasing the risk of osteoporosis or bone fracture (Barzel U & Massey L, 1998). On the other hand, some effect

could also be achieved with a lack of sufficient dietary protein level. The interaction of protein level and calcium was proven by Ryu et al. (1994). Dietary protein significantly increased calcium retention, and also highly affected calcium to phosphorus ratios in bone. In the light of the change in protein to calcium ratio in diet formulation in this study, it could be assumed that there was a retarding effect of lowering protein to calcium ratio on bone development.

Furthermore, future research can be conducted on the interactive effect of calcium and certain CAA on the bone mass, nutrient absorption in intestine, and mineral excretion over time.

6. CONCLUSIONS

In conclusion, dietary protein restriction by 6% units had an inhibitive effect on the growth and development of visceral organs, muscle tissues and bone. Despite a hypothesis that the supplementation of essential or limiting amino acids to low protein diets could satisfy the daily nutrient requirement of broilers, the effectiveness of CAA was evident only in early stages of growth. Adding CAA to low protein diets failed to increase the growth efficiency. No major difference was observed between two commercial strains, but Cobb broilers demonstrated heavier body weight gain, heart, muscles (Peroneus and Iliotibialis), and radius development than Ross broilers. However, Ross broilers produced significantly heavier pectoralis, and had a higher pectoralis yield when fed the control and low protein CAA added diets. Moreover, Cobb broilers exhibited more resistance to the protein restriction than Ross broilers for growth. Ross broilers had higher growth recover capacity in the low protein diets with added CAA.

REFERENCES

- Aarnink A. J. A., Hoeksma P., and Van Ouwerkerk E.N.J. 1993. Factors affecting ammonium concentration in slurry from fattening pigs. In: M.W.A Verstegen, L. A. den Hartog, G.J.M. van Kempen, and J.H.M. Metz (Ed.) Nitrogen Flow in Pig Production and Environmental Consequences. EAAP Publ. No. 69. pp 413–420. Pudoc, Wageningen, The Netherlands.
- Abeni F. and Bergogoglio G. 2001. Characterization of different strains of broiler chicken by carcass measurements, chemical and physical parameters and NIRS on breast muscle. *J Meat Sci.* 57:133–137.
- Adams G. R. and Haddad F. 1985. The relationships among IGF-1, DNA content, and protein accumulation during skeletal muscle hypertrophy. *J Appl Physiol.* 81:2509–16.
- Akhter S., Khan M., Anjum M., Ahmed S., Rizwan M., and Ijaz M. 2008. Investigation on the availability of amino acids from different animal protein sources in golden cockerels. *J Anim Plant Sci.* 18:53–4.
- Aletor V. A., I. Hamid I., Niess E., and Pfeffer E. 2000. Low protein amino acid-supplemented diets in broiler chickens: Effects on performance, carcass characteristics, whole-body composition and efficiencies of nutrient utilization. *J Sci Food Agric.* 80:547–554.
- Allison W. 1998. Poultry trade taken aback. *Richmond Times-Dispatch.* March 1. pp.7.
- Applegate T. J. and Lilburn M. S. 2002. Growth of the femur and tibia of a commercial broiler Line. *Poul Sci.* 81:1289–1294.
- Aschemann M., Lebzien P., Hüther L., Döll S., Südekum K. H., and Dänicke S. 2012. Effect of niacin supplementation on digestibility, nitrogen utilization and milk and blood variables in lactating dairy cows fed a diet with a negative rumen nitrogen balance. *Arch Anim Nutr.* 66:200–214.
- Baker D. H., Batal A. B., Parr T. M., Augspurger N. R., and C. M. Parsons. 2002. Ideal ratio (relative to lysine) of tryptophan, threonine, isoleucine, and valine for chickens during the second and third week post-hatch. *Poult Sci.* 81:485–494.
- Bake J., Balnave, D., and Dibner, J.J. 1998. Optimum dietary arginine: lysine ratio for broiler chickens in altered during heat stress in association with changes in intestinal uptake and dietary sodium chloride. *Br Poult Sci.* 39: 639–647.
- Baker D. H. 1997. Ideal amino acid profiles for swine and poultry and their applications in feed formulation. *BioKyowa Tech. Rev.* Vol. 9. Nutri-Quest, Inc., Chesterfield, MO.
- Bartov I. 1996. Interrelationship between the effects of dietary factors and feed withdrawal on the content and composition of liver fat in broiler chicks. *Poult Sci.* 75:632–641.

- Barzel U., Massey L. 1998. Excess dietary protein can adversely affect bone. *J Nutr.* 128:1051–1053.
- Bee G., Biolley C., Guex G., Herzog W., Lonergan S. M., and Huff-Lonergan E. 2006. Effects of available dietary carbohydrate and pre-slaughter treatment on glycolytic potential, protein degradation, and quality traits of pig muscles. *J Anim Sci.* 84:191–203.
- Benefield B. C., Patton R. A., Stevenson M. J., and Overton T. R. 2009. Evaluation of rumen-protected methionine sources and period length on performance of lactating dairy cows within Latin squares. *J. Dairy Sci.* 92:4448–4455.
- Blair R., Jacob J.P., Ibrahim S., and Wang P. 1999. A quantitative assessment of reduced protein diets and supplements to improve nitrogen utilization. *Appl Poult Res.* 8:25–47.
- Blaxter K. E. 1989. *Energy metabolism in animals and man.* pp. 254–289, Cambridge University Press, Cambridge, England.
- Bornstein, S. and Lipstein B. 1975. The replacement of some of the soybean meal by the first limiting amino acids in practical broiler diets. I. The value of special supplementation of chick diets with methionine and lysine. *Br Poult Sci.* 16: 177–188.
- Bregendahl K., Sell J.L., and Zimmerman D.R. 2002. Effect of low-protein diets on growth performance and body composition of broiler chicks. *Poult Sci.* 81:1156–1167.
- Brickett K. E., Dahiya J. P., Classen H. L., Annett C. B., and Gomis S. 2007. The Impact of nutrient density, feed form, and photoperiod on the walking ability and skeletal quality of broiler chickens. *Poult Sci.* 86(10): 2117–2125.
- Broiler Nutrition Specification. Ross broilers 708 broiler: nutrition specification. June 2007. Newbridge, Midlothian EH28 8SZ, Scotland, UK.
- Bruno L. D. G., Furlan R. L., Malheiros E. B., and Macari M. 2000. Influence of early quantitative food restriction on long bone growth at different environmental temperatures in broiler chickens. *Br Poult Sci.* 41(4):389.
- Bunchasak C., Tanaka K., Ohtani S., and Collado C. M. 1996. Effect of Met+Cys supplementation to low-protein diet on the growth performance and fat accumulation of broiler chicks at starter period. *J Anim Sci Technol.* 67:956–966.
- Buyse J., Decuypere E., Berghman L, Kuhn E. R., and Vandesande F. 1992. Effect of dietary protein content on episodic growth hormone secretion and on heat production of male broiler chickens. *Br Poult Sci.* 33:1101–1109.
- Cahaner A., Deeb N., and Gutman M., 1993. Effects of the plumage-reducing naked neck (*Na*) gene on the performance of fast-growing broilers at normal and high ambient temperatures. *Poult Sci.* 72:767–775.

- Cahaner A., Pinchasov Y., and Nir I. 1995. Effects of dietary protein under high ambient temperature on body weight, breast meat yield, and abdominal fat deposition of broiler stocks differing in growth rate and fatness. *Poult Sci.* 74:968–975.
- Cahaner A. and Leenstra F., 1992. Effects of high temperature on growth and efficiency of male and female broilers from lines selected for high weight gain, favorable feed conversion, and high or low fat content. *Poult Sci.* 71:1237–1250.
- Carew L. B., Evarts K. G., and Alster F. A. 1997. Growth and plasma thyroid hormone concentrations of chicks fed diets deficient in essential amino acids. *Poult Sci.* 76:1398–1404.
- Chung T. K. and Baker D. H. 1992. Ideal amino acid pattern for 10-kilogram pigs. *J Anim Sci.* 70:3102–3111.
- Cisneros F., Ellis M., Baker D. H., Easter R. A., and McKeith F. K. 1996. The influence of short-term feeding of amino acid-deficient diets and high dietary leucine levels on the intramuscular fat content of pig muscle. *Anim Sci.* 63:517–522.
- Collin A., Malheiros R. D., Moraes V. M. B., Van A. P., Darras V. M., Taouis M., Decuypere E., and Buyse J. 2003. Effects of dietary macronutrient content on energy metabolism and uncoupling protein mRNA expression in broiler chickens. *Br J Nutr.* 90:261–269.
- Colnago L., Penz A. M., and Jensen L. S. 1991. Effect of response of starting broiler chicks to incremental reduction in intact protein on performance during the grower phase. *Poult Sci.* 70 (Suppl. 1):153.
- Dairo F. A. S., Adesihinwa A. O. K., Oluwasola T. A., and Oluyemi J. A., 2010. High and low dietary energy and protein levels for broiler chickens. *Afr J Agric Res.* 5(15):2030–2038.
- Davidson S., Hopkins B. A., Odle J., Brownie C., Fellner V., and Whitlow L. W. 2008. Supplementing limited methionine diets with rumen-protected methionine, betaine, and choline in early lactation Holstein cows. *J Dairy Sci.* 91:1552–1559.
- Dean D. W., Bidner T. D., and Southern L. L. 2006. Glycine Supplementation to Low Protein, Amino Acid-Supplemented Diets Supports Optimal Performance of Broiler Chicks. *Poult Sci.* 85:288–296
- DeVol D. L., McKeith F. K., Bechtel P. J., Novakofski J., Shanks R. D., Carr T. R. 1988. Variation in composition and palatability traits and relationships between muscle characteristics and palatability in a random sample of pork carcasses. *J Anim Sci.* 66:385–395.
- Edmonds, M. S., Izquierdo O. A., and Baker D. H. 1985. Feed additive studies with newly weaned pigs: Efficacy of copper, antibiotics and organic acids. *J Anim Sci.* 60:462–

- Erbay E., Park I. H., Nuzzi P. D., Schoenherr C. J., Chen J. 2003. IGF-2 transcription in skeletal myogenesis is controlled by mTOR and nutrients. *J Cell Biol.* 163(5): 931–936.
- Fancher B. I. and Jensen L. S. 1989a. Male broiler performance during the starter and grower periods as affected by dietary protein, essential amino acids, and potassium levels. *Poult Sci.* 68:1385–1395.
- Ferguson N. S., Gates R. S., Taraba J. L., Cantor A. H., Pescatore A. J., Straw M. L., Ford M. J., and Burnham D. J. 1998. The effect of dietary protein and phosphorus on ammonia concentration and litter composition in broilers. *Poult Sci.* 77:1085–1093.
- Firman, J. D. and Boling, S. D. 1998. Ideal protein in turkeys. *Poult Sci.* 77:105–110.
- Fisher C. 1984. Fat deposition in broilers. In: *Fats in animal nutrition*. pp 437–470. Proceedings of the 37th Nottingham Easter School. Ed. Wiseman I., Nottingham, England
- Gerrard D. E. and Grant A. L. 2004. *Principles of animal growth and development*. ISBN13: 978-0-7575-2986-3.
- Giallongo F., Oh J., Frederick T., Weeks H., Hristov A. N., Lapierre H., Patton R. A., Gehman A., and Parys C. 2014. Effects of slow-release urea, rumen-protected methionine, and histidine on performance of dairy cows fed metabolizable protein-deficient diets. *J Dairy Sci.* 98:3292–3308
- Gong L. M., Lai C. H., Qiao S. Y., Li D.F, Ma Y. X., and Liu Y. L. 2005 Growth performance, carcass characteristics, nutrient digestibility and serum biochemical parameters of broilers fed low-protein diets supplemented with various ratios of threonine to lysine Asian-Aust. *J Anim Sci.* 18:1164–1170.
- Guzik A. C, Matthews J. O, Kerr B. J, Bidner T. D., and Southern L. L. 2006. Dietary tryptophan effects on plasma and salivary cortisol and meat quality in pigs. *J Anim Sci.* 84:2251–2259.
- Hahn J. D. and Baker D. H. 1995. Optimum ratio to lysine of threonine, tryptophan and sulfur amino acids for finishing swine. *J Anim Sci.* 73:482–489.
- Han Y., H. Suzuki, C. M. Parsons, and Baker D. H. 1992. Amino acid fortification of a low-protein corn and soybean meal diet for chicks. *Poult Sci.* 71:1168–1178.
- Heaney R. P. 2007. Effects of protein on the calcium economy. In: Burckhardt P, Heaney RP, Dawson-Hughes B, eds. *Nutritional aspects of osteoporosis 2006*, Lausanne, Switzerland. Amsterdam, Netherlands: Elsevier Inc. 191–7.
- Heaney R. P. and Layman D. K. 2008. Amount and type of protein influences bone health. *Am J*

Clin Nutr. 87:67–70.

- Jacob J. and Pescatore T. 2013(a). Avian skeletal system. Bulletin ASC-202, Cooperative Extension Service, University of Kentucky College of Agriculture, Food and Environment, Lexington, Kentucky. p 2.
- Jacob J. and T. Pescatore. 2013(b). Avian muscular system. Bulletin ASC-204, Cooperative Extension Service, University of Kentucky College of Agriculture, Food and Environment, Lexington, Kentucky. p 2.
- Kamran Z., Sarwar M., Nisa M., Nadeem M. A., Mahmood S., Babar M. E., and Ahmed S. 2008. Effect of low-protein diets having constant energy-to-protein ratio on performance and carcass characteristics of broiler chickens from one to thirty-five days of age. *Poult Sci.* 87:468–474.
- Kerr, B. J. and Easter R. A. 1995. Effect of feeding reduced protein, amino acid- supplemented diets on nitrogen and energy balance in grower pigs. *J Anim Sci.* 73:3000–3008.
- Kidd M. E. and Kerr B. J. 1996. Growth and carcass characteristic of broilers fed low-protein, threonine-supplemented diets. *J Appl Poult Res.* 5:180–190.
- Kleyn R. and Chrystal P. 2008. Feeding the young broiler chicken in practice: a review. 23rd World's Poultry Congress. Brisbane, Australia.
- Leclercq B., Chagneau A. M., Cochard T., and Khoury J. 1994. Parative responses of genetically lean and fat chickens to lysine, argentine and non-essential amino acid supply. *Br Poult Sci.* 35:687–696.
- Lee C., Hristov A. N., Heyler K. S., Cassidy T. W., Lapierre H., Varga G. A., and Parys C. 2012. Effects of metabolizable protein supply and amino acids supplementation on nitrogen utilization, production and ammonia emissions from manure in dairy cows. *J Dairy Sci.* 95:5253–5268.
- Leeson S., Summers J. D., and Caston L. 1993. Growth response of immature brown-egg strain pullet to varying nutrient density and lysine. *Poult Sci.* 72:1349–1358.
- Lemme A. 2003. The ideal protein concept in broiler nutrition. Experimental data on varying dietary ideal protein levels. *Amino News Degussa AG.* p 4 (2).
- Lenis N. P., Diepen J. T. M. V, Schutte J. B., and Jong J. 1993. The ideal pattern of ileal digestible amino acids in diets for growing pigs. In: M.W.A. Verstegen, L. A. den Hartog, G.J.M. van Kempen, and J.H.M. Metz (Ed.) *Nitrogen Flow in Pig Production and Environmental Consequences.* pp 253–258. Pudoc Scientific Publishers, Wageningen, The Netherlands.

- Liu S. K., Niu Z. Y., Min Y. N., Wang Z. P, Zhang J., He Z. F., Li H. L., Sun T. T., Liu F. Z., 2014. Effects of dietary crude protein on the growth performance, carcass characteristics and serum biochemical indexes of lueyang black-boned chickens from seven to twelve weeks of age. *Rev Bras Cienc Avic.* 17:103–108.
- MacDonald J. M. and McBride W. D, 2009. The transformation of U.S. livestock agriculture: scale, efficiency, and risks, economic information bulletin. No. 43. Economic Research Service, U.S. Dept. of Agriculture.
- Marcu A., Vacaru-Opri I., Dumitrescu G., Marcu A., Ciochina L. P., Nicula M., Dronca D., and Kelciiov B. 2013. Effect of diets with different energy and protein levels on breast muscle characteristics of broiler chickens. *J Anim Sci Biotechnol.* 46:333–340.
- Marcu A., Vacaru-Opriş I., Marcu A., Nicula M., Dumitrescu G., Nichita I., Dronca D., and Kelciiov B. 2011. The influence of feed protein and energy level on the meat chemical composition at Lohmann Meat hybrid. *J Anim Sci Biotechnol.* 44:439–443.
- Murakami A. E., Franco J. R. G., Martins E. N., Oviedo Rondon E. O., Sakamoto M. I., and Pereira M. S. 2003. Effect of electrolyte balance in low- protein diets on broiler performance and tibial dyschondroplasia incidence. *J Appl Poul Res.* 12:207–216.
- Nakajima T., Kishi H., Kusubae T., Wakamatsu H., and Y. Kusutani, 1985. Effect of L-threonine and DL-tryptophan supplementation to the low protein practical broiler finisher diet. *Japanese Poult Sci.* 22:1–6.
- Namroud N. F., Shivazad M., and Zaghari M. 2008. Effects of fortifying low crude protein diet with crystalline amino acids on performance, blood ammonia level, and excreta characteristics of broiler chicks. *Poult Sci.* 87:2250–2258.
- Ndegwa P. M., Hristov A. N., Arogo J., Sheffield R. E. 2008. A review of ammonia emission mitigation techniques for concentrated animal feeding operations. *Bioprocess Biosyst. Eng.* 100:453–469.
- Ngo A., Coon C.N. and Beecher G.R. 1997. Dietary glycine requirements for growth and cellular development in chicks. *J Nutr.* 107:1800–1808.
- Nieto R., Aguilera J. F., Fernandez-Figares I., and Prieto C. 1997. Effect of a low protein diet on the energy metabolism of growing chickens. *Arch. Tierernahr.* 50:105–109.
- NRC. 1994. Nutrient requirements of poultry. 9th rev. ed. National Academy Press, Washington, DC.
- NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th rev. ed. Natl. Acad. Sci., Washington, DC.

- Olmos Colmenero, J. J., and Broderick G. A. 2006. Effect of dietary crude protein concentration on milk production and nitrogen utilization in lactating dairy cows. *J Dairy Sci.* 89:1704–1712.
- Peng Y. and Harper A. E. 1970. Amino acid balance and food intake: Effect of different dietary amino acid patterns on the plasma amino acid pattern of rats. *J Nutr.* 100:429–437.
- Pinchasov Y., C. Mendonca X., and Jensen L. S. 1990. Broiler chick response to low protein diets supplemented with synthetic amino acids. *Poult Sci.* 69:1950–1955.
- Plavnik I. and Hurwitz S. 1985. The performance of broiler chicks during and following a severe feed restriction at an early age. *Poult Sci.* 64:348–355.
- Rerat A., Simous-Nunes C., Mendy F., Vaissade P., and Vaugelade P. 1992. Splanchnic fluxes of amino acids after duodenal infusion of carbohydrate solutions containing free amino acids or oligopeptides in the non-anaesthetized pig. *Br J Nutr.* 68:111–138.
- Rosebrough R. W., and Steele N. C. 1985. Effect of protein levels and feeding regimes on growth, body composition and in vitro lipogenesis in broiler chickens. *Poult. Sci.* 64:119–126.
- Roy B. C., Oshima I., Miyachi, H., Shiba N., Nishimura S., Tabata S., and Iwamoto H. 2006. Effects of nutritional level on muscle development, histochemical properties of myofibre and collagen architecture in the pectoralis muscle of male broilers. *Br Poult Sci.* 47:433–442.
- Ryu Y. S., Han. K. I., and Kim. I. B. 1994. Interaction of calcium, phosphorus and protein in broilers. *Asian-Aust J Anim Sci.* 7:583–598.
- Schimke R. T. 1963. Study on factors affecting the level of urea cycle enzymes in rat liver. *J Biol Chem.* 238:1012–1018.
- Schutte J. B., 1987. Utilization of synthetic amino acids in poultry. Pages RT11-12 *in:* World's Poultry Science Association 6th European Symposium on Poultry Nutrition. Konigslutter, Germany.
- Sell J. L., Jeffrey M. J., and Kerr B. J. 1994. Influence of amino acid supplementation of low-protein diets and metabolizable energy feeding sequence on performance and carcass composition of toms. *Poult Sci.* 73:1867–1880.
- Si J., Fritts C. A., Burnham D. J., and Waldroup P. W. 2001. Relationship of dietary lysine level to the concentration of all essential amino acids in broiler diets. *Poult Sci.* 80:1472–1479.

- Si J., Fritts C. A., Waldroup P. W., and Burnham D. J. 2004. Effects of tryptophan to large neutral amino acid ratios and overall amino acid levels on utilization of diets low in CP by broilers. *J. Appl. Poult Sci Res.* 13:570–578.
- Skinner J. T., Izat A. L., and Waldroup P. W. 1991. Research note: Fumaric acid enhances performance of broiler chickens. *Poultry Sci.* 70:1444–1447.
- Sleman S., Beski M., Swick R. A., and Paul A. 2015. Specialized protein products in broiler chicken nutrition: A review. *Anim Nutr.* 1:47–53.
- Suchy P., Jelínek P., Straková E., and Hucl J. 2002. Chemical composition of muscles of hybrid-broiler chickens during prolonged feeding. *Cze J Anim Sci.* 47:511–518.
- Summers J. D., Jackson S., and Spratt D. 1989. Weight gain and breast yield of large white male turkeys fed diets varying in protein content. *J Poult Sci* 68: 1547–1552.
- Summers J.D. and Leeson S. 1985. Broiler carcass composition as affected by amino acid supplementation. *Can J Anim Sci.* 65:717–723.
- Sutton A. L., Kephart K. B., Patterson J. A., Mumma R., Kelly D. T., Bogus E., Jones D. D., and Heber A. 1996. Manipulating swine diets to reduce ammonia and odor emissions. In: *Proc. 1st Int. Conf. Air Pollution from Agric. Operations, Kansas City, MO.* p 445–452.
- Swennen Q., G. Janssens P. J., Collin A., Bihan-Duval E. L., Verbeke K., Decuypere E., and Buyse J. 2006. Diet-induced thermogenesis and glucose oxidation in broiler chickens: Influence of genotype and diet composition. *J Poult Sci.* 85:731–742.
- Tang M. Y., Ma Q. G., Chen X. D., and Ji C. 2007. Effects of dietary metabolizable energy and lysine on carcass characteristics and meat quality in arbor acres broilers. *Asian-Aust J Anim Sci.* 12:1865–1873
- Temim S., Chagneau A. M., Peresson R., and Tesseraud S. 2000. Chronic heat exposure alters protein turnover of three different skeletal muscles in finishing broiler chickens fed 20 or 25% protein diets. *J Nutr.* 130:813–819.
- Tuitoek K., Young L. G., Lange C. F. de and Kerr B. J. 1997. The effect of reducing excess dietary amino acids on growing-finishing pig performance: an elevation of the ideal protein concept. *J Anim Sci.* 75:1575–1583.
- U.S. USDA. 2016. Poultry - Production and Value 2015 Summary. ISSN: 1949–1573.
- Uzu G. 1983. Broilers Feed Reduction of the Protein Level During Finishing Period and Effect on performance and fattening. A.E.C. Document No. 242. Commentary 03600, France.

- Waldroup P. W. 2000. Feeding programs for broilers: the challenge of low protein diets. Pages 119–134 in Proc. 47th Maryland Nutrition Conf. Univ. Maryland, College Park.
- Waldroup P. W., Jiang Q., and Fritts C. A. 2005. Effects of glycine and threonine supplementation on performance of broiler chicks fed diets low in CP. *Int. J Poult Sci.* 4:250–257.
- Widyaratne G. P. and Drew M. D., 2011. Effects of protein level and digestibility on the growth and carcass characteristics of broiler chickens. *Poultry Science* 90:595–603.
- Williams P. E. V. 1995. Animal production and European pollution problems. *Anim. Feed Sci Tec.* 53:135–144.
- Yamazaki M. Murakami, H., Nakashima K., Abe H., and Takemasa M. 2006. Effect of excess essential amino acids in low protein diet on abdominal fat deposition and nitrogen excretion of the broiler chicks. *J Poult Sci.* 43:150–155.
- Yu M. W. and Robinson E. E. 1992. The application of short-term feed restriction to broiler chicken production: a review. *J Appl Poult Res.* 1:147–153.

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