Genetic Analysis of Toxin-Induced Dilated Cardiomyopathy in the Turkey (*Meleagris gallopav*o)

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Thesis submitted to the Faculty of the Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

In

Animal and Poultry Sciences

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July 22, 2005

Blacksburg, Virginia

Keywords: Dilated cardiomyopathy, Turkeys, Genetics

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ABSTRACT

Dilated cardiomyopathy (DCM) or round heart disease is a muscle disease of the heart which is characterized by ventricular dilatation and abnormal systolic and diastolic left ventricular function. In animals, including turkeys and humans, DCM is the major cause of morbidity and mortality which results from heart failure. In the turkey, DCM can be idiopathic or induced. Since idiopathic or spontaneous DCM occurs in about 1-4% of normal turkeys, it is of significant concern to the poultry industry. In this study, it was proposed that the incidence and severity of DCM in the turkey may have a genetic basis. To test this hypothesis, I investigated differences in the incidence and severity of DCM in five domesticated turkey varieties including Blue Slate (BS), Bourbon Red (BR), Narragansett (N), Royal Palm (RP) and Spanish Black (SB). Preliminary investigations tested the reliability of echocardiography (ECHO) as a non-invasive and non-destructive technique for diagnosing DCM in a large number of birds from hatch to four weeks-of-age. One-day-old poults for both the preliminary and hypothesis testing investigations were obtained from Privett Hatcheries (Portales, New Mexico). The birds were raised under standard management conditions. In the preliminary investigation and to test my hypothesis, DCM was induced by feeding birds *ad libitum* standard diets containing 700 parts per million furazolidone. Results of the preliminary investigations showed that left ventricular end-diastolic dimension (LVEDD) and left ventricular end-systolic dimension (LVESD) were the most consistent ECHO indicators of DCM from hatch to 4 weeks-of-age. Variety differences in response to furazolidone were evaluated using these parameters as well as percent mortality. At 9

days-of-age, differences between control and treatment birds for percent mortality and LVESD were significant in the RP variety only but significant for LVEDD in RP and SB. At 29 and 33 days-of-age, all the pair-wise comparisons between control and treatment birds were significant for both LVEDD and LVESD. On average, the BR variety had the smallest dilatation of the heart and lowest mortality at 33 days-of-age when compared to other varieties. The results described in this thesis show, for the first time, variety differences in the turkey's response to diets containing furazolidone. They provide strong evidence that, like previous reports for idiopathic DCM, an animal's response to Fz-induced DCM has a strong genetic component.

ACKNOWLEDGEMENTS

I would like to express sincere thanks and gratitude to my adviser, Dr. Ed Smith, for his support and guidance, for being approachable and patient at all times, and for providing me the opportunity to work with an outstanding individual and a fanatical scientist like him.

I would also like to express my appreciation to all my committee members Drs. F. W. Pierson, R. L. Pyle, C. T. Larsen and D. P. Sponenberg for their comments and suggestions in conducting this research. Special thanks to D. F. Kamara, T. Geng, H. Hammade and Dr. JAP for their help in data collection and analysis.

I am grateful to the staff of the Virginia Tech turkey facility Dale Shumate and Curt Porterfield for their help and to Dr. Curtis Novak for his help in formulating the ration this experiment.

My deepest appreciation to my parents, N. Y. Boakye and M. T. Boakye, for giving me the gift of life, patience and encouragement through my academic endeavor. Special thanks to my brother and Sisters K. A. Boakye, N. A. Yeaboah and E. A. Boakye for being there when needed. I will finally like to express my wholehearted gratitude to G. N. Charles for always being by my side and her words of encouragement.

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CHAPTER 1

INTRODUCTION

The turkey, *Meleagris gallopav*o, is an agriculturally important bird which remains little studied and understood. This relatively low interest in studying this organism is surprising. Since 1970, to meet consumer demand for a healthy and lean protein source, turkey production in the United States has tripled. In recent years, the total annual gross receipt from the sale of turkey meat and meat products has exceeded $7.8 billion. In 2003, the U.S. raised 274 million birds with a combined live-weight of 7.55 billion pounds. This contribution from the turkey has helped to make the U.S. poultry industry (turkey, chicken, and duck) the world's largest. One consequence of the increased pressure to produce birds that meet the increasing consumer demand and preference for turkey meat and meat products is increased susceptibility to diseases caused by stress and pathogens.

Among DCM-related diseases of interest to the poultry industry is dilated cardiomyopathy (DCM) or round heart disease (RHD). This disease is characterized by left ventricular or biventricular dilation, cardiac hypertrophy, and severely depressed myocardial performance (Dec and Fuster, 1994). Cardiomyopathy has been shown to affect many animals including humans, cats, dogs, chickens, and turkey (Muders and Elsner, 2000). In humans, DCM affects approximately 4.7 million people annually. This results in health care cost of about 17.8 billion dollars annually in the United States.

In birds, DCM was first described by Adsersen (1948), and later by others (Sautter et al., 1968). Though the etiology of DCM in the turkey as well as in other animals remains largely unknown, DCM-affected birds have been clinically shown to have ruffled feathers, drooped wings, and an unthrifty appearance (Hunsaker, 1971). For Fz-induced DCM, mortality is usually

highest in two week-old birds (Sautter et al., 1968). Economic losses due to DCM, estimated at millions of dollars in the turkey and other birds, are due to mortality of between 1 to 4% and reduced body weights of birds that survive after 4 weeks-of-age (Frame et al., 1999).

Physiological factors are among the most investigated causes of DCM in animals. Myofibril loss has been described as one of the primary physiological changes in DCM affected animals like the rat. In some animals, this loss is accompanied by sarcomeric disarray (Schaper et al., 1991). Beltrami et al. (1995) reported that myocardial alterations in DCM affected hearts were due to myocyte loss, slippage of myocytes within the wall, segmental replacement, and interstitial fibrosis with hypertrophy of residual myocytes. Tagawa et al. (1996) previously suggested that the cytoskeleton, together with contractile proteins and the excitation-contraction coupling mechanisms of the heart represent major determinants of the intrinsic function of heart's myocyte. Furuoka et al. (2001) also reported cardiomyocyte hypertrophy and interstitial fibrosis to be the primary qualitative morphological changes in bovine DCM. In addition to these morphological changes, vacuolation of the cardiac muscle fibers and severe fibrosis were also observed. Other physiological changes associated with DCM that have been reported in animals include dilation of the chambers, thickening or thinning of the ventricular wall, pulmonary oedema, and occasionally ascities. In Holstein-Friesian cattle, Nart et al. (2004) reported that increased nuclear transverse cross-sectional area, and cardiomyocyte length, was the primary morphological characteristics observed in DCM-affected animals. As in other animals, fibrosis was found to be a consistent indicator of DCM. Though investigations into the physiological and morphological changes associated with DCM in the turkey have been limited, Marian and Roberts (1994) reported problems with the membrane transport mechanism in Fz-induced DCM-affected turkeys.

Biochemical factors have also been investigated as possible causes of DCM. Weekes et al. (1999) identified proteins with altered expressions in the left ventricles of DCM-affected bovine crossbreds. Of the thirty-five proteins with altered expressions, 24 and 11 were decreased and increased respectively. Though work is continuing to identify and further characterize the differentially expressed proteins, proteins with significantly altered expression in DCM-affected hearts include unbiquitin c and α 1 antiproteinase. Similar investigations in humans by Pleissner et al. (1997) identified myosin light chain 2, ventricular (MLC2) and heat shock proteins as those with altered expression in DCM-affected individuals. Also using 2-D gels, Heinke et al. (1999), described several differentially expressed proteins in DCM affected canine hearts. The differentially expressed proteins, including creatine kinase M, cytochrome *b*5 are known to function in energy metabolism and to be associated with mitochondria. The emerging data from proteomics investigations further support earlier work in diverse species that genetic differences significantly affect the incidence and severity of DCM. In one of the first investigations into the inheritance of DCM, Hunsakar (1971), showed that commercial turkeys from different genetic backgrounds respond differently when fed diets containing furazolidone. The differences among the genetic lines were especially significant for percent mortality.

Durand (1999), reviewed previous reports that evaluated factors that influence DCM including genetics and environment. Though a consensus is that DCM is a heterogeneous disease, several single genes and markers have been shown to be associated with cardiomyopathy in humans. The single genes shown to be associated with DCM in humans include actin and desmin (Olson and Keating, 1996), α-tropomyosin, and Troponin T and Troponin I (Kamisago et al, 2000). Genetic studies of DCM in the turkey, however, have been limited. A recent report by Smith et al. (2005) showed that five turkey varieties could be

classified into distinct molecular groups. This provides an opportunity to use these varieties to evaluate the genetic basis for furazolidone-induced DCM. The specific objectives of this thesis therefore include:

1. Determine differences among five turkey varieties in the incidence and severity of furazolidone-induced DCM based on echocardiographic parameters left ventricular end-diastolic dimension (LVEDD) and left ventricular end-systolic dimension (LVESD).
2. Evaluate variety differences in the severity of DCM based on percent mortality.

The rational for the thesis project is that development of resources will allow us to investigate the genetic factors that underlie DCM. One such question is whether DCM is a single gene or polygenic trait.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Turkeys

The domesticated turkey, which originated from North America, is reared throughout temperate parts of the World. Turkeys belong to the order *Galliformes* and the family *Meleagrididae*. Some, however, still designate the turkey as a member of the family *Phasianidae*. Two genera, *Agriocharis* and *Meleagris*, are recognized. While only one *Agriocharis* species, *A. ocellata* has been described, two have been reported within *Meleagris*, including *Meleagris gallopavo* and *Meleagris ocellata*. Phylogenetic classification of the turkey indicates that it is related to grouse, quail, pheasants and chickens (Turkey, Encarta Encyclopedia. 1993 Microsoft Corporation). North American turkeys, including the domesticated bird, belong to the single and highly variable species *Meleagris gallopovo*.

The American Poultry Association recognizes eight varieties of *Meleagris gallopovo* including Bronze, Narragansett, White Holland, Black, Slate, Bourbon Red, Beltsville Small White, and Royal Palm (Nesheim et al., 1986). The most widely raised commercial turkey, the Broad-Breasted White, is reported to have been developed from the White Holland. Knowledge of the genetic relatedness among the seven turkey varieties remains negligible. Recently, Smith et al. (2005) used genetic markers to distinguish among five turkey varieties. In their studies they used randomly amplified polymorphic DNA (RAPD), to express sequence tags based single nucleotide polymorphism and microsatellitie to evaluate for differences among the five varieties. Differences were evaluated for within and among varieties. They reported the Royal Palm to be closely related to the Narragansett variety as compared to the others. They suggested the two

varieties might have a common ancestry. Knowledge of the genetic relatedness of the varieties provides an opportunity to further evaluate whether these differences also influence their response to pathogens and toxins.

2.2 Turkey diseases and abnormalities

Both commercial and wild turkeys suffer diseases and abnormalities that result in minor or major economic losses. In general, mortality from stress or pathogens is reported to be about 7% in commercial birds. Diseases of the turkey can be nutritional (including rickets and vitamin deficiencies); parasitic (including coccidiosis, mites and lice) and metabolic (including dilated cardiomyopathy). Metabolic diseases affect internal organs such as liver, kidneys, and heart. They are believed to be one of the major causes of mortality in turkeys. Two of the most important metabolic diseases in the turkey are DCM and ascites. It is believed, however that DCM can cause ascites.

Because of their prevalence and potential economic consequences, cardiomyopathies are of strong interest to both animal scientists and the biomedical community. This disease is characterized by a heart muscle that doesn't pump blood efficiently. This disorder is the most common disease leading to cardiac transplantation in humans, with an associated cost of $200 million/year (Evans 1994). Cardiomyopathy can be classified as either primary or secondary (Towbin and Bowles, 2001). Primary cardiomyopathy cannot be attributed to a specific cause such as high blood pressure, heart valve disease, artery diseases or congenital heart defects (Richardson, 1996). On the other hand, secondary cardiomyopathy has been associated with diseases involving the heart as well as with abnormalities of other organs (Davies, 2000). Recently, the World Health Organization (WHO) redefined the general meaning of cardiomyopathy. Cardiomyopathy was said to be the major cause of ventricular dysfunction

which can result from a failure to correct volume or pressure overload in valve diseases of the heart (Davies, 2000). The loss of myocardium caused by coronary artery disease was shown to potentially lead to severe ventricular dysfunction. Another form of cardiomyopathy which was described is caused by intrinsic disorders of the myocardium and can be subdivided based on pathophysiological findings rather than an etiological classification.

Cardiomyopathies can be classified into four forms: dilated cardiomyopathy, hypertropic cardiomyopathy, restrictive cardiomyopathy, and arrhythmogenic right ventricular dysplasia/cardiomyopathy (Towbin and Bowles, 2001). In humans and other animals, DCM is the most prevalent of these cardiomyopathies. It is also the leading cause of mortality due to heart failure in humans and commercial turkeys (Czarnecki et al., 1974; Jankus et al., 1972).

2.3 Dilated cardiomyopathy

In DCM affected animals, the heart ventricles is enlarged and stretched, causing the heart to become weak and to lose its ability to pump blood efficiently (Durand, 1999). Abnormal rhythms known as arrhythmias which cause disturbances in the heart's electrical conduction have also been reported to be associated with DCM (Marian and Roberts, 1994). Histopathological changes associated with DCM typically include extensive areas of subendocardial, focal interstitial, and perivascular fibrosis as well as hypertrophic and atrophic myofibres. In humans heart failure due to DCM is lethal disease, with a 5-year mortality of about 75% (Frame et al., 1999). In the turkey, about 5% of spontaneous mortality in poultry has been attributed to DCM. Factors that influence DCM in both turkeys and humans include physiological, environmental (stress) and genetics (Poller et al., 2005; Michels et al. 1992; Czarnecki 1979).

In humans, DCM has been associated with certain cardiac or systemic abnormalities such as neuromuscular disorders, glycogen storage diseases, mucopolysaccharidosis, and disorders of

fatty acid metabolism (Poller et al., 2005). Michels et al. (1992), indicated that 20% of DCM is inherited. Inherited DCM is believed to be a highly heterogeneous disease. Several modes of inheritance of DCM including X-linked, autosomal recessive, and mitochondrial transmission, have been described in humans (Mestroni et al., 1999). Muntoni et al. (1993), showed that mutations in the cytoskeletal protein dystrophin gene were the cause of X-linked DCM as well as that of Duchenne and Becker type muscular dystrophy in humans. Three autosomal genes including cardiac actin, desmin, and lamin A/C have been shown to influence DCM (Komajda, 2000). Durand et al. (1995), analyzed a four generation family of 46 members for genes associated with DCM. Several candidate genes, including MEF-2, rennin, and helix loop helix DNA binding protein MYF-4 were localized to the 1q32 chromosomal region to be associated with DCM. Further characterization of these genes has been limited because of the lack of adequate animal models with the appropriate genetic background.

As heart failure remains a major clinical problem in humans, progress made in our understanding of the pathophysiology and treatment of heart failure in humans would not have been possible without a number of animal models. Each of these animals has its own unique advantages and disadvantages (Hasenfuss, 1998). According to Muders and Elsner (2000) and Towbin and Bowles (2001) the species and interventions used to model heart failure depends on the scientific question, ethics and economic considerations, accessibility, and reproducibility. Key factors to be considered when selecting an animal model for heart failure include closely mimicry of the human syndrome (Hasenfuss, 1998).

Several animal models for human DCM have been described including the rat (Sakai et al., 1996), dog (Wilson et al., 1987), pig (Muders and Elsner, 2000), cat (Tagawa et al., 1996), mouse (Wiesel et al., 1997) and the commercial turkey (Genao et al., 1996). Since rats are

inexpensive and have a relatively short gestation period combined with large average family size, and the ability to reproduce in a short period of time they have been extensively used to study the effect of long-term pharmacological interventions in DCM (Pfeffer et al., 1979; Sakai et al., 1996). The rat is, however, not an ideal model for human DCM. Its myocardial function is different from that of human and the myocardium also exhibits a very short action potential that lacks a plateau phase (Hasenfuss, 1998). Additionally, the resting heart rate of the rat is five times grater than that in humans (Hasenfuss, 1998). Dogs and other large animals including pigs and sheep have been used as alternative models since the left ventricular function and volumes more accurately reflect that of the human (Spinale et al., 1992). The disadvantage with dog and other large animal models is that they are costly and require resources such as caging and care.

The turkey has emerged as the best model of heart human DCM (Gwathmey and Davidoff, 1993). The mechanism by which furazolidone (Fz) induces DCM in the turkey remains little understood. It has been speculated that certain compounds may inhibit the conversion of pyruvate to acetyl coenzyme A to induce DCM (Czarnecki et al., 1975). In another study, Czarnecki (1979) evaluated the mechanisms that underlie cardiac hypertrophy and congestive heart failure in Fz-induced DCM in the turkey. In their study, myelin fibers and glycogen deposits were observed in mitochondria of the right ventricular wall and damaged myofibers of DCM-affected birds. It was postulated that Fz affected the membrane system of the inhibition that leads to alternation of the mitochondrial and myofibrillar components with a consistent increase in cytoplasmic glycogen. In a similar study of how Fz induces DCM, Gwathmey and Hamlin (1983), looked at the effects of furazolidone, propranolol, and digoxin on the dilation of the heart of the turkey. Unlike propranolol and digoxin, Fz caused significant effect on the dilation of the heart.

Spontaneous or idiopathic DCM, which occurs in about 2% of turkeys, is reported to have characteristics similar to those observed in furazolidone-induced cardiomyopathy (Gwathmey, 1991). Both forms of DCM are characterized by cardiac hypertrophy and dilatation, systemic hypotension as well as depressed contractility. The etiology of spontaneous cardiomyopathy in turkeys is unknown. Physiological changes that have been reported in idiopathic DCM include increased (calcium-transport ATPase activity of the sarcoplasmic reticulum) biochemical changes are consistent with suggestions that ischemia significantly influences the pathogenesis of spontaneous cardiomyopathy in turkeys (http://www.merckvetmanual.com/mvm/index.jsp?cfile=htm/bc/200600.htm).

Most studies of treatment of DCM have focused on idiopathic DCM. Treatments of affected turkeys as human model used the turkey to test the efficiency of pharmacological drugs. For example, Chapados et al. (1992), demonstrated that physiological agents such as nifedipine and propranolol alter two transmembrane signaling pathways in DCM affected birds. They administered 3 propranolol, 3 atenolol, 2 phenoxybenzamine, 4 nifedipine, 4 verapamil, and 0.5 digoxin once a day or no treatment to 8-day-old birds. Propranolol and atenolol treated animals were found to have higher creatine content, lactate dehydrogenase and creatine kinase activities, thus demonstrating energy reserves in the birds. Nefedipine treated birds showed upregulation in both β-adrenergic and dihydropyridine receptors. Gwathmey and Hamlin (1983) also reported that turkeys fed propranolol prior to feeding diets containing Fz did not develop cardiomyopathy. Gwathmey et al. (1999) investigated the effects of Carteolol, a β-adrenergic blocking agent, in control and DCM affected turkey poults. They administrated Carteolol twice a day for 4 weeks to both control and DCM affected birds. At the end of the study, there was 59% mortality in non-treated DCM group and 22% mortality in the group treated with the carteolol. The Carteolol-

treated group also showed a significant decrease in left ventricular size and a significant restoration of ejection fraction and left ventricular peak systolic pressure. Carteolol treatment also increased β-adrenergic receptor density and restored sarcoplasmic reticulum Ca^{2+}-ATPase and myofibrillar ATPase activities to normal. Kim et al. (1999) also examined in this same model for DCM, the effect of pranidipine, a new dihydropyridine calcium antagonist, on the gross and microscopic morphology of the heart and overall contractile performance of the heart myocardium in DCM-affected. In their study, they found myocyte hypertrophy regression in DCM-affected animals treated with pranidipine and a reduction in the size of left ventricular dilation. These studies provide a good example in DCM affected animals or the value in understanding the action of pharmacological agents on the heart. More recently, Washington et al. (2001) showed that Carteolol also improves the contraction of myopathic hearts of DCM-affected birds. The drug was shown to significantly reduce mortality in turkeys fed Fz-containing diets. Okafor et al. (2003) tested whether chronic treatment with high and low doses β-blockers such as Carvedilol decreases apoptosis in DCM affected and non affected birds. They showed that Carvedilol at any dose significantly improved fractional shortening and reduces the number of apoptotic nuclei found in DCM-affected birds.

CHAPTER 3

Echocardiography as a diagnostic tool for dilated cardiomyopathy in the turkey (*Meleagris gallopavo*)

3.1 ABSTRACT

The use of the turkey, *Meleagris gallopavo*, as an effective animal model for dilated cardiomyopathy (DCM) is limited by the lack of a consensus diagnostic tool that does not involve necropsy. This lack of a widely tested non-necropsy method makes it difficult for a large-scale study of the genetic factors that underlie DCM, which is a concern both to the agricultural and biomedical industries. Here, an investigation was conducted to investigate the use of echocardiography (ECHO) as a non-invasive and non-destructive technique for identifying a large number of DCM-affected turkeys from hatch to four weeks-of-age. To induce DCM, 700 ppm of Furazolidone (Fz) was fed to turkey poults from one day-of-age until four weeks-of-age. Among the ECHO measurements evaluated, the left ventricular end-diastolic dimension (LVEDD) and left ventricular end-systolic dimension (LVESD) were the most consistent indicators of DCM. The average difference between control and Fz-fed birds in LVEDD ranged from 25% in 7-day-old to 80% in 28-day-old poults. At similar ages, average differences between control and Fz-treated birds in LVESD were 74 and 326% respectively. Necropsy of the birds still alive at the end of the 4-week study confirmed the ECHO measurements that identified birds as either DCM or normal. Our data suggest that ECHO is a reliable and consistent tool for identifying DCM turkeys. This will help investigators more rapidly and efficiently evaluate genetic or molecular factors that influence DCM in turkeys and other birds.

3.2 Introduction

The turkey industry continues to be one of the most successful in livestock and poultry. This success is primarily a result of increased consumption of turkey as a non-holiday meat item. In response to this increased demand, there has been a 20% average increase in the growth rate and body weight of commercial turkeys using genetic and non-genetic approaches. These gains in production characteristics appear to have been made at the expense of other important physiological traits leading to increased susceptibility to diseases such as dilated cardiomyopathy (DCM) or round heart disease (RHD).

In commercial turkeys, RHD is believed to be responsible for almost 10% of poult mortality from hatch to 4 weeks-of-age (Frame et al., 1999). Despite these economic losses to the turkey industry, the etiologies of this abnormality remain very poorly understood (The Merck Veterinary Manual, on line version 2004). Understanding the etiology of RHD in the turkey may also contribute to our understanding of human DCM, a major cause of heart attacks (Genao et al., 1996). RHD is a disease condition characterized by weakness of the heart muscle and the inability to pump blood efficiently (The Merck Veterinary Manual, on line version 2004). It is distinguished by a rounding of the heart and enlargement of the ventricles. In the turkey, two types have been described: idiopathic or spontaneously occurring (IDCM) and Fz-induced DCM (Genao et al., 1996). The Fz-induced DCM (Fz-DCM) mimics the physiological characteristics of IDCM and can therefore be used as a model to define the genetic and molecular basis of this abnormality (Czarnecki, 1979).

While the etiology of DCM remains unknown, specific characteristics shown to be associated with Fz-DCM in the turkey include metabolic defects, early rapid growth, and lack of oxygen to the heart muscle (Liao et al., 1996, 1997; Gwathmey et al., 1999). IDCM is reported

to be a major cause of mortality in poults between 0 and 6 weeks-of-age (Roberson et al., 2003). Since there is currently no available treatment for DCM, understanding the cause offers a unique opportunity to discover possible treatments.

Diagnostic tools that have been used to identify turkeys with DCM have primarily been necropsy and to a limited extent electrocardiography (Czarnecki, 1979; Czarnecki and Good, 1980; Hunsaker et al., 1971). Both tools are of limited practical use in the field and in investigations into the etiology of DCM (The Merck Veterinary Manual, on line version, 2004). In addition, diagnosis using these tools limits genetic studies which are helpful in defining the etiology of DCM. In the present work, we evaluated the use of echocardiography (ECHO) for the diagnosis of DCM in poults from hatch to 4 weeks-of-age, the critical period for both IDCM and Fz-DCM. While ECHO has been widely used for diagnosis of DCM in mammalian species (Jawad, 1996), its use in birds has been limited. Studies that have used ECHO involving birds include only a limited number of animals, often less than 10 (Wu et al., 2004). To be useful, ECHO requires the establishment of baseline parameters that can be referenced in the diagnosis of the incidence and severity of DCM. In the present study, several ECHO-based measurements were assessed for their consistency relative to necropsy in the diagnosis of DCM in the turkey.

3.3 Materials and Methods

Fifty day-old poults obtained from a commercial hatchery were used. The birds were randomly divided into control and treatment group of 25 each and raised according to standard protocols (Nesheim et al., 1986). The treatment group was fed a standard turkey poult diet containing 700 parts per million furazolidone. Both groups of birds were fed *ad libitum* throughout the four-week study. Body weight was recorded weekly for all birds.

A portable Aloka ECHO machine with a 7.5MHz transducer was used to obtain weekly readings of heart measurements on unsedated, resting animals. The ECHO readings were made in the M-Mode. This mode generates a one-dimensional view of small portions of the heart which allow for the detection of axial motion of structures parallel to the beam (Kienle and Thomas, 1995). The different dimensions measured by the ECHO were: the left ventricular end-diastolic (LVEDD), left ventricular end-systolic (LVESD), interventricular septum end-diastolic (IVSED), interventricular septum end-systolic (IVSES), left ventricular wall end-systolic (LVWES), left ventricular wall end-diastolic (LVWED), and right ventricular end-diastolic diameter (RVEDD).

The *in vivo* contractile performance of the heart of each bird, control and experimental, was evaluated using the fractional shortening (%) according to Genao et al. (1996) as follows:

(LVEDD – LVESD)/ LVEDD) X 100.

At 4 weeks-of-age the birds were euthanized by cervical dislocation and body weights obtained. Hearts were dissected, atria and large vessels removed and the remaining left and right ventricles were trimmed off and weighed separately. Histopathology was conducted after

fixation in 10% buffer formalin for twenty-four hours. Tissue sections were stained with hematoxylin and eosin and viewed using standard light microscopy.

Statistical analysis was conducted in SAS using general linear model (GLM) procedure to evaluate differences between control and Fz-treatment means. The student t-test was used to evaluate pair wise differences with significance set at $P < .05$ (SAS Inst., Inc., Cary, NC).

3.4 Results and Discussion

Birds on Fz-containing diet had, on average, lower body weight but larger LVEDD and LVESD (Table 1). The difference in body weight increased with age; though at the end of the 2nd week the treatment birds were 92%, at the end of the 4th week they were only 64% of the weight of the control birds. Using ECHO, signs of Fz-induced DCM were detectable and apparent though not significantly different from control, as early as one week-of-age. As shown in Table 1 and Figure 1, LVEDD of Fz-fed poults increased by 25, 32, 47, and 80% in week 1, 2, 3 and 4, respectively, compared to control birds. The LVESD showed even larger increases compared to controls. The DCM detection was highly correlated with mortality beginning in week 1 through week 4 (data not presented). In week 1, 2, 3, and 4, mortality of birds on the Fz-fed diet was 0, 4, 24, and 52% respectively, while in the control group mortality was 0, 2, 8 and 10% respectively. The fractional shortening in the Fz-fed birds decreased consistently with respect to the age of the birds when compared to that of the control group (Table 1 and Figure 2).

Necropsy were consistent with those of Hunsaker et al. (1971), Birds identified by ECHO to be affected by DCM had distended hearts with the apex being rounded instead of conical. The hearts of Fz-fed birds also showed significant dilation of the left ventricle and thinning of the left ventricle free wall and ventricular septum. At week 4, the dimensions of the hearts of Fz-fed poults were several-fold larger than those of normal birds (Table 1). This difference in dilation may account for the differences in the weights of the right ventricle, left ventricle, whole heart, and the measurement from the apex to the thorax obtained from necropsy (Table 2).

Histopathological examination of poults with DCM revealed degeneration myocytes, B-cells with vacuoles, necrotic cells deep in the left ventricle, and extensive inflammation with a significant number of lymphocytes (Figure 3). In the control group, however, only minimal

degeneration of myocytes and necrosis of the right ventricle were apparent (Figure 4). These observations confirmed the diagnosis made by ECHO in identifying birds as DCM or normal. The gross morphological observations were also consistent with characteristics defined by others, including Hunsaker et al. (1971), for DCM-affected birds.

Here it has been shown that ECHO consistently identifies birds with Fz-induced DCM from 14 days- of-age, but can detect the development of DCM in birds as young as 7 day-olds. This can be useful in commercial turkey production and management. Furthermore, the use of ECHO eliminates the impracticality of using necropsy and electrocardiography for the diagnoses of DCM. This tool, though relatively expensive, will allow diverse investigations including genetic and molecular research to define the etiology of DCM. ECHO is a relatively easy tool to use and appears to yield measurements of dimensions indicative of DCM that are consistent with necropsy.

CHAPTER 4

Differences Among Turkey (*Meleagris gallopavo)* Varieties for the Incidence and Severity of Furazolidone-Induced Dilated Cardiomyopathy.

4.1 ABSTRACT

Dilated cardiomyopathy (DCM) or round heart disease is economically important to both the agricultural and biomedical industries. It is characterized by dilatation of the left ventricles and is often a major cause of heart disease in both humans and animals. Despite the economic losses caused by DCM, its etiology remains little understood. In this study, it was hypothesized that the turkey's response to Fz-induced DCM is genetic. To test this hypothesis, an investigation was conducted to determine if five unique turkey varieties including Blue Slate (BS), Bourbon Red (BR), Narragansett (N), Royal Palm (RP) and Spanish Black (SB) differed in the incidence and severity of experimentally-induced dilated cardiomyopathy. These genetically distinct turkey populations were randomly divided into control and treatment groups consisting of 50 birds each. They were fed either a standard commercial starter diet (control) or a starter-diet containing 700 ppm furazolidone (treatment) *ad libitum* to 33 days-of-age. The incidence and severity of DCM in control and treatment birds were evaluated based on percent mortality and echocardiography using left ventricular end-diastolic dimension (LVEDD), left ventricular end-systolic dimension (LVESD), and fractional shortening as primary indicators. Mortality within the varieties ranged from 40 to 70% in the BR and SB, respectively. Similarly, SB and N had the highest LVEDD and LVESD measurements while the BR was the lowest, though not significantly different from the left ventricular dimensions for RP. These data suggest, for the

first time, the response of turkeys to Fz-induced dilated cardiomyopathy may have a genetic basis.

4.2 Introduction

The turkey (*Meleagris gallopavo)* industry is one of the most rapidly growing industries in agriculture. This growth is partly due to increased consumption that is a result of consumer preference for turkey meat and meat products. The increased consumption has led to an increase in turkey production (National Turkey Federation-Statistic, 2003). In addition to domesticated turkey consumption, export of turkey meat has also risen over the past decade. In 2001, the United States exported about 8.5% of its total turkey production (National Turkey Federation-Statistic, 2003). Selection of turkeys for agriculturally important traits such as carcass quality, egg number, and rapid growth to meet the rise in consumer preference for turkey meat may have contributed to the increase in dilated cardiomyopathy (DCM) or round heart disease (RHD) (Frame et al., 1999).

Though previous studies (Gwathmey, 1991 and others discussed in Chapter 2) have investigated the effect of physiological and biochemical factors on the occurrence of DCM in commercial turkeys. Knowledge of whether genetics has played a role in the increase in DCM in turkeys however is limited. For example, investigations conducted by Hunsaker (1971) that evaluated genetic differences in DCM, used only two commercial lines that originated from genetic foundation sire stock. As reviewed by Durand (1999) and described here in Chapter 2, several reports have also described single mutations and altered expression of different proteins in DCM-affected animals, which suggest a genetic basis for idiopathic DCM. Lacking, however, are data about the genetic influence on Fz-induced DCM. The investigation conducted here will begin to address the gap in our knowledge of the effects of genetics on DCM. The primary objective of this study was to determine if differences do exist among varieties of domesticated

turkeys in their response to diets containing furazolidone, an agent known to induce DCM. The variety differences will be based on percent mortality and echocardiographic parameters including left ventricular end-diastolic dimension (LVEDD) and left ventricular end-systolic dimension (LVESD).

4.3 Materials and Methods

One hundred day-old birds of each variety, Royal Palm, Bourbon Red, Spanish Black, Blue Slate and Narragansett, were obtained from a commercial hatchery (Privett Hatcheries, Portales, New Mexico). Within each variety, the birds were randomly and evenly divided into two groups, treatment and control; and raised according to standard management practices (Nesheim et al., 1986). Both groups were fed either a standard commercial starter-diet (control) or a starter diet containing 700 parts per million of furazolidone (Fz) *ad libitum* up to 33 days-of-age. Both control and treatment diets also contained bacitracin methylene disacliylate (BMD 50) and 400 mg/gal of terramycin.

A portable Aloka Echocardiography (ECHO) machine with a 7.5MHz transducer was used to obtain LVEDD and LVESD measurements of control and treatment birds. Echocardiography measurements were carried out at 9, 18, 23, 29, and 33 days-of-age.

Fractional shortening: The *in vivo* contractile performance or fractional shortening was determined within each variety using the average values for LVEDD and LVESD as indicated in Chapter 3.

At 33 days-of-age, birds were taken off treatment and a few affected and unaffected birds from the control and treatment groups were sacrificed for standard necropsy as also described in Chapter 3.

Statistical analysis was conducted in SAS using the general linear model procedure to evaluate differences between control and Fz-treatment means as well as differences among varieties within treatment and control groups (SAS Inst., Inc., Cary, NC, 1998). Duncan's multiple range test and Waller-Duncan K-ratio t-test were used to assess significance ($P<.05$). Results were reported as mean ± standard deviation (SD).

4.4 Results and Discussion

Variety differences of nine-day-old birds control groups were significant ($P< 0.05$) for LVEDD but not for percent mortality and LVESD (Table 3). The differences were most significant between BS and N at 33%. Differences among varieties within the treatment group, on the other hand, were significant for all the three traits. The LVEDD ranged from 0.28 to 0.41 for BS and RP, respectively. The LVESD measurement also ranged from 0.18 to 0.27, respectively or 35% differences between RP and the other varieties. Within variety comparisons of control and treatment birds for all the three traits, show the beginning effects of furazolidone. The effect appears to be greatest in RP.

Results of variety comparisons for mortality, LVESD, and LVEDD in 18-day-olds are presented in Table 4. The percent mortality for BR, BS and RP was significantly lower than that for N and SB birds on the diet containing furazolidone. The differences in mortality between control and treatment birds were also significant in N and SB varieties. The trend in LVESD and LVEDD was consistent with the differences observed in percent mortality. Both SB and N birds within the treatment group had larger average LVEDD and LVESD measurements. Additionally, with the exception of an anomaly observed in BS where control LVEDD measurement was the same as treatment, control birds had significantly lower LVEDD and LVESD and larger fractional shorting in N and SB varieties. At 23 (Table 5) 29 (Table 6) and 33 days-of-age (Table 7) differences observed among varieties and between treatment and control birds within-varieties were consistent with those of 18-day-old birds (Table 4). At these ages, differences among the varieties for LVESD, LVEDD and fractional shortening continued to increase and to be statistically significant. Briefly, within the treatment group, SB, N and BS had significantly larger LVEDD, LVESD and higher mortality than the RP and BR birds (Table 5-8). Additionally,

the cumulative mortality, as well as the percent mortality compare to control was highest for SB and lowest for RP at 66 and 36%, respectively (Table 9).

As expected, the dimensions of the hearts of treatment birds were several-fold larger than that of normal birds. The difference in dilation may account for the differences in the weights of the right ventricle, left ventricle, whole heart, and the measurement from the apex to the thorax obtained from necropsy (Table 8). From the necropsy results, the treated hearts of birds showed significant dilation of the left ventricle and thinning of the left ventricle free wall and ventricular septum (Table 8). Specifically, N and BR had the smallest and largest LVW, respectively. In control birds, however, N and RP birds had the smallest and largest LVW. Right ventricular weights of treatment birds ranged from 0.31 to 0.44 grams.

Here, it was shown for the first time that different varieties of turkeys differ in their susceptibility to Fz-induced DCM. The data are useful for further analysis of the nature of the genetic factors responsible for the differences among turkeys fed diets containing furazolidone. The work supports earlier investigations by Hunsaker (1971), which reported that DCM in the turkey has a genetic basis. Consistent with the current results, Hunsaker (1971) showed that two commercial turkey lines significantly differed in percent mortality caused by DCM as well as for dilation of the heart. Unlike the current work however, the commercial inbred lines evaluated were all derived from the Broad Breasted Bronze.

In summary, the results of this study suggest that the BR variety was the most resistant to Fz-induced DCM. The SB and N varieties were the most affected with Fz-induced DCM through the feeding of Fz containing diet. The study of variety difference or susceptibility to Fz-induced DCM will allow for studies to evaluate potential genetic factors that contribute to the resistance and susceptibility of varieties to Fz-induced DCM.

CHAPTER 5
SUMMARY OF THESIS

This thesis research investigated the hypothesis that genetic differences among five turkey varieties influence their resistance and susceptibility to DCM caused by furazolidone. In preliminary studies, it was shown that echocardiography can be used to accurately and efficiently diagnose DCM in the turkey. The preliminary studies established that left ventricular end-diastolic and systolic dimensions from hatch to 4 weeks-of-age were the most consistent indicators of DCM. Based on these findings, ECHO was used to evaluate differences among five varieties of the domesticated turkey including Blue Slate, Bourbon Red, Narragansett, Royal Palm and Spanish Black for the incidence and severity of Fz-induced DCM. Consistent with previous work, the incidence and severity of DCM was highest at two weeks of age. Additionally, mortality declined in all varieties after the third week.

Specific conclusions are:

1. Echocardiography can be used to accurately diagnose and distinguish between DCM-affected and non-affected birds. Left ventricular end-diastolic and systolic dimension were the most consistent ECHO parameters in diagnosing DCM.
2. Based on ECHO parameters and mortality, Bourbon Red had the lowest incidence of Fz-induced DCM while the Spanish Black and Narragansett had the highest. In comparison, however, the data from this investigation suggested that the commercial turkeys were the most susceptible.

Future Work

The present work has established that genetics is a factor in the incidence and severity of Fz-induced cardiomyopathy. The nature of the genetic basis, however, requires further investigation. Specific investigations that could help answer this question include:

1. Determine the heritability of DCM.
2. Evaluate the effect of heterosis on DCM
3. Use structural, functional and comparative genomics approaches to identify candidate genes that influence furazolidone-induced DCM in the turkey.
4. Evaluate biomarkers, especially those associated with oxidative Fz, for DCM incidence and severity.

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Table 1. Average weekly weight and echocardiography measurements in control (CTL) and Furazolidone-fed turkeys (TRT).

Week	Body weight(g)		LVEDD(mm)*		LVESD(mm)*		Fractional shortening (%)	
	$CTL_{(N=25)}$	$TRT_{(N=25)}$	CTL	TRT	CTL	TRT	CTL	TRT
1	137.16^{a}	133.04^{a}	0.40^{a1}	0.50^{a1}	0.15^{a1}	0.26^{a1}	63^{a}	48^{b}
2	303.61^{a}	281.03^{a}	0.46^{a1}	0.61^{b2}	0.17^{a1}	0.33^{b}	63^{a}	46^{b}
3	521.40^{a1}	400.44^{b1}	0.57^{a2}	0.84^{b}	0.21^{a2}	0.62^{b2}	63^{a1}	26^{b2}
4	880.17^{a1}	564.06^{b2}	0.70^{a1}	1.26^{b2}	0.27^{a1}	1.15^{b2}	61^{a1}	9^{b2}

*LVEDD and LVESD are left ventricular end-diastolic dimension and left ventricular end-systolic dimension, respectively, as determined from ultrasound measurements by echocardiography.

^{a,b}Measurements in the same column with similar numeric superscript are not significantly different (P>.05).

^{1,2}Values in the same row for the same measurement with similar alphabetic superscript are not different (P>.05).

Table 2. Necropsy measurements of control and Fz-fed birds at 4 weeks-of- age.

Week 4	Body weight (g)	RVW (g)*	LVW (g)*	WOH (g)*	Apex-Thorax(mm)
Control(18)	839.02 ± 12.00^{a}	0.70 ± 0.13^{a}	3.08 ± 0.48^{a}	3.87 ± 0.66^{a}	21.12 ± 1.19^{a}
Treatment(9)	545.89 ± 79.76^{b}	0.56 ± 0.18^{b}	2.67 ± 0.68^{a}	3.37 ± 0.86^{a}	19.99 ± 2.22^{a}

*RVW, LVW, and WOH represent the right ventricular weight, left ventricular weight, and whole heart weight, respectively.

^{a,b}Means ± S.E. with the same superscript are not different ($p > .05$).

Table 3. Echocardiographic measurements and mortality in 9-day-old turkeys fed normal (CTL) or furazolidone (TRT) containing diet.

Variety[†]	Mortality		LVEDD[*]		LVESD[*]		Fractional Shortening (%)	
	$CTL_{(n=50)}$	$TRT_{(n=50)}$	CTL	TRT	CTL	TRT	CTL	TRT
BR	1	3	0.30^{bc2}	0.32^{b1}	0.23^{a1}	0.22^{ab1}	27	33
BS	6	3	0.27^{c1}	0.28^{b1}	0.17^{a1}	0.20^{b1}	37	29
N	1	1	0.39^{a1}	0.32^{b2}	0.19^{ab1}	0.18^{b1}	47	43
RP	1	7	0.34^{b2}	0.41^{a1}	0.21^{a2}	0.27^{a1}	38	34
SB	1	3	0.31^{bc2}	0.40^{a1}	0.18^{a1}	0.21^{b1}	42	48

[*]LVEDD and LVESD are left ventricular end-diastolic dimension and left ventricular end-systolic dimension, respectively, as determined from ultrasound measurements by echocardiography.

[a,b]Measurements in the same column with similar alphabetic superscript are not different (P>.05).

[1,2]Values in the same row with similar numeric superscript are not significantly (P>.05).

[†]BR, BS, N, RP and SB represent Bourbon Red, Blue Slate, Narragansett, Royal Palm, and Spanish Black, respectively.

Table 4. Echocardiographic measurements and mortality in 18-day-old turkeys fed normal (CTL) or furazolidone (TRT) containing diet.

Variety[†]	**Mortality**		**LVEDD**[*]		**LVESD**[*]		Fractional Shortening (%)	
	$CTL_{(n=50)}$	$TRT_{(n=50)}$	CTL	TRT	CTL	TRT	CTL	TRT
BR	1	1	0.47^{b1}	0.50^{bc1}	0.21^{a1}	0.23^{b1}	55	54
BS	0	3	0.57^{a1}	0.49^{b2}	0.23^{a1}	0.25^{ab1}	59	43
N	0	9	0.51^{b1}	0.69^{a2}	0.17^{b1}	0.32^{ab2}	67	54
RP	0	1	0.46^{b1}	0.48^{bc1}	0.22^{ab1}	0.25^{ab1}	52	48
SB	1	11	0.46^{b2}	0.59^{ab1}	0.17^{b2}	0.38^{a1}	63	36

[*]LVEDD and LVESD are left ventricular end-diastolic dimension and left ventricular end-systolic dimension, respectively, as determined from ultrasound measurements by echocardiography.

[a,b]Measurements in the same column with similar alphabetic superscript are not different (P>.05).

[1,2]Values in the same row with similar numeric superscript are not significantly (P>.05).

[†]BR, BS, N, RP and SB represent Bourbon Red, Blue Slate, Narragansett, Royal Palm, and Spanish Black, respectively.

Table 5. Echocardiographic measurements and mortality in 23-day-old turkeys fed normal (CTL) or furazolidone (TRT) containing diet.

Variety[†]	Mortality		LVEDD[*]		LVESD[*]		Fractional Shortening (%)	
	$CTL_{(n=50)}$	$TRT_{(n=50)}$	CTL	TRT	CTL	TRT	CTL	TRT
BR	0	9	0.55^{b1}	0.56^{b1}	0.18^{b1}	0.23^{a1}	67	59
BS	1	8	0.63^{a1}	0.63^{ab1}	0.20^{ab2}	0.29^{a1}	68	54
N	0	11	0.58^{b2}	0.62^{ab1}	0.20^{ab2}	0.24^{a1}	68	61
RP	0	15	0.56^{b1}	0.59^{ab1}	0.23^{a1}	0.26^{a1}	59	56
SB	0	5	0.58^{b2}	0.67^{a1}	0.17^{b2}	0.29^{a1}	71	57

[*]LVEDD and LVESD are left ventricular end-diastolic dimension and left ventricular end-systolic dimension, respectively, as determined from ultrasound measurements by echocardiography.

[a,b]Measurements in the same column with similar alphabetic superscript are not different (P>.05).

[1,2]Values in the same row with similar numeric superscript are not significantly (P>.05).

[†]BR, BS, N, RP and SB represent Bourbon Red, Blue Slate, Narragansett, Royal Palm, and Spanish Black, respectively.

Table 6. Echocardiographic measurements and mortality in 29-day-old turkeys fed normal (CTL) or furazolidone (TRT) containing diet.

Variety[†]	Mortality		LVEDD[*]		LVESD[*]		Fractional Shortening (%)	
	$CTL_{(n=50)}$	$TRT_{(n=50)}$	CTL	TRT	CTL	TRT	CTL	TRT
BR	0	7	0.74^{a2}	0.84^{a1}	0.19^{a2}	0.36^{a1}	74	57
BS	0	11	0.78^{a2}	0.93^{ab1}	0.21^{a2}	0.34^{a1}	73	63
N	1	8	0.72^{a2}	0.92^{ab1}	0.23^{a2}	0.41^{b1}	68	55
RP	0	5	0.72^{a1}	0.78^{a1}	0.21^{a2}	0.32^{a1}	71	59
SB	0	14	0.72^{a2}	0.94^{ab1}	0.18^{a2}	0.32^{a1}	75	66

[*]LVEDD and LVESD are left ventricular end-diastolic dimension and left ventricular end-systolic dimension, respectively, as determined from ultrasound measurements by echocardiography.

[a,b]Measurements in the same column with similar alphabetic superscript are not different (P>.05).

[1,2]Values in the same row with similar numeric superscript are not significantly (P>.05).

[†] BR, BS, N, RP and SB represent Bourbon Red, Blue Slate, Narragansett, Royal Palm, and Spanish Black, respectively.

Table 7. Echocardiographic measurements and mortality in 33-day-old turkeys fed normal (CTL) or furazolidone (TRT) containing diet.

Variety[†]	Mortality		LVEDD[*]		LVESD[*]		Fractional Shortening (%)	
	CTL(n=50)	TRT(n=50)	CTL	TRT	CTL	TRT	CTL	TRT
BR	0	0	0.75^{ab2}	0.86^{cd1}	0.29^{bc2}	0.65^{bc1}	61	24
BS	0	2	0.62^{cd2}	0.96^{bc1}	0.21^{c2}	0.72^{c1}	66	25
N	0	3	0.75^{ab2}	1.05^{bc1}	0.33^{ab2}	0.83^{b1}	56	21
RP	0	1	0.54^{d2}	0.74^{d1}	0.24^{bc2}	0.54^{c1}	56	27
SB	0	2	0.68^{bc2}	1.08^{ab1}	0.21^{c2}	0.78^{cb1}	69	28
COM[§]	0	0	0.70^{a1}	1.26^{b2}	0.27^{a1}	1.15^{b2}	61	9

[*]LVEDD and LVESD are left ventricular end-diastolic dimension and left ventricular end-systolic dimension, respectively, as determined from ultrasound measurements by echocardiography.

[a,b]Measurements in the same column with similar alphabetic superscript are not different (P>.05).

[1,2]Values in the same row with similar numeric superscript are not significantly (P>.05).

[†]BR, BS, N, RP and SB represent Bourbon Red, Blue Slate, Narragansett, Royal Palm, and Spanish Black, respectively.

[§]Data obtained from pilot studies and reported in Chapter 3.

Table 8. Necropsy measurements of 33-day-old turkeys from different varieties fed normal (CTL) or furazolidone (TRT) containing diet.

Variety†	Body weight(g)		LVW(g)*		RVW(g)*		WOH(g)*		APEX - THORAX	
	CTL	TRT	CTL	TRT	CTL	TRT	CTL	TRT	CTL	TRT
BS	584.88^{a}	366.46^{ab}	1.88^{ab}	1.24^{b}	0.54^{a}	0.40^{ab}	2.57^{a}	1.76^{b}	20.17^{ab}	17.84^{bc}
N	621.83^{a}	305.96^{cd}	2.08^{a}	1.11^{b}	0.48^{ab}	0.42^{ab}	2.64^{a}	1.71^{b}	20.73^{a}	17.97^{bc}
RP	456.19^{b}	280.57^{d}	1.49^{c}	1.19^{b}	0.44^{bc}	0.36^{b}	1.99^{b}	1.58^{a}	18.38^{c}	16.46^{c}
SB	570.82^{a}	350.78^{bc}	1.68^{bc}	1.39^{ab}	0.46^{abc}	0.31^{b}	2.38^{ab}	1.86^{b}	19.72^{abc}	18.46^{bc}
BR	456.97^{b}	353.85^{bc}	1.50^{c}	1.45^{a}	0.38^{c}	0.44^{ab}	2.13^{b}	2.04^{bc}	18.67^{bc}	19.95^{ab}

*LVW, RVW, and WOH represent the right ventricular weight, left ventricular weight, and whole heart weight, respectively.

^{a,b,c,d}Measurements in the same column with similar superscript are not different (P>.05).

†BR, BS, N, RP and SB represent Bourbon Red, Blue Slate, Narragansett, Royal Palm, and Spanish Black, respectively.

Table 9. Cumulative total mortality in turkey varieties fed normal diet and feed containing furazolidone.

Variety[†]	Total mortality CTL	TRT	ΔMort (%)[*]
BR	2	20	36^{a}
BS	9**	27	50^{b}
N	2	32	58^{c}
RP	5	29	56^{c}
SB	2	35	66^{d}
COM[††]	2	16	28^{*}

[†]BR, BS, N, RP and SB represent Bourbon Red, Blue Slate, Narragansett, Royal Palm, and Spanish Black, respectively.

[*]Percent change in mortality above control at 33 days-of-age. Significance is at (P<.05).

[††]Data from commercial turkeys used in preliminary studies described in chapter 3.

*Percent change represents that at week 4.

**The relatively high total mortality of control birds may have been due to stress in transit.

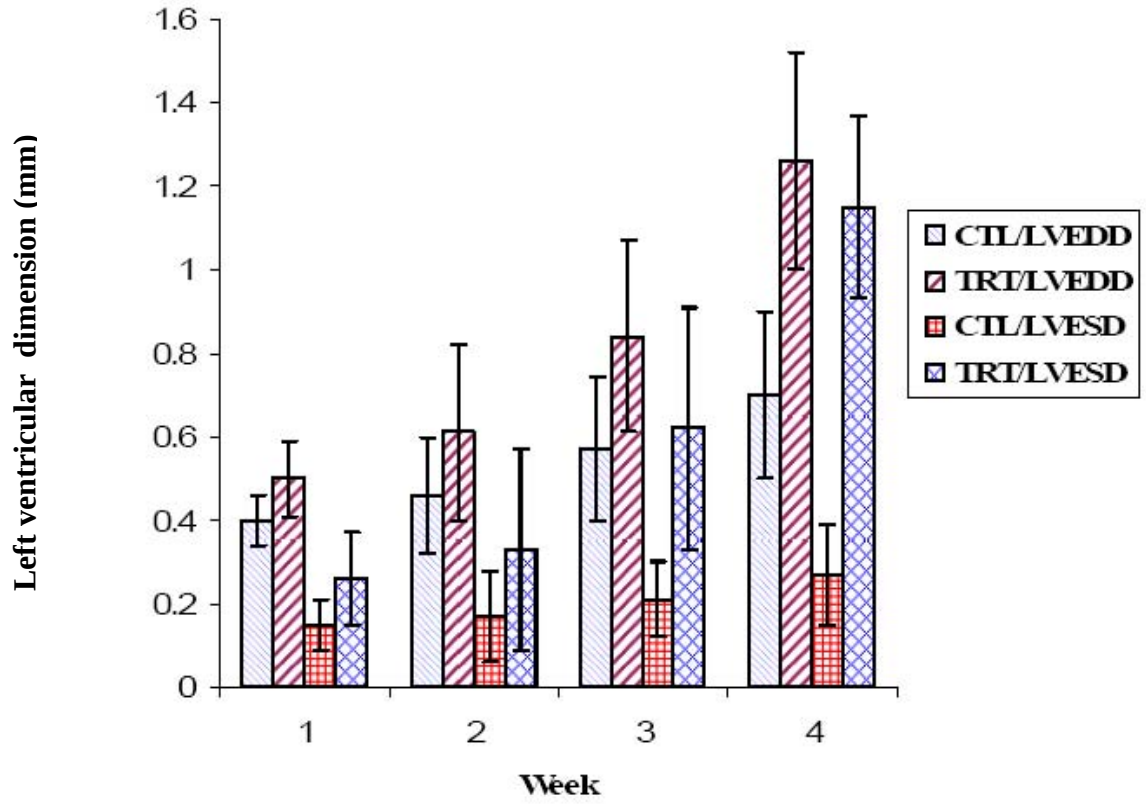

Figure 1. Left ventricular end diastolic (LVEDD) and systolic (LVESD) dimensions in turkeys fed normal (CTL) and furazolidone-containing diet (TRT). Means ± S.E.

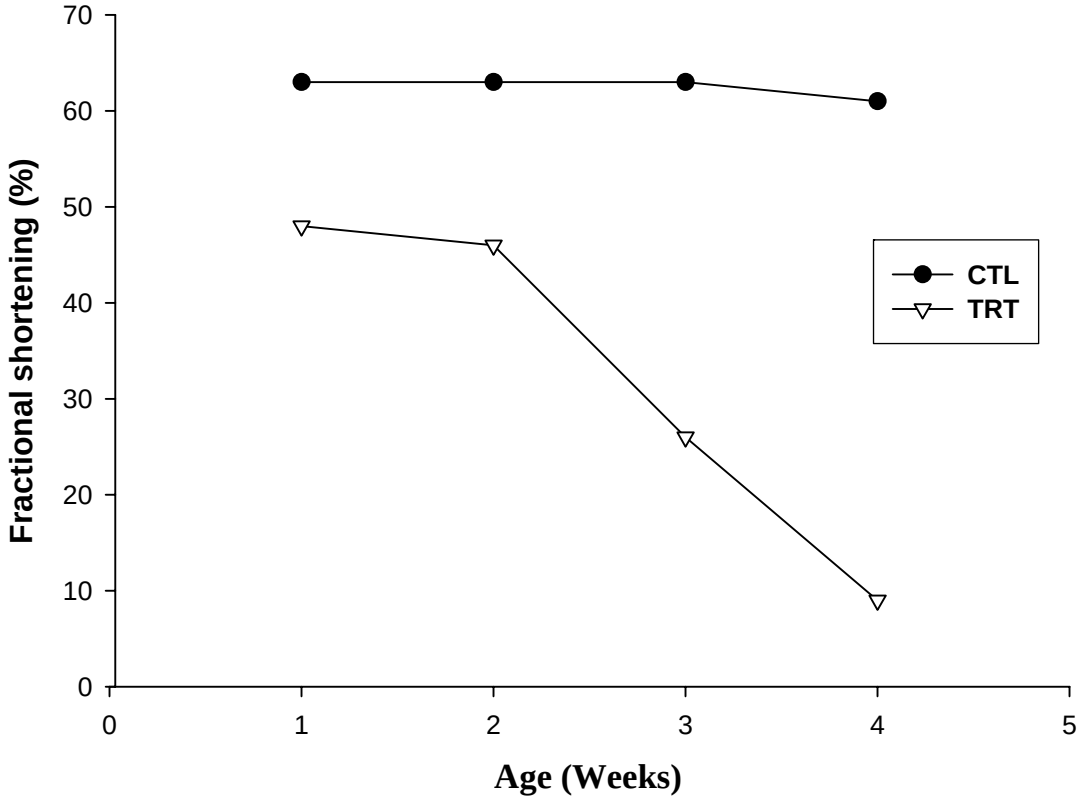

Figure 2. Fractional shortening (ejection fraction) of the heart from control (CTL) and furazolidone-fed turkey poults (TRT). Significant decrease in TRT birds after 2 weeks-of-age. Means ± S.E.

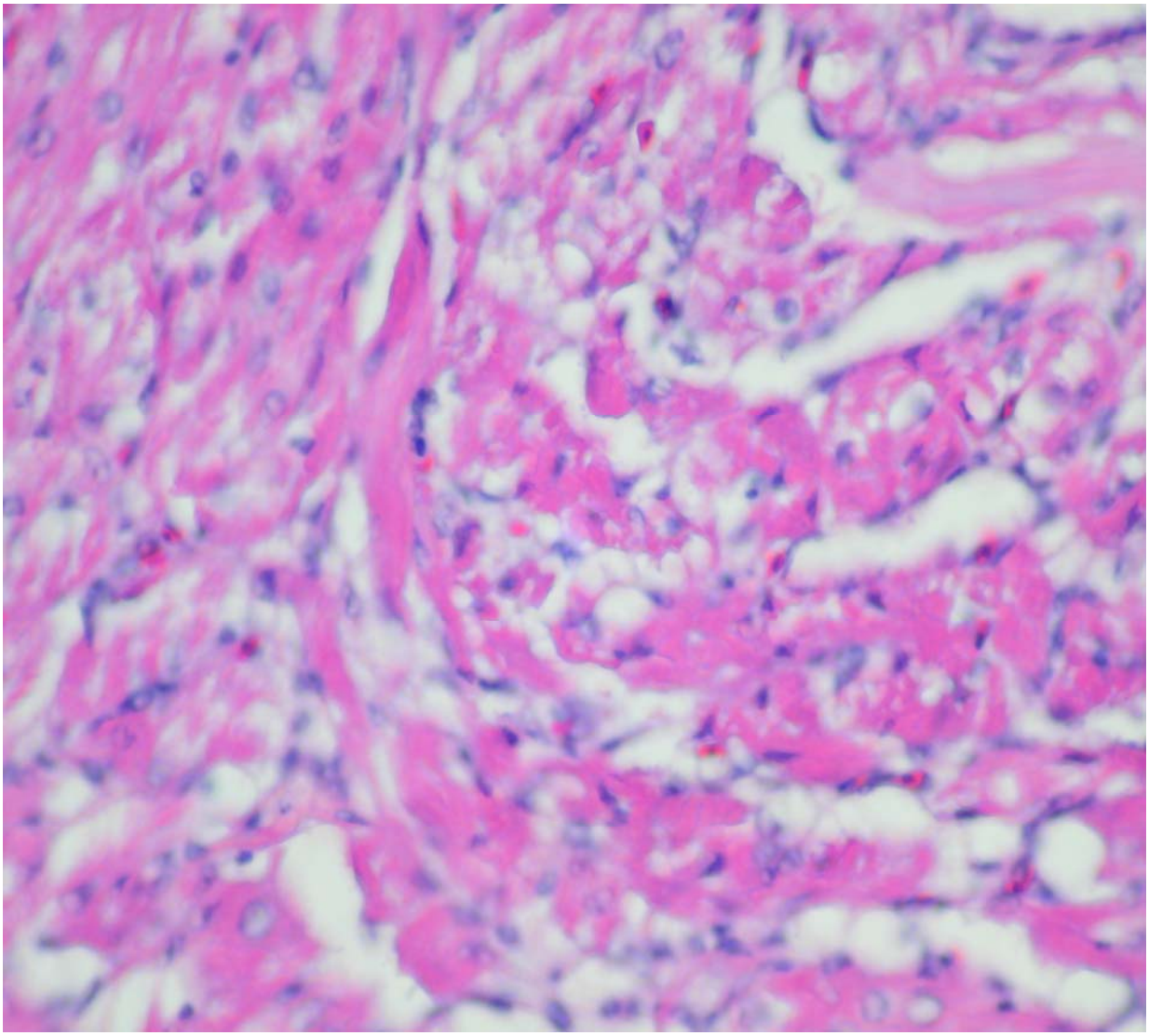

Figure 3. Cross section of the heart of a furazolidone-fed bird (diagnosed as dilated cardiomyopathic using echocardiography) showing increased necrosis and vacuolation as well as significant degeneration and increased inflammation of cells.

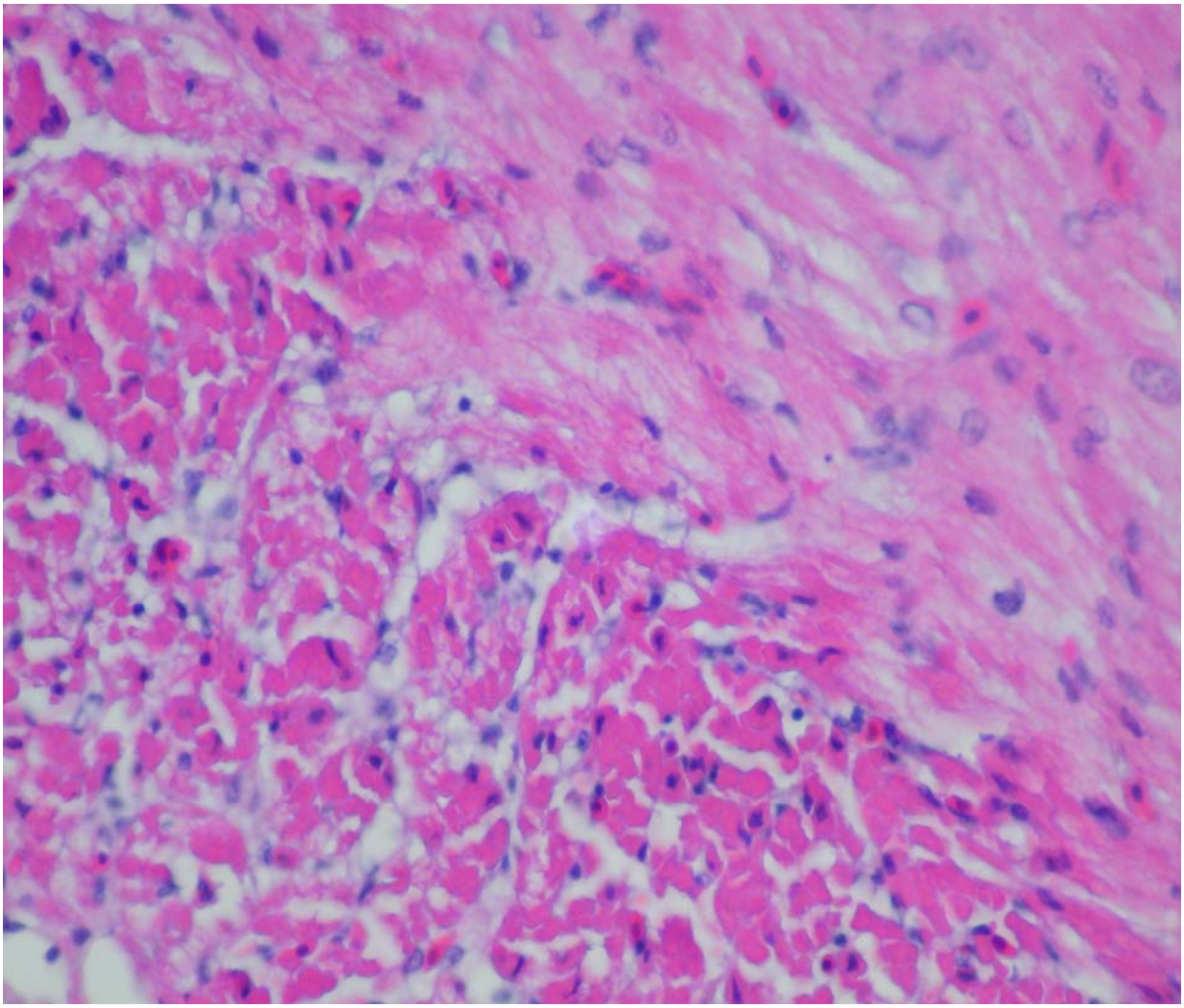

Figure 4. Cross section of the heart of control (normal) bird showing minimal vacuolation and degeneration of myocytes with relatively slight inflammation.

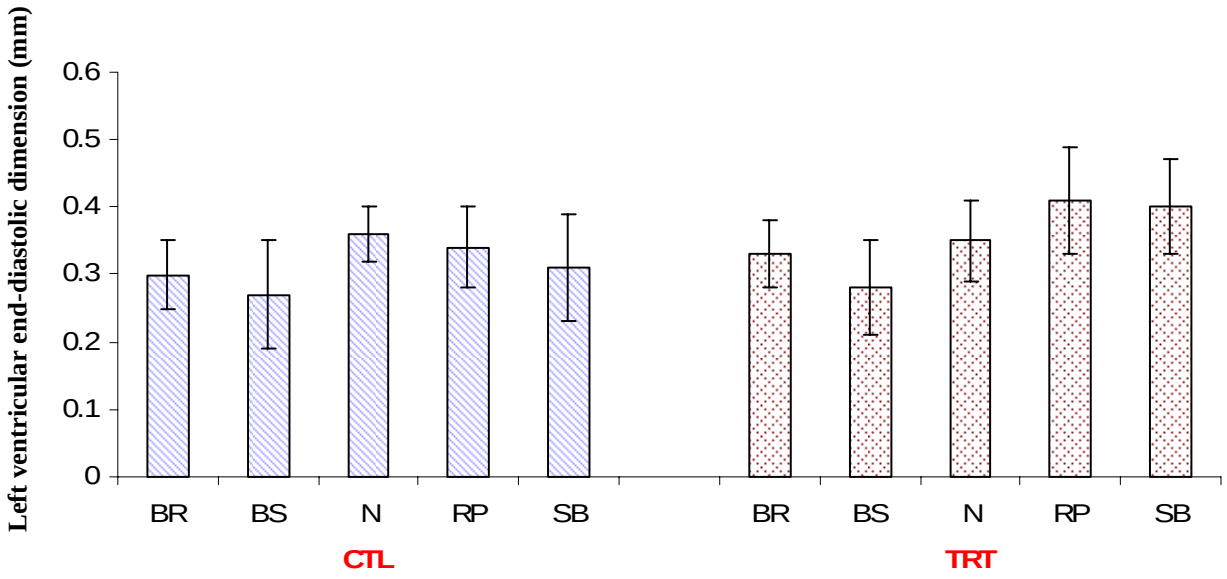

Figure 5. Left ventricular end-diastolic measurements in 9-day-old turkeys fed normal (CTL) and furazolidone-containing diet (TRT). Values are expressed as mean ± SD.

BR, BS, N, RP and SB represent Bourbon Red, Blue Slate, Narragansett, Royal Palm, and Spanish Black, respectively.

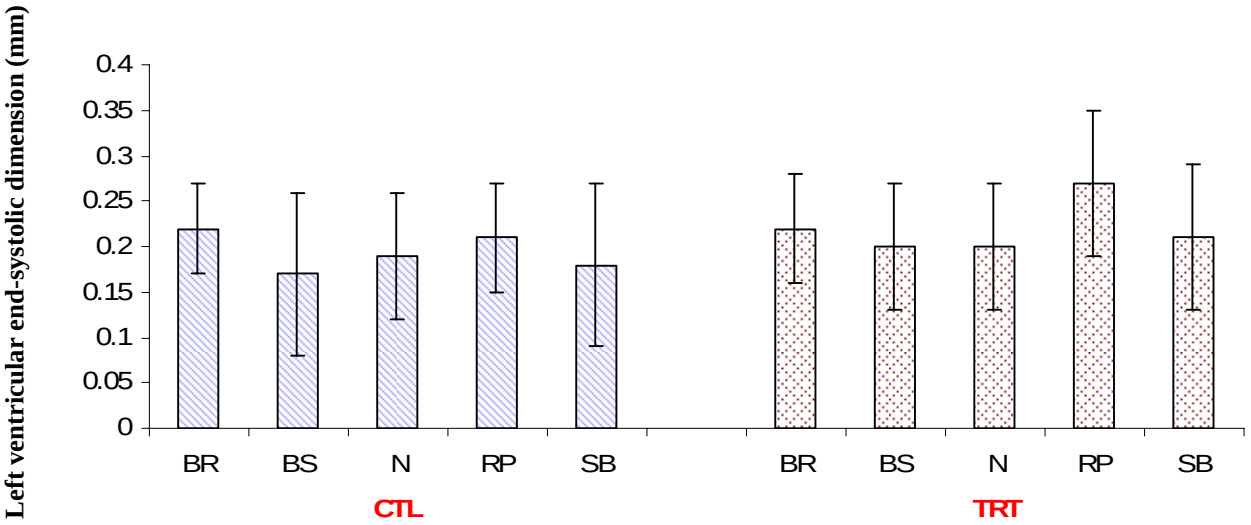

Figure 6. Left ventricular end-systolic measurements in 9-day-old turkeys fed normal (CTL) and furazolidone-containing diet (TRT). Values are expressed as mean ± SD.

BR, BS, N, RP and SB represent Bourbon Red, Blue Slate, Narragansett, Royal Palm, and Spanish Black, respectively.

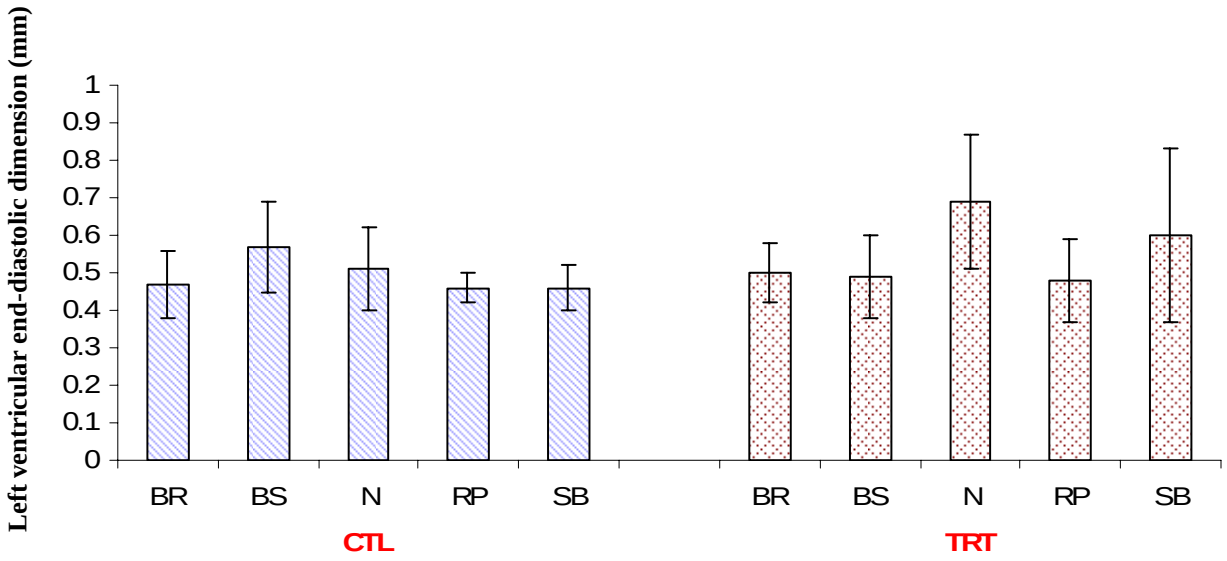

Figure 7. Left ventricular end-diastolic measurements in 18-day-old turkeys fed normal (CTL) and furazolidone-containing diet (TRT). Values are expressed as mean ± SD.

BR, BS, N, RP and SB represent Bourbon Red, Blue Slate, Narragansett, Royal Palm, and Spanish Black, respectively.

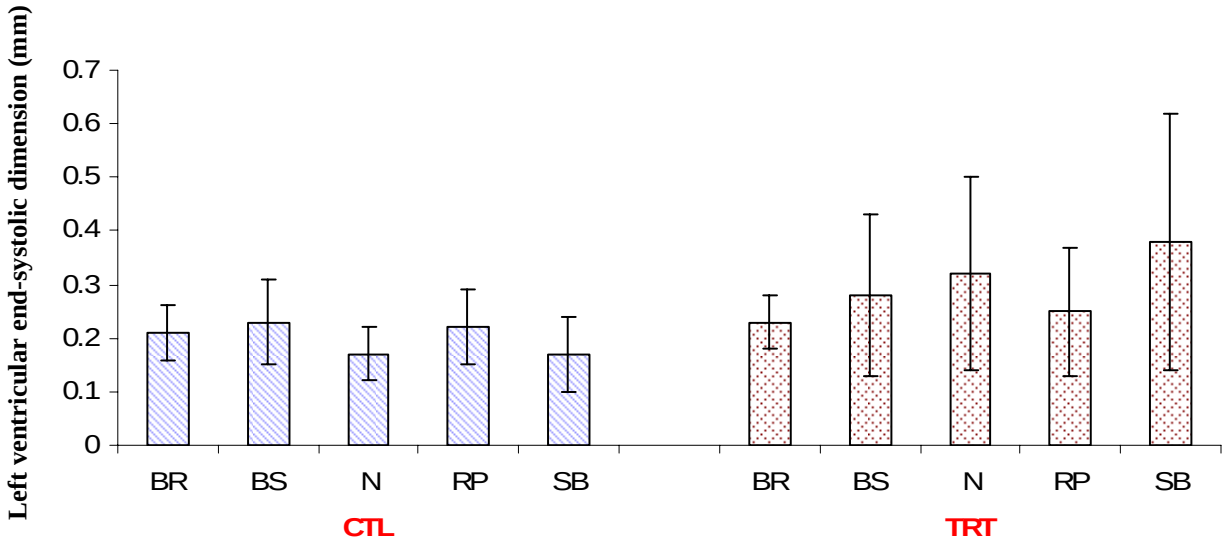

Figure 8. Left ventricular end-systolic measurements in 18-day-old turkeys fed normal (CTL) and furazolidone-containing diet (TRT). Values are expressed as mean ± SD.

BR, BS, N, RP and SB represent Bourbon Red, Blue Slate, Narragansett, Royal Palm, and Spanish Black, respectively.

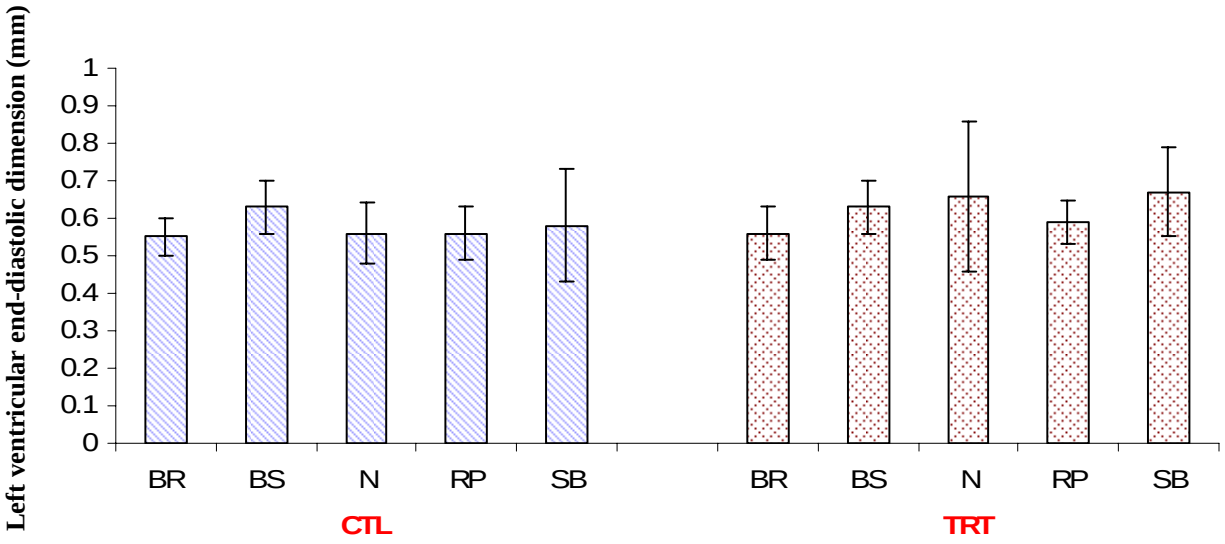

Figure 9. Left ventricular end-diastolic measurements in 23-day-old turkeys fed normal (CTL) and furazolidone-containing diet (TRT). Values are expressed as mean ± SD.

BR, BS, N, RP and SB represent Bourbon Red, Blue Slate, Narragansett, Royal Palm, and Spanish Black, respectively.

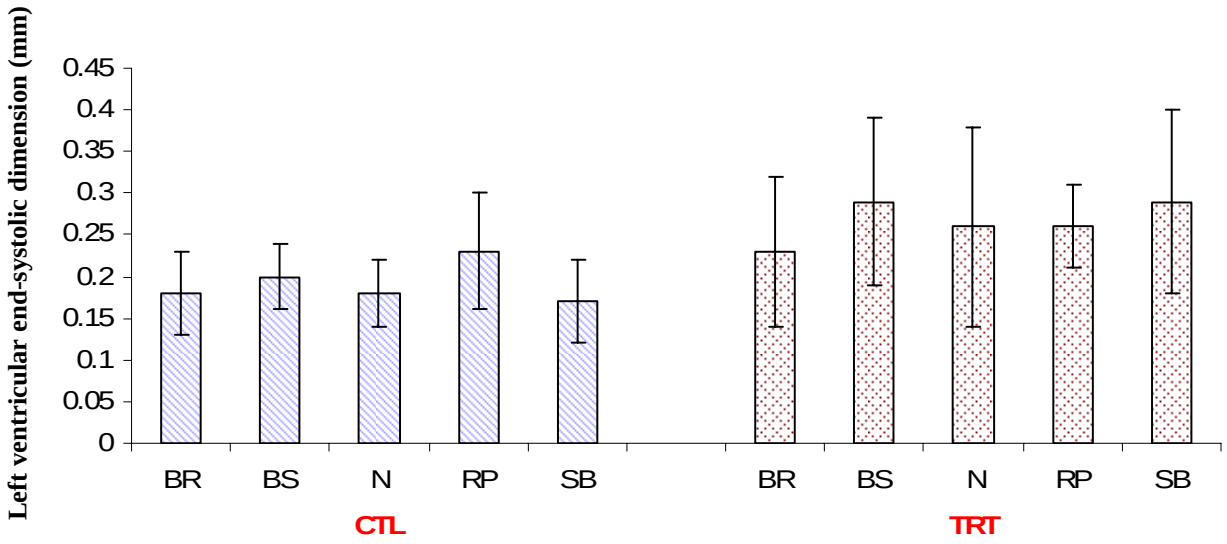

Figure 10. Left ventricular end-systolic measurements in 23-day-old turkeys fed normal (CTL) and furazolidone-containing diet (TRT). Values are expressed as mean ± SD.

BR, BS, N, RP and SB represent Bourbon Red, Blue Slate, Narragansett, Royal Palm, and Spanish Black, respectively.

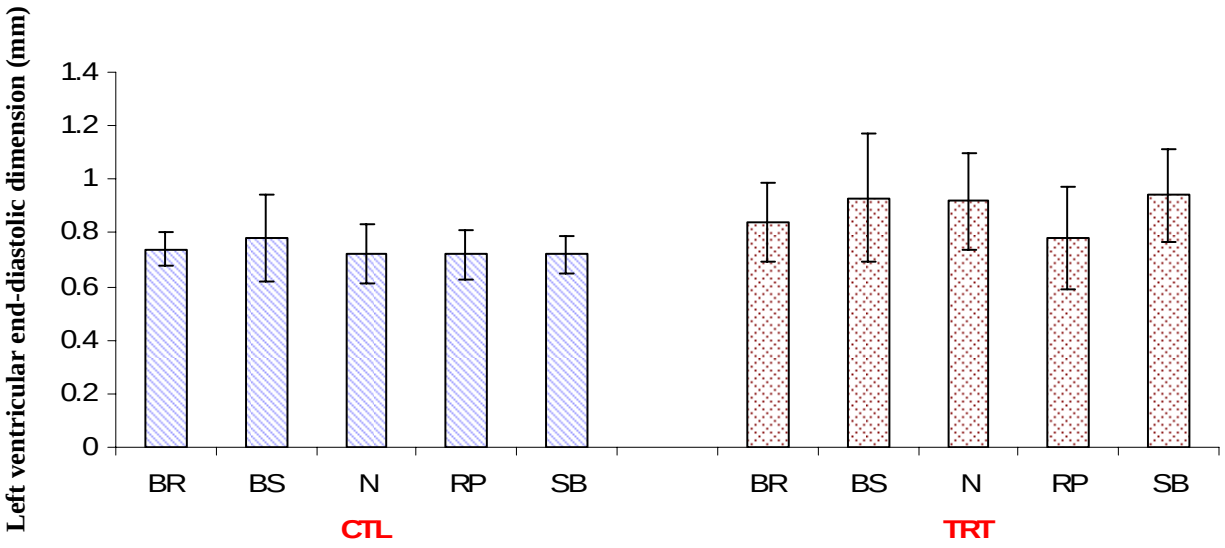

Figure 11. Left ventricular end-diastolic measurements in 29-day-old turkeys fed normal (CTL) and furazolidone-containing diet (TRT). Values are expressed as mean ± SD.

BR, BS, N, RP and SB represent Bourbon Red, Blue Slate, Narragansett, Royal Palm, and Spanish Black, respectively.

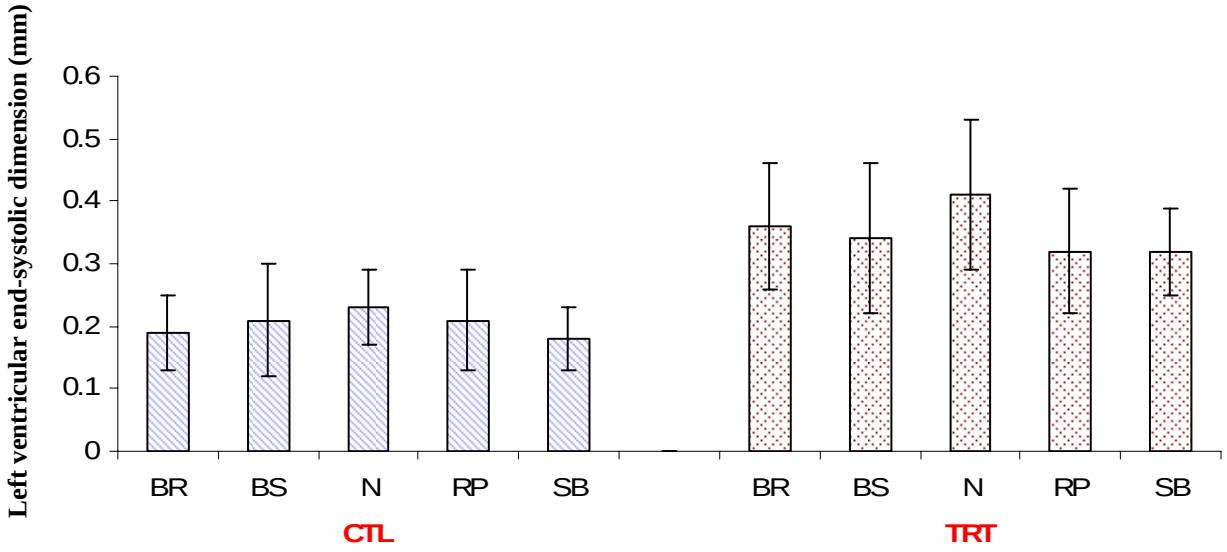

Figure 12. Left ventricular end-systolic measurements in 29-day-old turkeys fed normal (CTL) and furazolidone containing diet (TRT). Values are expressed as mean ± SD.

BR, BS, N, RP and SB represent Bourbon Red, Blue Slate, Narragansett, Royal Palm, and Spanish Black, respectively.

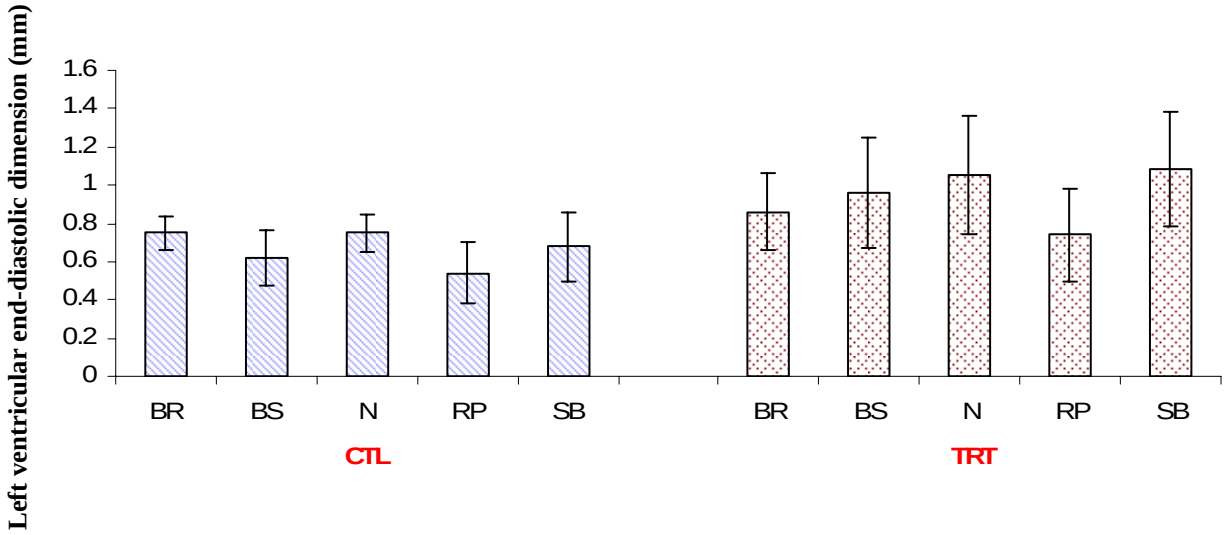

Figure 13. Left ventricular end-diastolic measurements in 33-day-old turkeys fed normal (CTL) and furazolidone-containing diet (TRT). Values are expressed as mean ± SD.

BR, BS, N, RP and SB represent Bourbon Red, Blue Slate, Narragansett, Royal Palm, and Spanish Black, respectively.

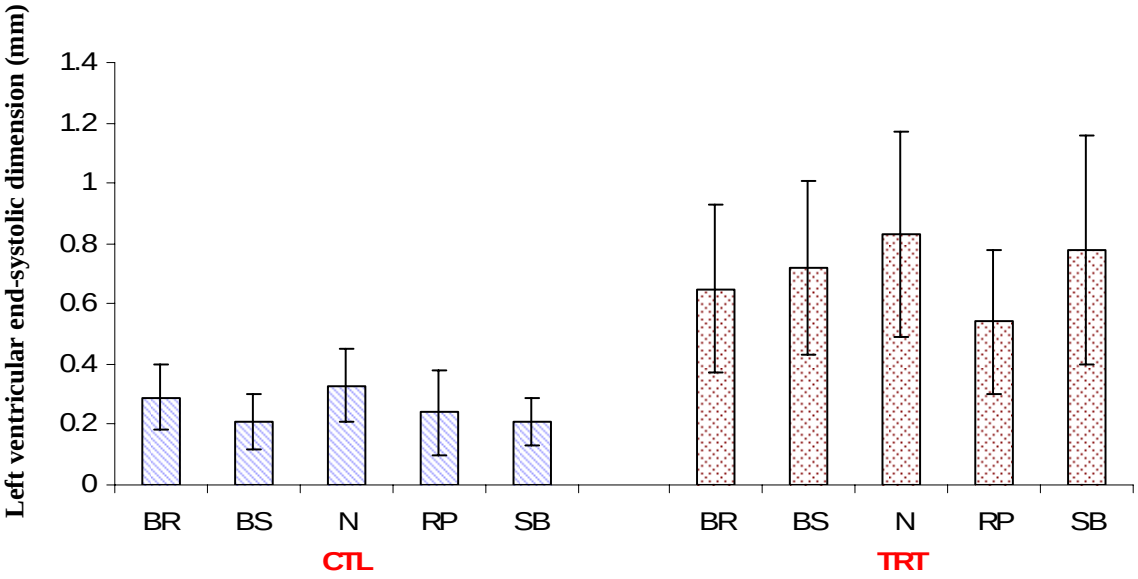

Figure 14. Left ventricular end-systolic measurements in 33-day-old turkeys fed normal (CTL) and furazolidone-containing diet (TRT). Values are expressed as mean ± SD.

BR, BS, N, RP and SB represent Bourbon Red, Blue Slate, Narragansett, Royal Palm, and Spanish Black, respectively.

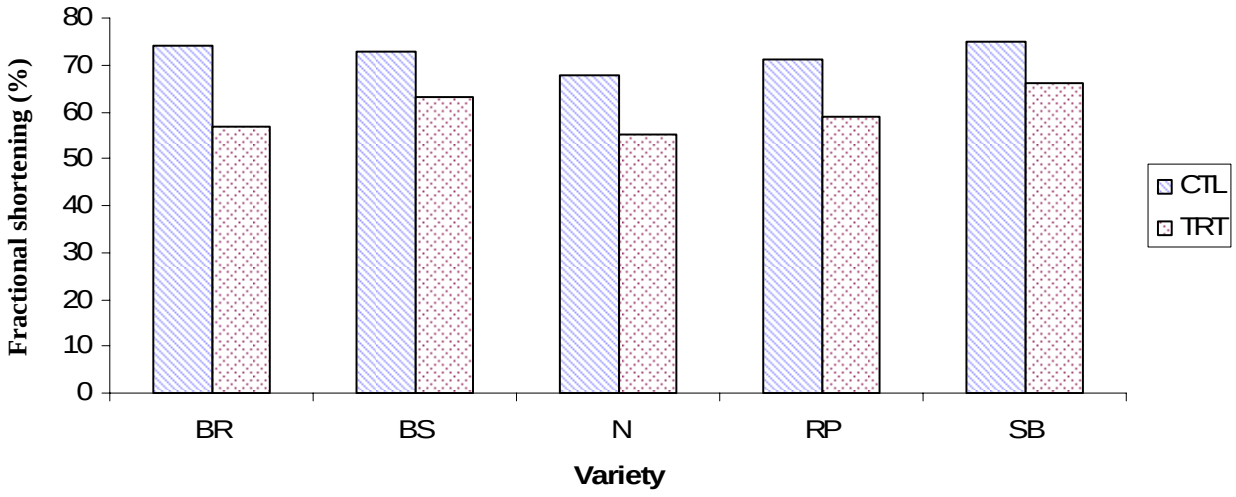

Figure 15. Fractional shortening in 33-day-old turkeys fed normal (CTL) or furazolidone-containing diet (TRT).

BR, BS, N, RP and SB represent Bourbon Red, Blue Slate, Narragansett, Royal Palm, and Spanish Black, respectively.

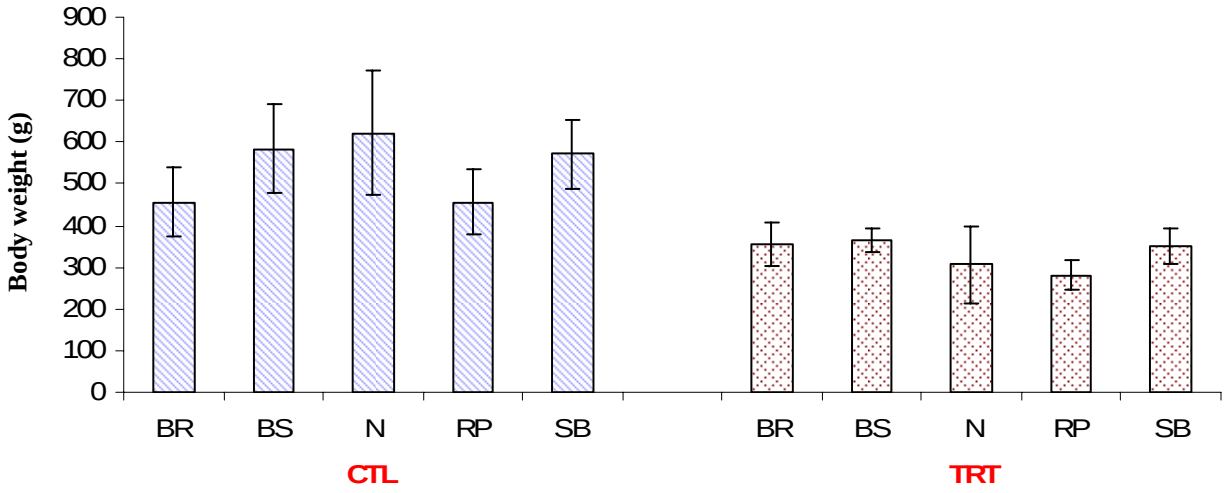

Figure 16. Variety differences in body weight in 33-day-old turkeys fed normal (CTL) and furazolidone-containing diet (TRT). Values are expressed as mean ± SD.

BR, BS, N, RP and SB represent Bourbon Red, Blue Slate, Narragansett, Royal Palm, and Spanish Black, respectively.

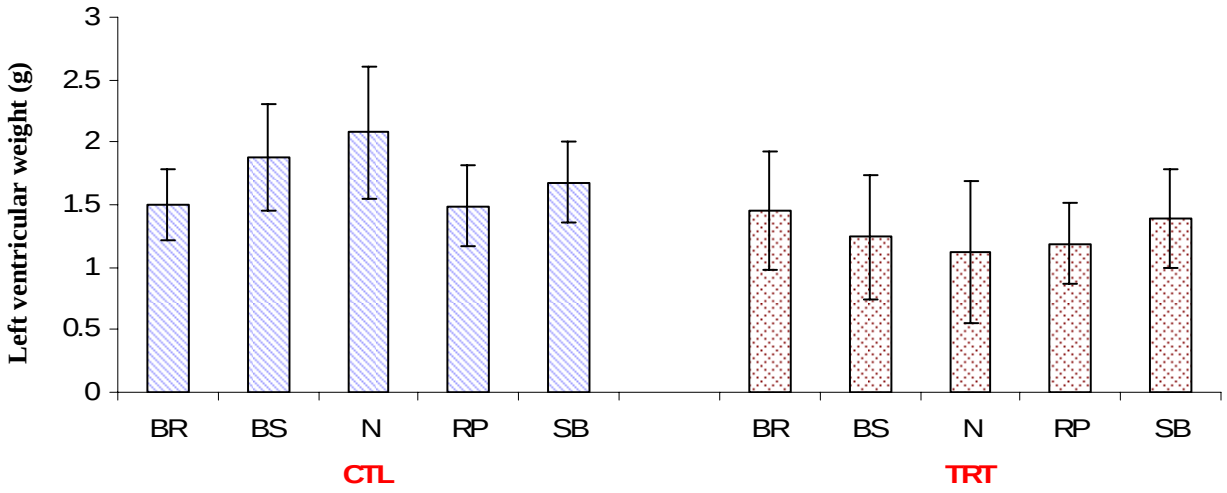

Figure 17. Variety differences in left ventricular weight in 33-day-old turkeys fed normal (CTL) and furazolidone-containing diet (TRT). Values are expressed as mean ± SD.

BR, BS, N, RP and SB represent Bourbon Red, Blue Slate, Narragansett, Royal Palm, and Spanish Black, respectively.

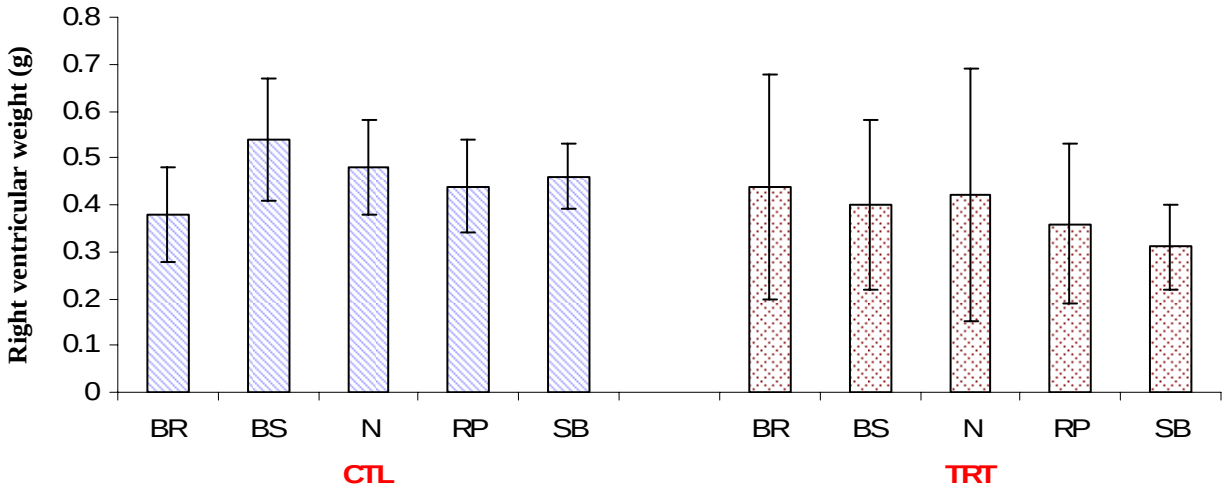

Figure 18. Variety differences in right ventricular weight in 33-day-old turkeys fed normal (CTL) and furazolidone-containing diet (TRT). Values are expressed as mean ± SD. BR, BS, N, RP and SB represent Bourbon Red, Blue Slate, Narragansett, Royal Palm, and Spanish Black, respectively.