AN EXAMINATION OF BROILER GROWTH

by

JONATHAN ANDREW MORRIS

(Under the Direction of Robert B. Beckstead and Samuel E. Aggrey)

ABSTRACT

With rising feed costs and increasing concern about environmental impact, the broiler industry must continue to improve in order to meet the ever changing demands of consumers and government entities. To this effect, a focus on improving feed efficiency phenotypes will likely be the most effective long-term solution to mitigate these concerns. The objectives of this study are to characterize differences in broiler lines divergently selected against feed efficiency to identify the role of avian TOR in feed efficiency phenotypes as well as to investigate the growth patterns of broiler chickens utilizing CT technology. The phenotypic response of avTOR to stimulation or inhibition in pedigreed broiler lines. Both treatments were successful in eliciting an effect on feed efficiency traits. With the goal of developing a predictive equation, the body surface area of broilers was examine by CT scan. A growth curve was developed and existing predictive equations were assessed. Due to the high amount of variation and low accuracy of the results, a new equation must be developed that incorporates additional parameters to aid in the accuracy of prediction.

INDEX WORDS: avTOR, mTOR, rapamycin, leucine, chicken, computed tomography, surface area, growth

AN EXAMINATION OF BROILER GROWTH

by

JONATHAN ANDREW MORRIS

B.S.A., The University of Georgia, 2012

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment

of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2015

AN EXAMINATION OF BROILER GROWTH

by

JONATHAN ANDREW MORRIS

Major Professor: Robert B. Beckstead
Sammy E. Aggrey

Committee: Franklin D. West
Ajay Sharma

Electronic Version Approved:

Julie Coffield
Interim Dean of the Graduate School
The University of Georgia
May 2015

DEDICATION

To my mom and dad, thank you for your years of encouragement and support. None of this would have been possible without you. To Tiffany, Elena, Avery, and Cora, thank you for keeping me focused and motivated to keep working and reaching for a better tomorrow. With love and appreciation, I dedicate this body of work to you.

ACKNOWLEDGEMENTS

I would first like to thank Adam Davis for all of his patience and suffering to help in bringing me this far and to Dalyn Guinn for all of her hard work going through the tedium of research. Lastly, I would like to thank Robert Beckstead and Sammy Aggrey for their encouragement to pursue this degree and for all of their patience and assistance since.

TABLE OF CONTENTS

Page

ACKNOWLEDGEMENTS v

LIST OF TABLES vii

LIST OF FIGURES viii

CHAPTER

1 INTRODUCTION 1

2 LITERATURE REVIEW 3

3 CHARACTERIZATION OF THE ROLE OF THE AVIAN MECHANISTIC TARGET OF RAPAMYCIN (avTOR) IN FEED INTAKE AND GROWTH 14

4 ASSESSMENT OF THE ESTIMATION OF TOTAL SURFACE AREA OF BROILER CHICKENS BY COMPUTED TOMOGRAPHY 30

5 CONCLUSIONS 46

LIST OF TABLES

Page

Table 3.1: Production Data by Line 29

Table 3.2: P values for Sex, Line, and Treatment 29

Table 4.1: Average Body Weight and Body Surface Area by Sex 42

Table 4.2: Assessment of Existing k Values 45

LIST OF FIGURES

Page

Figure 3.1: mTOR Signaling Pathway....................26

Figure 3.2: Average Body Weight Gain by Line and Treatment....................26

Figure 3.3: Average Feed Intake by Line and Treatment....................27

Figure 3.4: Average Feed Conversion Ratio by Line and Treatment....................27

Figure 3.5: Percent Deviation from Control for HRFI Line....................28

Figure 3.6: Percent Deviation from Control for LRFI Line....................28

Figure 4.1: Growth Curve by Body Weight....................43

Figure 4.2: Growth Curve by Body Surface Area....................43

Figure 4.3: Body Surface Area Correlation Matrix....................44

CHAPTER 1

INTRODUCTION

Initially domesticated for sport and entertainment purposes, the chicken has become a major food product worldwide. In fact, its popularity in the market has grown significantly. In the US alone, per capita consumption of chicken has overtaken beef as the preferred protein source. This increase has been made possible by advancements in production processes. Namely, broilers today are growing much larger in a much shorter period of time. A broiler in the 1920's would take roughly 16 weeks to reach a market weight of 4 pounds. Modern broilers can attain a market weight in excess of 6 pounds in as little as 6 weeks. This massive transformation can mostly be attributed to very efficient selective breeding. Genetics accounts for 90-95% of these changes with improvements in nutrition, housing, and husbandry making up the rest. Historically the selection process has primarily focused on gross production traits such as growth rate and body weight and currently entails extensive quantification and data collection on production phenotypes. More recently, this focus has begun to shift to address efficient growth or feed efficiency. The most common method employed to assess the efficiency of growth is feed conversion ratio (FCR), although growing consideration is being given to utilizing residual feed intake (RFI) as a measure of efficiency. Little is known about the molecular and genetic components of feed efficiency. Recently, the mechanistic target of rapamycin (mTOR), a key cellular sensor, and members of the mTOR pathway have been correlated with growth and feed efficiency. Further characterization of this pathway could help to delineate those genes which underlie the molecular regulation of efficiency. This information could help to enable a breeding selection process based on genotype rather than phenotype and to reduce both the cost and environmental impact of production.

Trying to discern the molecular machinery controlling growth traits is in actuality an attempt to further understand broiler metabolism. As such, it is important when trying to comprehend the nuances of growth to examine metabolic rate. Directly measuring the metabolic rate of an individual is not a simple task and involves specialized equipment and can be quite laborious. It would be far easier to measure a physical value or trait that is highly correlated with the metabolic rate of an animal and to deduce relevant metabolic information from the data acquired. Body surface area has been shown to be highly correlated with many physical traits including basal metabolic rate. Measuring body surface area allows the extrapolation of the information desired. In fact, it has become quite common to determine drug dosage, especially for antineoplastic drugs, based on surface area as a result of this correlation. There are many methods for determining the surface area of an individual. However, many of these require euthanasia and all have their pitfalls regarding the accuracy of the measurement.

The overall goal of these studies is to investigate broiler growth at both the molecular and organismal level. This will involve characterizing differences in feed efficiency of pedigreed broiler lines that have been divergently selected for RFI. More specifically, the objective here is to qualify changes in feed efficiency phenotypes through the stimulation or inhibition of the avTOR pathway. Identifying changes brought on by altering the activity of this pathway will assist in determining the role that this pathway plays in feed efficiency. Additionally, this study examines methods for measuring body surface area as a correlated estimator of metabolic rate with the idea that an accurate estimate of metabolism begins with an accurate estimate of surface area.

CHAPTER 2

LITERATURE REVIEW

Feed efficiency is a measure of an animal's ability to convert food into saleable product. Feed conversion ratio (FCR), the ratio of feed consumed to productive gain, is one of the most common ways by which feed efficiency is assessed. For laying hens this is pounds of feed consumed per dozen eggs laid. For meat-type animals this is pounds of feed consumed per pound of meat produced. Therefore, lower ratios of feed consumption to body weight gain indicate higher levels of efficiency. While FCR remains the canonical measure of efficiency, the use residual feed intake (RFI) is increasing due to its independence from growth rate and body size [1-3]. First proposed in 1963, RFI segregates feed efficiency from body size and growth and is, therefore, independent of development patterns [4]. It is defined as the difference between observed feed intake and the expected feed intake at a given body weight. As such, genetic selection based on RFI should result in offspring that consume less feed without negatively impacting growth traits.

Historically, breeding selection has been primarily focused on output traits such as body size and growth rate. More recently, there has been an attempt to reduce input costs through genetic selection. This is achieved through improving feed efficiency traits. Consequently, the heritability of feed efficiency traits has been demonstrated. Feed efficiency traits, namely FCR and RFI, have been shown to exhibit moderately high heritability estimates ranging from about 0.3 to around 0.5 in multiple species, including cows and poultry[1, 4-8]. They have also demonstrated fairly strongly negative genetic correlations with feed intake. While FCR retains strong correlations with body weight gain (BWG), RFI exhibits little or no genetic correlation with growth rate. Interestingly, data suggests that there is an age effect on the correlation of RFI to BWG [5]. In their 2010 study, Aggrey *et al.* found the two traits to be moderately positively correlated at days 28-35. The correlation was negligible at days 35-42. Consequently, the efficacy of selection may, in part, rely on the time or age at which selection pressure is applied.

While selection based on either FCR or RFI will result in improved feed utilization, the use of FCR obfuscates the results of selection [2, 5, 8]. Being a ratio dependent on growth traits, the calculated FCR can be highly similar for two individuals with vastly different sizes, feed intakes, and growth rates. RFI allows the delineation of each of these traits individually. This is the reason for its growing popularity despite the difficulties of measurement [2, 4]. Still, the improvement on nutrient utilization and retention is achievable by both methods. It is interesting to note that the opposite is also true. Nitrogen and phosphorus have come to the forefront of concern over the environmental impact of the broiler industry [9]. Phosphorus, which is largely unavailable to broilers, and nitrogenous waste, from protein metabolism, are shed in the excreta which is subsequently sold as fertilizer to nearby farms and fields. The minerals, carried by rainwater, are then able to enter and pollute neighboring bodies of water. Selecting for higher bioavailability of both nitrogen and phosphorus may improve production while reducing costs and environmental impact [8].

Only recently have studies attempted to elucidate the underlying molecular basis for anabolic protein synthesis. The protein commonly referred to as mammalian Target of Rapamycin (mTOR) and gene of the same name has arisen as a major target of these studies. mTOR was first discovered in 1994 by Dr. David Sabatini who was working with rapamycin, a macrolide antibiotic, at the time [10]. mTOR acts as a sensor of cellular input and an activator of downstream targets that stimulate and support protein synthesis [11-17]. Also called FK506 binding protein-

12 rapamycin associated protein (FRAP1), it is encoded by a gene of the same name. mTOR is a serine/threonine protein kinase that integrates upstream input, including insulin, growth factors, and AAs, while also sensing cellular nutrient levels and redox states. Its activity regulates cell growth, proliferation, motility, survival, autophagy, transcription, and protein and ribosomal synthesis [18]. To accomplish this, mTOR stimulates the phosphorylation of substrates that direct translation while preventing proteosomal and autophagic degradation. mTOR is found in the cellular matrix in two distinct multiprotein complexes, mTORC1 and mTORC2. mTORC1 is comprised of mTOR, DEPTOR, GβL (LST8) and Raptor (Figure 1). Raptor acts as a scaffolding protein and stabilizes the mTORC1 [14]. Activity of this complex results in the initiation of protein anabolism and cell proliferation and growth. Less is known about the activity of mTORC2, but it is thought to play a role in production and maintenance of the cytoskeleton while also acting to further stimulate the activity of mTORC1 [19].

Rapamycin, an antibiotic discovered in *Streptomyces hygroscopicus*, forms a complex with FK506 binding protein-12 (FKBP12) that inhibits mTORC1 by preventing the association of mTOR to Raptor [14, 20]. This disassociation prevents the formation of the complex thus inhibiting its activity. Once used as an antifungal agent, rapamycin was discovered to possess potent immunosuppressant properties [10, 21]. This immunosuppression led to its use for organ transplant patients. Owing to its low nephrotoxicity, it has become an important medication to prevent rejection particularly for kidney transplants. Principally due to its inhibition of mTOR activity, rapamycin continues to be a drug involved in numerous studies. Most of these involve the treatment of cancers and cognitive disorders such as Alzheimer's disease. While studying Alzheimer's disease in mice, Spilman et. al. (2010) found that a group of mice fed rapamycin maintained BWG statistically similar to those of a control fed group despite their consumption of significantly more feed [22]. This study helped to implicate mTOR and the mTOR pathway as the putative regulator of growth and feed intake at the molecular level.

In contrast, the branched chain amino acids (BCAA) have been shown to stimulate protein synthesis by triggering the activity of mTORC1, especially in conjunction with exercise, as well as to improve feed efficiency in broilers[13, 23-31]. Leucine has come to the forefront of these investigations. The essential amino acid acts as a nutrient signal to regulate protein synthesis and has been seen to be highly active in skeletal muscle, particularly in conjunction with exercise. Leucine stimulates the translocation of mTORC1 to the lysozyme through interactions with the regulator complex [25, 26, 28-31]. Given this, the BCAAs, especially leucine, are being investigated to determine their correlations and roles in protein synthesis.

Rapamycin (for patients weighing less than 40 kg) and numerous other drugs are currently being prescribed at dosages based on body surface area (BSA) rather than weight [32]. This is because of the high correlation of BSA to metabolic rate. BSA also exhibits strong correlations with glomerular filtration rate, heart rate, lifetime heartbeat number, and even blood volume [33-45]. Since muscle and fat have drastically different effects on metabolism, BSA provides a more accurate estimate of metabolic rate by taking into account body condition. The most commonly used method for estimating BSA for humans is the DuBois and DuBois formula: $BSA = (W^{0.425} \times H^{0.725}) \times 0.007184$; where W is weight in kilograms, H is height in centimeters, and BSA is in cm^2 [46].

Because it can be used as a measure of metabolic rate, BSA has also been correlated with heat production which is critical to the broiler industry. From Kleiber’s Surface Law, surface area increases at a rate of 2/3 to the power of the volume [43, 44]. Therefore, as organisms grow, their volume increases more quickly than their surface area and their surface area to volume ratio decreases. Lower surface area to volume ratios result in less heat being lost to convection. For the broiler industry, this is an aid in cold climates and during winter months while it is an obstacle in warmer climates and during the summer months.

Developed in 1879, the Meeh formula: $BSA = k \times W^{2/3}$; where k is a species specific constant, W is body weight in grams, and BSA is in cm^2; is the most commonly used method to predict surface area values of various animal species [47]. As it is, Meeh's equation lacks consistency and accuracy. There is no consensus on a single k value for a species. Numerous k values exist even within a species and the accuracy of the estimates are questionable. The estimates for individuals can exhibit deviations from the actual measurements by over 40%. As such, several attempts have been made to improve upon the original Meeh formula. Each improvement has proven to be very species specific while still exhibiting similar ranges in accuracy concerning the estimates.

This calls into question, not only the method for calculating the estimates, but also the methods for measuring the body surface area of an individual. Numerous studies have utilized the pelts and skins of animals that have been euthanized or some type of wrap, such as medical gauze, that was affixed to the animal [35, 37, 48]. Cut into regular shapes, the skin or fixed gauze was placed on a planimeter to assess BSA. Depending on the elasticity of the pelt and length of time it remained exposed, these pelts are subject to stretching, shrinking, or even tearing. Others have used a roller of known surface area adjoined to a revolution counter [35, 49]. The device is then rolled along the entirety of the animal's surface and the number of revolutions is used to calculate BSA. The most commonly employed method for small animals, such as rodents and small birds, is the use of a clear plastic pouch [33, 50, 51]. The animal is anesthetized or euthanized, then placed inside the pouch. An outline is drawn, cut out, then placed on the planimeter to measure

BSA. These measurements of BSA rely heavily on an individual's ability to accurately maintain the morphology of the samples used for measurement. They also frequently omit certain sections of the body and also typically assume symmetry for an individual. For instance, the ears of mice and combs of chickens are frequently measured once and the SA doubled. The feet and toes of birds are often omitted. The numerous advances in technology fortunately supply new methods that mitigate these problems and can provide highly accurate information.

Computed tomography (CT) or computed axial tomography (CAT) utilizes x-ray technology to provide high resolution images that can be assessed in two or three dimensions. Developed in 1972 by G. N. Hounsfield, CT is 100 times more sensitive than conventional x-ray and has the capability of distinguishing between tissues that differ by less than one percent [52, 53]. These scans also allow for the visualization and analysis of the complete animal without changing the morphology of the dermal layers and is not subject to shrinkage, tearing, or exclusion of parts, such as feet. Therefore, CT technology is an excellent method to employ for the analysis of current methods for estimating BSA.

References

1. Herd, R.M. and P.F. Arthur, *Physiological basis for residual feed intake*. Journal of Animal Science, 2009. **87**(14 suppl): p. E64-E71.

2. Sainz, R.D. and P.V. Paulino, *Residual Feed Intake*. 2004.

3. Santana, M.H., et al., *Genome-wide association analysis of feed intake and residual feed intake in Nellore cattle*. BMC Genet, 2014. **15**: p. 21.

4. Koch, R.M., et al., *Efficiency of Feed Use in Beef Cattle*. Journal of Animal Science, 1963. **22**(2): p. 486-494.

5. Aggrey, S.E., et al., *Genetic properties of feed efficiency parameters in meat-type chickens*. Genet Sel Evol, 2010. **42**: p. 25.

6. Case, L.A., B.J. Wood, and S.P. Miller, *The genetic parameters of feed efficiency and its component traits in the turkey (Meleagris gallopavo)*. Genet Sel Evol, 2012. **44**: p. 2.

7. de Verdal, H., et al., *Improving the efficiency of feed utilization in poultry by selection. 1. Genetic parameters of anatomy of the gastro-intestinal tract and digestive efficiency*. BMC Genet, 2011. **12**: p. 59.

8. Ankra-Badu, G.A., G.M. Pesti, and S.E. Aggrey, *Genetic interrelationships among phosphorus, nitrogen, calcium, and energy bioavailability in a growing chicken population*. Poult Sci, 2010. **89**(11): p. 2351-5.

9. The PEW Charitable Trusts *Big Chicken: Pollution and Industrial Poultry Production in America - Pew Environment Group*. 2011.

10. Sabatini, D.M., et al., *The rapamycin and FKBP12 target (RAFT) displays phosphatidylinositol 4-kinase activity*. J Biol Chem, 1995. **270**(36): p. 20875-8.

11. Ballou, L.M. and R.Z. Lin, *Rapamycin and mTOR kinase inhibitors*. J Chem Biol, 2008. **1**(1-4): p. 27-36.

12. Borders, E.B., C. Bivona, and P.J. Medina, *Mammalian target of rapamycin: Biological function and target for novel anticancer agents.* American Journal of Health-System Pharmacy, 2010. **67**(24): p. 2095-2106.

13. Cota, D., et al., *Hypothalamic mTOR Signaling Regulates Food Intake.* Science, 2006. **312**(5775): p. 927-930.

14. Dowling, R.J.O., et al., *Dissecting the role of mTOR: Lessons from mTOR inhibitors.* Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics, 2010. **1804**(3): p. 433-439.

15. Frost, R.A. and C.H. Lang, *mTor Signaling in Skeletal Muscle During Sepsis and Inflammation: Where Does It All Go Wrong?* Physiology, 2011. **26**(2): p. 83-96.

16. Gibbons, J.J., R.T. Abraham, and K. Yu, *Mammalian Target of Rapamycin: Discovery of Rapamycin Reveals a Signaling Pathway Important for Normal and Cancer Cell Growth.* Seminars in Oncology, 2009. **36, Supplement 3**(0): p. S3-S17.

17. You, J.S., J.W. Frey, and T.A. Hornberger, *Mechanical stimulation induces mTOR signaling via an ERK-independent mechanism: implications for a direct activation of mTOR by phosphatidic acid.* PLoS One, 2012. **7**(10): p. e47258.

18. Zaytseva, Y.Y., et al., *mTOR inhibitors in cancer therapy.* Cancer Letters, 2012. **319**(1): p. 1-7.

19. Hou, X., E.W. Arvisais, and J.S. Davis, *Luteinizing hormone stimulates mammalian target of rapamycin signaling in bovine luteal cells via pathways independent of AKT and mitogen-activated protein kinase: modulation of glycogen synthase kinase 3 and AMP-activated protein kinase.* Endocrinology, 2010. **151**(6): p. 2846-57.

20. Vezina, C., A. Kudelski, and S.N. Sehgal, *Rapamycin (AY-22,989), a new antifungal antibiotic. I. Taxonomy of the producing streptomycete and isolation of the active principle.* J Antibiot (Tokyo), 1975. **28**(10): p. 721-6.

21. Huber, T.B., G. Walz, and E.W. Kuehn, *mTOR and rapamycin in the kidney: signaling and therapeutic implications beyond immunosuppression.* Kidney Int, 2011. **79**(5): p. 502-511.

22. Spilman, P., et al., *Inhibition of mTOR by Rapamycin Abolishes Cognitive Deficits and Reduces Amyloid-β Levels in a Mouse Model of Alzheimer's Disease.* PLOS ONE, 2010. **5**.

23. Dreyer, H.C., et al., *Leucine-enriched essential amino acid and carbohydrate ingestion following resistance exercise enhances mTOR signaling and protein synthesis in human muscle.* American Journal of Physiology - Endocrinology And Metabolism, 2008. **294**(2): p. E392-E400.

24. Du, M., et al., *Leucine stimulates mammalian target of rapamycin signaling in C2C12 myoblasts in part through inhibition of adenosine monophosphate-activated protein kinase.* Journal of Animal Science, 2007. **85**(4): p. 919-927.

25. Gran, P. and D. Cameron-Smith, *The actions of exogenous leucine on mTOR signalling and amino acid transporters in human myotubes.* BMC Physiol, 2011. **11**: p. 10.

26. Lynch, C.J., *Role of Leucine in the Regulation of mTOR by Amino Acids: Revelations from Structure–Activity Studies.* The Journal of Nutrition, 2001. **131**(3): p. 861S-865S.

27. Norton, L.E. and D.K. Layman, *Leucine Regulates Translation Initiation of Protein Synthesis in Skeletal Muscle after Exercise.* The Journal of Nutrition, 2006. **136**(2): p. 533S-537S.

28. Pimentel, G. and J. Zemdegs. *Leucine stimulates mTOR and muscle protein synthesis in both animal and human*. 2009; 131:[Available from: http://www.efdeportes.com/efd131/leucine-stimulates-mtor-and-muscle-protein-synthesis.htm.

29. Suryawan, A., et al., *Leucine stimulates protein synthesis in skeletal muscle of neonatal pigs by enhancing mTORC1 activation.* Am J Physiol Endocrinol Metab, 2008. **295**(4): p. E868-75.

30. Suryawan, A., et al., *TRIENNIAL GROWTH SYMPOSIUM: Leucine acts as a nutrient signal to stimulate protein synthesis in neonatal pigs.* Journal of Animal Science, 2011. **89**(7): p. 2004-2016.

31. Vianna, D., et al., *Protein synthesis regulation by leucine.* Brazilian Journal of Pharmaceutical Sciences, 2010. **46**: p. 29-36.

32. Wyeth Pharaceuticals Company, *Rapamune [package insert]*. 2012, Pfizer Inc.: Philadelphia, PA.

33. Dawson, N.J., *The surface-area-body-weight relationship in mice.* Australian Journal Of Biological Sciences, 1967. **20**(3): p. 687-690.

34. Economos, A.C., *On structural theories of basal metabolic rate.* Journal of Theoretical Biology, 1979. **80**(4): p. 445-450.

35. Elting, E.C., *A Formula for Estimating Surface Area of Dairy Cattle.* Journal of agricultural research, 1926. **33**(3): p. 269-279.

36. Gray, B.F., *On the "surface law" and basal metabolic rate.* Journal of Theoretical Biology, 1981. **93**(4): p. 757-767.

37. Mitchell, H.H., *The Significance of Surface Area Determinations.* The Journal of Nutrition, 1930. **2**(5): p. 437-442.

38. Murlin, J.R., *On the Law of Surface Area in Energy Metabolism.* Science, 1921. **54**(1392): p. 196-200.

39. Peters, A.M., B.L. Henderson, and D. Lui, *Indexed glomerular filtration rate as a function of age and body size.* Clin Sci (Lond), 2000. **98**(4): p. 439-44.

40. Pinkel, D., *The Use of Body Surface Area as a Criterion of Drug Dosage in Cancer Chemotherapy.* Cancer Research, 1958. **18**(7): p. 853-856.

41. Redal-Baigorri, B., K. Rasmussen, and J.G. Heaf, *Indexing glomerular filtration rate to body surface area: clinical consequences.* J Clin Lab Anal, 2014. **28**(2): p. 83-90.

42. Spaargaren, D.H., *Metabolic rate and body size: a new view on the 'surface law' for basic metabolic rate.* Acta Biotheor, 1994. **42**(4): p. 263-9.

43. Kleiber, M., *Body size and metabolism.* ENE, 1932. **1**: p. E9.

44. Kleiber, M., *Body size and metabolic rate.* Physiol. rev, 1947. **27**(4): p. 511-541.

45. Kleiber, M., *Metabolic rate and food utilization as a function of body size*, in *Metabolic rate and food utilization as a function of body size.* 1961, Missouri Agric. Exp. Stat.

46. Du Bois, D. and E.F. Du Bois, *A formula to estimate the approximate surface area if height and weight be known. 1916.* Nutrition, 1916. **5**(5): p. 303-11; discussion 312-3.

47. Meeh, K., *Oberflächenmessungen des menschlichen Körpers.* Zeitschrift Fur Biologie, 1879(15): p. 425–58.

48. Mitchell, H.H., *The Surface Area of Single Comb White Leghorn Chickens.* The Journal of Nutrition, 1930. **2**(5): p. 443-449.

49. Wang, J. and E. Hihara, *A unified formula for calculating body surface area of humans and animals.* Eur J Appl Physiol, 2004. **92**(1-2): p. 13-7.

50. Diack, S.L., *The determination of the surface area of the white rat.* The Journal of Nutrition, 1930. **3**(3): p. 289-296.

51. Perez, C.R., J.K. Moye, and C.A. Pritsos, *Estimating the surface area of birds: using the homing pigeon (Columba livia) as a model.* Biology Open, 2014.

52. Hounsfield, G.N., *Computerized transverse axial scanning (tomography): Part I. Description of system. 1973.* The British Journal Of Radiology, 1973. **68**(815): p. H166-H172.

53. Ambrose, J., *Computerized transverse axial scanning (tomography). 2. Clinical application.* The British Journal Of Radiology, 1973. **46**(552): p. 1023-1047.

CHAPTER 3

CHARACTERIZATION OF THE ROLE OF THE AVIAN TARGET OF RAPAMYCIN (avTOR) IN FEED INTAKE AND GROWTH

Morris, J. A., Beckstead, R. B., Aggrey, S. E. To be submitted to *Poultry Science*.

Abstract

With ever changing consumer demands and rising input costs, the challenges facing the broiler industry are mounting. At the core of these challenges are increasing concern over environmental impact and the immense increases in the cost of corn for feed. While numerous techniques are being employed to help to mitigate these obstacles, improving the feed efficiency of chickens has the potential to ameliorate a significant portion of this additional stress. As such, a better understanding of the molecular machinery governing feed efficiency traits is crucial. The mammalian target of rapamycin (mTOR) gene and protein have been implicated as putative regulators of metabolism and growth. Avian TOR (avTOR) exhibits over 97% homology to its mammalian counterpart. To aid in the characterization of the role avTOR plays in feed efficiency, we treated pedigreed broiler lines divergently selected for residual feed intake with rapamycin, a potent mTOR inhibitor, and leucine, a stimulator of mTOR signaling, while measuring their feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR). While gender played a significant role on BWG, the only significant factor contributing to differences in FI was feed efficiency line. As such, the high efficiency line exhibited a much improved FCR over the low efficiency line. While treatments did not have a statistically significant impact, they did elicit an effect in the low efficiency line by lowering both BWG and FI. Even so, FCR was slightly worse as compared to the control group. Though there appeared to be a physiological affect due to treatment, variation within the control impeded statistical significance. To further implicate avTOR as a putative regulator of feed efficiency traits, additional research with increased N and additional replicates will be required to determine the significance of the observed changes.

Introduction

Despite the extraordinary gains in broiler production that have already been achieved, market and consumer demands are ever shifting. Significant changes in the market have come as a result of a mounting concern over environmental impact and carbon emissions. The use of corn for the production of ethanol and biofuels has diverted a significant portion of the grain from incorporation in animal feeds. This has contributed to the approximately threefold increase in the price of corn since 2005 [1]. The impact on the poultry industry is huge as feed costs represent approximately 70% of the total cost of production [2-4]. Simultaneously, there has been an increasing demand for a diminished environmental impact. A large portion of this concern is focused on the release of free nitrogen (N) and phosphorus (P) into the environment. This N and P is excreted by the animal in the feces and deposited into the litter which is frequently sold to nearby farms as fertilizer. This provides significant potential for runoff contamination of nearby rivers, lakes, and estuaries. The poultry industry must certainly address these problems in order to maintain economic and social viability [5].

Improving the feed efficiency of the animals is likely the most efficient means to accomplish these tasks. Feed efficiency is commonly assessed as feed conversion ratio (FCR) which is the amount of feed consumed per production gain or kilograms consumed per kilograms of body weight gain (BWG) for broilers. There is a recent trend toward assessing efficiency by Residual Feed Intake (RFI) which takes into account nutritional needs for maintenance and growth separately at a given weight and can be expressed as $RFI = RFI_M + RFI_G$, where RFI_M represents maintenance requirements and RFI_G represents requirements for growth [6-8]. Numerous methods have been employed to improve feed efficiency in chickens including genetic selection, feed additives such as enzymes and oils, lighting colors and schedules, rearing temperature, and litter quality [2, 4, 9-13]. The heritability of feed efficiency traits, particularly RFI, has been demonstrated in broilers and breeding selection is primarily responsible for the improvements already attained [4]. Though highly efficacious, it is thought that room for genetic improvement through selection still exists. Efficiency has also been successfully improved by increasing the crude protein in the diet with increasing interest in the dietary amino acid (AA) profile. This focus has begun to narrow onto the branched chain amino acids (BCAA), particularly leucine, as stimulators of protein synthesis [9-11, 14-18]. This stimulation is intended to increase N and P retention and nutrient utilization by improving feed efficiency, thereby reducing the amount of free N and P excreted into the environment [3, 4].

Only recently have studies attempted to elucidate the underlying molecular basis for feed efficiency and its relationship with protein anabolism. The protein commonly referred to as mechanistic Target of Rapamycin (mTOR) and the gene of the same name has arisen as a major target of these studies. mTOR, is a highly conserved serine/threonine kinase that exhibits over 97% homology between the human mTOR protein and avian TOR (avTOR) in chickens according to alignment analysis performed using NCBI's BLASTp tool [19]. It acts as a sensor of cellular inputs such as ATP and ADP levels, oxidative stress, insulin and glucose levels, hormones, growth factors, and amino acids. It integrates these signals then disseminates the information to stimulate and support processes such as cell growth and proliferation, autophagy, lipid metabolism, transcription, and translation [14, 17, 20-24]. It exists in the cell in two distinct complexes, mTOR complex 1 (mTORC1) and complex 2 (mTORC2). mTORC1, comprised of mTOR, regulatory associated protein of mTOR (RAPTOR), G protein beta subunit-like (GβL), and DEP domain containing mTOR-interacting protein (DEPTOR), is the key regulator of cell growth and proliferation and of translation. mTOR regulates protein metabolism primarily through the activation or inhibition of cap-dependent translation through the phosphorylation of downstream targets such as ribosomal protein S6 kinase beta-1 (p70S6K), eukaryotic translation initiation factor 4 gamma (EIF4G), and EIF 4E binding protein 1 (4E-BP1), among others. Its activity is potently inhibited by the macrolide antibiotic rapamycin through the inhibition of the interaction between mTOR and RAPTOR in mTORC1 whereas leucine and other BCAAs stimulate mTORC1. mTORC2 is primarily involved in the regulation and maintenance of the cytoskeleton.

The link between the genotype and phenotype of feed efficiency may be elucidated by the investigation of the mTOR protein and its encoding and regulatory genes as well other members involved in the mTOR pathway. This knowledge would provide a rapid method for breeding selection based on a genotype for feed efficiency, thus replacing more costly and laborious selections based on phenotype. The objectives of this study are to characterize differences in feed efficiency phenotypes in pedigreed lines of broiler chickens and to identify changes in those phenotypes generated by oral treatment with either leucine or rapamycin. We hypothesize that there will be notable differences in the phenotypic expression of feed efficiency between broiler strains and that a correlation will exist between phenotype and genotype as evidenced in the mTOR kinase cascade and gene pathway.

Materials and Methods

Pedigreed broiler lines divergently selected against feed efficiency (residual feed intake from day 35 to 42, RFI_{35-42}) and maintained at The University of Georgia Poultry Research Center were used for this study [4]. From the resulting high (HRFI, low efficiency) and low (LRFI, high efficiency) lines, approximately 135 chicks were hatched, vent sexed, and tagged with uniquely numbered tags. All birds were raised according to conditions detailed in Aggrey et al. (2010). Initially reared in floor pens, birds were randomly placed into individual cages at 28 days and switched to a grower/finisher ration. Blocking for feed efficiency line, they were randomly assigned to one of four treatment groups: Baseline, Control, Leucine, and Rapamycin. The Baseline group was terminated and processed at day 35 to serve as a reference point for future studies. The Control group was given a placebo treatment of 1 ml of 25% ethanol. The Leucine group was provided supplemental leucine at 4x the NRC recommendation which was solubilized in ethanol and diluted in water [25]. The Rapamycin group was given a treatment of rapamycin at a dosage of 1.5 mg/kg solubilized in water with ethanol added to a concentration of 25%. These inclusion rates enabled the incorporation of ethanol into all treatment groups while preventing the precipitation of the solute. All treatments were administered orally by syringe once daily. The feed for each pen was weighed at days 28, 35, and 42. Feed consumption was calculated for the periods from day 28-35 and day 35-42. Body weights were recorded at day 28 and daily from day 35-42. All remaining birds were terminated and processed at day 42. At processing, the mass of the wings, thighs, pectoralis major, pectoralis minor, and abdominal fat pad were measured. Using clean instruments treated with AbSolve™ Glassware Cleaner, tissue samples were collected from the pectoralis major, distal portion of the duodenum, liver, and hypothalamus. Tissues were snap frozen in liquid nitrogen and stored at -80°C for later use. Data were analyzed by using ANOVA procedures in JMP® Statistical Discovery from SAS. All analyses of BWG, FI, and FCR considered only the day 35-42 time period.

Results

Body weight gain did not differ significantly ($p \leq 0.05$) for between any treatments or lines. Only sex had a significant effect on body weight gain with males outgaining females by an average of 56.29g. Feed intake (FI) and abdominal fat pad mass differed significantly between the LRFI and HRFI lines. Individuals from the HRFI line consumed an average of 134.4g more feed and deposited an average of 7.5g more fat into the abdominal fat pad than those from the LRFI line. Similarly, individuals from the LRFI line exhibited a 1.3 fold increase in breast muscle yield to abdominal fat pad mass ratio over those from the HRFI line. FCR was also improved in the LRFI line over the HRFI line by a factor of 0.82 with mean values of 2.07 and 2.51 respectively.

The treatments did elicit an effect on the performance of the lines as compared to the control. For both the leucine and rapamycin treatments, BWG and FI exhibited similar decreases in the HRFI line. However, despite these decreases, FCR increased from the control mean for both leucine and rapamycin treatments by 1.9% or 4.7% respectively. These results were drastically different from those of the LRFI line with BWG and FI increasing over those of the control. Most significantly, in contrast to the increases seen in BWG and FI, FCR for the leucine group decreased by 4.9% below that of the control. Treatment with rapamycin had the opposite effect on FCR in this line resulting in an increase of 4.5%. All other processing data was statistically similar.

Conclusions

With BWG being similar for both lines, the selection process against RFI has had no discernable effect on the rate of growth for these broiler lines. Neither BWG nor body weight differed between the lines. In contrast, FI was higher in the HRFI line as compared to the LRFI line. Furthermore, the HRFI line is less efficient at utilizing and depositing the energy and nutrition provided in the feed in a desirable manner. This is evidenced by the increase in fat mass in the abdominal fat pad. In effect, selecting for RFI selects for feed consumption traits independently of growth traits. Given the maintenance of similarity of body weight and rate of gain and the differences seen in feed consumption, it is logical that FCR was significantly improved in the LRFI line. Based on these parameters alone, it is reasonable to conclude that feed efficiency is a heritable and selectable trait and that improvements in this trait are attainable through basic selection techniques.

Discussion

The individual treatments did not elicit as dramatic an effect as hypothesized. All treatments lacked statistical significance in all parameters measured. Therefore the deviation from the control means was examined for the leucine and rapamycin groups. While these deviations did not generally differ between treatment groups within a given line, they were markedly different from the controls. For both the leucine and rapamycin treatments, BWG and FI were diminished from the control means for the HRFI line by an average of 5.8% and 0.8% respectively. This resulted in an increased FCR for both groups. This was contrary to the hypothesis that leucine should improve FCR through the stimulation of avTOR activity. Both treatments resulted in the already inefficient line becoming even less efficient. The LRFI line exhibited results more similar to the expected outcomes. BWG increased from the control by 8.8% and 1.5% for the leucine and rapamycin groups respectively while FI increased by slightly more than 4% for both treatments. The ratio of change between BWG and FI resulted in FCR improvement by 4.9% in the leucine group and a worsening by 4.5% in the rapamycin group. The contrasting results suggest a difference in the metabolic machinery between the HRFI and LRFI lines given how differently the two responded to the treatments.

All of the data indicate that improvement in efficiency is both reasonable and attainable by standard selective breeding processes. Given the lack of statistical significance, additional study will be required to determine the validity of the physiological effects observed. This will be achieved by increasing the number of birds and the number of replicates. The data from the LRFI group provide additional support for the involvement of avTOR and the avTOR signaling pathway in feed efficiency phenotypes. As such, it would be advisable that the industry begin to focus on selecting for feed efficiency in order to ameliorate some of the upcoming and rising concerns facing it. Still, the molecular machinery requires further study to identify all of those genes directly involved in feed efficiency. To this end, this study will be followed by molecular analyses of those tissues collected. These will include characterizing the transcriptional activity of key members of the pathway as well as correlating transcriptional activity to translational activity. This will be achieved by performing qRT-PCR and ELISA assays against those key members. These studies can help to correlate activity at the cellular level to actual feed efficiency phenotypes. The ultimate goal is to identify specific SNPs and alleles that encode for feed efficiency.

References

1. USDA National Agricultural Statistics Service, *Prices Received fo Corn by Month - United States*. 2014, USDA-NASS: Washington D.C.

2. de Verdal, H., et al., *Improving the efficiency of feed utilization in poultry by selection. 1. Genetic parameters of anatomy of the gastro-intestinal tract and digestive efficiency.* BMC Genet, 2011. **12**: p. 59.

3. Ankra-Badu, G.A., G.M. Pesti, and S.E. Aggrey, *Genetic interrelationships among phosphorus, nitrogen, calcium, and energy bioavailability in a growing chicken population.* Poult Sci, 2010. **89**(11): p. 2351-5.

4. Aggrey, S.E., et al., *Genetic properties of feed efficiency parameters in meat-type chickens.* Genet Sel Evol, 2010. **42**: p. 25.

5. The PEW Charitable Trusts *Big Chicken: Pollution and Industrial Poultry Production in America - Pew Environment Group*. 2011.

6. Case, L.A., B.J. Wood, and S.P. Miller, *The genetic parameters of feed efficiency and its component traits in the turkey (Meleagris gallopavo).* Genet Sel Evol, 2012. **44**: p. 2.

7. Koch, R.M., et al., *Efficiency of Feed Use in Beef Cattle.* Journal of Animal Science, 1963. **22**(2): p. 486-494.

8. Sainz, R.D. and P.V. Paulino, *Residual Feed Intake*. 2004.

9. Suryawan, A., et al., *Leucine stimulates protein synthesis in skeletal muscle of neonatal pigs by enhancing mTORC1 activation.* Am J Physiol Endocrinol Metab, 2008. **295**(4): p. E868-75.

10. Suryawan, A., et al., *TRIENNIAL GROWTH SYMPOSIUM: Leucine acts as a nutrient signal to stimulate protein synthesis in neonatal pigs.* Journal of Animal Science, 2011. **89**(7): p. 2004-2016.

11. Vianna, D., et al., *Protein synthesis regulation by leucine.* Brazilian Journal of Pharmaceutical Sciences, 2010. **46**: p. 29-36.

12. Cao, J., et al., *Effect of combinations of monochromatic lights on growth and productive performance of broilers.* Poult Sci, 2012. **91**(12): p. 3013-8.

13. Weber, G.M., et al., *Effects of a blend of essential oil compounds and benzoic acid on performance of broiler chickens as revealed by a meta-analysis of 4 growth trials in various locations.* Poult Sci, 2012. **91**(11): p. 2820-8.

14. Borders, E.B., C. Bivona, and P.J. Medina, *Mammalian target of rapamycin: Biological function and target for novel anticancer agents.* American Journal of Health-System Pharmacy, 2010. **67**(24): p. 2095-2106.

15. Norton, L.E. and D.K. Layman, *Leucine Regulates Translation Initiation of Protein Synthesis in Skeletal Muscle after Exercise.* The Journal of Nutrition, 2006. **136**(2): p. 533S-537S.

16. Pimentel, G. and J. Zemdegs. *Leucine stimulates mTOR and muscle protein synthesis in both animal and human*. 2009; 131:[Available from: http://www.efdeportes.com/efd131/leucine-stimulates-mtor-and-muscle-protein-synthesis.htm.

17. Frost, R.A. and C.H. Lang, *mTor Signaling in Skeletal Muscle During Sepsis and Inflammation: Where Does It All Go Wrong?* Physiology, 2011. **26**(2): p. 83-96.

18. Dreyer, H.C., et al., *Leucine-enriched essential amino acid and carbohydrate ingestion following resistance exercise enhances mTOR signaling and protein synthesis in human muscle.* American Journal of Physiology - Endocrinology And Metabolism, 2008. **294**(2): p. E392-E400.

19. Altschul, S.F., et al., *Gapped BLAST and PSI-BLAST: a new generation of protein database search programs.* Nucleic Acids Res, 1997. **25**(17): p. 3389-402.

20. Ballou, L.M. and R.Z. Lin, *Rapamycin and mTOR kinase inhibitors.* J Chem Biol, 2008. **1**(1-4): p. 27-36.

21. Cota, D., et al., *Hypothalamic mTOR Signaling Regulates Food Intake.* Science, 2006. **312**(5775): p. 927-930.

22. Dowling, R.J.O., et al., *Dissecting the role of mTOR: Lessons from mTOR inhibitors.* Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics, 2010. **1804**(3): p. 433-439.

23. Gibbons, J.J., R.T. Abraham, and K. Yu, *Mammalian Target of Rapamycin: Discovery of Rapamycin Reveals a Signaling Pathway Important for Normal and Cancer Cell Growth.* Seminars in Oncology, 2009. **36, Supplement 3**(0): p. S3-S17.

24. You, J.S., J.W. Frey, and T.A. Hornberger, *Mechanical stimulation induces mTOR signaling via an ERK-independent mechanism: implications for a direct activation of mTOR by phosphatidic acid.* PLoS One, 2012. **7**(10): p. e47258.

25. Nutrition;, N.R.C.S.o.P., *Nutrient Requirements of Poultry: Ninth Revised Edition, 1994*. 1994, The National Academies Press.

Tables and Figures

Figure 3.1 The PI3K/mTOR signaling pathway, adopted from Huber *et al*. [21].

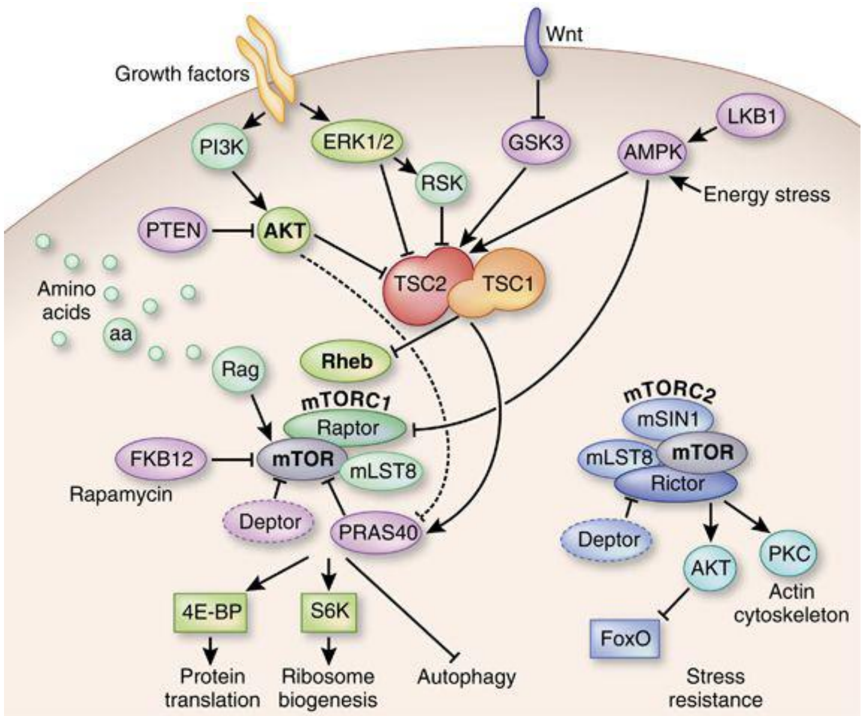

Figure 3.2 Average body weight gain (BWG) with SEM by line and treatment.

Figure 3.3 Average feed intake (FI) with SEM by line and treatment.

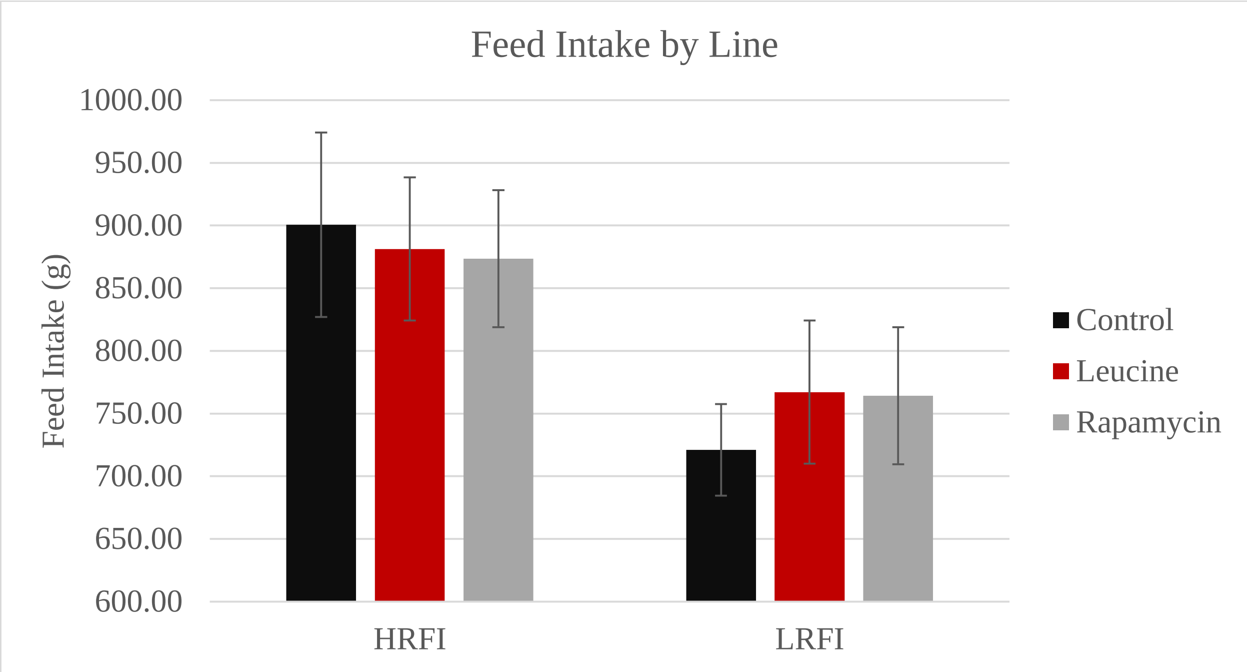

Figure 3.4 Average feed conversion ratio (FCR) with SEM by line and treatment.

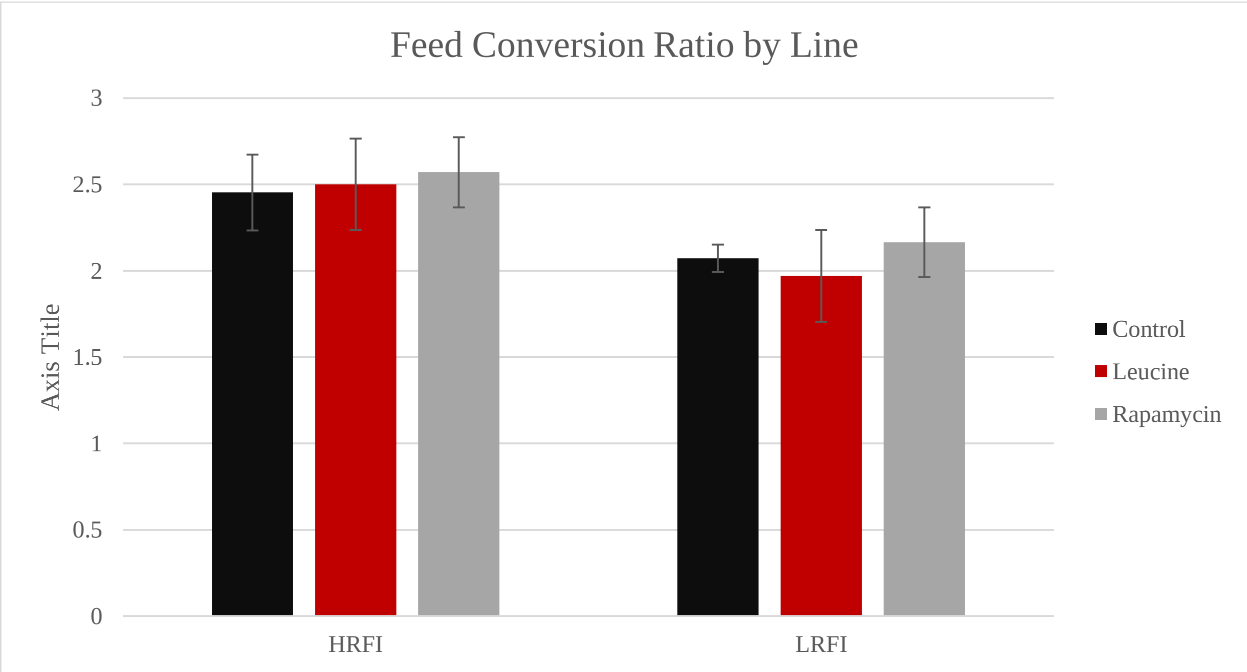

Figure 3.5 Percent deviation from Control means for BWG, FI, and FCR by treatment for the HRFI line. All p values were greater than 0.05.

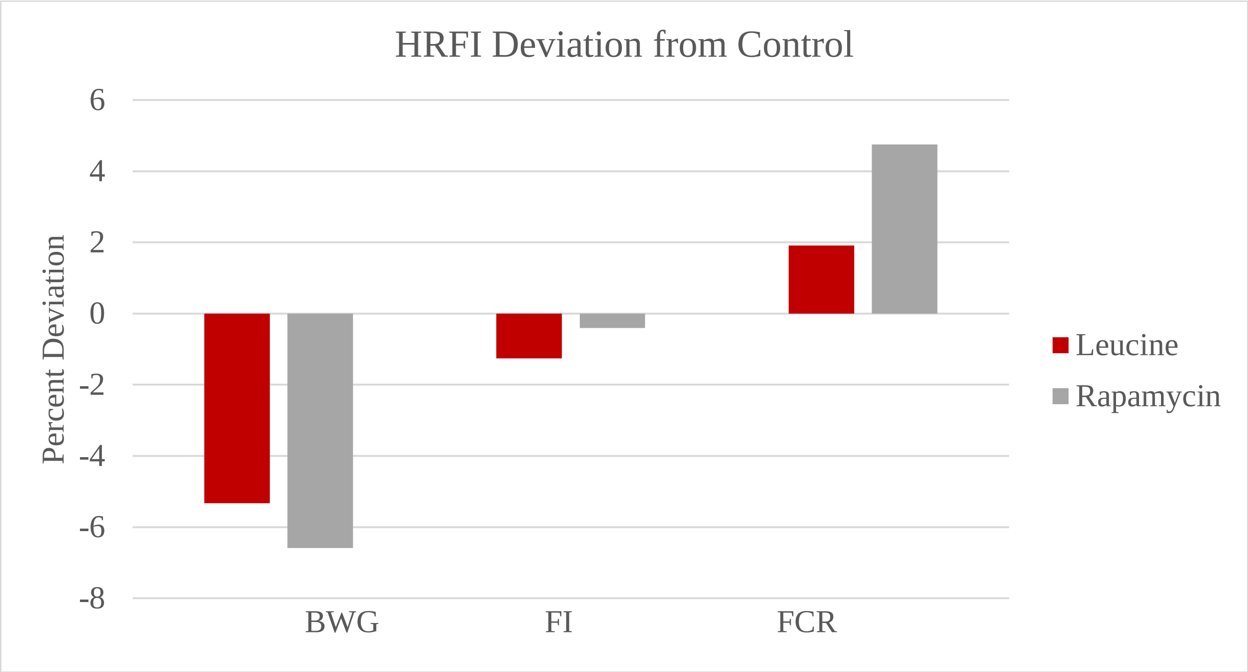

Figure 3.6 Percent deviation from Control means for BWG, FI, and FCR by treatment for the LRFI line. All p values were greater than 0.05.

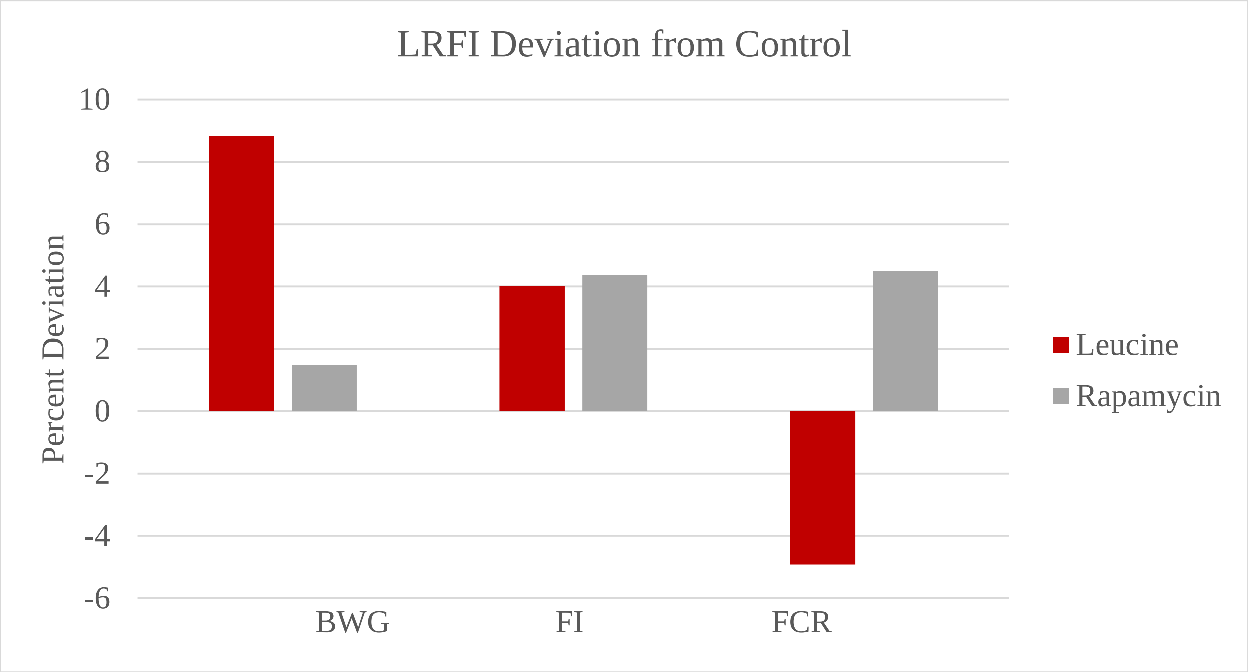

Table 3.1 Production data in grams for total breast meat yield, abdominal fat pad, and total yield (breast, wings, and thighs) by line and treatment.

		Control	Leucine	Rapamycin
HRFI	Breast Yield	213	206.46	214.38
	Abdominal Fat	27.22	29.38	29.23
	Breast/Fat Ratio	7.82	7.03	7.33
	Total Yield	558.22	547.85	555.92
LRFI	Breast Yield	202	206.85	210.86
	Abdominal Fat	22.05	20.54	20.67
	Breast/Fat Ratio	9.16	10.07	10.2
	Total Yield	535.95	547.15	546.19

Table 3.2 P values indicating the significance of the effect of Sex, Line, and Treatment on each production trait.

Trait	Sex	Line	Treatment
BWG	6.34e-07	0.432	0.787
FI	0.330	4.14e-06	0.890
FCR	7.66e-04	1.56e-05	0.404
Breast Yield	2.97e-04	0.144	0.581
Abdominal Fat	0.051	6.85e-05	0.997
Breast/Fat Ratio	0.007	0.003	0.486

CHAPTER 4

ASSESSMENT OF THE ESTIMATION OF TOTAL SURFACE AREA OF BROILER CHICKENS BY COMPUTED TOMOGRAPHY

Morris, J. A., Sharma, A., Daley, W., Beckstead, R. B. To be submitted to *Poultry Science*.

Abstract

Poultry production is an industry whose primary concern is growth. While the majority of focus is on saleable yield, other oft overlooked aspects of growth are of great import. Body surface area (BSA) is one such characteristic. Rarely considered, it has enormous implications on heat loss and retention as well as metabolic rate. There exist a number of methods to predict the BSA of an animal with Meeh's formula ($S = kW^{2/3}$, where S is surface area, W is body weight, and k is a species specific constant) being the canonical methodology. Unfortunately, there is no consensus on the value k should take for avian species. Numerous k values and variations of the formula have been employed, each yielding highly variable results. Our assessment found BSA estimates with over 50% ranges in variation and over 27% deviations from the actual BSA. Therefore, we suggest that a new formula that incorporates additional parameters is essential to improving the quality of the BSA estimates.

Introduction

The goal of the poultry industry, as with most animal production industries, is to maximize saleable yield. At its core, it is an industry of growth. Its chief concern is overall body growth as well as the deposition of lean muscle mass and fat. Therefore, body weight has been the primary focus of the industry since its inception. While there is little doubt that body weight and lean muscle mass should and will continue to be of utmost importance for consumers, there remain other aspects of growth that, better understood, could prove useful to producers.

With correlations to heat production, metabolism, and blood volume among others, body surface area (BSA) is one such trait [1-9]. Thermoregulation, a critical aspect of broiler growth and welfare, is greatly impacted by the surface area. Birds are better able to maintain their body temperature as the surface area to volume ratio decreases. Their large surface area to volume ratio, which results in a high rate of heat loss by convection, is the primary reason that newly hatched chicks require supplemental heat during the brooding period. This physical characteristic provides assistance in cooler climates and is a challenge that must be overcome in warmer climates. This is further impacted by basal metabolic rate and heat production which have also been shown to be correlated with BSA [10-12]. Given the close relationships to these traits that affect production, there is a need to better comprehend the role of BSA in broiler production.

Unrelated to poultry production, BSA has several applications in human and veterinary medicine. For both human and veterinary pharmacology, BSA has long been used to calculate dosages for certain medications, particularly for antineoplastic drugs [3, 7, 13-17]. This is due to the correlation between surface area and metabolism and helps to account for variations in patient body composition when calculating drug doses. Similarly, many drugs are metabolized in the kidneys. This is relevant to BSA as glomerular filtration rate has been shown to be correlated with BSA [6, 8]. For obvious reasons, the assessment of surface area is critical for burn victims [14]. For these conditions, segmented surface area (hands, torso, legs, etc.) as well as total BSA is of high interest.

The issue of calculating or estimating BSA is not a recent one. Meeh, for example, published his predictive equation $S = k*BW^{2/3}$, where S is surface area, k is a constant, and BW is body weight, in 1879 [18]. While developed for use in humans and animals, other formulas have since replaced the Meeh formula for the estimation of BSA in humans. However, the Meeh formula is still the standard for use in veterinary medicine and requires the use of a species specific k constant. Numerous iterations of k values have been published for various species including chickens [19-22]. Similarly, several attempts have been made to update and improve the Meeh formula. While each has yielded some fairly accurate estimates, almost all have exhibited a rather large range in deviation of the predicted values from the measured BSA. In most cases, the range of values was more than forty percent. There are some where the range in values is over fifty percent. Further complicating the matter, most of the model species have continued to undergo some type of selection pressure. This is certainly the case for broilers. As such, k constants suggested even ten years ago may no longer be relevant. Values over fifty years old are undoubtedly anachronistic. Given that these values rely on a single predictor value, body weight, there is room for significant improvement without the addition of much tedium or labor by simply including other predictor values.

Furthermore, the historic methods for measuring BSA may also be a source of error. Some earlier methods have involved removing the skin or pelt from the animal [23]. Others involved making a cast or mold using medical gauze and some fixative agent [20]. The skins or gauze were then measured using a planimeter. Depending on the animal, methods employed during removal, and delay between removal and measurement, the samples may have been stretched or torn or even allowed to dry and shrink thereby reducing the reliability of the measurements taken. Others have placed the specimen inside a plastic pouch, traced the outline of the animal, then used a planimeter to measure the surface area of the tracings on the pouches. Fortunately, this material is unlikely to exhibit issues of stretching or shrinking [24-26]. It is, however, prone to variations based on body positioning as well as the issue of ensuring a proper fit of the material to the body to ensure that the pouch was neither too loose nor too tight thus altering the measurement. Still, others employed the use of a cylinder of known surface area and a revolution counter for their measurements [23]. The cylinder was rolled along the body (chalk was often used to mark the path of travel of the cylinder to ensure no areas were missed or overlapped) and the revolutions tallied. The surface area of the cylinder was then multiplied by the revolutions to yield the measurement of BSA. This was not only time consuming and laborious, it was incredibly difficult around areas of the body that were not flat. Those using this method frequently only measured a single side of the animal and wrongly assumed perfect body symmetry.

X-ray computed tomography (CT) technology can provide a remedy for these issues. Sharing the 1979 Nobel Prize for the invention, Godfrey N. Hounsfield and Allen M. Cormack began developing their idea for computerized tomography in 1973 [27, 28]. CT has the ability to provide high resolution, three dimensional images from which BSA as well as many other measurements may be assessed. These images, while still subject to the issues of consistent positioning, are immune to issues regarding changes to the morphology of the animals [29, 30]. Likewise, since a full 3-D image is provided, the issue of assumed symmetry is negated. Furthermore, even at its inception in 1972, CT is able to differentiate tissues that differ by less than one percent in physical density [27, 31]. As such, even a single scan can provide a wealth of data at a level of accuracy and detail that is unparalleled by any manual methodology. These highly accurate measurements will enable an assessment of those equations in use to estimate BSA from BW. It is our hypothesis that the existing predictive equations will exhibit significant deviations from true BSA measurements taken from CT images.

Materials and Methods

At day of hatch, 200 Ross 708 broiler chicks were obtained, feather sexed, and placed into floor pens separated by sex. Birds were raised according to conditions specified by the Broiler Management Handbook (available from http://en.aviagen.com/ross-708/). They were provided feed and water *ad libitum* beginning with a starter ration. At day 10 they were moved to a grower ration and finally to a finisher diet at day 25 (Table 3.1). At 1 day of age, ten birds were removed, five male and five female, euthanized by CO_2 gas and body weights recorded. Each bird was imaged by CT scan at the University of Georgia College of Veterinary Medicine. This was repeated each week until the birds reached 8 weeks of age with a total of 90 birds being imaged.

Measurements were taken of the lengths of the right and left humerus, radius, femur, and tibia using the length measurement tool in OsiriX DICOM viewer. Measurements were taken so as to approximate the length of the section as it would be measured on a live bird. In order to account for variability based on technique and individual measurements, each length was measured in triplicate by two individuals. The studies were then processed in Mimics® (Materialise) to refine the image surfaces. Threshold values were adjusted individually for each bird to reduce interference from the feathers and to maximize the visibility and integrity of the skin. The region growing tool was used to calculate a 3D model which was then exported to Geomagic Studio®. A solid mesh was created and defects were repaired using the fill all button and the tangent setting. Some larger holes were filled using the fill single option also using the tangent setting. The mesh was verified using the mesh doctor tool and the resulting image was exported into the 3D CAD software SolidWorks (Dassault Systèmes). The mass properties tab provided the surface area measurement of the imported image in mm^2 which was converted to cm^2. Statistical analyses were performed following ANOVA procedures in JMP® Software from SAS.

Results

Neither body weight (BW) nor body surface area (BSA) differed significantly ($p < 0.05$) between the sexes as can be seen in Table 1 as well as in Figures 1 and 2. While variation tended to increase each week, this was not always the case. As such, both males and females exhibited enough similarity to examine both groups together. All parameters measured showed very high correlations with r^2 values ranging from 0.9522 to 0.9976 (Figure 3, Table 2). It is interesting to note that the leg measurements exhibited the highest r^2 values of all. Even more so, the tibia tended to be the most highly correlated with all other measurements and yielded the highest average r^2 value of 0.9839.

To evaluate the accuracy of existing BSA prediction equations, five different values were considered for Meeh's k constant as well as Mitchell's proposed revised equation (Table 3). To more accurately examine the deviation of each prediction from the actual BSA, the absolute value of the percent deviation were also calculated. The predictions yielded estimates of varying accuracy with values as low as 0.09% deviation or as high as 31.68% deviation and ranges of over 50%. Mitchell's revised equation gave results with the lowest average accuracy and widest range, whereas using Meeh's formula with a k value of 9.64 provided the closest predictions overall. Still, even this value provided results with almost a 28% deviation from the actual value. All in all, none of the predictive equations consistently gave estimates of acceptable accuracy.

Conclusions

The lack of variation based on sex suggests that gender does not play a significant role when trying to estimate BSA, thus indicating that any the accuracy, or lack thereof, of any predictive equation will not be greatly influenced by gender consideration. Therefore, it suggests that it may not be necessary to include a factor to account for a gender effect in any such equation. The data indicate that there is a lack of any consensus on any one best method for estimating the BSA of a broiler or other avian species. It also shows that there is a severe lack of accuracy in the estimates of the existing equations when applied to a modern broiler. This illustrates the need for the development of a method by which BSA can be readily estimated while rendering consistent results with moderate accuracy.

Discussion

The lack of significant differences between male and female BSA measurements is not altogether unexpected. In accordance with the surface law, all are of similar shape, density, and weight. Thus significant differences here could be considered surprising. Even so, there was a great degree of variation in the body weights of the birds. This variation was only exaggerated as the birds continued to grow. This variation could obfuscate any significance of a gender effect; therefore, it would be unreasonable to completely discount the possibility of a significant gender effect in the estimation of BSA. The use of more genetically pure samples, such as broiler breeders or especially grandparent stock, would alleviate this issue and help to truly delineate the presence or absence of a gender effect.

It is significant to note the pattern of growth exhibited when considering both BW and BSA. The line for BW is curved for broiler chickens (Figure 1). This exponential growth will likely necessitate the incorporation of a term raised to some power or a mathematical transformation of the data to improve the fit of a predictive equation. In contrast, our data show that BSA grows in a more linear fashion (Figure 2).

The lack of accurate BSA estimates from the various equations that exist has long been noted in the literature. Our data simply serve to echo those sentiments and illustrate the highly variable results obtained by the current methodologies. A confounding effect is a lack of consistent positioning of live animals as positioning can change the shape and, therefore, the perceived surface area of an animal. For these reasons, the canonical use of the Meeh formula may be erroneous. This is certainly the case for avian species. While easy to use, Meeh's formula may be overly simplistic where highly accurate estimates are desirable while also assuming some standard position. It thus becomes necessary to employ and develop a new method to improve the reliability of the estimates yielded by the current methods. The incorporation of additional parameters in such an equation is essential to improving the quality of the predictions. As seen here, there are several measurements that would be relatively easy to take, even on a live specimen, that are highly correlated with both BW and BSA. Furthermore, the correlations observed were highest with the leg measurements, especially the tibia. Therefore, the incorporation of tibia or femur length into such a formula has the potential to greatly improve its accuracy. Similarly, it becomes apparent that some consideration for the position of the animal may be useful. As has been shown, there is a significant difference in the surface area of a bird who is standing and one whose wings are extended. While this difference will have little effect on basal metabolic rate, it is certainly relevant to the issue of thermoregulation. Because of this, it could prove useful to incorporate a method to distinguish the two and to establish a standard for taking measurements.

References

1. Economos, A.C., *On structural theories of basal metabolic rate.* Journal of Theoretical Biology, 1979. **80**(4): p. 445-450.

2. Gray, B.F., *On the "surface law" and basal metabolic rate.* Journal of Theoretical Biology, 1981. **93**(4): p. 757-767.

3. Miller, A.A., *Body surface area in dosing anticancer agents: scratch the surface!* Journal Of The National Cancer Institute, 2002. **94**(24): p. 1822-1823.

4. Mitchell, H.H., *The Significance of Surface Area Determinations.* The Journal of Nutrition, 1930. **2**(5): p. 437-442.

5. Murlin, J.R., *On the Law of Surface Area in Energy Metabolism.* Science, 1921. **54**(1392): p. 196-200.

6. Peters, A.M., B.L. Henderson, and D. Lui, *Indexed glomerular filtration rate as a function of age and body size.* Clin Sci (Lond), 2000. **98**(4): p. 439-44.

7. Pinkel, D., *The Use of Body Surface Area as a Criterion of Drug Dosage in Cancer Chemotherapy.* Cancer Research, 1958. **18**(7): p. 853-856.

8. Redal-Baigorri, B., K. Rasmussen, and J.G. Heaf, *Indexing glomerular filtration rate to body surface area: clinical consequences.* J Clin Lab Anal, 2014. **28**(2): p. 83-90.

9. Spaargaren, D.H., *Metabolic rate and body size: a new view on the 'surface law' for basic metabolic rate.* Acta Biotheor, 1994. **42**(4): p. 263-9.

10. Kleiber, M., *Body size and metabolism.* ENE, 1932. **1**: p. E9.

11. Kleiber, M., *Body size and metabolic rate.* Physiol. rev, 1947. **27**(4): p. 511-541.

12. Kleiber, M., *Metabolic rate and food utilization as a function of body size*, in *Metabolic rate and food utilization as a function of body size.* 1961, Missouri Agric. Exp. Stat.

13. Du Bois, D. and E.F. Du Bois, *A formula to estimate the approximate surface area if height and weight be known. 1916.* Nutrition, 1916. **5**(5): p. 303-11; discussion 312-3.

14. Gilpin, D.A., *Calculation of a new Meeh constant and experimental determination of burn size.* Burns, 1996. **22**(8): p. 607-611.

15. Gouma, E., et al., *A simple procedure for estimation of total body surface area and determination of a new value of Meeh's constant in rats.*

16. Jones, P.M., S. Wilkinson, and P.W. Davies, *A revision of body surface area estimations.* European Journal of Applied Physiology and Occupational Physiology, 1985. **53**(4): p. 376-379.

17. Mathijssen, R.H.J., et al., *Flat-Fixed Dosing Versus Body Surface Area–Based Dosing of Anticancer Drugs in Adults: Does It Make a Difference?* The Oncologist, 2007. **12**(8): p. 913-923.

18. Meeh, K., *Oberflächenmessungen des menschlichen Körpers.* Zeitschrift Fur Biologie, 1879(15): p. 425–58.

19. J¨²nior, Y., et al., *Digital surface area assessment of broiler chickens.* Engenharia Agr¨ªcola, 2011.

20. Mitchell, H.H., *The Surface Area of Single Comb White Leghorn Chickens.* The Journal of Nutrition, 1930. **2**(5): p. 443-449.

21. Thomas, N.L., *Observations of the relationship between the surface area and weight of eviscerated carcases of chickens, ducks and turkeys.* International journal of food science & technology, 1978. **13**(2): p. 81-86.

22. WALSBERG, G.E., *The Relationship of the External Surface Area of Birds to Skin Surface Area and Body Mass.* The Journal of Experimental Biology, 1978. **76**(1): p. 185-189.

23. Elting, E.C., *A Formula for Estimating Surface Area of Dairy Cattle.* Journal of agricultural research, 1926. **33**(3): p. 269-279.

24. Dawson, N.J., *The surface-area-body-weight relationship in mice.* Australian Journal Of Biological Sciences, 1967. **20**(3): p. 687-690.

25. Diack, S.L., *The determination of the surface area of the white rat.* The Journal of Nutrition, 1930. **3**(3): p. 289-296.

26. Perez, C.R., J.K. Moye, and C.A. Pritsos, *Estimating the surface area of birds: using the homing pigeon (Columba livia) as a model.* Biology Open, 2014.

27. Hounsfield, G.N., *Computerized transverse axial scanning (tomography): Part I. Description of system. 1973.* The British Journal Of Radiology, 1973. **68**(815): p. H166-H172.

28. Nobelprize.org. *The Nobel Prize in Physiology or Medicine 1979*. 2015; Available from: http://www.nobelprize.org/nobel_prizes/medicine/laureates/1979/.

29. Ambrose, J., *Computerized transverse axial scanning (tomography). 2. Clinical application.* The British Journal Of Radiology, 1973. **46**(552): p. 1023-1047.

30. Heilbrun, M.P., et al., *Preliminary experience with Brown-Roberts-Wells (BRW) computerized tomography stereotaxic guidance system.* J Neurosurg, 1983. **59**(2): p. 217-22.

31. Beckmann, E.C., *CT scanning the early days.* The British Journal of Radiology, 2006. **79**(937): p. 5-8.

Tables and Figures

Table 4.1 Average body weight (g) and average body surface area (cm^2) with standard deviations for males, females, and overall for both sexes combined.

	Male		Female		Overall	
Age (weeks)	BW (g)	BSA (cm^2)	BW (g)	BSA (cm^2)	BW (g)	BSA (cm^2)
0	46.5 ± 4.1	144.89 ± 7.3	47.9 ± 2.3	139.72 ± 5.7	47.2 ±3.4	142.3 ± 7.1
1	166.0 ± 21.3	313.45 ± 29.6	166.8 ± 10.0	310.65 ± 25.2	166.4 ± 16.7	312.05 ± 27.6
2	388.8 ± 37.5	551.67 ± 85.9	423.2 ± 33.3	551.45 ± 30.2	406.0 ± 39.4	551.56 ± 64.4
3	775.2 ± 90.2	838.86 ± 43.8	792.0 ± 70.9	819.59 ± 47.1	783.6 ± 81.5	829.22 ± 46.5
4	1321.0 ± 113.7	1147.09 ± 30.3	1304.6 ± 135.6	1164.34 ± 88.0	1312.8 ± 125.4	1155.71 ± 66.4
5	1988.5 ± 173.2	1588.64 ± 256.3	1740.8 ± 223.3	1324.43 ± 101.3	1864.7 ± 235.1	1456.53 ± 235.4
6	2810.9 ± 143.2	1994.62 ± 300.4	2774.7 ± 298.2	1808.67 ± 406.0	2788.3 ± 253.8	1878.40 ± 363.6
7	3298.1 ± 351.8	2131.30 ± 290.3	3156.9 ± 162.8	2001.56 ± 56.6	3227.5 ± 283.1	2066.43 ± 219.0
8	3780.8 ± 574.9	2367.07 ± 103.2	3536.6 ± 174.9	2381.83 ± 387.0	3658.7 ± 442.1	2374.45 ± 283.3

Figure 4.1 Growth curve showing average weekly body weight (g) by sex and overall.

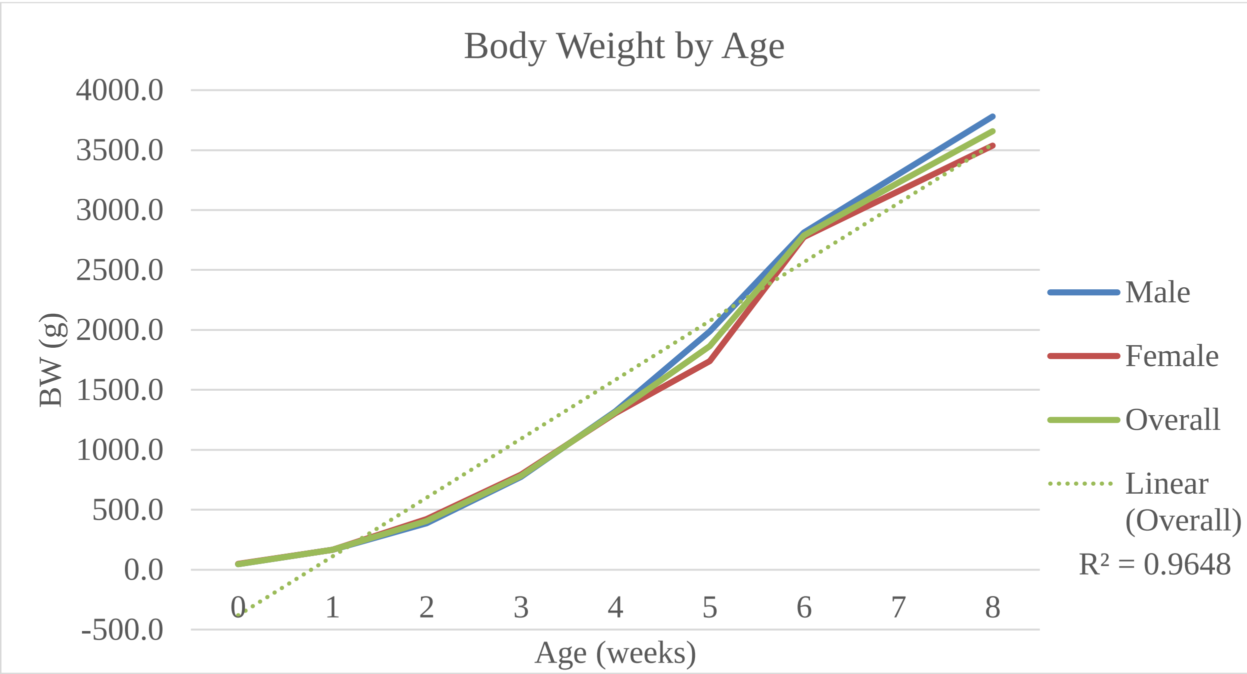

Figure 4.2 Growth curve showing average weekly body surface area (cm^2) by sex and overall.

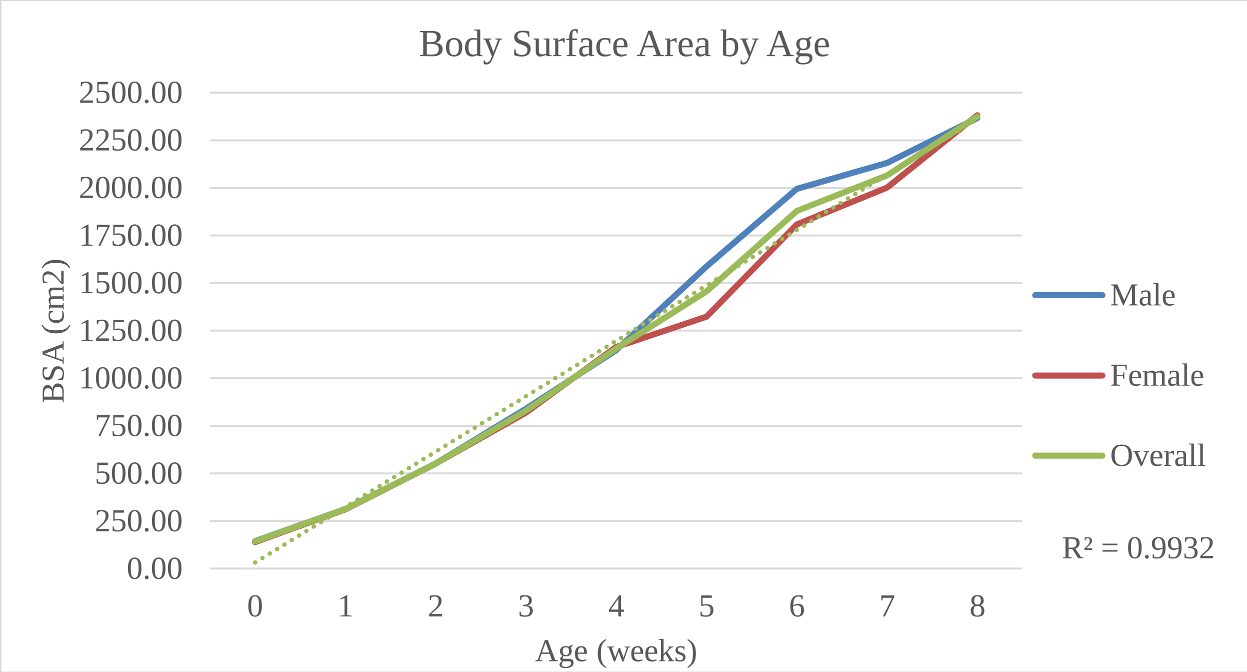

Figure 4.3 Scatterplot matrix indicating correlations of each parameter measured. r^2 values ranged from 0.9522 to 0.9976.

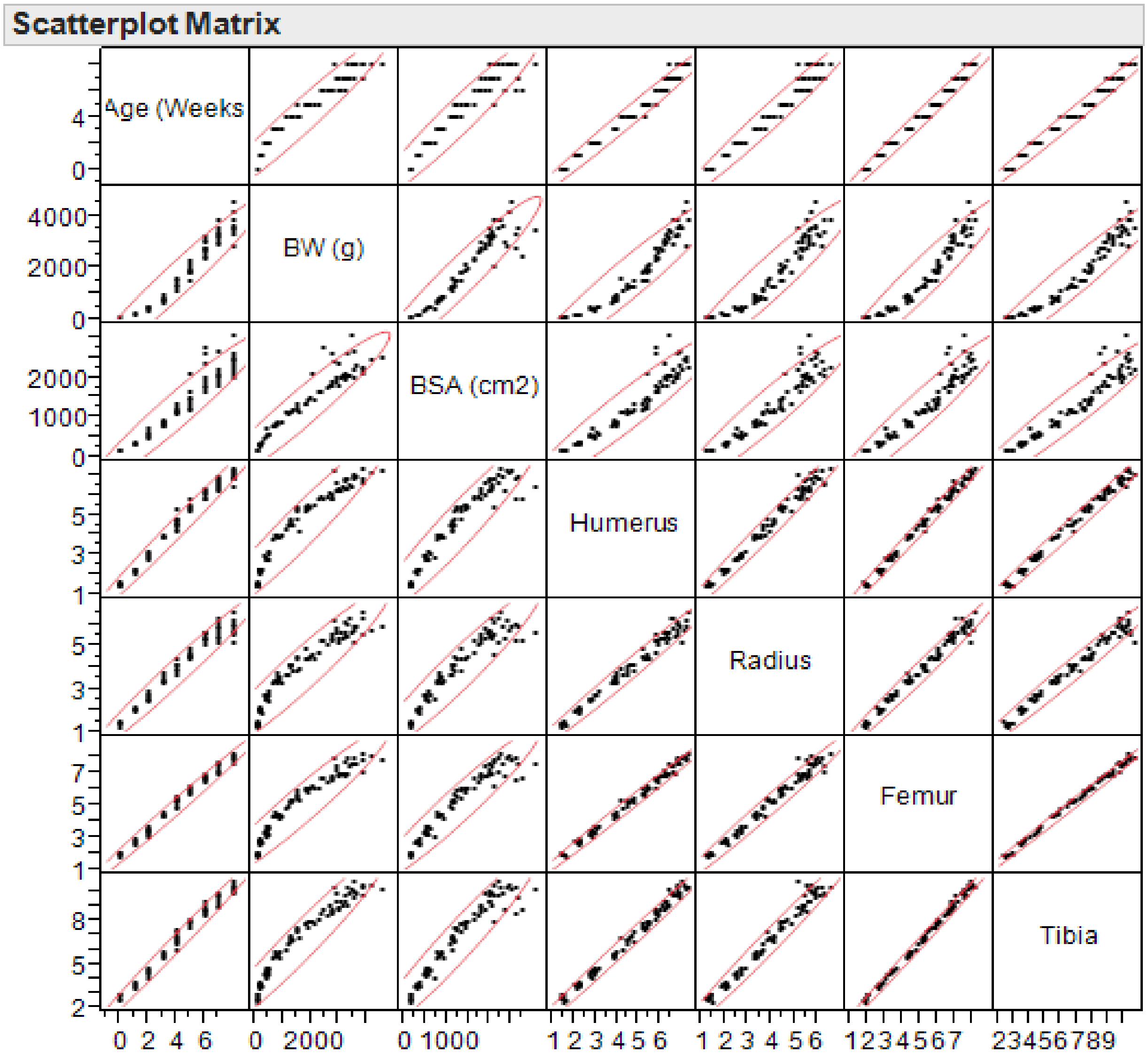

Table 4.2 Assessment of predictive equations using published and experimentally determined k values as well as Mitchell's revised equation.

	Average	Male	Female	Median	1931	Mitchell
k	9.94	10.10	9.77	9.64	10.64	$8.19*W^{0.705}$
Minimum	-25.29	-22.33	-26.59	-27.56	-20.06	-20.68
Maximum	16.90	18.69	14.49	13.36	25.09	31.68
Range	42.20	41.02	41.08	40.92	45.15	52.36
Mean	0.85	0.88	0.56	-2.21	7.91	7.76
Median	3.13	2.59	3.01	0.00	10.35	9.68
Minimum*	0.09	0.38	0.22	0.30	0.27	0.28
Maximum*	25.29	22.33	26.59	27.56	25.09	31.68
Mean*	7.16	7.84	6.31	6.49	10.90	13.18

*These values were calculated using the absolute values of the deviations from measured BSA.

CHAPTER 5

CONCLUSIONS

Feed efficiency is a critical component of growth and will continue to grow as a key trait for genetic selection. Improvements in efficiency will result in reduced cost of production and, therefore, the maintenance of current market pricing despite rising production costs. Simultaneously, improving feed efficiency and utilization will help to reduce the environmental impact of broiler production. Already some producers are beginning to give increased focus on feed efficiency as a selection criteria. Still, a better understanding of the mechanisms responsible for regulating feed efficiency. Previous studies have implicated the avTOR pathway as a key component to efficiency traits. The study presented here provides additional support. Ongoing molecular studies will help to further clarify its role in efficiency. The ultimate goal of these of studies is to enable selecting for feed efficiency genotypes rather than engaging in the laborious and time consuming process of data collection based on phenotypic expression. Shifting to a selection process based on genotype will speed the gains attained through selective breeding and will reduced the cost of breeding selection.