The Potential for Refeeding Syndrome in Broiler Breeders

by

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Thesis Abstract

Sudden death at the initial point of lay in broiler breeders has long been an under-researched issue that results in a mortality of 0.5%-2% from 5%-30% egg production in breeder females. This mortality has been labeled as sudden death syndrome (SDS) in literature and has been characterized by low phosphorous and potassium concentrations. The goal of these studies is to show that, based on available evidence, the refeeding syndrome, rather than SDS, is the source of mortality at the initial point of lay in breeders.

Two experiments were performed so that the potential for refeeding syndrome in breeder females could be determined. One experiment determined the validity of a hand-held glucometer as a tool for rapid serial blood glucose monitoring in maturing breeder females. The other experiment monitored plasma electrolyte concentrations in maturing breeder females to determine whether changes in feed intake produced changes in electrolyte concentrations indicative of the refeeding syndrome.

From these experiments, it was determined that the glucometer is an acceptable tool for serial monitoring of blood glucose in chickens. Furthermore, it was determined that electrolytic shifts indicative of the refeeding syndrome occur within breeder females and that more research is necessary to determine if these shifts are evidence of a lager breeder welfare issue.

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Table of Contents

List of Tables

List of Figures

Introduction

In maturing breeder females, an increase in mortality often occurs between week 24 and week 26 coinciding with the initial point of lay. In the research literature and within the breeder industry, this mortality is referred to as SDS (Leeson and Summers, 2000). A different form of sudden death is commonly associated with broilers, and is described as a cardiomyopathy in which normal, seemingly healthy broilers experience a sudden paroxysm followed by death (Newberry et al., 1987). While they appear to be similar, SDS in broilers and sudden death in breeders are brought on by different circumstances. SDS in broilers is associated with an intrinsic physiological defect in cardiac function that leads to ventricular fibrillation and death (Olkowski et al., 2008). The rise in SDS occurrence has paralleled the increase in body weight and decrease in feed conversion ratio (FCR) that has occurred within the broiler industry in the last 50 years and is attributable to the inadequate growth and function of the heart and lungs of the modern broiler (Havenstein et al., 2003a).

Sudden death associated with a period of significant nutrient restriction followed by a rapid transition to a higher level of feeding, as occurs in breeders, is suggestive of refeeding syndrome. Refeeding syndrome is a condition characterized by electrolyte depletion occurring when malnourished individuals receive sudden nutrient repletion (Marinella, 2005). The primary electrolyte deficiency is phosphorous while the secondary deficiency is potassium (Skipper, 2012) (Lambers et al., 2015). Hypophosphatemia and hypokalemia have been reported in broilers at the initial point of lay in breeders, giving further evidence that the refeeding syndrome

is potentially the source of sudden mortality at that time (Aviagen Breeder Management Handbook, 2013) (Hopkinson et al., 1990).

Due to the similarities of the source of mortality at the initial point of lay in breeders and the refeeding syndrome, further evaluation is required. In addition to the refeeding syndrome, two other prominent forms of sudden death within broilers will be evaluated as the potential source of mortality at the initial point of lay: SDS and ascites syndrome. Through the examination of literature concerning these three syndromes, elucidation of the source of mortality at the initial point of lay can hopefully be achieved.

Literature Review

Trends and Changes within the Broiler Industry

The poultry industry has changed drastically in the last 60 years. Due to improvements in feeding methods and intensive genetic selection, productivity of poultry has nearly tripled (Grandin and Deesing, 1988). A comparison between broilers of a strain similar to those that would be found in 1957 was compared to a modern broiler strain. Modern broilers were found to have a significantly higher body weight and a significantly lower FCR than did the 1957 broilers when measured at 21, 42, 56, 70, and 84 days of age (Havenstein et al., 2003b). Similar trends have been seen in turkeys. Modern strains are twice the size of 1966 strains when they reach market age while maintaining a FCR of 20% less than the 1966 strains (Havenstein et al., 2004). While these trends have been a boon for the poultry industry, animal welfare concerns have been raised regarding genetic selection for rapid growth and production, (Grandin and Dessing, 1988).

Although studies of contemporary broiler lines found higher body weight, higher breast meat yield, and a lower FCR as compared to broilers from 1957, the modern birds exhibited negative trends in organ growth. When compared to broilers of 1957, not only did modern broilers have significantly lower lung and heart weight percentages in relation to total body weight, but the total lung weight of modern broilers was significantly lower as well (Havenstein et al., 2003a). This study is consistent with another study in which Ross 308 broilers were compared to broilers that had not been selected for fast growth since 1972 along with chickens

that had never been selected for fast growth. This comparison showed that there were significant strain effects on the sizes of pectoral muscle, leg muscle, gizzard, liver, heart, lungs, and brain with everything being larger in the birds selected for faster growth (Gavin and McDevitt, 1999). Interestingly though, a significant plateau was observed in organ growth in the Ross 308 broilers, suggesting that organs reach a set size and will not grow in a parallel manner with muscle growth. This leaves significantly increased metabolic demand on the organs which have to service the rapid growth of the musculature of the broiler (Hafez and Hauck, 2004). Because of the increased metabolic demand relative to organ capacity, broilers are susceptible to metabolic diseases. Two cardiomyopathies are of particular interest commercially: SDS and ascites syndrome. These metabolic diseases represent significant causes of mortality within the poultry industry and, as such, are issues that need to be explored further.

SDS

Acute flip-over syndrome, also known as SDS is a cardiomyopathy in which seemingly normal, healthy broilers experience an acute attack prior to death that is characterized by vigorous wing flapping, muscle contraction, and a sudden loss of balance followed by death (Newberry et al., 1987). These episodes typically last 40-70 seconds and are accompanied by an audible cry. The disease receives its name due to how the birds are found after they die; birds are typically found lying on their backs or sides after death due to SDS (Newberry et al., 1987). Other symptoms used to diagnose a death by SDS are as follows: a full intestinal tract with

recently ingested feed in the crop and gizzard with a small or empty gall bladder; contracted, empty ventricles with clotted blood filling dilated atria; left ventricular and/or right atrial hypertrophy; lungs and other organs congested with mottled muscles; and a lack of specific lesions that may accompany other metabolic/cardiovascular diseases (Oninwu et al., 1979) (Bowes and Julian, 1988). It is further clarified that though heart tissue may appear ruptured and massively contracted, heart tissues appear otherwise healthy and the cardiac event is not indicative of congestive heart failure as is common in left heart failure (Moghadam et al., 2005). In opposition to these findings, lung congestion, which typically indicates left heart failure, is the most common pathological finding surrounding SDS (Kittelsen et al., 2015). Although the pathology of SDS is fairly well known, the behavior of broilers prior to SDS episodes is inconsistent. One study reports that no SDS death was preceded by strenuous activity or human handling and that the birds' behavior prior to death was variable including feeding, drinking, lying, walking, and sitting (Newberry et al., 1987). In contrast, it has been reported that stress can make broilers more vulnerable to a SDS episode (Kittelsen et al., 2015). Even more unknown is the etiology of SDS. Currently, the prevalent theory as to the cause of SDS induction is elevated dietary quantity combined with higher nutritional content that leads to cardiac dysfunction via an intrinsic physiological defect within the broiler (Afolayan et al., 2016).

The dietary portion of the theory assumes that it is the content and quantity of feed consumed by broilers that leads to the cardiac dysfunction that results in an increase in mortality attributed to SDS. However, it may be that it is the specialization and production requirements of modern broilers that is placing strain on poultry metabolism. Any disturbance of energy

metabolism or mineral balance can have a significant impact on performance and mortality (Decuypere and Vergstegen, 1999). Modern broilers are currently fed in an *ad libitum* state in order to maximize growth rate and efficiency. However, as previously discussed, this allows for a significant increase in muscle size without a parallel increase in organ size which presumably leads to metabolic disease such as SDS. In an experiment where broilers' dietary intake was restricted to varying amounts during the second weeks of age, results were consistent with the previous statement. When feed intake was restricted by 30-40%, there was a statistically significant decrease in mortality due to SDS within the broilers; however, there was a correlated reduction in body weight with birds that were restricted in their feed. When broiler feed was restricted by 20%, an observational (not statistically significant) decrease was observed in SDS mortality without the accompanying correlated reduction in body weight (Gonzales et al., 1998). These results suggest that a slight feed restriction could reduce the mortality attributable to SDS without impacting the weight of the bird. In contrast, another study showed that while feed restriction significantly reduced body weight and fat pad size while increasing feed efficiency in broilers, there was no significant decrease seen in SDS related mortality (Mollison et al., 1984).

While the role that the quantity of feed plays in the mortality rates due to SDS is well defined, many experiments examining a role for feed composition in SDS mortality have been performed with mixed results. It has been shown that reducing dietary fat decreases incidence of SDS (Chung et al., 1993). While less fatty diets can lead to lower incidence of SDS, these diets also reduce initial growth rates, increase FCR, and increase abdominal fat at 49 days of age (Mollison et al., 1984). This same study showed that broilers given finisher diets with higher

protein content experienced a reduction in SDS related mortality. Another additive shown to have positive effects on SDS mortality is omega-3 fatty acids derived from canola oil. Fish oil could also be used as a source for omega-3 fatty acids, which have anti-arrhythmic properties in many mammals including humans, but fish oil has been associated with negative flavors of chicken meat as well as unfavorable costs for inclusion into diets (Gregory et al., 2014). When canola oil is added to broiler feed, broilers experience a 24% increase in eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA) in heart phospholipids, the two primary omega-3 fatty acids with cardioprotective properties, suggesting the potential to decrease SDS related mortality. While the above listed additives and alterations to feed content have an overall positive effect on the cardiac health of broilers, others can have neutral or negative impacts upon broiler health. Due to the inherent importance of calcium and phosphorous in cardiac function, experiments have been performed using calcium and phosphorous additives to assess impact on SDS related mortality. In one study, the addition of calcium and phosphorous to the feed led to a metabolic imbalance that led to an increase of SDS within broilers while resulting in a decrease in feed efficiency (Scheideler et al., 1995). In a separate experiment in which calcium, phosphorous, and magnesium were added to the grower and finisher diets of broilers, there were no significant effects of the mineral additions to incidences of SDS, however; the mineral additives led to an increase of leg-weakness incidence due to a metabolic imbalance that resulted in improper bone and muscle growth within the legs (Julian, 1986). Finally, lactate has been reported to have a potential role in the etiology of SDS. An intravenous application of lactate to a broiler results in 100% mortality due to SDS although the effects are negligent when lactate is

supplied through feed (Jacob et al., 1990). Furthermore, dietary glucose does not exacerbate the effects of dietary lactate. While feed composition and quantity play an important role in the etiology of SDS, it is the intrinsic metabolic defects within the broiler that predispose broilers to SDS.

Broilers that are susceptible to SDS are indistinguishable from their healthy counterparts showing no significant indicator of metabolic events that would predispose the broiler to SDS (Bowes et al., 1989). Due to this lack of external indication and to the previously stated increase in metabolic demand on the fast growing broiler, an intrinsic physiologic dysfunction is likely important in the etiology of SDS. Two main conditions pave the way for intrinsic physiological dysfunction: tissue hypoxia, or an insufficient supply of oxygen, combined with hypercapnia, or an elevated partial pressure of carbon dioxide within the blood (Olkowski, 2007). Broilers with higher carbon dioxide tension at two weeks of age were shown to be predisposed to ventricular arrhythmias and SDS at an age of 5-7 weeks (Korte et al., 1999). Furthermore, it is noted that while broilers with higher carbon dioxide tension displayed higher concentrations of premature ventricular contractions including varying concentrations of ventricular tachycardia during ECG examination; all broilers examined displayed some level of premature ventricular contractions regardless of carbon dioxide tension (Korte et al., 1999). This shows that while hypercapnia can exacerbate ventricular arrhythmias and aid in the predisposition of a broiler to SDS related death, it is hypoxia, in combination with reduced lung and heart weight percentages, which result in the myocardial irritability that leads to SDS related death.

The action potential within the heart is unlike a typical neuro-muscular action potential. There are four steps that result in the myocardial action potential which are critical to proper function: 1. Voltage regulated sodium channels open and result in a massive influx of sodium with a minor influx of calcium to depolarize the myocardial membrane; 2. Inward rectifier voltage regulated potassium channels open and cause a slight repolarization of the membrane; 3. Slow voltage regulated calcium channels open and allow an influx of calcium into the myocardium, resulting in a plateau of the action potential with both calcium and potassium channels open; 4. Slow voltage regulated calcium channels close and a separate subpopulation of voltage regulated potassium channels called delayed rectifiers open and the membrane is repolarized to resting membrane potential (Goodwin and Wit, 2012). The action potential will cause a contraction of the myocardium through a series of steps that is referred to as excitation contraction coupling (Whittow, 1994). When the myocardial membrane depolarizes, the slow calcium channels on the myocardial surface undergo a conformation change and slowly open to allow the influx of calcium (Guyton and Hall, 2015). This calcium will interact with ryanodine receptors on the sarcoplasmic reticulum (SR) within the cell causing a release of calcium stored within the SR (Whittow, 1994). Free calcium within the cell then reacts with troponin C and allow for the myosin-actin interaction that results in myocardial contraction (Guyton and Hall, 2015). The relaxation of myocardial muscle proceeds in a similar yet contrasting stepwise manner to relaxation: Voltage gated calcium channels close preventing new extracellular calcium from entering the cell, calcium ATPases work to pump free calcium within the cell back into the SR, and sodium-potassium ATPases work in concert with sodium-calcium co-transporters to

pump excess intracellular calcium into the interstitial fluid (Guyton and Hall, 2015). When the excitation-contraction coupling process works as it should, the length of the action potential and the process of contraction allow myocardial contraction to proceed in a wave-like manner across the heart and prevent further myocardial depolarization, particularly in the ventricles, until the entire heart has been repolarized (Goodwin and Wit, 2012). In broilers susceptible to SDS, calcium ATPase activity and general calcium uptake during the relaxation phase are depressed (Chung et al., 1993) (Imaeda, 2000). This delay in the repolarization of some of the ventricular myocardial cells during the relaxation causes the phenomenon of re-entry, which results in ventricular myocardial cells depolarizing and repolarizing at different rates leading to premature ventricular contractions (PVC) (Guyton and Hall, 2015). Depending on the severity of the PVC, the heart can enter ventricular tachycardia and, eventually, ventricular fibrillation resulting in the death of the broiler.

When compared to slower growing birds such as leghorn layers, fast growing broilers experience increased myocardial irritability that leads to myocardial fibrillation (Greenless et al., 1989). Typically, myocardial irritability manifests in the form of PVC. In a study in which broiler flocks were subjected to ECG testing, all of the broilers tested exhibited some level of PVC formation, however; only birds exposed to some kind of stressor developed ventricular tachycardias or ventricular fibrillations (Nain et al., 2007). Furthermore, atrial contractions were as strong as ventricular contractions at some points with abnormally close P and T waves. In a separate study, it was reported that P and T waves of broilers susceptible to SDS fused; the P and T wave fusion was the most common abnormality associated with SDS occurrence within

broilers and is likely due to the prolonged ventricular repolarization period experienced in broilers (Olkowski et al., 1997). In a broiler flock maintained to industry standards, 3-6% of the broilers succumbed to SDS while 17-35% of the broilers tested exhibited some sort of cardiac arrhythmia with PVC and ventricular tachycardias being the most commonly exhibited arrhythmias (Olkowski et al., 2008). Unifocal PVC were noted to be more common than multifocal PVC. Arrhythmias in general and PVC plus P and T wave fusion in particular are likely at the core of the pathogenesis of SDS (Olkowski and Classen, 1998). PVC may arise within the broiler due to hypokalemia, stress, exercise, electrolyte imbalance, hypoxia, cardiomyopathy, or any combination of these factors. It is likely that an external stressor is the trigger of SDS. 158 broilers were injected with epinephrine to simulate a stress response within the bird: 8% showed no arrhythmia; 74% showed moderate, episodic, short lasting ventricular arrhythmias; and 18% had life threatening ventricular arrhythmias (Olkowski et al., 2008). Of the 158 birds tested, 5 birds died (3.16%) of SDS related attacks within 3 days. During a SDS episode that leads to death, broilers show no identifiable P, T, or QRS waves during ECG; broilers will exhibit ventricular flutter followed by ventricular fibrillation that ultimately results in a cessation of heart activity approximately 4-6min after the onset of the clinical signs of SDS (Olkowski and Classen, 1997). With the previous information taken into account, the pathology of SDS is thought to be this: an arrhythmia is stimulated by an external or internal stressor, then, the heart is unable to recover from the arrhythmic event due to pathological changes within the myocardium that allow PVC to devolve into ventricular fibrillation which ultimately leads to death (Olkowski et al., 2008). Although diet quantity can exacerbate or depress SDS occurrence,

it is believed that SDS pathogenesis is not a direct result of diet concerning feed composition, as changes to broiler diet composition have proven to be mostly ineffective (Greenless et al., 1989)

The mortality percentage attributable to SDS within broiler flocks has remained fairly consistent with a slight decrease in overall percentage over the last 30 years. In the 1980s, mortality due to SDS ranged from 0.5-4% within select broiler flocks (Bowes et al., 1988). In 2016, that percentage ranged from 0.67-2.49% when measured between 18 different broiler flocks (Kumar et al., 2016). While the true absolute percentage of broiler mortality due to SDS is difficult to pinpoint, trends within broiler flocks that are associated with SDS prevalence are easily observable. When compared to broilers similar to 1957 birds, strains of broilers that would have been used in 2001, also known as modern broilers, had almost double the total mortality than did their 1957 counterparts, with much of that increase being attributable to the prevalence of metabolic diseases such as SDS or ascites within the modern broiler (Havenstein et al., 2003b). It has also been reported that broilers that are highly productive exist in a hypothyroid state and are more sensitive to metabolic disorders (Gonzales et al., 1999). In an experiment in which broilers were raised to 42 days, the total percentage of mortalities was highest amongst the most productive strains of broilers with over 50% of total mortality being attributable to SDS and ascites syndrome (Gonzales et al., 1998). The mortality percentage do to SDS or ascites was especially high if subjected to factors such as low temperature, a risk factor for ascites, or high nutritional density, a risk factor for SDS.

Though trends and mortality percentages concerning broiler flocks have remained consistent and observable, the question surrounding heritability of SDS predisposition is still

unanswered. Studies either confirming that SDS predisposition is a heritable condition (Moghadam et al., 2005) or is not a heritable condition (Afolayan et al., 2016) are both present and provide no clear answer to the question of heritability. Determining the heritability of SDS could allow for the potential of genetic selection against birds predisposed to SDS, a prospect that could significantly lower SDS related mortality without altering common industry practices. While many theories have been tested concerning how to lower SDS related mortality, only one currently available possibility has been truly successful. In a study in which feed was restricted by 25%, SDS related mortality was reduced from 3.3% to 0% (Bowes, 1988). Indeed, it has been widely agreed upon that the only way to limit or eliminate the impact of SDS on broiler flocks is by limiting the growth of broilers by either restricting feed or reducing nutrient density within the feed (Afolayan et al., 2016).

Ascites Syndrome

Ascites syndrome, also known as pulmonary hypertension syndrome, is a metabolic disorder that is characterized by a cascade of events stemming from the broiler being unable to satisfy the oxygen demands for proper maintenance and growth (Hassanzadeh, 2010). Unlike SDS, the pathology and etiology of ascites syndrome is fairly well known. When the metabolism of the broiler rises to the point where oxygen demand cannot be met, an internal hypoxic condition develops. At this stage, the bird will enter what can be termed “compensated heart failure.” During compensated heart failure, three differing mechanisms will be utilized by the

bird in order to increase blood flow through the heart in an attempt to satisfy the metabolic demands of the vasculature: 1. Sympathetic activation will occur to both cause vasoconstriction of the blood vessels and increase the cardiac output of the heart for a short term increase in blood pressure. 2. Activation of the renin-angiotensin system results in fluid retention, an increase in venous return, and a more permanent increase in blood pressure. 3. Cardiac remodeling will occur and will result in hypertrophy of the heart (Guyton and Hall, 2015). This compensation, when talking specifically about the ascites syndrome, results in increased lung arterial pressure and right ventricular hypertrophy (Wideman et al., 2013). If the hypoxic state persists after compensation occurs, the broiler will enter into decompensated heart failure which will eventually lead to death (Wideman et al., 2013). The first stage of the decompensated heart failure is the continued dilation of the right ventricle. When systemic blood pressure and venous return increase, more volume is pumped into the right side of the heart which causes a dilation to occur. Progressive dilation will result in a weakening of the right ventricle and an inability to pump 100% of the blood from the ventricle to the pulmonary artery (Wideman et al., 2013). Dilation in combination with increasing pulmonary blood pressure, which makes it more difficult to pump blood from the right ventricle to the pulmonary artery, causes the right atrioventricular valve to fail and for fluid volume to build within systemic circulation (Balog, 2003). Increased systemic fluid retention will eventually cause congestion in the circulatory system; liver congestion and edema; and finally, fluid will be exuded into the lungs, pericardium, and abdominal cavities which will eventually result in death (Balog, 2003).

Clinically, there are several external signs that a broiler might be in the early stages of ascites development that can be observed: cyanosis of the skin of the head (comb or wattle) and body, dilated and prominent veins, shrunken combs and wattles, abdomen dilated with fluid, and increased respiration rate with decreased exercise tolerance (Julian, 1993). Post-mortem indications of ascites syndrome include accumulation of fluid in the peritoneal cavities, hydropericardium, right ventricular hypertrophy, venous congestion, and diagnostic lesions occurring in the heart, lungs, kidneys, and liver (Kalmar et al., 2013). In some cases, the left ventricle of the heart has also been found to be undersized and dilated (Julian, 1993) (Olkowski, 2007). Birds susceptible to ascites syndrome display bradycardia which contributes to hypoxemia and hypoxia of the tissues by delaying oxygen delivery (Olkowski and Classen, 1998). Broilers that succumb to ascites syndrome frequently have higher hematocrit values than normal counterparts, leading to an increase in blood volume, with erythrocytes that are highly rigid (Mirsalimi and Julian, 1993). Interestingly, there is no relationship between ascites formation and electrolyte/metabolite concentrations including sodium, calcium, potassium, and glucose (van As et al., 2010). With all of these indicators considered, it is right ventricular hypertrophy along with fluid in the abdomen that are the factors most closely associated with the diagnosis of ascites syndrome.

The etiology of ascites syndrome is well known, with many endogenous and exogenous factors playing a role in its potential development. One of the most prominent environmental exogenous factors to play a role in ascites formation is lighting regimen. Current industrial practices have broilers growing on a nearly continuous lighting schedule (typically 23 hours of

light with one hour of dark) to maximize feed consumption and, subsequently, growth rate. In order to reduce the occurrence of ascites syndrome, an intermittent lighting schedule at an early age should be utilized (Hassanzadeh, 2010). Although intermittent lighting schedules tend to depress the growth rates of broilers, switching to a continuous lighting schedule after one or two weeks of intermittent lighting allows for compensatory growth to occur and for broilers to reach a body weight that is not significantly different than broilers that are raised on a purely continuous lighting regimen (Hassanzadeh, 2010). Altitude is another environmental exogenous factor that impacts broiler susceptibility to ascites syndrome. At higher altitudes, there is less oxygen available for consumption which exacerbates the hypoxemia and hypoxic tissue conditions experienced by broilers by causing greater resistance to blood flow, resulting in higher incidence of ascites syndrome (Scheele, 1991). In fact, it was in high altitude locations that ascites syndrome was first observed (Julian, 1998). Colder temperatures will also increase the incidence of ascites syndrome by increasing the metabolic rates of broilers, causing a rise in oxygen demand in the tissues (Julian, 1988). One study hypothesized that chickens in general are ill adapted to deal with colder temperatures due to being derived from jungle fowl which are tropical species (Huchzermeyer, 2012). Since their ancestors did not have to deal with the increase in metabolism that accompanies the thermoregulatory response to a cold environment and with broilers already being pushed to their metabolic limits, modern broilers do not have the capabilities to deal with the hypoxic tissue conditions rendered by colder temperatures. A final environmental exogenous condition to consider is proper ventilation. Improper house ventilation can cause a decrease in the available oxygen in the air and, much in the same way high altitude

effects broilers, cause an increase of the hypoxemic and hypoxic conditions experienced by broilers resulting in a much higher incidence of ascites syndrome (Currie, 1999).

An exogenous factor that is non-environmental in nature but is still of vital importance in the formation of ascites syndrome is the feed of the bird. One feed component that must be monitored is nutritional density. The more nutritionally dense feed is, the harder the broiler's metabolism must work in order to digest the feed and utilize the nutrients; increased metabolism leads directly to increased oxygen consumption which results in higher incidence of ascites formation (Balog, 2003). Another component of feed that can affect the incidence of ascites is feed form. Pelleted or crumbled feed is easier for birds to consume and is more digestible than mash feed which allows more food to be consumed (Julian and Squires, 1995). Furthermore, pelleted feed has a higher nutrient density than mash feed (Julian and Squires, 1995). The higher nutrient density, ability to consume feed more easily, and the ability to digest feed more easily result in an increase in metabolism and oxygen consumption, making mash feed the ideal feed form if the goal is to limit ascites formation (Balog, 2003). Feed additives can impact ascites syndrome formation as well. Antioxidants, such as vitamin E, vitamin C, and nitrous oxide, act as vasodilators and can serve to lessen the pulmonary hypertension that accompanies ascites (Hassanzadeh, 2010). Quantity of feed, more than any other component of feed previously mentioned, has the largest impact on incidence of ascites within broilers. In fact, feed restriction is one of the most common commercial treatments for ascites syndrome (Balog, 2003). An experiment performed in which broiler feed was restricted by 30-40% observed a multiple week delay in the development of ascites in birds from the 4th week of age to around the 7th week of

age, pushing the onset of ascites (if the bird experienced this onset) closer to or behind processing age for the broiler (Gonzales et al. 1998). Feed restriction is a successful treatment and preventative tool as the restriction will limit the growth rate of broilers at young ages which will lessen the metabolic oxygen demand to an achievable level (Hassanzadeh, 2010). Unlike alternative lighting regimens which will allow for compensatory growth so that broilers do not experience a reduction in market weight, feed restriction does not allow for the same compensatory growth and will result in a significant decrease from the body weight of a broiler that was fed *ad libitum* (Hassanzadeh, 2010). Therefore, it is always important to consider the economic feasibility of feed restriction and whether the mortality reduction due to ascites formation is of greater economic value than a potentially lower average body weight amongst the broiler flock (Balog, 2003).

One of the more prominent endogenous factors that plays a role in ascites formation is the insufficiency of the cardiopulmonary system to supply oxygen to the tissues of the broiler to satisfy the metabolic demands of the bird (Decuypere et al., 2000). At sea level, fast growing broilers typically have lower oxygen saturation within their hemoglobin than slow growing counterparts. This indicates that broilers are not efficiently transferring oxygen from their lungs to their tissues, resulting in hypoxic tissue conditions (Julian, 1998). One reason for the insufficiency of the cardiopulmonary system is the nature of bird lungs. Avian lungs account for a smaller percentage of body mass than a typical mammal and are firm and fixed with no ability to expand (Julian, 1998). The avian lung is ideal for flying, but the rigid nature of the avian lung proves incapable of adapting to the metabolic needs of a fast growing broiler chicken. The

primary cause of cardiopulmonary insufficiency; however, relates back to the rapid growth of musculature in the fast growing broiler without parallel growth within the lungs and heart (Havenstein et al., 2004) (Havenstein et al., 2003a) (Havenstein et al., 2003b). Without this parallel growth within the cardiopulmonary vasculature, oxygen supply will not meet metabolic demand in the presence of some external factor (cold temperature, low air oxygen supply, exercise, etc.) and will result in the formation of ascites. An experiment in which fast growing broilers were grown out to 16 weeks of age supports this claim, with 26% of fast growing broilers being fed *ad libitum* dying due to ascites syndrome with another 10.5% of birds exhibiting right ventricular hypertrophy when processed (Julian et al., 1987). Failure of the cardiopulmonary system to properly supply the tissues of the broiler with oxygen is the underlying cause of ascites syndrome with exogenous factors serving as enhancers of the condition.

Another endogenous factor that could be playing a role in ascites formation is muscle fiber type. Most consumers prefer “white meat” as opposed to “dark meat” which is evidenced by the selection trend of broiler chickens having exclusively “white meat” in their breast muscle (Decuypere et al., 2000). The breast muscle of the chicken is solely comprised of type IIb muscle fibers (Decypere et al., 2000). Type II muscle fibers are glycolytic in nature, have a low myoglobin content, low capillary density, and few mitochondria (Guyton and Hall, 2015). Due specifically to the low capillary density, a selection for increased breast meat yield would not result in a proportionate increase in cardiopulmonary mass, leaving the breast muscle particular undersupplied in relation to oxygen demand (Decuypere et al., 2000). For comparison, ducks and

geese have type I muscle fibers within their breast muscle. Type I fibers are oxidative in nature with high myoglobin content, high capillary density, and many mitochondria (Guyton and Hall). While broilers struggle with metabolic diseases such as SDS and ascites syndrome, there are almost no reported cases of SDS or ascites syndrome within geese and ducks (Decuypere et al., 2000). While muscle fiber composition is likely not the sole cause for the prevalence of these cardiopulmonary conditions, enough of a relationship exists that further research is warranted.

Unlike SDS susceptibility which seemingly has low heritability, ascites syndrome susceptibility has been proven to be highly heritable (Moghadam et al., 2005). It is believed that ascites susceptibility manifests in the late stages of incubation without impacting the internal quality of the egg (Tona et al., 2005). While susceptibility manifests during incubation, ascites sensitivity could not be predicted by embryonic evaluation (Tona et al., 2005). Ascites susceptibility is a genomic condition; in fact, a series of cross-breeding experiments were able to determine that two major genes are responsible for the difference between a strain of broilers that were ascites resistant versus a strain of birds that were ascites susceptible (Druyan and Cahaner, 2007). Ascites susceptible birds have key differences from ascites resistant birds: the right ventricle was seen to be hypertrophic in susceptible birds, susceptible birds have significantly lower body weights in comparison to resistant birds after 19 days of age, susceptible birds had significantly higher hematocrit values after 19 days of age in comparison to resistant birds, and mean heart rate was lower in susceptible birds (Druyan et al., 2007a). These aforementioned traits are all highly heritable though none of them serve as a primary indicator of ascites syndrome (Druyan et al., 2007a). These traits serve, rather, as diagnostic traits of ascites

syndrome (Druyan, 2012). Due to the genomic nature of ascites susceptibility, it is entirely possible that susceptibility to ascites syndrome could be bred out of broiler lines (Druyan et al., 2007b). More genomic information is needed in order for effective and efficient selection against ascites syndrome to be implemented into breeding programs (Druyan 2012).

Refeeding Syndrome and Phosphate concentrations

Refeeding syndrome is characterized by electrolyte depletion, fluid retention, and altered glucose homeostasis that occurs when malnourished individuals receive nutrient repletion via oral, enteral, or parenteral means (Marinella, 2005). While deficiencies of several electrolytes can be connected to the refeeding syndrome, no electrolyte deficiency is more closely correlated with refeeding syndrome than hypophosphatemia (Lambers et al., 2015). Other nutrient deficiencies that have been connected to refeeding syndrome include hypomagnesemia, hypokalemia, hyponatremia, hypocalcemia, hypoglycemia, and vitamin deficiencies but only hypophosphatemia has been reported as a consistent symptom (Skipper, 2012). Hypophosphatemia typically occurs within 2 or 3 days of nutrition support although some sources indicate that it occurs between 2 and 5 days of nutrition support (Marinella, 2005) (Abed et al., 2014). In a human, starvation for as little as 48 hours could predispose an individual to refeeding syndrome (Khardori, 2005). If left untreated, refeeding syndrome can result in cardiac arrhythmia, cardiac arrest, hemolytic anemia, delirium, seziures, coma, and sudden death (Garber

et al., 2016). A supplemental phosphate source can be utilized to combat the effects of hypophosphatemia (Marinella, 2005).

The etiology of refeeding syndrome is fairly well known. The precursor of refeeding syndrome is malnutrition; the malnutrition can take many forms including anorexia nervosa, food strikes, inadequate nutrient concentrations, etc. (Rohrer and Dietrich, 2014). Several conditions accompanying malnourishment can serve to enhance the effects of refeeding syndrome. If an individual already has low plasma concentrations of potassium, phosphate, or magnesium prior to nutrient repletion then they are particularly susceptible to the effects of refeeding syndrome (Machado et al., 2009). Children and adolescents are also particularly susceptible to the effects of refeeding syndrome due to reduced nutrient reserves and increased metabolic demands that are indicative of that stage of life (Raj et al., 2012). Malnourishment sets the stage for refeeding syndrome to take place, but it is upon repletion of nutrients where the effects and symptoms can truly be seen.

When an individual is in a starved state, stores of body potassium, magnesium, and phosphate are depleted; at the same time, insulin concentrations decrease which results in the body using fat stores as its primary energy source (Khardori, 2005). It is important to note that potassium, magnesium, and phosphate store depletion refers to an electrolytic shift of these stores from an intracellular environment to an extracellular environment in order to maintain the tight regulation of plasma concentrations required for many different cellular functions (Marinella, 2005). It is because of these shifts that the speed of nutrient repletion and what is being used to replete nutrients becomes vital; when the body is refed carbohydrates and proteins,

insulin concentrations will increase, resulting in a cellular uptake of glucose and the inward movement of phosphate and potassium from an extracellular environment to an intracellular environment (Khardori, 2005). If this sudden decrease in extracellular phosphate concentrations is not accounted for by either controlling the speed of repletion or ensuring that adequate phosphate supplementation occurs upon repletion, hypophosphatemia, which is characteristic of refeeding syndrome, will occur upon nutrient repletion (Lambers, 2015).

Phosphorous (phosphate) is an incredibly important element in the body that serves a variety of functions including maintaining DNA integrity and the formation of ATP, creatine phosphokinase, 2.3 diphosphoglycerate, and glucose-6-phosphate (Khardori, 2005). ATP is a vitally important molecule that is ubiquitous within the body and is responsible for energy used to catalyze many bodily reactions (Sturkie, 1976). Creatine phosphokinase is an enzyme used to catalyze the conversion of creatine to phosphocreatine which is a high energy molecule stored in muscle and the brain (Guyton and Hall, 2015). Finally, 2.3 diphosphoglycerate and glucose-6-phosphate are both important components of the glycolytic pathway (Nelson and Cox, 2012). Furthermore, 2.3 diphosphoglycerate plays an important role in oxygen loading and unloading from hemoglobin; an increase in the amount of 2.3 diphosphoglycerate will cause a right shift in the hemoglobin saturation curve and favor oxygen unloading while a decrease would cause a left shift in the curve and favor oxygen loading onto hemoglobin in erythrocytes (Guyton and Hall, 2015). Given the wide variety of important bodily functions it is involved in, phosphorus is commonly used as an indicator of metabolic well-being, especially within avian species (Alonso-

Alvarez and Ferrer, 2001) (de le Court et al., 1995). ATP as well as 2.3 diphosphoglycerate will play important roles in the pathology of refeeding syndrome.

Hypophosphatemia is the most highly associated electrolyte deficiency that occurs at the onset of refeeding syndrome. Typically, human plasma phosphate concentrations are tightly regulated and range from 2.5-4.5 mg/dL (Marinella, 2005). In broiler hybrids, plasma phosphate concentrations are reported to occur in the range of 7.47mg/dL while brown leghorn's phosphate plasma concentrations occurred in the range of 8.34mg/dL (Ross et al., 1976) (Ross et al., 1978). Hypophosphatemia occurs in stages, ranging from mild which indicates a very slight derivation from normal phosphate ranges to severe which indicates large derivations from normal phosphate ranges and can be life threatening (Marinella, 2005). Generally, mild hypophosphatemia does not present any clinical symptoms, while moderate to severe hypophosphatemia can lead to arrhythmias, seizures, cardiac failure, respiratory failure, coma, and sudden death (O'Connor, 2013). These aforementioned problems arise due to phosphate depletion. The primary casualty of phosphate depletion is the synthesis of new ATP as well as the synthesis of 2.3 diphosphoglycerate (Korbonits et al., 2007). The lack of new ATP results in an overall lack of energy throughout the body and causes a suppression of ATP-powered pump activity such as sodium-potassium ATPases and calcium ATPases, etc. (Marinella, 2005). The suppression of these and other ATP-powered pumps can have critical effects on the heart, immune cells, and skeletal muscle (Marinella, 2005). A decrease in 2.3 diphosphoglycerate results in a left shift of the hemoglobin saturation curve, resulting in unfavorable conditions for oxygen unloading at the tissues; this left shift can result in dyspnea or fatigue depending on

where the decrease in 2.3 diphosphoglycerate occurs (Fiaccordori et al., 1990). When it begins to present clinically, hypophosphatemia is a potentially fatal electrolyte deficiency with the potential to impact all bodily systems.

While hypophosphatemia is the primary nutrient deficiency associated with refeeding syndrome, many other nutrient deficiencies can occur. In a clinical study in which 27 patients with symptoms of refeeding syndrome were examined, 70% of individuals experienced at least one other nutrient deficiency in addition to hypophosphatemia (Skipper, 2012). In this same experiment, 51% of patients exhibited hypomagnesmia, 46% exhibited hypokalemia, 27% experienced hypocalcemia, and 8% experienced hyponatremia (Skipper, 2012). Hypoglycemia has also been shown to be a potential deficiency associated with starvation; however, it is seen as a late stage symptom that occurs when gluconeogenesis from fat stores is no longer a possibility (Shimizu, 2014). The hypoglycemic state is typically more indicative of someone who could be at risk for refeeding syndrome upon nutrient repletion rather than a symptom of the refeeding syndrome as it has been documented that plasma glucose concentrations remain fairly constant during refeeding syndrome (Korbonits et al., 2007). Indeed it is hypokalemia and hypomagnesmia that are seen as the secondary nutrient deficiencies to hypophosphatemia within the refeeding syndrome, although the association of either secondary deficiency with refeeding syndrome is loose. In a study in which 63 patients were examined for nutrient deficiencies associated with symptoms of refeeding syndrome, only hypophosphatemia was seen to have a statistically significant association with the presentation of refeeding syndrome (Skipper, 2012).

The refeeding syndrome, when it progresses to severe stages which lead to arrhythmias, cardiac arrest, and sudden death, presents in a similar manner to SDS. As previously discussed, calcium ATPases and sodium-potassium ATPases are imperative to proper myocardial relaxation (Guyton and Hall). In the hypophosphatemic state, all ATPase activity is depressed, causing calcium ATPases and sodium-potassium ATPases to work slower which slows the sequestration of calcium into the SR and the removal of excess calcium into extracellular spaces via sodium co-transporters respectively (Korbonits et al., 2007). When this process is slowed the phenomenon of re-entry can occur which causes PVC and potentially ventricular tachycardia (Guyton and Hall, 2015). Electrocardiographic evidence taken from individuals experiencing refeeding syndrome symptoms shows a prolonged Q-T interval which is indicative of an elongated myocardial relaxation period (Abed, 2014). If proactive treatment is not taken, ventricular arrhythmias can devolve into ventricular fibrillation and result in acute heart failure and sudden death which is commonly associated with severe refeeding syndrome (Garber et al., 2007). While the end result of refeeding syndrome is similar of that to SDS, it is important to note a key difference: SDS is brought about by an intrinsic defect due to the disparate growth rates of cardiopulmonary vasculature and muscle which leads to hypoxic conditions in the birds' tissues while refeeding syndrome is brought about by a nutrient deficiency, specifically hypophosphatemia, upon being refed after a period of starvation. This key difference will be important in attempting to distinguish between SDS and sudden death experienced by breeders.

Potassium and the Refeeding Syndrome

Hypophosphatemia is the most commonly associated electrolyte deficiency associated with the refeeding syndrome, occurring in almost 100% of cases. While not as closely associated, another electrolyte deficiency, hypokalemia, has a strong enough association with the refeeding syndrome that it merits closer investigation (Skipper, 2012) (Korbonits, 2007). Potassium concentrations are subject to variation during refeeding syndrome presentation but does not follow a predictable pattern (Korbonits, 2007). Potassium is one of the most important electrolytes within the body; similarly to phosphates and other electrolytes, potassium concentrations are tightly regulated within the body, with human ranges occurring between 3.5mmol/L and 5.0mmol/L (Alfonzo et al., 2006). In chickens, potassium concentrations are maintained at higher concentrations. In brown leghorns, potassium concentrations were recorded at 7.53 mEq/L (Ross et al., 1978). In broiler hybrids, potassium concentrations were recorded at 9.08 mEq/L (Ross et al., 1976). Finally, in a breeder flock, plasma potassium concentrations were evaluated between 10 and 60 weeks of age, with potassium concentrations remaining roughly around 5 mmol/L until the 20^{th} week in which concentrations began to fall until they reached their lowest average concentration of around 4.1 mmol/L at week 26 coinciding with the initial point of lay/5% hen lay day (Hopkinson et al., 1990). Due to potassium's tight regulation and overall importance in metabolic function, it is a common index of metabolic and physiological well-being (Ferrerand Dobado-Berrios, 1998) (Hollmén et al., 2001) (de le Court et al., 1995).

One of the most important functions potassium serves within the body is serving as the electrolyte used for membrane repolarization (Guyton and Hall, 2015). Potassium is able to function as a repolarizing electrolyte due to intracellular potassium concentrations being far greater than extracellular potassium concentrations. This potassium gradient contributes to the excitability of nerve, muscle, and cardiac membranes (Alfonzo et al., 2006). Most excitable membrane action potentials proceed as follows: 1. Ligand gated sodium channels activated by acetylcholine; 2. Threshold potential is reached and voltage gated sodium channels are activated allowing for an influx of sodium causing depolarization; 3. Potassium channels are opened and an efflux of potassium occurs causing membrane repolarization; 4. Potassium and sodium channels are opened while Sodium-Potassium ATPases pump sodium extracellularly and potassium intracellularly against their respective concentration gradients in order to maintain homeostatic balance (Guyton and Hall, 2015). This is the process for nervous and muscular depolarization and repolarization, however; cardiac muscle differs from typical depolarization and repolarization (Faggioni and Knollmann, 2015). As noted previously, cardiac action potentials are much longer than muscular or nervous action potentials due to the action of slow opening calcium channels. Potassium remains important in the repolarization of the heart through the action of the inward rectifiers and delayed rectifiers (Goodwin and Wit, 2012). When plasma potassium concentrations are outside of normal ranges, the ability of the body to repolarize excitable membranes is altered with potentially damaging consequences as a result (Alfonzo et al., 2006).

Hypokalemia is a condition in which plasma potassium concentrations are lower than the accepted normal range of concentrations. The condition, like many other electrolyte imbalances, is varied in its severity, with mild conditions presenting as asymptomatic or with very mild symptoms such as weakness or muscle fatigue while severe cases present with muscle cramps, pain, respiratory arrest, and cardiac arrhythmias including ventricular tachycardia, ventricular fibrillation, and cardiac arrest (Dongilli et al., 2016) (Alfonzo et al., 2006). While tending to be asymptomatic, even mild potassium depletion can result in cardiac impairment; dogs with potassium depletion suffer a reduced maximal rate of filling in response to volume expansions within the heart while humans have a decrease in overall blood flow associated with lower potassium concentrations (Laragh and Sealey, 2001). Other issues that can arise from more severe cases of hypokalemia include lassitude, constipation, muscle necrosis, and impairment of respiratory function (Gennari, 1998). Hypokalemia is common in individuals who are ill or have a fever, who are malnourished via an eating-disorder or a lack of potassium within the diet, and within individuals who have specific diseases such as AIDS that impact potassium regulation (Unwin, 2011). Hypokalemia is typically treated via potassium supplementation, with supplements being used including potassium canrenoate, potassium chloride, potassium phosphate, and potassium bicarbonate (Gennari, 1998) (Dongilli et al., 2016). Cardiopulmonary weakening is the one of the most dangerous symptoms of hypokalemia and it is central to the refeeding syndrome pathogenesis making hypokalemia a potentially deadly secondary condition associated with refeeding syndrome (Abed et al., 2014).

Hypokalemia is associated with several ECG abnormalities. These include U waves which can indicate a delayed repolarization of the Purkinje fibers and are inversely proportional to heart rate, T wave flattening which indicates a prolonged ventricular repolarization period, and/or ST segment changes which represent changes between the duration of ventricular depolarization and repolarization (Alfonzo et al., 2006). These derivations from normal rhythmicity which are attributed to prolonged ventricular repolarization, slow conduction, and abnormal pacemaker activity lead to the arrhythmias mentioned above, including ventricular tachycardia, ventricular fibrillation, long QT syndrome, and cardiac arrest (Osadchii, 2010). Prolongation of ventricular repolarization leads to cardiac arrhythmias due to the phenomenon of re-entry which was discussed earlier. This prolongation of ventricular repolarization due to hypokalemia is caused by an inhibition of outward potassium currents. (Osadchii, 2010). This results in a reduction of "repolarization reserve" due to a reduction of extracellular potassium (Faggioni and Knollmann, 2015). This inhibition of the outward potassium channels impacts the rapid components of the delayed and inward rectifying channels causing ventricular action potentials to take longer (Faggioni and Knollmann, 2015). At the same time sodium-potassium ATPases are inhibited which further decrease the repolarization reserve and leads to an increase in intracellular sodium; the increase in intracellular sodium prevents calcium removal via the sodium-calcium exchanger causing intracellular calcium overload and an increase in delayed afterdepolarizations (Faggioni and Knollman, 2015). Slowed conduction speed across the myocardium is attributed to hyperpolarization of the membrane as well as an increase in the excitation threshold (Osadchii, 2010). Finally, abnormal pacemaker activity occurs due to

calcium overload described previously (Osadchii, 2010). All of the aforementioned factors are responsible for creating the conditions that make individuals susceptible to cardioarrhythmias in the hypokalemic state.

While hypokalemia is the predominant potassium imbalance associated with refeeding syndrome, hyperkalemia could have a role in the syndrome as well. In one study that evaluated a patient that was being refed after a period of starvation, potassium supplementation resulted in potassium toxicity during replacement (Vemula et al., 2015). While the patient being monitored was still in a hypokalemic state (human with potassium concentration of 2.9 mEq/L), the patient's ECG showed no R or P wave which is indicative of hyperkalemia as opposed to hypokalemia (Vemula et al., 2015). Hyperkalemia typically does not present with any symptoms until cardiac arrhythmias occur (Dunn et al., 2015). Typical ECG findings associated with hyperkalemia are as follows: a widened QRS indicative of a weaker than normal ventricular contraction, tall T waves, flattened or absent p waves indicative of reduced to absent atrial contraction, and a prolonged PR interval which is indicative of an increase in duration between the beginning of atrial contraction and the end of ventricular contraction (Alfonzo et al., 2006). It follows that bradycardia is the most common arrhythmia associated with hyperkalemia and that, if left untreated or if the case is severe enough, the arrhythmia can devolve into ventricular fibrillation (Alfonzo et al., 2006). Risk for hyperkalemia increases in individuals who are older or have chronic heart failure, chronic kidney disease, and/or diabetes (Dunn et al., 2015). Acute hyperkalemia can be treated with beta-2 agonists, insulin-glucose, calcium gluconate, sodium

bicarbonate, or loop diuretics while chronic hyperkalemia is treated with low potassium diets (Dunn et al., 2015).

During a refeeding state, the potential for potassium imbalance is significant. Potassium supplementation, except on the rare occasion that an individual presents hyperkalemic symptoms due to the development of potassium toxicity, is a common practice and one that proves effective in correcting against potassium deficiencies (Gennari, 1998). Potassium chloride is a very common supplement and has been shown to have positive effects on reducing heart arrhythmias with the exception of atrial flutter and atrial fibrillation regardless of the presence of hypokalemia (Bettinger et al., 1956). Similarly, a separate study showed that when potassium infusion was given to a patient, ventricular rate, ventricular ectopic beats, and atrio-ventricular conduction were all reduced in some manner (Fisch et al., 1958). An increase in dietary potassium has been shown to lower blood pressure, suppress sympathetic activity, suppress the renin-angiotensin system, and prevent development of vascular injury such as a stroke or sudden death (Packer, 1990). Potassium supplementation is the easiest and best course of action when correcting for hypokalemia, however; potassium supplement administration should be gradual, like all electrolytes being restored to a malnourished individual, in order to avoid refeeding syndrome and, in the case of potassium, the potential for potassium toxicity.

General Breeder Management

Breeder management is, by its very nature, a counterintuitive process. Broiler chickens have been heavily selected for high rates of body mass growth and low feed conversion ratios (FCR) over the last 40 years (Ricklefs, 1985). In fact, genetic selection of broilers has halved the time required for a single broiler to reach 2kg in weight (Gavin and McDevitt, 1999). While high growth and low FCR are optimal for achieving maximal profit from each bird, these same factors work to directly inhibit the reproductive viability of breeder strains (Leeson and Summers, 2000). Due to the paradoxical relationship of maintaining high growth rates, low FCR, and high reproductive viability, managing broiler breeder flocks is an intensive and strictly regulated process.

The first stage of a breeder's life is the brooding phase, which occurs from day of hatch until 7 to 10 days of age (Aviagen Breeder Management Handbook, 2013). During this stage of life, chicks are given feed and water *ad libitum*, temperature is gradually reduced from 90°F to 80°F, relative humidity should be maintained between 60-70%, and lighting is gradually reduced from 23 hours of daylength at day of hatch to 8 hours of daylength at 10 days of age (Aviagen Breeder Management Handbook, 2013). The management goal for this time period is to successfully transition the chicks from egg to pen in such a manner as to reduce potential stressors to the bird. The most important developmental goal for the chick in this time period is to ensure that proper appetite development occurs (Aviagen Breeder Management Handbook, 2013). Improper management techniques could severely impact the chick's ability to grow in a

uniform manner and ultimately result in a significant decrease of peak fertility (North and Bell, 1990).

From 7 days of age until approximately 168 days of age, breeders will be in the growing phase of its lifecycle (Aviagen Breeder Management Handbook, 2013). During this phase of the breeder's life, lighting will be maintained at an 8 hour daylength until 147 days of age, where photoperiods will be gradually increased to 15 hours to photostimulate egg production in breeder hens (North and Bell, 1990). Temperature and humidity are to be maintained around room temperature (75°F) and approximately 40-50% relative humidity respectively (Aviagen Breeder Management Handbook, 2013). The management component under the most intense regulation is content and quantity of feed. Crude protein content, available phosphorous, methionine, methionine + cysteine, lysine, tryptophan, choline, iron, and copper content all appear in reduced amounts in the grower diet when compared to the brooder diet (Leeson and Summers, 2000). In addition to the reduction of these elements within the pullet diet, feed restriction is implemented to help maintain flock body weight uniformity, delay egg production, produce larger first eggs, increase egg production, reduce laying house mortality, reduce feed cost of growing a pullet, reduce feed cost of producing a dozen hatching eggs, increase fertility of hatching eggs, and increase hatchability of hatching eggs (North and Bell, 1990). These practices are maintained until the flock begins to reach the initial point of lay around 24-26 weeks of age (Aviagen Breeder Management Handbook, 2013).

Starting at approximately 18 weeks of age, birds enter what is known is the pre-breeder phase. During this stage, several changes are made to prepare birds for egg production (North,

and Bell, 1990). The first change that is made is transitioning birds from a rearing facility to a laying facility. This change occurs between 18 and 23 weeks of age. Males are typically moved into the facilities first so they can find feed and water (Aviagen Breeder Management Handbook, 2013). Typically, the transition to the laying house is performed at approximately 21 weeks of age so that it coincides with the mixing of sexes. Otherwise termed "mating up," sex mixing occurs at 21 weeks of age to ensure that all birds within the flock have reached sexual maturity (Leeson and Summers, 2000). This sex mixing is a gradual process; the most sexually mature males are introduced to the flock at 21 weeks of age while the next group of males will be introduced a week later and so on until the desired mating ratio (usually around 10%) is reached (Aviagen Breeder Management Handbook, 2013). Lighting is also changed during this time. Starting at 21 weeks of age, the photoperiod is increased from 8 hours of light to 11 hours of light (Aviagen Breeder Management Handbook, 2013). Light is then increased to a 12 hour photoperiod at week 22 and then to a 13 hour photoperiod at week 23 where lighting is maintained until depletion (Aviagen Breeder Management Handbook, 2013). Finally, feeding equipment is modified so that male and female feed remains separate as the nutrition requirements of each sex are not equal (North and Bell, 1990).

During weeks 24-26 of age, hens of a breeder flock should reach 5% hen-day production meaning that the ratio of number of eggs laid to number of hens in the house is equal to 0.05 or 5% (North and Bell, 1990). At this point, feed restriction is decreased to a 25-50% restriction and feed nutrition is modified to support egg production (Grandin and Deesing, 2013). Feed will continue to be incrementally increased based on the hen-day egg production until peak

production is achieved, typically between 80-90% hen-day production (Aviagen Breeder Management Handbook, 2013). At this point, 2 factors are used to assess proper feed totals and the nutritive intake of the flock: daily egg weight and feed clean-up trends (Aviagen Breeder Management Handbook, 2013). Feed clean-up trends deal with how quickly it takes a flock to consume its daily feed allotment. If feed clean-up times are longer than what is considered standard, birds may be overfed while shorter clean-up times might indicate an underfeeding of birds; both overfeeding and underfeeding birds can have significant short-term and long-term impacts on hen-day production (Aviagen Breeder Management Handbook, 2013). Daily egg weights are similarly impacted by underfeeding; underfed birds will produce lighter than normal eggs (Leeson and Summers, 2000). Overfeeding of birds will not significantly affect daily egg weight although total hen-day production will suffer if birds are overfed (North and Bell, 1990).

Peak production in broiler breeders is typically achieved at 31 weeks of age (Aviagen Breeder Management Handbook, 2013). After the window of peak production passes, several steps are taken to help maintain the flock until depletion. When the peak of production passes, the number of males per females is reduced gradually from 9.5-10 to 7-9 males per 100 females (Aviagen Breeder Management Handbook, 2013). After the peak production window passes, less males are needed in order to maintain diminishing flock production so non-working/less viable males can be removed from the breeder flock (Leeson and Summers, 2000). Accompanying the depopulation of males from the breeder flock is a decrease in feed allotments to the breeder flock. As the birds age, they are more likely to turn feed into fat deposits which can significantly impact hen-day production (North and Bell, 1990). Gradual decreasing feed allotments from

peak production to depletion aids in maintaining lean birds and optimal hen-day production (Aviagen Breeder Management Handbook, 2013). It is important to monitor the effects of feed reduction in the aging broiler breeder flock. Monitoring body weight and egg weights can inform when the next feed reduction should occur and whether or not a feed reduction may have occurred too soon depending on the trends of the daily egg weight and overall body weight of the breeder (Aviagen Breeder Management Handbook, 2013). Male breeders, unlike females, will continue to get small feed increases throughout the life of the flock (Aviagen Breeder Management Handbook, 2013). These management practices are followed until the hen-day production of the flock is no longer financially viable at which time the flock is depleted.

Feed Restriction in Breeders

Feed restriction is most commonly accomplished via physical reduction in the amount of feed that is administered to each bird (Leeson and Summers, 2000). During the grower phase, feed is restricted between 60-80% of normal feed consumption (Grandin and Deesing, 2013). One study found that when breeders are fed *ad libitum*, the birds are on average 700g heavier, laid an average of 40 fewer eggs, and were 10% fatter than were breeders maintained on restricted feed diets (Robinson et al., 1991). Feed Restriction is also accompanied by potential animal welfare concerns. One study showed that feed restriction leads to behavior stereotypes in breeder hens (Grandin and Deesing, 2013). Another study described these stereotypes; describing breeders by noting they spent a large portion of time scratching and pecking litter and

that breeders had lower plasma viscosity while also showing higher evidence for physiological stress between 8 and 16 weeks of age when feed is typically most restricted (Hocking et al., 1993). While it is widely accepted that feed restriction produces breeders with the highest potential fertility, the method of feed restriction is less agreed upon. The two main strategies for physical feed restriction are every day feed restriction and skip-a-day feed restriction (North and Bell, 1990). In every day restricted feeding, birds are given a predetermined, small amount of food every day while birds on a skip-a-day regimen are given approximately double the amount of feed as every day feeders but are only fed every other day. Both methods have positives and negatives that are associated with their use.

The skip-a-day feeding regimen is a very popular method of feed restriction. The primary benefit of this method is that by delivering double the feed on feed days, more feed is available so that all birds may have an opportunity to eat (North and Bell, 1990). While this access seems ideal, breeder gorging becomes a prevalent issue when skip-a-day feeding is utilized (North and Bell, 1990). When birds, breeders or broilers, are deprived of feed during a fasting period, these birds will tend to consume greater amounts of feed during a refeeding period (Chamblee et al., 1989). Another issue with skip-a-day feeding centers around water consumption. Specifically on non-feed days, birds tend to consume excess amounts of water in an attempt to satiate hunger (Chamblee et al., 1989). Excess water consumption can result in loose droppings and wet litter which can lead to disease (North and Bell, 1990). While these problems remain ever present, easier access to feed for breeders results in high use of the skip-a-day feeding regimen.

A common alternative to the skip-a-day feeding regimen is a feeding regimen in which birds are fed restricted amounts of feed every day, also known as every day restricted feeding. With every day restricted feeding, many of the common problems that arise with the use of skip-a-day feeding, including excess water consumption and feed gorging, are not present; however, other problems arise which lead to non-uniform growth (North and Bell, 1990). The primary problem with this alternative is ready access to feed (Leeson and Summers, 2000). Especially early in the feeding regimen, birds on an everyday restricted feeding schedule can be fed as little as 24 g of feed per bird, resulting in a very small amount of food being provided to a breeder flock on a given day (Aviagen Breeder Management Handbook, 2013). For larger commercial flocks where an automated feeder is utilized, feed dominant birds can consume feed quickly enough and in large enough quantities that non-feed dominant birds may not have feed left to consume (Leeson and Summers, 2000). Theoretically, an everyday restricted feeding regimen would serve to provide greater uniformity and cause fewer potential issues than a skip-a-day feeding regimen; however, depending on methods of feed distribution within the breeder house, an everyday restricted feeding regimen may be less practical than a skip-a-day feeding regimen (North and Bell, 1990).

Metabolic Diseases in Broiler Breeders

Mortality in broiler breeders, especially when compared to broilers, is low. Heavy breed birds, such as broilers, have a reduced ability to deal with overfeeding; overfeeding in these

heavy breed birds causes these birds to approach their genetic potential for weight gain which stresses the bird and causes an alteration to linear growth and the potential for disease (Nir et al., 1978). Breeders are still susceptible to two major sources of mortality in their life: during the brooding phase and during the point of initial lay (North and Bell, 1990). The source of brooding phase mortality is due to the fragility of the newly hatched chick. Young chicks cannot produce their own body heat and will sometimes struggle to find food and water sources within their brooding pen (Aviagen Breeder Management Handbook, 2013). Any failure to provide optimal feed, water, temperature, lighting, humidity, or ventilation can cause an increase in breeder flock mortality.

A main source of breeder flock mortality in an otherwise healthy flock occurs between 24-26 weeks of age at the initial point of lay for the breeder hens. At this stage, breeder hens are susceptible to death via sudden death in which healthy looking birds are found lying on their backs dead (Aviagen Breeder Management Handbook, 2013). It is important to note that the sudden death experienced by breeder flocks is distinct from SDS experienced in broiler flocks; sudden death in breeder flocks is more commonly associated with a nutrient deficiency while sudden death in broiler flocks can be attributed to a number of things including hypokalemia, stress, exercise, electrolyte imbalance, hypoxemia, cardiomyopathy, or any combination of these factors that lead to premature ventricular contractions, ventricular fibrillations, and ultimately death (Olkowski and Classen, 1998) (Leeson and Summers, 2000). While the pathology of sudden death in broilers and sudden death in breeders is similar, the etiology behind the two are

different. Discovering the pathogenesis behind breeder sudden death is key to determining a management technique for limiting sudden death breeders.

During post-mortem examinations, breeders exhibit a large flaccid heart, congested lungs, and congested pericardium (Aviagen Breeder Management Handbook, 2013). Sudden death has been a major source of mortality for breeder flocks (Hopkinson et al., 1990) (Hopkinson et al., 1984) (Hopkinson, 1991) (Pass, 1983) and represents a significant loss of egg production and resources. Mortality percentage of a breeder flock at the point of lay is variable, although rates as high as 30% have been previously recorded (Hopkinson et al., 1984). Typically, in flocks that are susceptible to sudden death, mortality rates occur between 0.5-2% per week from 5% hen-day production to 20-30% hen-day production (Leeson and Summers, 2000). In examining sudden death in breeders, deficiencies in plasma phosphate/phosphorous concentrations and plasma potassium concentrations were observed in birds that had succumb to sudden death (Hopkinson et al., 1990). A common result of both the hypophosphatemic and hypokalemic states are tachycardia and arrhythmias leading to potential ventricular fibrillation and death (Gennari, 1998) (Garber et al., 2016). Not only has the heart rate of feed restricted birds been shown to be higher than that of *ad libitum* fed birds, but post-mortem examinations of breeders that had died due to sudden death related complications were, in addition to the previously listed symptoms, shown to have died acutely and with no obvious lesions. The lack of lesions is indicative of a death due to heart failure via tachycardia/heart arrhythmia as opposed to ascites syndrome or some other metabolic disorder (Olkowski and Classen, 1998) (Pass, 1983). Finally, in a study where sudden death was induced via changes in diet composition, the role of

potassium and phosphorous was slightly elucidated. It was found that breeders on diets producing 30% flock mortality due to sudden death had significantly lower plasma potassium concentrations than when compared to control flocks (Hopkinson, 1991). In addition, flocks on diets producing 30% mortality had lower available phosphorous in the diet in comparison to a diet with similar available potassium concentrations that caused a mortality of 5% in separate flocks (Hopkinson, 1991). Supplementation of potassium and/or phosphorous can help reduce mortality within a breeder flock that is susceptible to sudden death (Aviagen Breeder Management Handbook, 2013).

Manuscript 1

Abstract

The objectives of this study were to verify the use of a glucometer for chicken blood glucose analysis as well as to verify the comb as a site for obtaining serial blood samples from chickens. Longitudinal sampling of blood borne substances at short sampling intervals in poultry is complicated by the difficulty in obtaining serial blood samples in a way that is safe and expeditious. Drawing blood via the venipuncture method from the wing (Alar) vein is complicated by the formation of a hematoma over the puncture site. Glucometers of the type used for routine monitoring of human blood glucose require as little as 0.3µl of sample for analysis and allows sampling by pricking the skin to obtain capillary blood. The comb, which is bare of feathers and highly vascularized, would appear to be an ideal site for obtaining serial blood. Twenty leghorn hens were sampled utilizing an Agamatrix Wavesense Presto glucometer to analyze glucose concentration values of whole blood from the wing vein and from the comb in hens as well as concentration values of plasma from the wing vein. An O-Toluidine assay was utilized as a standard bench assay for validating glucose values obtained with the glucometer. A one-way ANOVA procedure was utilized for data analysis. Glucose concentration means (mg/dL) of 269.33, 280.32, 179.03, and 189.43 were obtained for the bench assay (BA), glucometer assay of blood plasma via the wing vein, glucometer assay of whole blood via the

comb (GC), and glucometer assay of whole blood via the wing vein (GV) respectively. Blood glucose concentrations analyzed from plasma, either using the glucometer or the bench assay, showed insignificant differences ($P > .05$) between means. Likewise, whole blood analyzed from the wing vein and the comb using the glucometer showed insignificant differences between means. However, significant differences ($P < .05$) were observed between blood glucose concentrations in blood plasma analyzed treatments versus whole blood analyzed treatments. This study shows that glucometers are suitable tools for tracking blood glucose concentration changes over time while the measurement of absolute values of blood glucose would require an adjustment factor to match any bench assay results.

Introduction

Within poultry research, there lies a problem concerning the serial sampling of blood borne substances such as blood glucose. Current blood sampling methods, not involving chronic cannulation of veins, are not amenable to taking serial blood samples over short time intervals in a manner that is both safe for the bird and convenient for the researcher. The most common method of obtaining blood samples from chickens and other birds is the venipuncture method in which a needle and syringe are used to withdraw blood from the wing vein (alar/brachial vein) of the bird. This technique is the safest, quickest, and most convenient technique for both chicken and researcher. However, this method is not suitable for obtaining serial blood samples over time intervals shorter than about one week. The problem with alar vein venipuncture is that upon

removal of the needle from the wing vein, a hematoma will form around the venipuncture site and under the skin. This obscures the wing vein, preventing further sampling from this site for approximately a week (Kelly and Alworth, 2013). Another method used for blood sampling is known as the cardiac puncture method. In this procedure, a needle is inserted into the heart of an anesthetized bird and blood is directly withdrawn. This procedure is rarely used in the modern setting due to the risk factors associated with the procedure including elevated stress concentrations within the bird, the risk of hemorrhage within the pericardial sac which can result in death, and other risk factors associated with anesthetization (Johnson, 1981). In addition to the risk factors, the cardiac puncture method has also been associated with detrimental effects on the body weight of broilers and leghorn pullets (Buckland et al., 1973). The final procedure used for obtaining serial blood samples is the use of an indwelling cannula. In this procedure, a cannula is inserted into a vein of the bird, usually located in the neck or the wing, and maintained so that blood can be withdrawn at any time. This is the best available procedure for obtaining serial samples over short time intervals but the procedure is complicated by the inherent difficulty in maintaining the cannula in a non-restrained animal. Furthermore, when the cannula is removed from the vein, the vein tends to collapse preventing further sampling from the same site in the bird (Nordstrom and Smith, 1969). With the three most common blood sampling methods having inherent drawbacks, a new method is needed for repeated blood sampling over short time intervals.

A potential solution to the aforementioned problem as related to serially measuring blood glucose is the handheld glucometer of the type used for at-home blood glucose monitoring in

humans. Glucometers are inexpensive, easy to use, and require as little as 0.3μL of blood which would allow for the use of a skin prick as opposed to drawing multiple mL of blood for analysis. This is a solution that has been adopted for use in research studies of common laboratory mammals. Handheld glucometers have been used to monitor blood glucose values in mammalian species such as rats (Weitgasser et al., 1999) and dogs (Cohen et al., 2009), however; relatively few studies have been conducted with avian species (Lieske et al., 2002). The lack of work with avian species necessitates that the glucometer be validated as a tool for monitoring blood glucose within chickens before proper research can be conducted using this tool.

With regards to time course studies of blood glucose in avians, the primary advantage of using glucometers as a tool for analyzing blood glucose concentrations is the lack of blood volume that is required for analysis. The skin prick is the commonly used method for obtaining blood for glucose analysis in humans (Tamada et al., 2002). When examining chickens, there are very few possibilities for a skin prick site due to the nearly complete covering of the body with feathers and the relatively low level of vascularization of chicken skin. One site that is bare of feathers and is also highly vascularized is the comb, an essential thermoregulatory body part of the chicken. For chickens, the lack of feathers on the comb, in combination with the relatively high level of vascularity, would seem to make the comb an ideal location to obtain serial blood samples for glucose analysis via a handheld glucometer.

Due to the nature of circulation within a single animal, there lies the potential for venous blood glucose concentrations to differentiate from capillary blood glucose concentrations (Kempe et al., 2009). Depending on the metabolic needs of the bird, glucose could be taken up

from the bloodstream or released into the bloodstream at the capillaries which could alter the concentrations of blood glucose downstream in the veins in relation to the capillary beds. Furthermore, it should be noted that, within chickens, reported literature values of blood glucose concentrations range greatly from as little as 176 mg/dL (Simon and Leclercq, 1982) to as high as 263 mg/dL (Belo et al., 1976) depending on the sampling site and the assay employed by the researcher. All of these variables establish the importance of validating the glucometer as a tool to measure blood glucose concentrations in multiple ways: comb blood glucose concentrations versus venous blood glucose concentrations, whole blood glucose concentrations versus plasma glucose concentrations, and the glucometer readings versus reading from an accepted and widely used bench assay.

The two main objectives of this study are as follows: 1) validate the use of a handheld glucometer as a tool for analysis of chicken blood glucose concentrations and 2) evaluate the comb as a site for obtaining serial blood samples. Four treatment groups were used in this experiment and are coded as follows: GC = Glucometer analyzed comb whole blood, GV = Glucometer analyzed wing vein whole blood, GP = Glucometer analyzed wing vein blood plasma, and BA = Glucose Oxidase bench assay. The Agamatrix Wavesense Presto (Agamatrix Inc., Salem, NH) glucometer was utilized in accordance with the work of Zhao et al. (2014). The bench assay employed to validate the glucometer results (Eton Bioscience, San Diego, CA*)* utilizes the same glucose oxidase chemistry that the Agamatrix Wavesense Presto glucometer employs.

Materials and Methods

Sample Collection

Blood samples were collected from 20 Single-Comb White Leghorn hens. Sampled hens were sexually mature and were fed *ad libitum*. Hen selection was not predetermined and each bird was chosen randomly from a larger population. For the first 10 hens, an 18 ga needle was used to prick the comb and the blood droplet was analyzed immediately via glucometer. A venipuncture was then performed on the wing vein of the same hen utilizing a 25 ga needle (coated in saturated EDTA) and syringe to extract 3 mL of whole blood. Blood samples were stored on ice until analysis. When the needle was removed from the venipuncture sight, a glucometer test strip was applied to the puncture site to collect a sample for glucometer analysis. These steps were reversed for the second 10 hens (venipuncture then comb prick) in order to control for a potential glucocorticoid stress response (Ralph et al., 2015). All hens' combs were massaged prior to pricking to allow for maximal blood flow to the capillary bed.

Upon return to the lab, whole blood samples were immediately centrifuged at 800 g_n for 30 min and plasma was extracted utilizing a 1000 µL micropipette. No hemolysis of erythrocytes was observed and there was no apparent contamination of plasma samples. Glucometer was used to assay plasma samples immediately after harvest. Plasma was then stored in a -20 °C freezer for analysis via glucose oxidase assay the following day. Glucose oxidase bench assay was carried out following the manufacturer's instructions for the assay (Eton Bioscience, San Diego,

CA). A Biotrak II microplate reader (GE Healthcare, Chicago, IL) with an absorbance filter of 490 nm was used to generate absorbance readings for each sample and the glucose standard solutions.

Statistical Analysis

Simple linear regression analysis was utilized to obtain a standard curve for the bench assay. This yielded a regression equation into which sample absorbance values were plugged to obtain the predicted blood glucose values. A one way ANOVA was conducted to assess potential differences between the 4 treatment means. Tukey comparison of individual means was conducted to determine where significant differences, if they existed, could be found amongst treatment means. SAS 9.4 (SAS Institute, Cary, NC) was used for statistical analysis.

Results and Discussion

Standard Curve

The standard curve (Fig. 1) produced using the glucose standard solution was statistically significant ($P < .0001$) which suggests that this curve is a good predictive model for determining blood glucose concentrations of the samples from absorbance readings. This conclusion is further validated by the achieved R-squared statistic of .9996. A regression equation of

Blood_Glucose = -31.09 +354.34(Absorbance) was obtained and yielded our predicted blood glucose concentrations for the bench assay.

Comparison of Treatment Means

Upon calculation of the predicted blood glucose concentrations for the bench assay, data from all four treatments were analyzed in SAS. Means and standard deviation values for the four treatments are found in Table 1 while graphical representations are found in Figure 2. Upon conducting the ANOVA, a significant difference between at least one treatment groups ($P < .05$) was yielded. The Tukey comparison of individual means test revealed that the assay treatment and the GP treatment were not significantly different from one another and the GV and GC treatments were not significantly different from one another. There was a significant difference between the GV/GC group of treatments and the BA/GP group of treatments.

Given the above results, there is enough evidence to support the validation of the glucometer as a tool to monitor blood glucose concentrations in a serial manner over short time intervals within chickens. The primary evidence lies in the lack of a significant difference in blood plasma concentrations between the blood plasma tested via the glucometer versus the blood plasma tested via the glucose oxidase bench assay. Furthermore, we can see evidence that the glucometer was more precise than the bench assay with a standard deviation of 39.99 for BA while GC, GV, and GP treatments had standard deviations ranging from 10.00 to 13.50. The precision of the glucometer and the statistically insignificant difference between the GP and BA

treatments would suggest that the glucometer is a precise and accurate tool for assessment of blood plasma concentrations.

An interesting and expected result was the wide significant difference in glucose concentrations between the GP/BA and the GV/GC group of treatments. In previous studies (Cohen et al., 2009, Lieske et al., 2002, Weitgasser et al., 1999), similar differences between plasma glucose analysis and whole blood glucose analysis were found across multiple species including rats, dogs, seabirds, etc. These differences also seemed to be present irrespective of the bench assay chemistry employed. Therefore, a reasonable and likely conclusion to explain the differences in glucose concentrations is the level of interference experienced in whole blood sampling versus plasma sampling. In blood samples that have been centrifuged and plasma extracted, erythrocytes and other formed elements of whole blood are removed as potential interference for glucose analysis. This results in blood glucose being more concentrated in plasma though there is not an increase in total glucose amount. This conclusion is supported by the lack of significant difference between the GP and BA treatments while the GV and GC whole blood treatments differed significantly from our plasma treatments. Were the difference due to something other than the level of interference in whole blood versus plasma such as bench assay chemistry, significant differences would have occurred elsewhere within the study.

The second objective of this study was to validate the comb of the chicken as a site for safe, convenient, and accurate sampling of whole blood. First, it can be concluded that comb is a valid site for sampling due to the lack of difference between the GV and GC treatment groups. The comb was also a much more convenient site of sampling in comparison to the wing vein.

When sampling from the wing vein, there was always a possibility of missing or damaging the vein and clotting of blood, resulting in an insufficient or poor quality sample. Furthermore, the chicken has to be restrained and feathers removed from the venipuncture site causing some discomfort and stress for the chicken. When sampling from the comb, the chicken remains upright and requires less restraint. A simple prick of the comb skin produces ample blood for testing via glucometer and seems to be a safer and more humane practice for the bird. Furthermore, with the comb prick, blood loss is minimal and no visible hematoma forms.

In considering the above results, the glucometer satisfied the two objectives of this study and can be considered a suitable tool for tracking changes in blood glucose concentrations over time in chickens. This will allow researchers to monitor changes in blood glucose, an extremely important physiological parameter more effectively. It must be kept in mind that this validation was conducted with only one make and model of glucometer, the Agamatrix Wavesense Presto glucometer. Before using glucometers from other manufacturers, validation must be employed in a similar manner to the experiment above before use. These tools will increase researchers' abilities to monitor blood glucose and should be used in future studies where serial sampling over short time intervals is required.

Treatment	N	Blood Glucose (mg/dL)	
		Mean	Std Dev
ASSAY	60	269.33	39.99
GC	60	179.03	10.18
GP	60	280.32	13.30
GV	60	189.43	12.87

Table 1. Depiction of Means and Standard Deviations for the four treatments

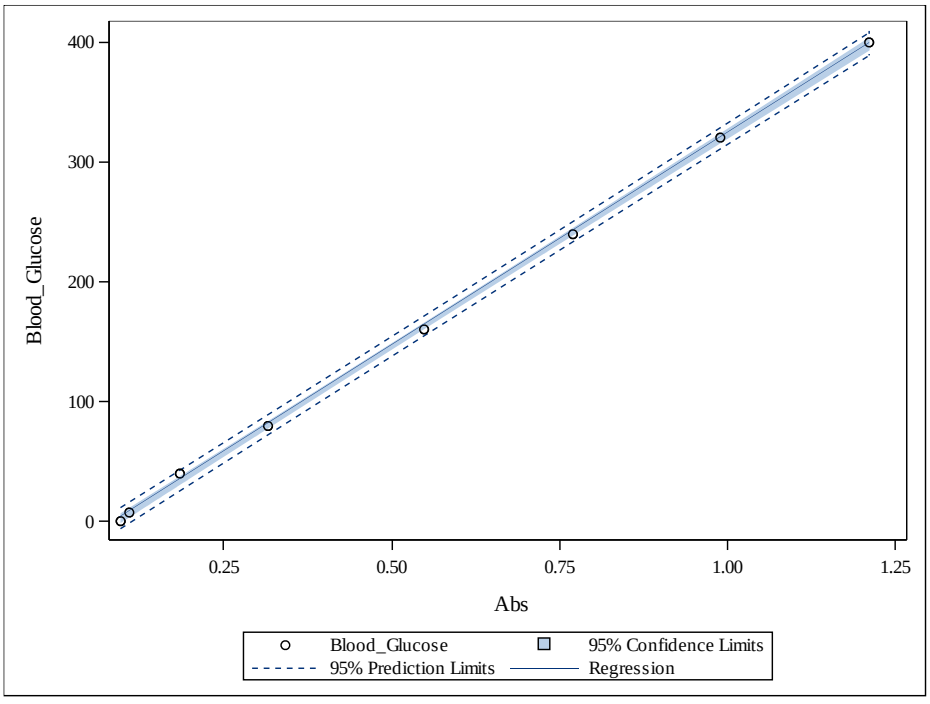

Fig. 1. Standard Curve of Glucose Oxidase Bench Assay. Blood glucose concentrations in μM.

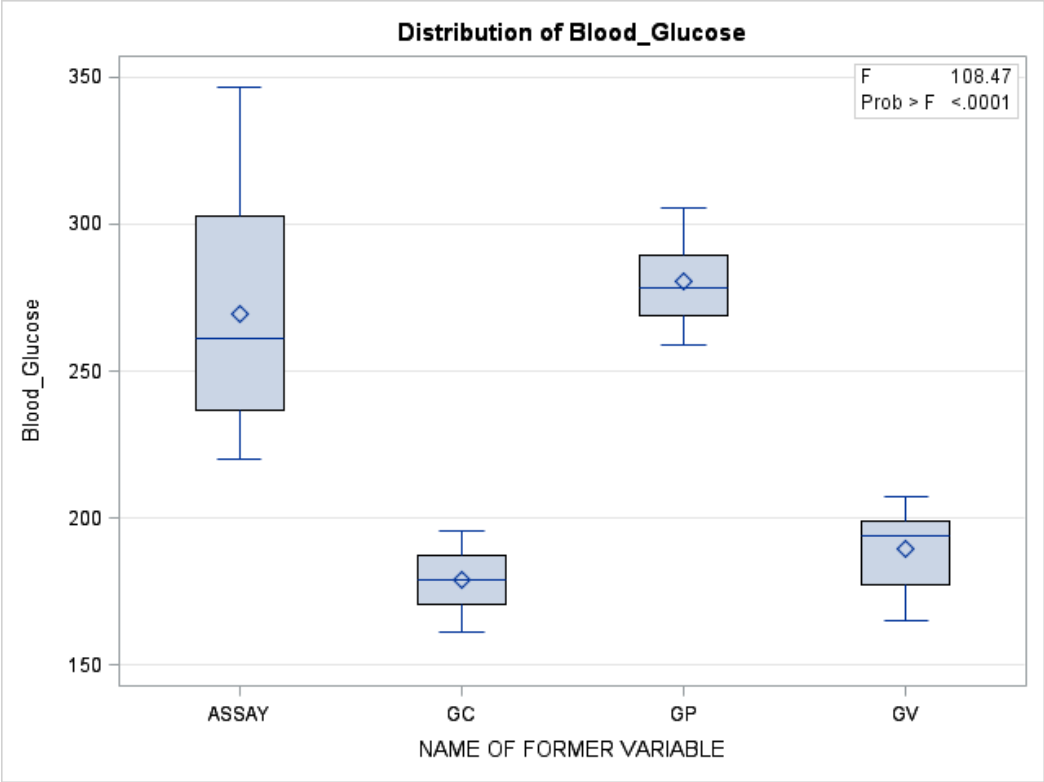

Figure 2. Means (diamonds) and standard deviations (boxes) with error bars

Manuscript 2

Abstract

In maturing breeder females, a transient increase in mortality often occurs between week 24 and week 26 coinciding with the initial point of lay. In the research literature and within the breeder industry, this mortality is referred to as Sudden Death Syndrome (SDS). A different form of sudden death is commonly associated with broilers, and is described as a cardiomyopathy in which normal, seemingly healthy broilers experience a sudden paroxysm that is characterized by vigorous wing flapping, muscle contraction, and a loss of balance followed by death. While sudden death in breeders and SDS in broilers appear to be similar, the circumstances preceding these sudden death events are quite different. Unlike SDS in broilers, sudden death in breeders has been connected to a decrease in plasma phosphate and potassium concentrations, particularly around the time that breeder feed allotments are increased. Hypophosphatemia and hypokalemia occurring upon nutrient repletion is indicative of the refeeding syndrome. Refeeding syndrome is described as a condition characterized by electrolyte depletion, fluid retention, and altered glucose homeostasis occurring when malnourished individuals receive sudden nutrient repletion via oral, enteral, or parenteral means. The purpose of this study was threefold: 1) To determine whether maturing female breeders exhibit changes in electrolyte concentrations characteristic of refeeding syndrome; 2) To determine the effect of feeding regimen (skip-a-day versus every day feeding) upon the potential for refeeding syndrome and; 3) To determine the impact of low level,

long-term potassium supplementation to reduce the potential for refeeding syndrome. 100 Ross 708 breeder females were reared for 26 weeks using standard practices recommended by the Avaigen Breeder Management Handbook (2013). Birds were divided equally into four separate treatment groups: TRT A = Every day feeding, no supplementation; TRT B = Every day feeding with potassium supplementation, TRT C = Skip-a-day, no supplementation; and TRT D = Skip-a-day with potassium supplementation. Birds were bled on weeks 16, 20, and 22 weeks of age and were bled again after 5% production was achieved. A Nova 8 electrolyte analyzer was used to assess calcium, sodium, and potassium concentrations in plasma; a glucometer was used to assess glucose concentrations within plasma; a Quantichrom phosphate assay kit was used to assess phosphate concentrations within plasma. Two way ANOVAs were used to determine mean differences between treatment means over successive blood samplings and to determine overall flock trends. Factorial analyses were performed on postproduction bleeding concentrations to determine the effects of treatments upon concentrations. Factorial analyses yielded no significant results ($p < .05$); however, overall flock plasma phosphate and potassium concentrations dropped significantly over the late grower phase and into the initial point of lay. While the treatment effects were not significant, the overall flock depletion of phosphate and potassium plasma concentrations provide sufficient evidence to merit further research into the potential for refeeding syndrome within breeder females.

Introduction

Broiler parent-stock birds, hereafter referred to as breeders, have long presented the paradoxical challenge of obtaining high reproductive efficiency from an animal that has been extensively selected for growth. Delaying sexual maturation and controlling body weight and conformation through a combination of photoperiod control and feed restriction is critical to productivity in breeders and is universally practiced among breeder managers (Leeson and Summers, 2000). While feed restriction has been a critical tool for maintaining egg productivity, welfare concerns regarding feed restrictions have been noted. In particular, a source of mortality referred to as Sudden Death Syndrome (SDS) by the Aviagen Ross 708 Parentstock Management Handbook (2013) occurs around 5% production, also known as the initial point of lay, and has become more commonplace in breeder flocks. Typically, in flocks that are susceptible to sudden death, mortality rates occur between 0.5-2% per week from 5% production to 20-30% production (Leeson and Summers, 2000). The defining characteristic of sudden death in breeders is the apparent depletion of plasma potassium and plasma phosphate concentrations (Hopkinson et al., 1984). While labeled SDS in available literature, sudden death in breeders does not present like the more prominently known SDS in broilers. SDS in broilers is a syndrome that is brought upon by metabolic insufficiency, particularly the inability of the heart and lungs to supply enough oxygen for the fast-growing bird which leads to ventricular arrhythmias and eventually death (Havenstein et al., 2003b) (Olkowski et al., 2008) (Chung et al., 1993) (Imaeda, 2000). While overall feed restriction remains the solely effective way to combat SDS in broilers, changing diet

composition through supplementation has widely been regarded as the least effective way to treat SDS within broilers (Greenless et al., 1989). In breeders, the opposite is true; it is recommended that potassium and/or phosphate supplementation is the most effective way to combat sudden death (Aviagen Breeder Management Handbook, 2013). Through the work of Hopkinson (1991), it was determined that insufficiency of available potassium and phosphate in the diet was likely the cause of sudden death in breeders rather than metabolic insufficiency. Therefore, the primary objective of this study is to present a new syndrome as the cause behind breeder mortality at the initial point of lay: the refeeding syndrome.

Refeeding syndrome is a condition characterized by electrolyte depletion, fluid retention, and altered glucose homeostasis that occurs when malnourished individuals receive nutrient repletion via oral, enteral, or parenteral means (Marinella, 2005). The primary electrolyte deficiency associated with the refeeding syndrome is hypophosphatemia (Lambers et al., 2015). Within human subjects (only available source of data), hypophosphatemia typically presents between 2 to 5 days after the refeeding period has begun (Marinella, 2005) (Abed et al., 2014). Hypokalemia is a secondary condition associated with the refeeding syndrome but only presents in approximately 50% of cases (Skipper, 2012). If left untreated, refeeding syndrome can result in cardiac arrhythmia, cardiac arrest, hemolytic anemia, delirium, seizures, coma, and sudden death (Garber et al., 2016). Supplemental phosphate and/or potassium can be used to reduce the effects of the refeeding syndrome (Marinella, 2005).

When an individual is in a starved state, an electrolytic shift of potassium, magnesium, and phosphate occurs from the intracellular environment to the extracellular environment; at the

same time, insulin concentrations decrease which results in the body using fat stores as its primary energy source (Khardori, 2005). It is because of these shifts that the speed of nutrient repletion and what is being used to replete nutrients becomes vital; when the body is refed carbohydrates and proteins, insulin concentrations will increase, resulting in a cellular uptake of glucose and the inward movement of phosphate and potassium from an extracellular environment to an intracellular environment (Khardori, 2005). If this sudden decrease in extracellular phosphate concentrations is not accounted for by controlling the speed of repletion, or ensuring that adequate phosphate and/or potassium supplementation occurs upon repletion, hypophosphatemia and/or hypokalemia can occur (Lambers, 2015).

The refeeding syndrome, when it progresses to severe stages leading to arrhythmias, cardiac arrest, and sudden death, presents in a similar manner to SDS. Pumps within the heart such as calcium ATPases and sodium-potassium ATPases are imperative to proper myocardial relaxation (Guyton and Hall). In the hypophosphatemic state, all ATPase activity is depressed due to the lack of new ATP synthesis, causing calcium ATPases and sodium-potassium ATPases to work slower which slows the sequestration of calcium into the sarcoplasmic reticulum and the removal of excess calcium into extracellular spaces via sodium co-transporters respectively (Korbonits et al., 2007). When this process is slowed, the phenomenon of re-entry can occur (Guyton and Hall, 2015). Re-entry occurs when myocardial cells do not repolarize in a uniform manner, allowing for the next electrical signal to cause non-uniform myocardial depolarizations. When this occurs in the ventricular myocardium, pre-mature ventricular contractions (PVC) result and, if left untreated, PVC can devolve into ventricular tachycardia (Guyton and Hall,

2015). When hypokalemia occurs in concert with hypophosphatemia, additional delaying of ventricular action potentials can occur due to the inhibition of outward potassium channels impacting the rapid components of the delayed and inward rectifying channels in the myocardium (Osadchii, 2010) (Faggioni and Knollman, 2015). Electrocardiographic evidence taken from individuals experiencing refeeding syndrome symptoms shows a prolonged Q-T interval indicative of an elongated myocardial relaxation period (Abed, 2014). If proactive treatment is not taken, ventricular arrhythmias can devolve into ventricular fibrillation and result in acute heart failure and sudden death (Garber et al., 2016).

In order for a phenomenon similar to refeeding syndrome to be considered as the potential source of mortality at the initial point of lay within breeders, current management practices must be explored to determine the potential for breeder malnourishment during the pullet grower phase. During the grower phase, feed is restricted to between 60-80% of normal feed consumption (Grandin and Deesing, 2013). While this restriction leads to greater flock egg productivity and less flock obesity, it also leads to stereotyped behaviors such as scratching and pecking the litter as well as higher evidence of physiological stress (Robinson et al., 1991) (Hocking et al., 1993). The two most common methods of feed restriction are the "skip-a-day" feeding regimen and the everyday feeding regimen (Leeson and Summers, 2000). In a skip-a-day feeding regimen, birds are fed approximately twice the amount as if they were fed every day. Flocks fed with this regimen tend to have greater uniformity at the initial point of lay due to greater feed access during on-feed days; however, negatives for skip-a-day feeding include increased water consumption to satiate hunger, the tendency for birds to gorge themselves and

eat food too quickly during on-feed days, and the need to switch back to everyday feeding at the initial point of lay which can serve as an additional physiological stressor (Chamblee et al., 1989) (North and Bell, 1990). In an everyday feeding regimen, birds are fed a small amount of feed every day. The greatest drawback to everyday feeding is decreased flock uniformity (North and Bell, 1990). Due to the small amounts of feed given to the bird, especially during the early stages of the grower phase, feed dominant birds will more easily consume more than their allotment of feed while non-feed dominant birds may not get any feed at all which can result in a wide range of body weights within a breeder flock at the initial point of lay (Leeson and Summers, 2000).

Both of the aforementioned feeding regimens have their benefits and disadvantages, but both feeding regimens allow for the potential of refeeding syndrome occurrence. Around the initial point of lay, feed allotments are greatly increased and diet composition is changed to ready breeders for egg production (Aviagen Breeder Management Handbook, 2013). At the same time, breeder females are experiencing the physical action of mating as well as laying their first eggs, all of which serve to increase physiological stress within the breeder female that only increases the chance of sudden death, especially if refeeding syndrome is present. Therefore, it is the objective of this study to determine the potential for the refeeding syndrome-like phenomenon within breeder females by examining plasma electrolyte concentrations throughout the later stages of the growing phase and through the initial point of lay. Furthermore, the skip-a-day feeding regimen will be compared to the everyday feeding regimen in order to determine the effect of the feeding regimen on the potential for refeeding syndrome. Finally, the effectiveness

of the recommendation of potassium supplementation by the Aviagen Breeder Management Handbook (2013) will be assessed.

Methods

Breeder Management

One hundred Ross 708 parent stock breeders were received on 1 day of age and were placed in 4 pens holding 25 birds per pen. House temperature upon chick arrival was 86°F and was gradually reduced to ambient temperature in accordance with Table 1 of the Ross 708 Parentstock Management Handbook (2013). Humidity was maintained at approximately 55% during the first week and was reduced to roughly 40% for the remainder of the study. Lighting regimens suggested by the Ross 708 Parentstock Management Handbook were strictly followed, with chicks receiving 23h of light on day 1 with gradual reduction to 8h by day 10. Birds remained on an 8h lighting schedule until 22 weeks of age, after which lighting duration was gradually increased to 15h.

Upon reaching 3 weeks of age, chicks were redistributed from the initial pens so that, at 4 weeks of age, the final experimental set up was 20 pens across two rooms containing 5 birds per pen (Figure 3). Birds were wingbanded at 4 weeks of age after being redistributed into their new pens. Each pen contained one hanging feeder and a nipple drinker with six nipples. Birds had *ad libitum* access to water for the duration of the study and to food for the first week, after which feed restriction began. Birds fed on a skip-a-day restricted feeding regimen began the regimen at

2 weeks of age and were fed every other day until 5% egg production was achieved. Feed amounts were calculated using the Ross 708 parent stock performance objectives as a guide. Bird weight was taken every week or every other week to ensure proper weight gain and uniformity was achieved. From 1-16 weeks of age, birds were weighed 4 hours after commencement of feeding. From 16 weeks until the end of the study, birds were weighed just prior to being bled and before feed was administered for that day.

Treatments

At 4 weeks of age, birds were divided into four separate treatment groups based upon feed composition and feed restriction technique. Treatment A birds were fed a standard breeder diet every day. Treatment B birds were fed a breeder diet supplemented with potassium every day. Treatment C birds were fed a standard breeder diet every other day. Treatment D birds were fed a breeder diet supplemented with potassium every other day. Potassium chloride was used as the potassium supplement raising available potassium in the feed from 0.6% to 0.8%. Birds were spread amongst the two available rooms in such a way as to minimize the effect of the rooms upon experimental factors.

Blood sampling

On weeks 16, 20, and 22 of age, blood samples were collected from each individual bird to establish a baseline of electrolytes and glucose. Blood was collected via the venipuncture method from the wing vein of the bird. Lithium Heparin was used to prevent blood coagulation. After collection, samples were centrifuged and plasma was extracted and stored in a -20°C freezer until assays could be conducted. Starting on day 172 of age (approximately 24.5 weeks of age) after 5% egg production was achieved, a rotational bleeding schedule was utilized in which 1 bird/pen/day was bled over 5 days to yield blood electrolyte and glucose concentrations over the period of time in which breeder hens are reported to be most susceptible to sudden death and in which refeeding syndrome is most likely to occur. Sample collection and analysis for the post-production blood sampling occurred in the same manner as the baseline blood sampling listed above.

Assays and Statistical Analysis

Phosphate concentrations were determined using a Quantichrom Phosphate Assay kit (BioAssay Systems, Hayward, CA). A Biotrak II microplate reader (General Electric, Chicago, IL) was used at an absorbance of 620nm to determine phosphate concentrations within breeder plasma. Sodium, potassium, and calcium concentrations were determined utilizing a Nova 8 Electrolyte Analyzer (NOVA Biomedical, Waltham, MA). Glucose concentrations were obtained

with a Presto Agamatrix Wavesense glucometer (Agamatrix Inc., Salem, NH). All electrolyte and glucose concentrations were obtained from extracted plasma and all samples were tested in duplicate. For analysis of room, feeding regimen, and potassium supplementation effects upon electrolyte and glucose concentrations, a 2x2 factorial analysis was run in SAS 9.4 (SAS Institute, Cary, NC). To determine trends in electrolyte and glucose concentrations, two-tailed ANOVAs were run in SAS 9.4 (SAS Institute, Cary, NC). For the purposes of the results and discussion baseline bleed 1 (BB1) will refer to the bleed performed at 16 weeks of age, baseline bleed 2 (BB2) will refer to the bleed performed at 20 weeks of age, baseline bleed 3 (BB3) will refer to the bleed performed at 22 weeks of age, and Refed will refer to the rotational bleed started at 172 days of age.

Mortality and Removal

In this study, 7 of the starting one hundred birds were either removed from the study or died during the course of the study. In week 9, bird 8 (TRT B) died with no recorded cause of death. In week 16, bird 98 (TRT C) died; because of the timing of the death (less than 10 h after a blood sampling event), it is believed the bird died due to adverse effects attributable to the blood sampling event. In week 18, 3 birds (49, 82, and 94), all from TRT D, died from a crop impaction/dehydration due to issues with waterers. Finally, birds 65 and 74 (TRT B) were removed from the study due to being males. There was no mortality in this flock directly attributable to sudden death during the initial point of lay.

Results and Discussion

Sodium, Glucose, and Calcium

Sodium concentrations remained fairly consistent from the first to the last bleed with the only significant difference occurring during BB1 ($p < .05$) in the overall sodium trends (Table 2) (Figure 4). Intra-treatment sodium analysis followed the same patterns as the overall trends (Tables 3-6, Figure 12, Figure 17, Figure 22, Figure 27, Figure 32). The factorial analysis revealed no significant differences between sodium concentrations based on treatment (Table 7). Glucose concentrations were fairly consistent as well, with the only significant difference occurring during BB2 within the overall glucose trends and intra-treatment analysis following similar patterns (Tables 2-6, Figure 5, Figure 13, Figure 18, Figure 23, Figure 28, Figure 33). Glucose factorial analysis did not reveal significant differences either (Table 7). This is consistent with findings that hypoglycemia is a late stage symptom of refeeding syndrome that only occurs when fat stores are depleted and gluconeogenesis is no longer a possibility (Shimizu, 2014). Plasma calcium concentrations also presented as expected both within intra-treatment analysis and overall trends by remaining steady and then increasing significantly as the birds approached the initial point of lay (Tables 2-6, Figure 6, Figure 11, Figure 16, Figure 21, Figure 26, Figure 31). Again, factorial analysis revealed no significant differences attributable to treatment (Table 7).

Potassium

Potassium plasma concentrations dropped significantly with increasing bird age as seen in the overall analysis and within the intra-treatment analysis (Tables 3-6, Figure 10, Figure 15, Figure 20, Figure 25, Figure 30). Means taken during BB1, BB2, BB3, and Refed were all significantly different from each other, with mean concentrations falling with each successive bleed (Table 2) (Figure 7). While the overall ANOVA showed significance, factorial analysis showed that both feeding regimen and potassium supplementation level had an insignificant effect on differences in potassium plasma concentrations (Table 7). While statistically insignificant, there was an observable difference between treatment C potassium concentrations and the other three treatment concentrations, with treatment C concentrations being the lowest potassium plasma concentrations across all blood samplings. It is possible that, if the sample size were to be increased in future testing, that birds fed on skip-a-day, non-potassium supplemented diets could prove to have significantly lower plasma potassium concentrations across the later stages of the grower phase and into the beginning of egg production. Further testing with larger sample sizes is required to determine if the observational differences seen in potassium plasma concentrations in this study were coincidental or indicative of a larger trend.

Plasma potassium concentrations are tightly regulated and, in humans, do not appear to experience much change in relation to age (Alfonzo et al., 2006). Indeed, humans are one of the few species where information is available concerning plasma potassium concentrations in relation to age. Due to the lack of available information concerning patterns of plasma potassium

concentration changes in relation to age of breeders, it is hard to make any definitive statements concerning the significant drops in plasma potassium concentrations that were seen in this study when examining overall trends of the flock. The results of this study are generally consistent with one of the few studies that examined plasma potassium concentration changes over time within breeders in which potassium concentrations tended to drop during the late grower phase into the initial point of lay (Hopkinson et al., 1991). While the falling plasma potassium concentrations could validate the idea of the refeeding syndrome being present within breeders, the falling concentrations could very well be the physiological norm within breeders. In the absence of comprehensive studies that establish biochemical parameters within breeders, it is impossible to state whether the fall in potassium plasma concentrations is directly indicative of the presence of the refeeding syndrome. However, the overall decrease in potassium plasma concentrations over time warrants future study and is consistent with the presence of refeeding syndrome.

Phosphate

Phosphate plasma concentrations, in a similar manner to potassium concentrations, decreased significantly from BB1 to the Refed blood sampling within overall flock analysis and intra-treatment analysis (Tables 2-6, Figure 8, Figure 9, Figure 14, Figure 19, Figure 24, Figure 29). In addition to the similarities to potassium plasma concentrations in reference to the overall flock trends, phosphate plasma concentrations showed no significant differences during factorial

analysis (Table 7). The parallels between potassium and phosphate plasma concentrations in relation to treatment effects continued, with an observed difference showing treatment C phosphate plasma concentrations to be noticeably lower than the other treatments even though statistical significance was not achieved. Again, further testing with an increase in sample size could show that the observational difference seen in this study could prove to be statistically significant.

Within humans, phosphate plasma concentrations tend to be highest during young childhood and decrease until the end of growth during late adolescence, decreasing approximately 1mg/dL over that time (Skipper, 2012). Phosphate concentrations remain high during concentrations of increased somatic growth to compensate for increased ATP and other phosphate compound usage (Guyton and Hall, 2015). This plasma phosphate concentration trend is contradictory to what was seen in this study. In the current study, overall phosphate plasma concentrations had the largest numerical decrease from BB3 at approximately 22 weeks of age to the Refed bleed at approximately 24.5 weeks of age. Typically, during this time, feed allotments are increasing significantly and somatic growth is expected to be occurring at the highest rate that it will occur within the commercial breeder lifecycle. Therefore, it is expected that phosphate concentrations would either be unchanged or increasing during this time to facilitate growth. However, the current study shows significant decreases in phosphate plasma concentrations across all treatments. Especially in the presence of increased feed allotments, the decrease in phosphate plasma concentrations is consistent with the presence of the refeeding syndrome in breeders.

Conclusion

The results of this study suggest that the potential for the refeeding syndrome in breeders is high. Phosphate concentrations and potassium concentrations fall throughout the late stages of the grower phase and into the initial point of lay with no other tested metabolite showing any adverse effects. While the overall flock trend seems to indicate the presence of the refeeding syndrome, not enough information was gathered to make a definitive statement concerning the treatments that were tested. In particular, treatment C breeders (skip-a-day fed birds with no potassium supplementation) had obvious observational differences from the other 3 treatments even though statistical significance was not achieved. This difference is important to note, as treatment C represents breeders fed on a skip-a-day diet with no supplementation which is the most common diet and feeding regimen used within the poultry industry today. If the effects of skip-a-day feeding are shown to be significant through further testing, it is possible that new feeding regimens would need to be explored in order to minimize the potential for the refeeding syndrome without impacting flock body weight uniformity. It is therefore necessary that this study be repeated in the future with a larger sample size. While examination of the feeding regimens and feed supplementation was inconclusive, overall flock trends including phosphate plasma concentrations decreasing at a time when they should be unchanged or increasing along with the parallels between phosphate and potassium plasma concentration trends within each treatment provide enough evidence for the presence of the refeeding syndrome within breeders warrant future research.

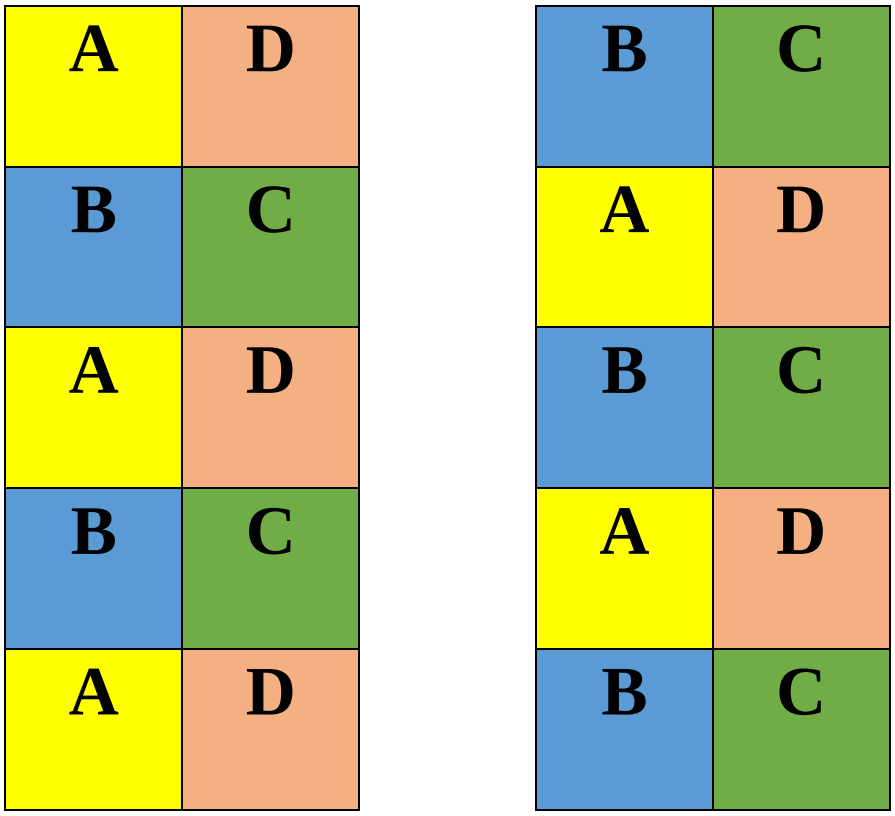

Figure 3. Depiction of treatment layout amongst pens between two rooms. Each pen contained 5 birds apiece.

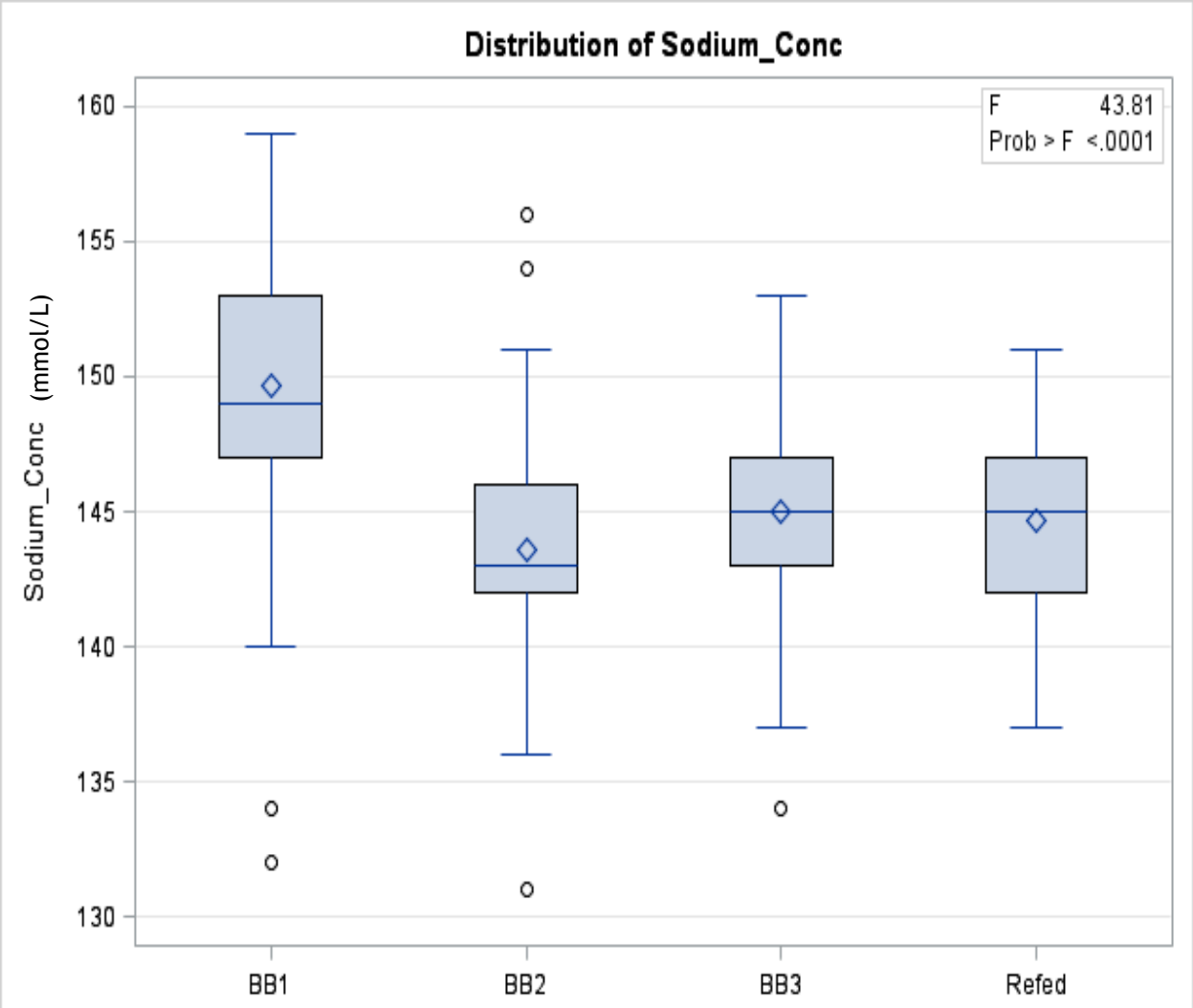

Figure 4. Means (diamonds) and standard deviations (boxes) for overall sodium plasma concentrations

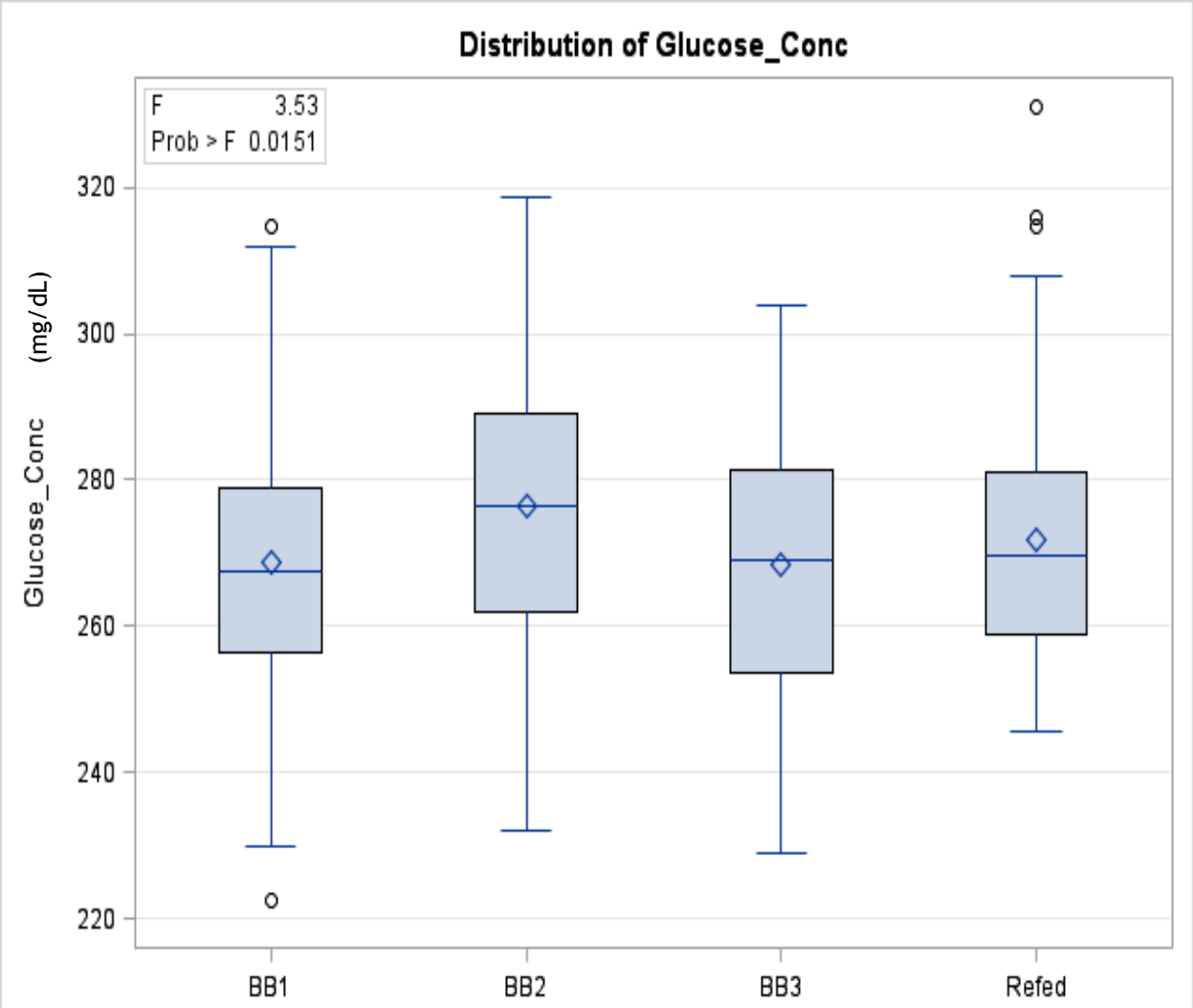

Figure 5. Means (diamonds) and standard deviations (boxes) for overall glucose plasma concentrations

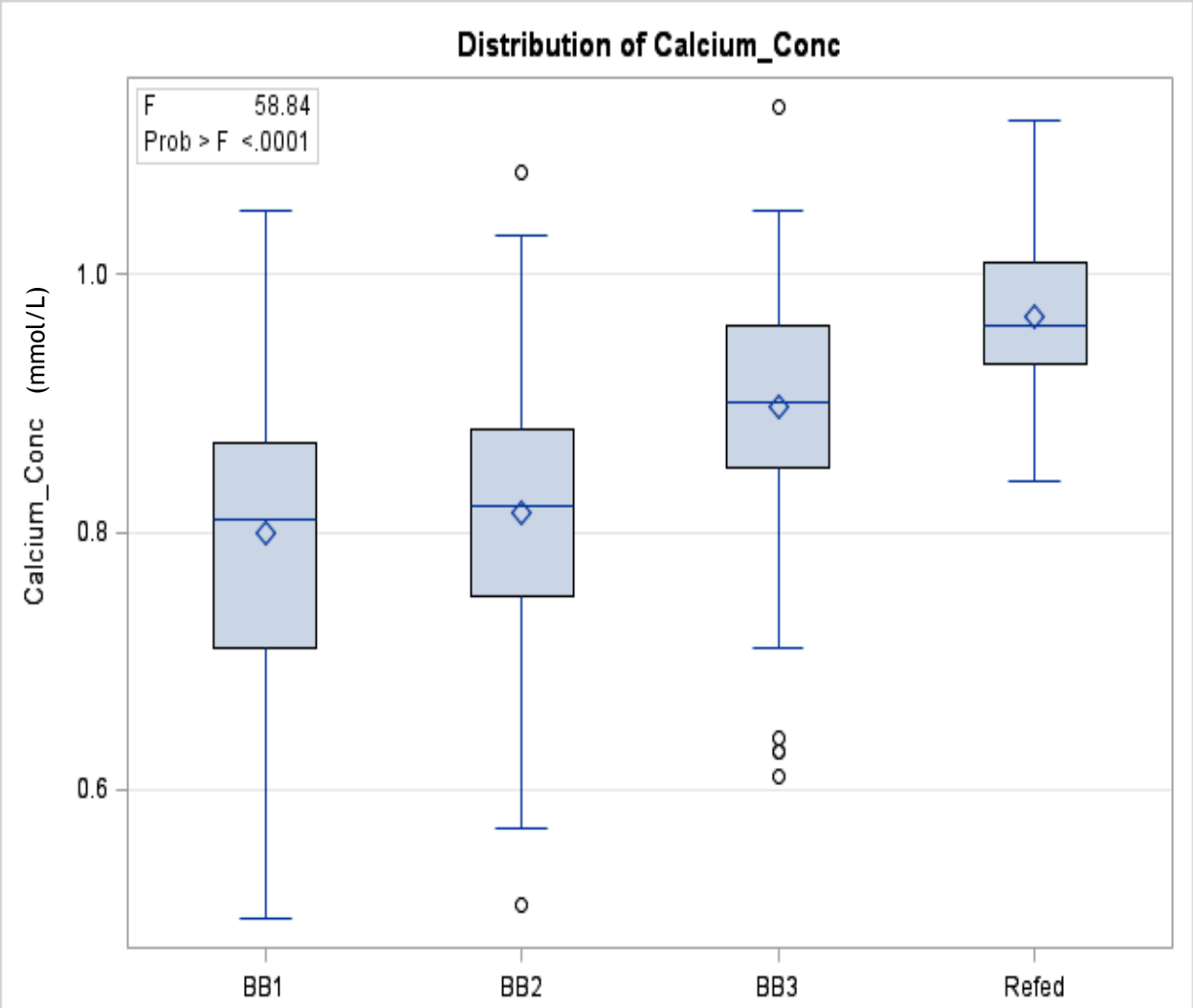

Figure 6. Means (diamonds) and standard deviations (boxes) for overall calcium plasma concentrations

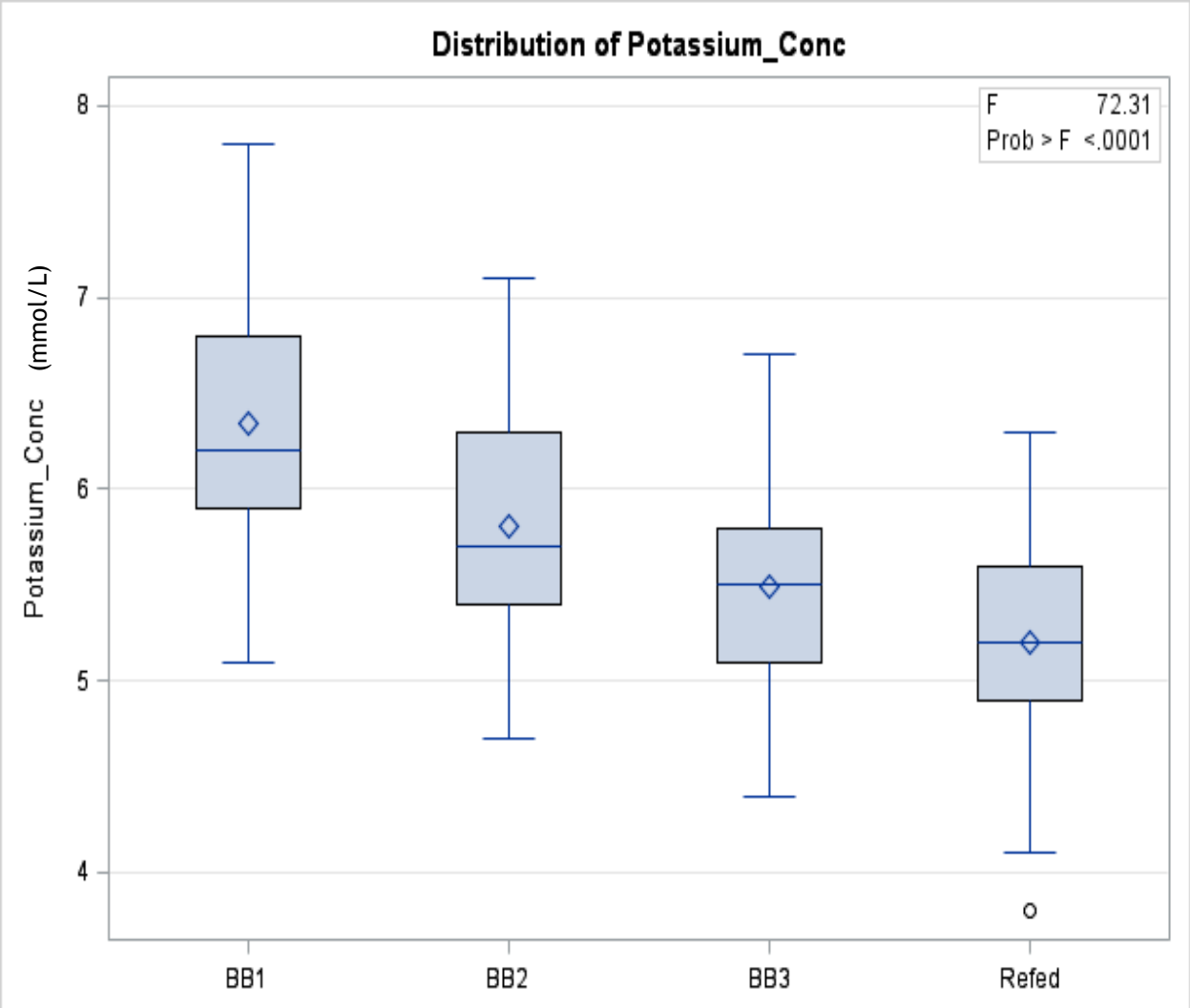

Figure 7. Means (diamonds) and standard deviations (boxes) for overall potassium plasma concentrations

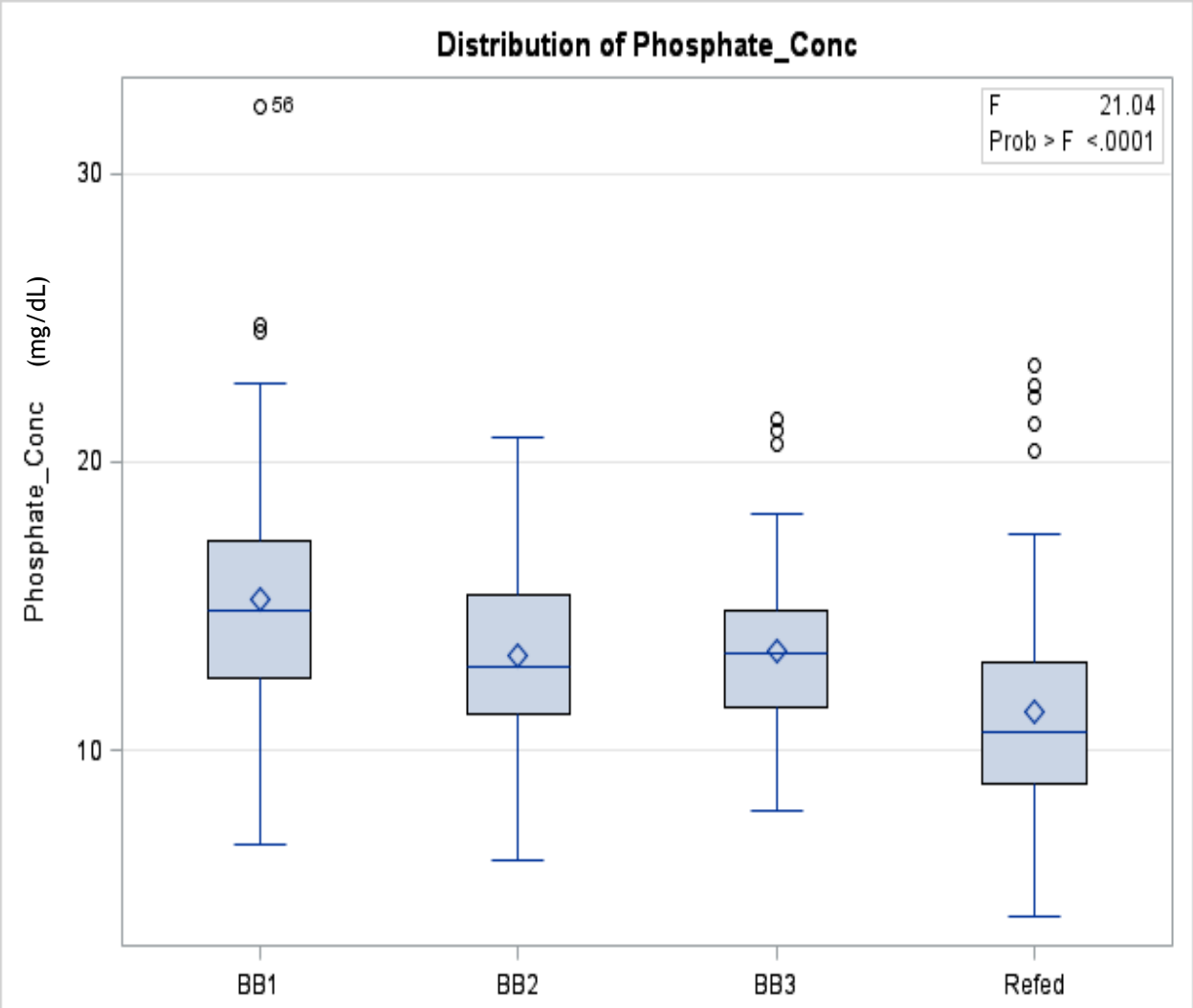

Figure 8. Means (diamonds) and standard deviations (boxes) for overall phosphate plasma concentrations

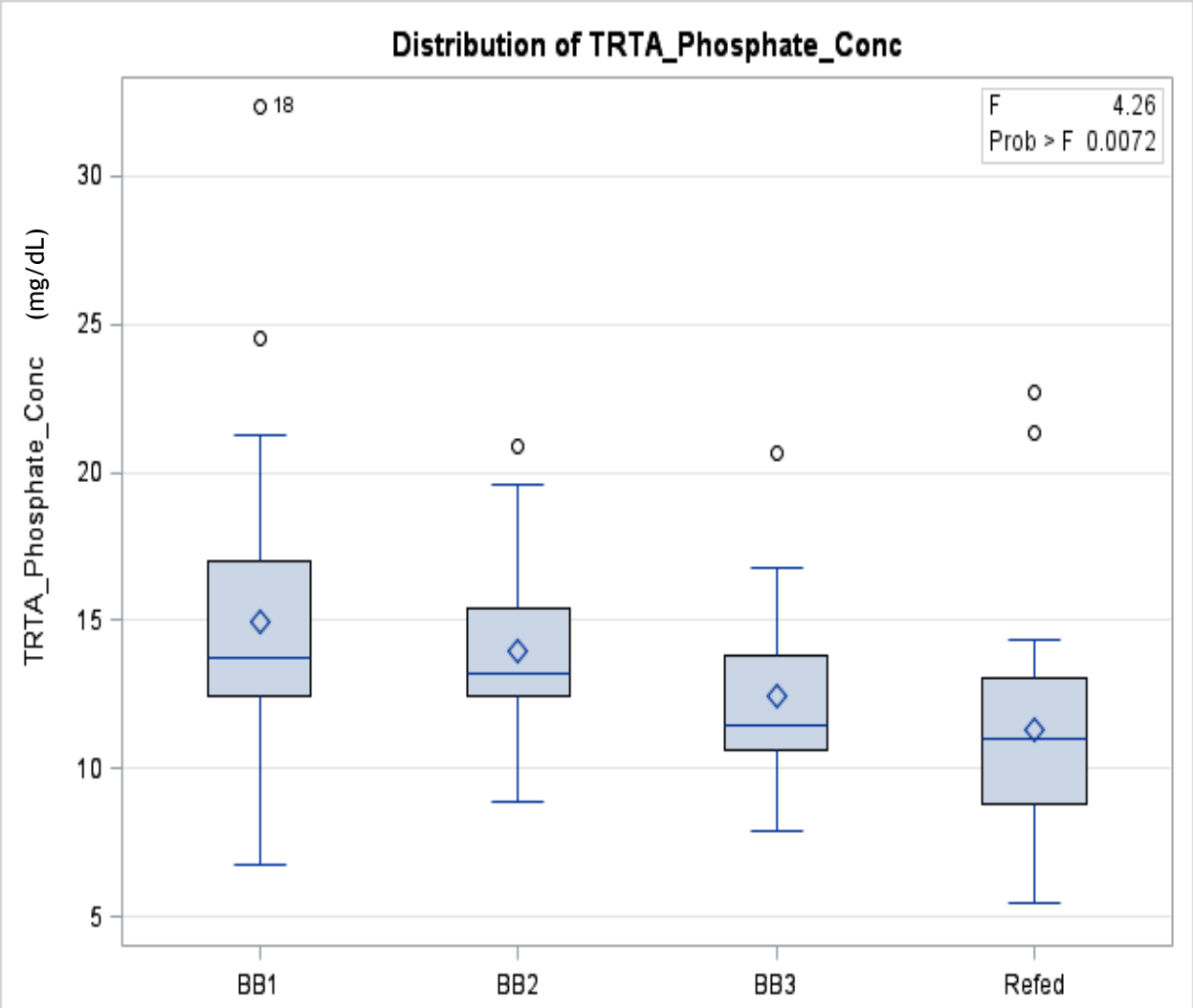

Figure 9. Means (diamonds) and standard deviations (boxes) for phosphate plasma concentrations of TRT A

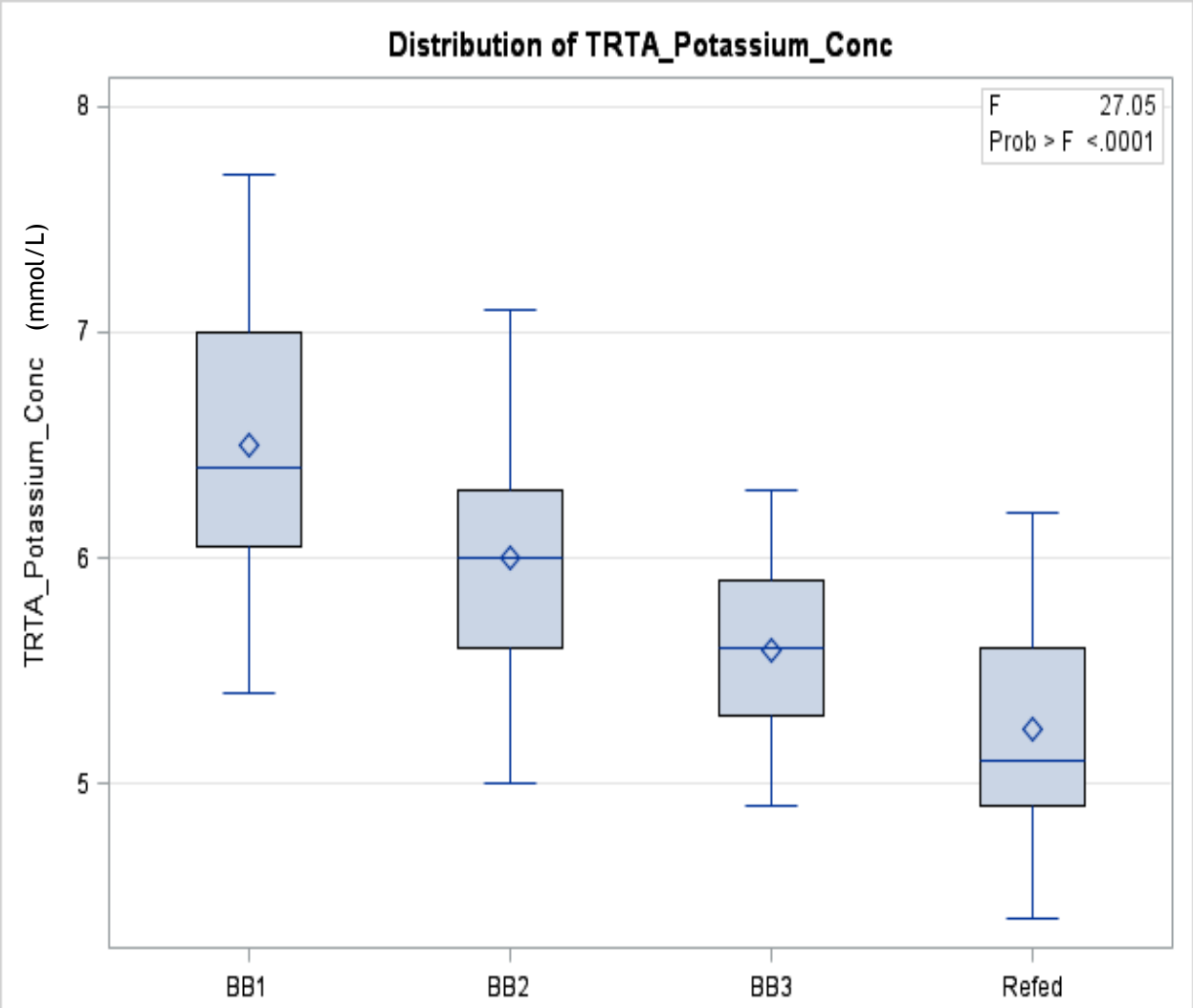

Figure 10. Means (diamonds) and standard deviations (boxes) for potassium plasma concentrations of TRT A

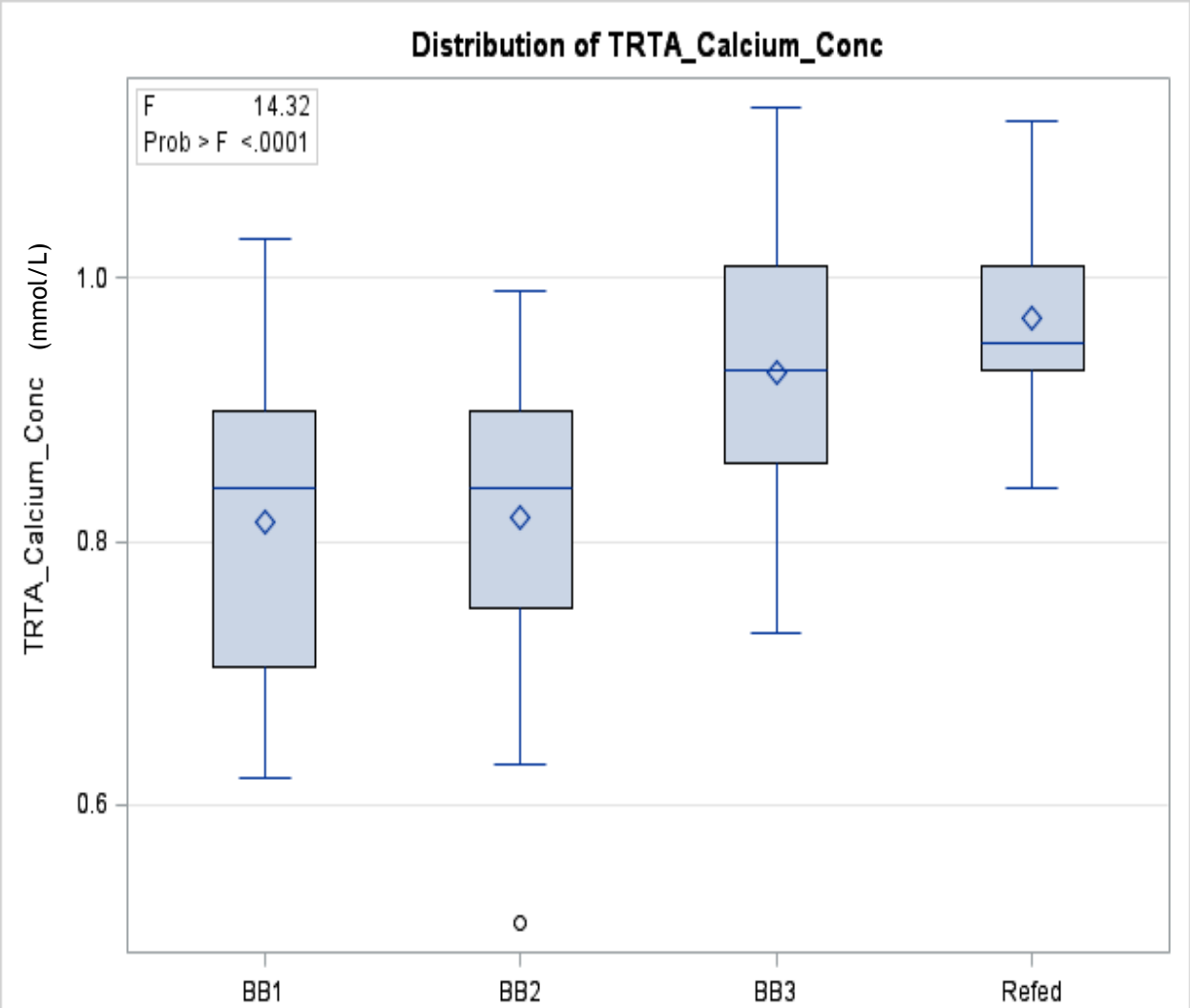

Figure 11. Means (diamonds) and standard deviations (boxes) for calcium plasma concentrations of TRT A

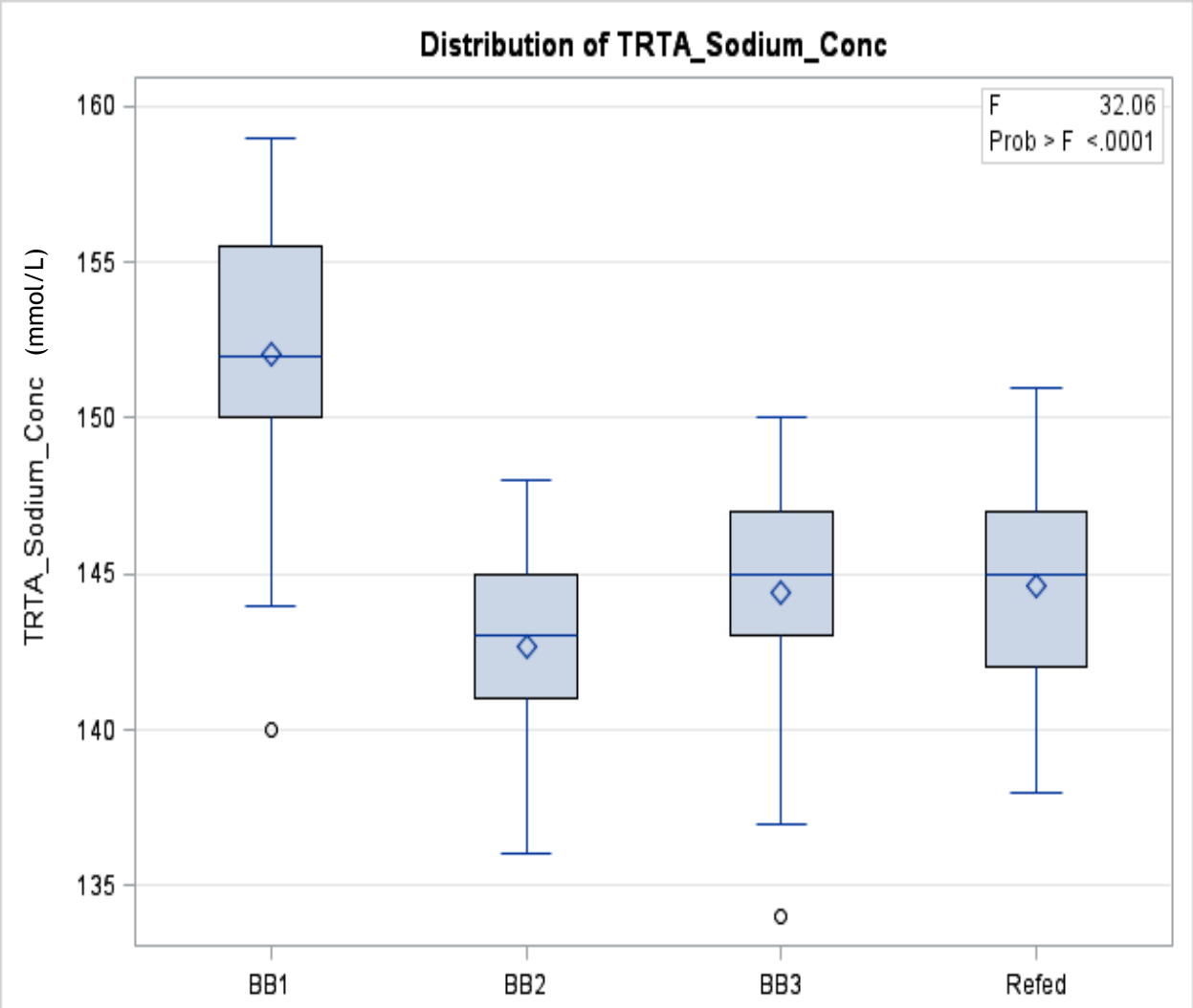

Figure 12. Means (diamonds) and standard deviations (boxes) for sodium plasma concentrations of TRT A

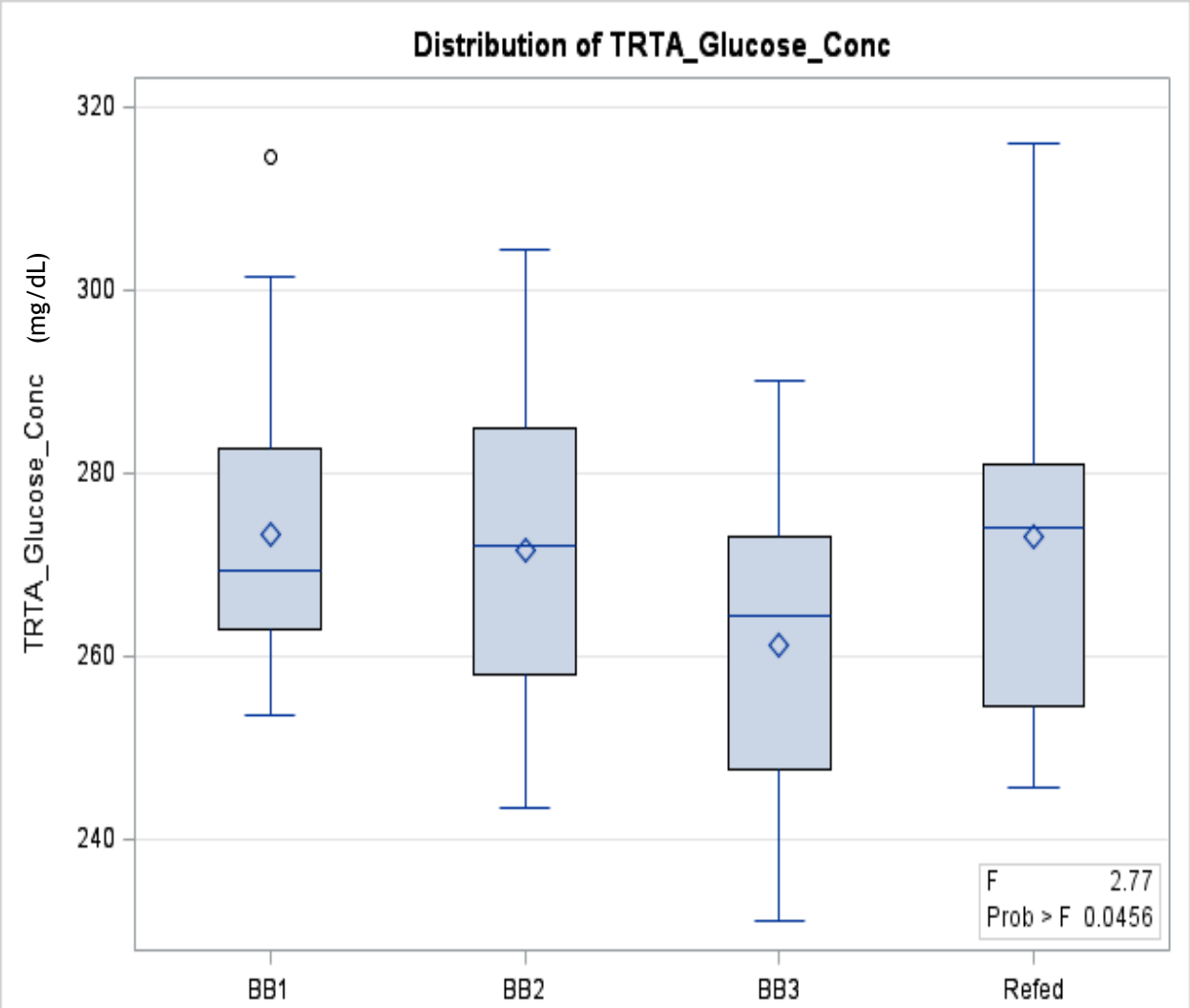

Figure 13. Means (diamonds) and standard deviations (boxes) for glucose plasma concentrations of TRT A

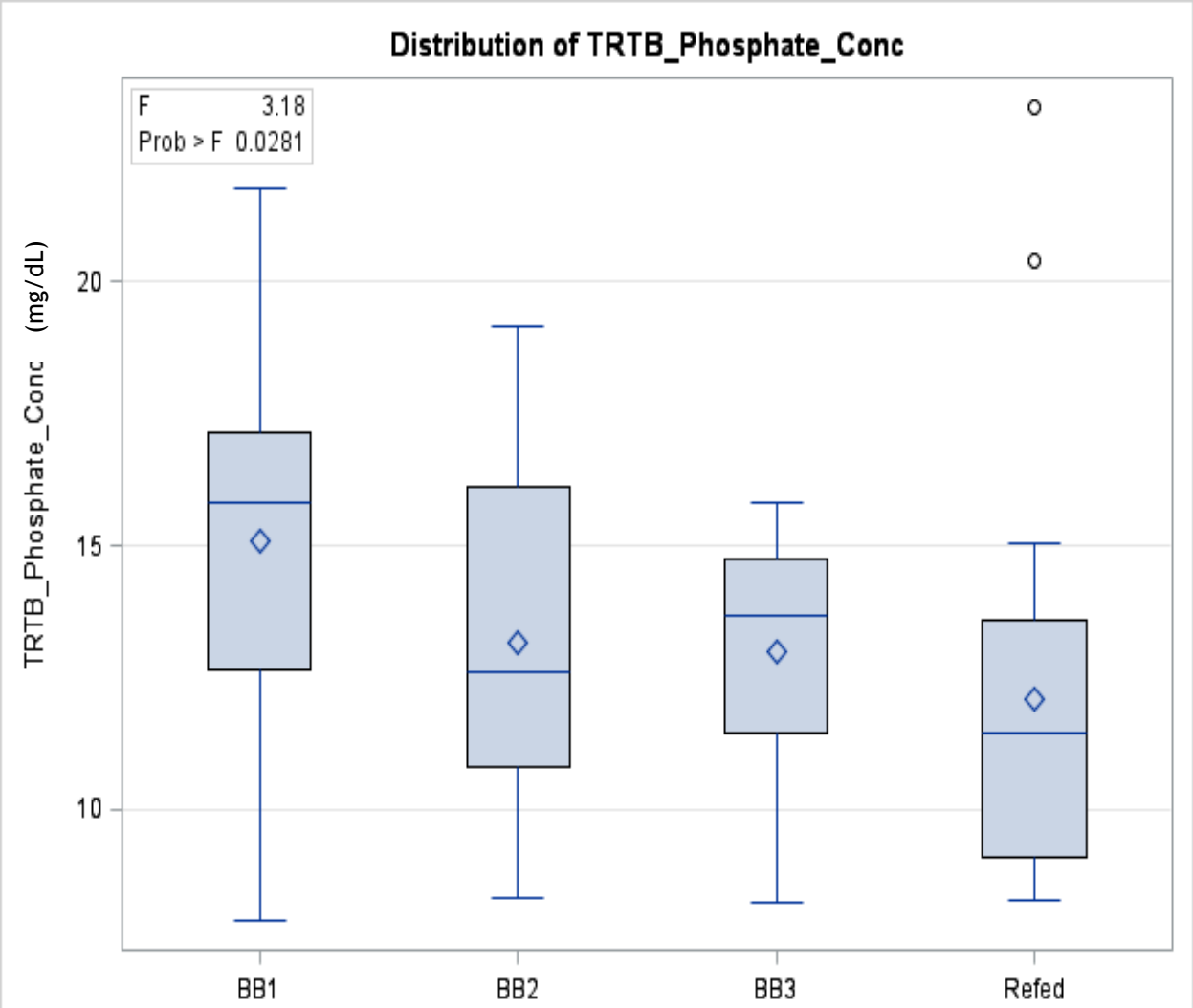

Figure 14. Means (diamonds) and standard deviations (boxes) for phosphate plasma concentrations of TRT B

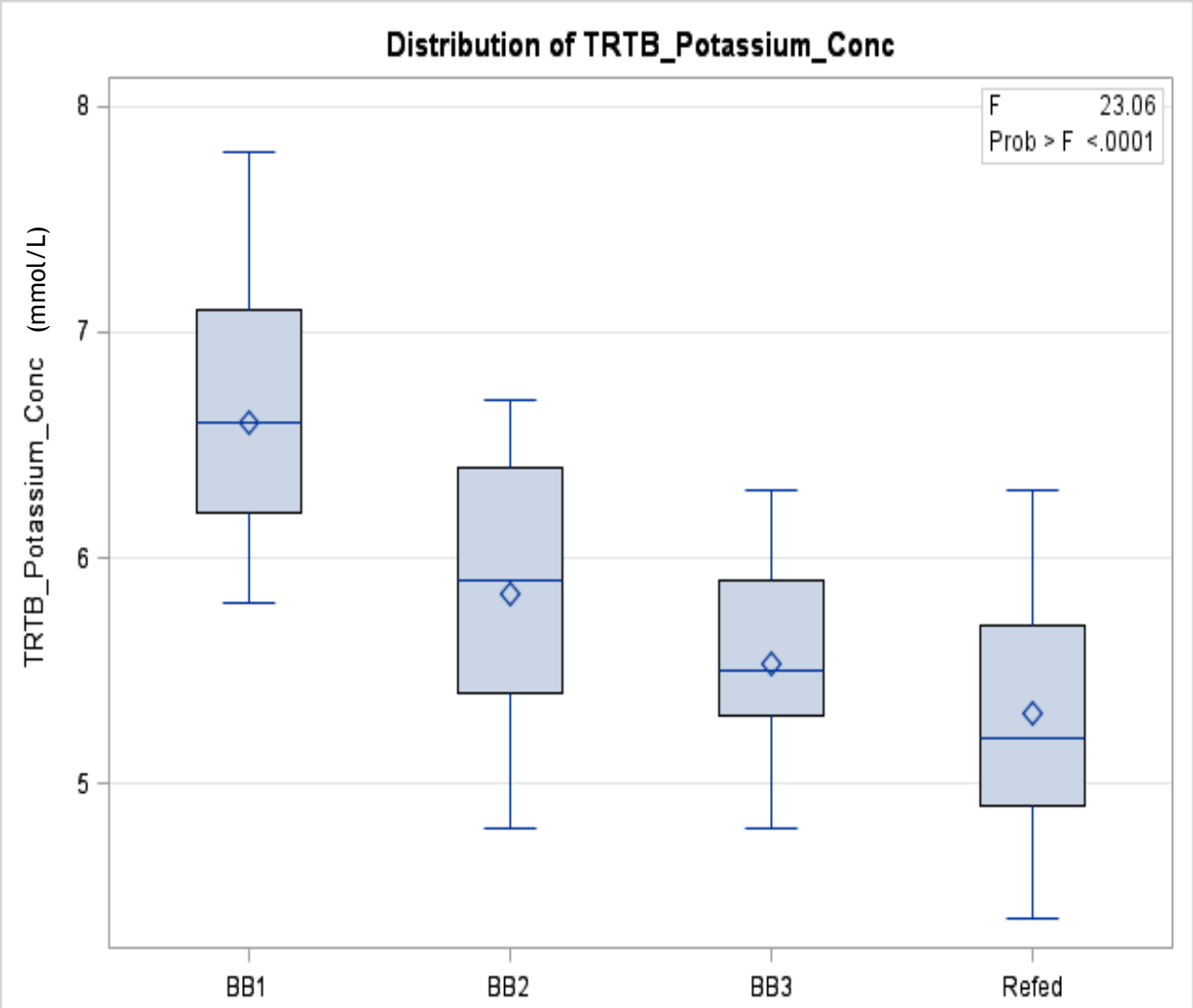

Figure 15. Means (diamonds) and standard deviations (boxes) for potassium plasma concentrations of TRT B

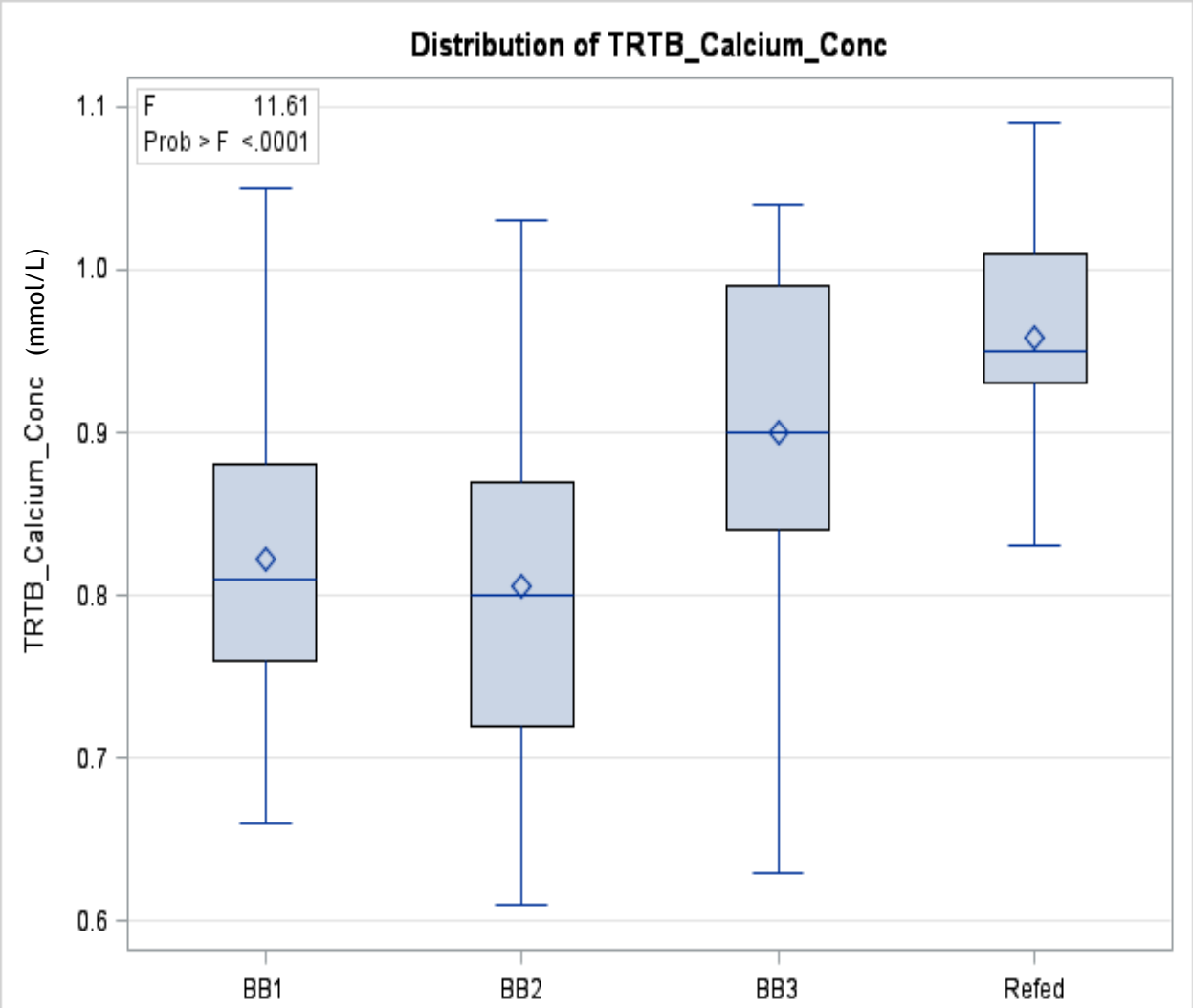

Figure 16. Means (diamonds) and standard deviations (boxes) for calcium plasma concentrations of TRT B

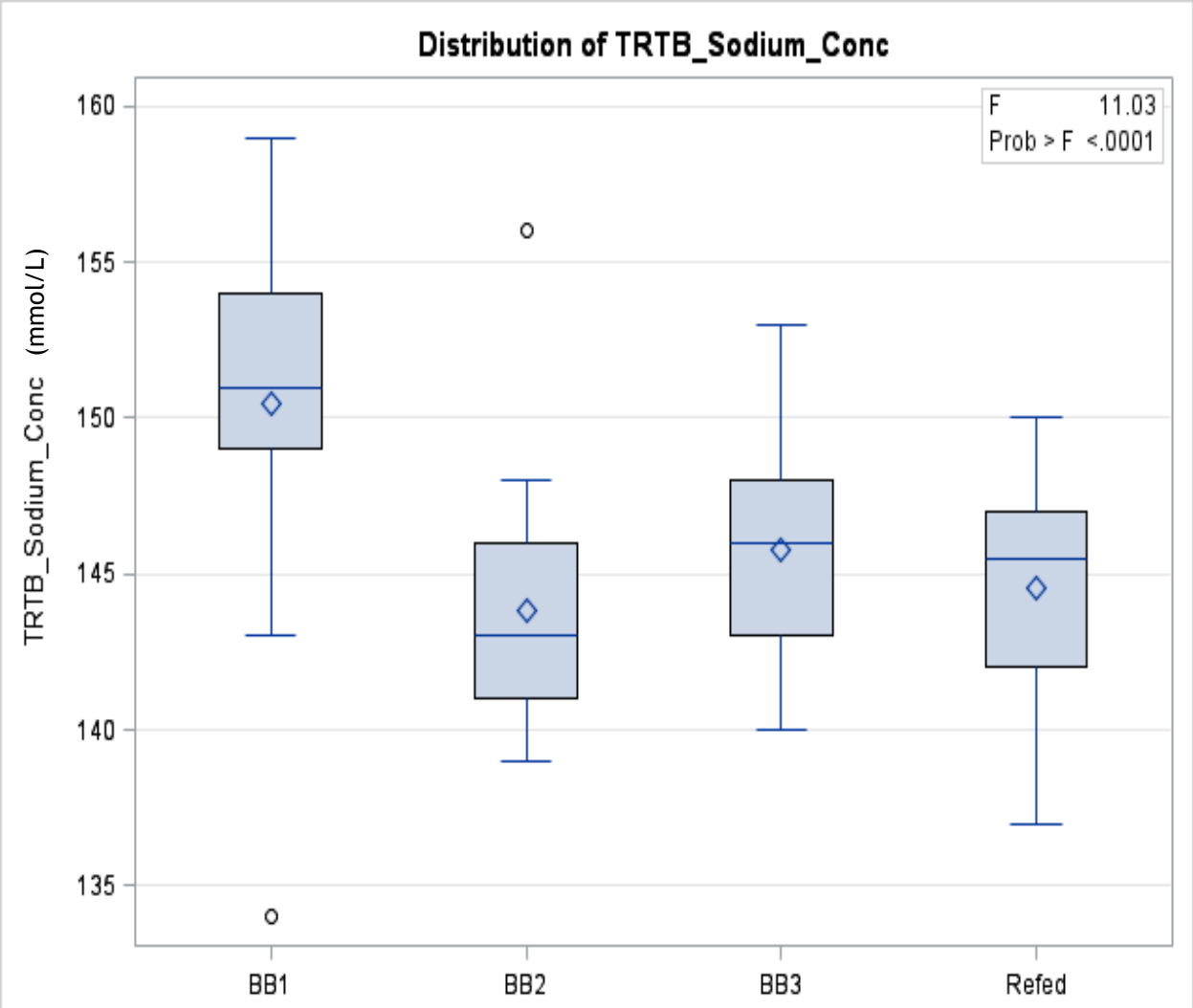

Figure 17. Means (diamonds) and standard deviations (boxes) for sodium plasma concentrations of TRT B

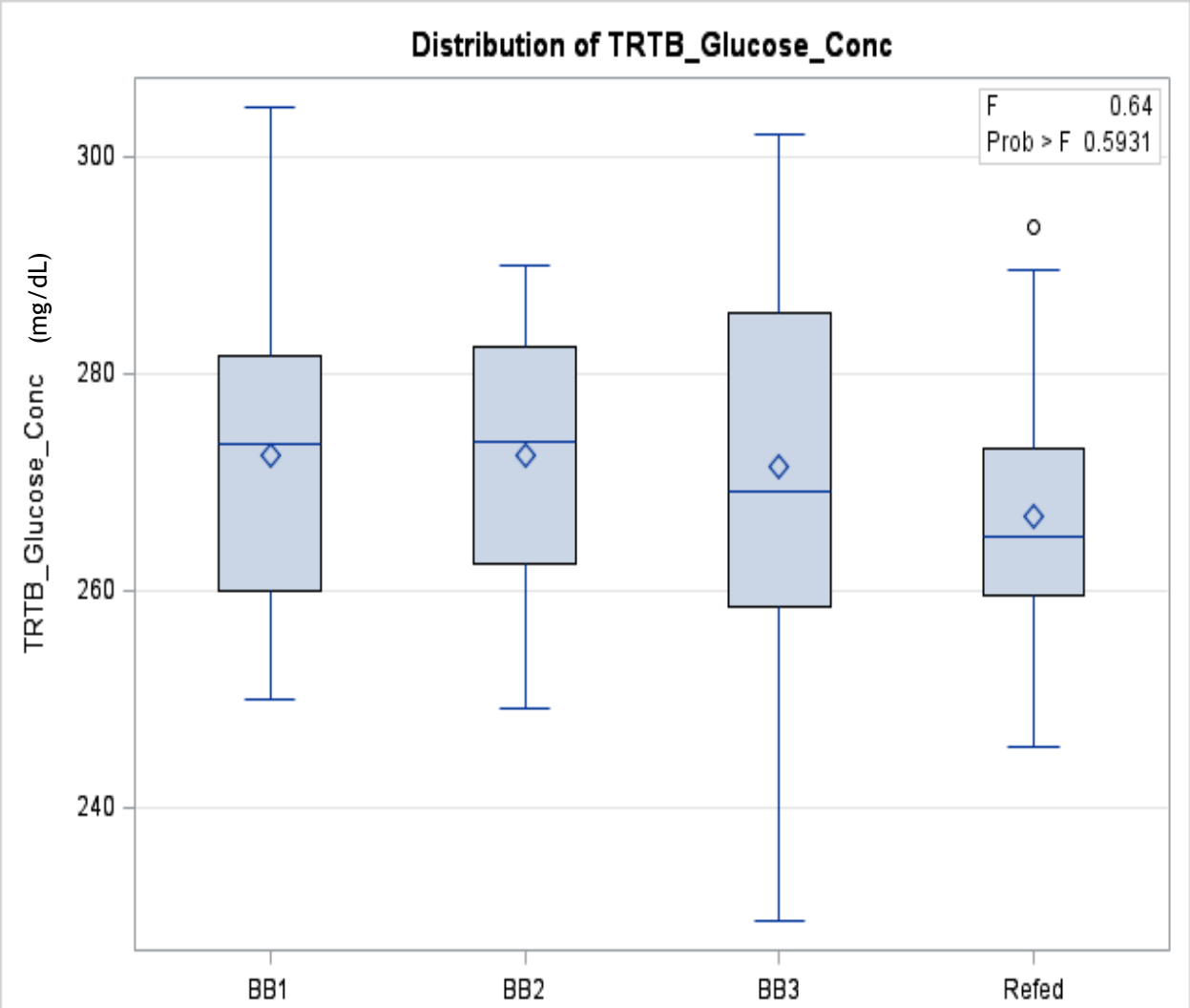

Figure 18. Means (diamonds) and standard deviations (boxes) for glucose plasma concentrations of TRT B

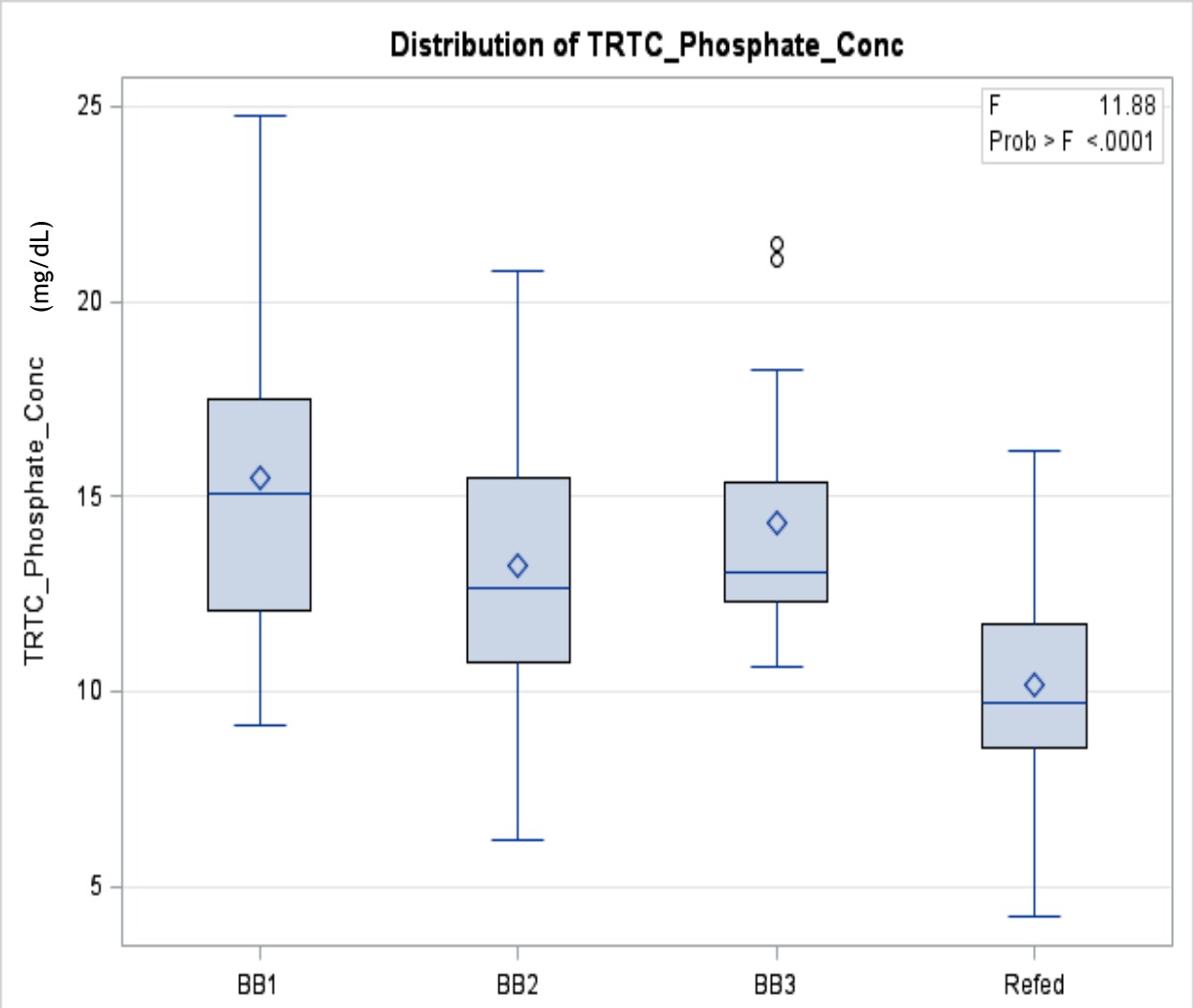

Figure 19. Means (diamonds) and standard deviations (boxes) for phosphate plasma concentrations of TRT C

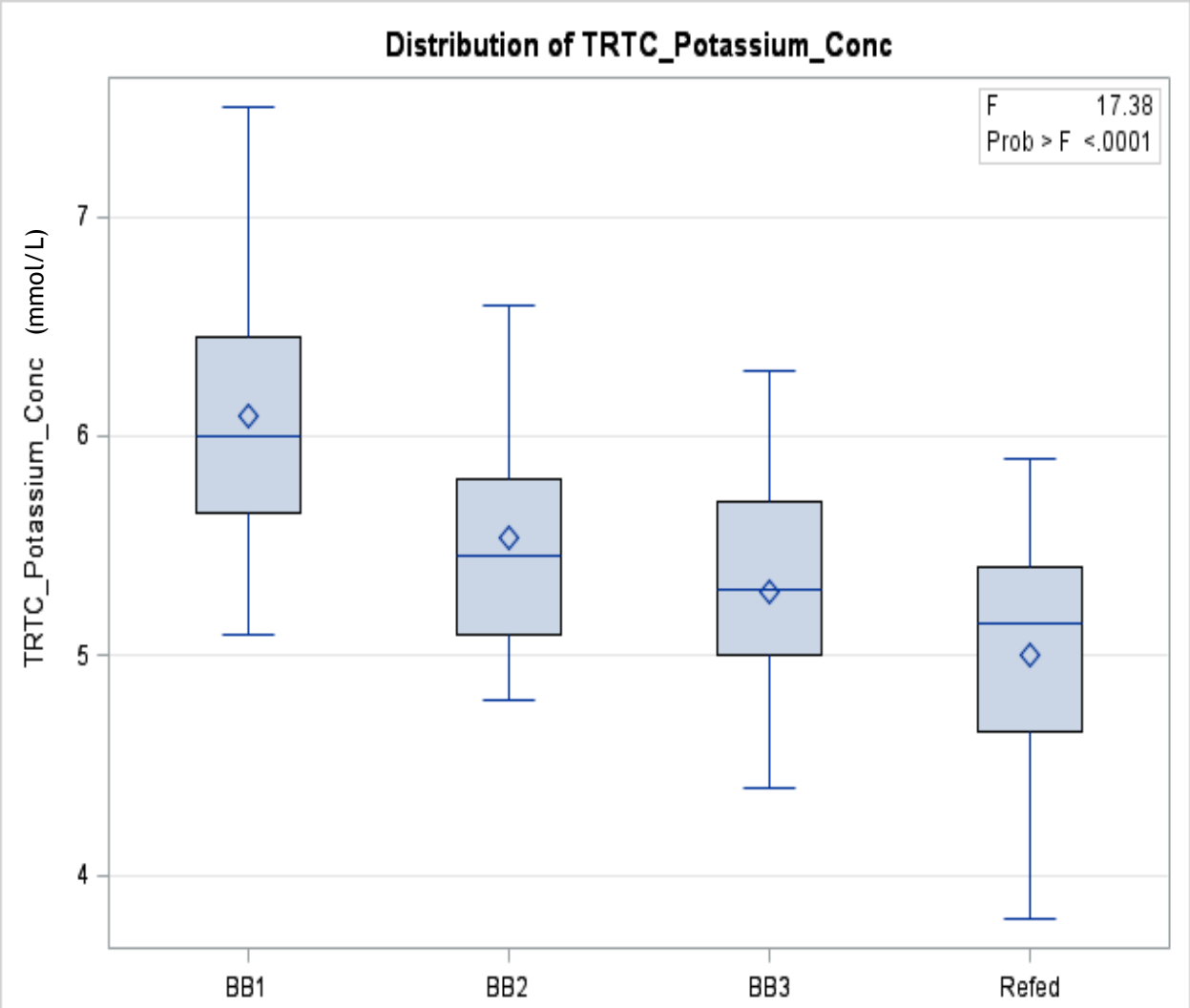

Figure 20. Means (diamonds) and standard deviations (boxes) for potassium plasma concentrations of TRT C

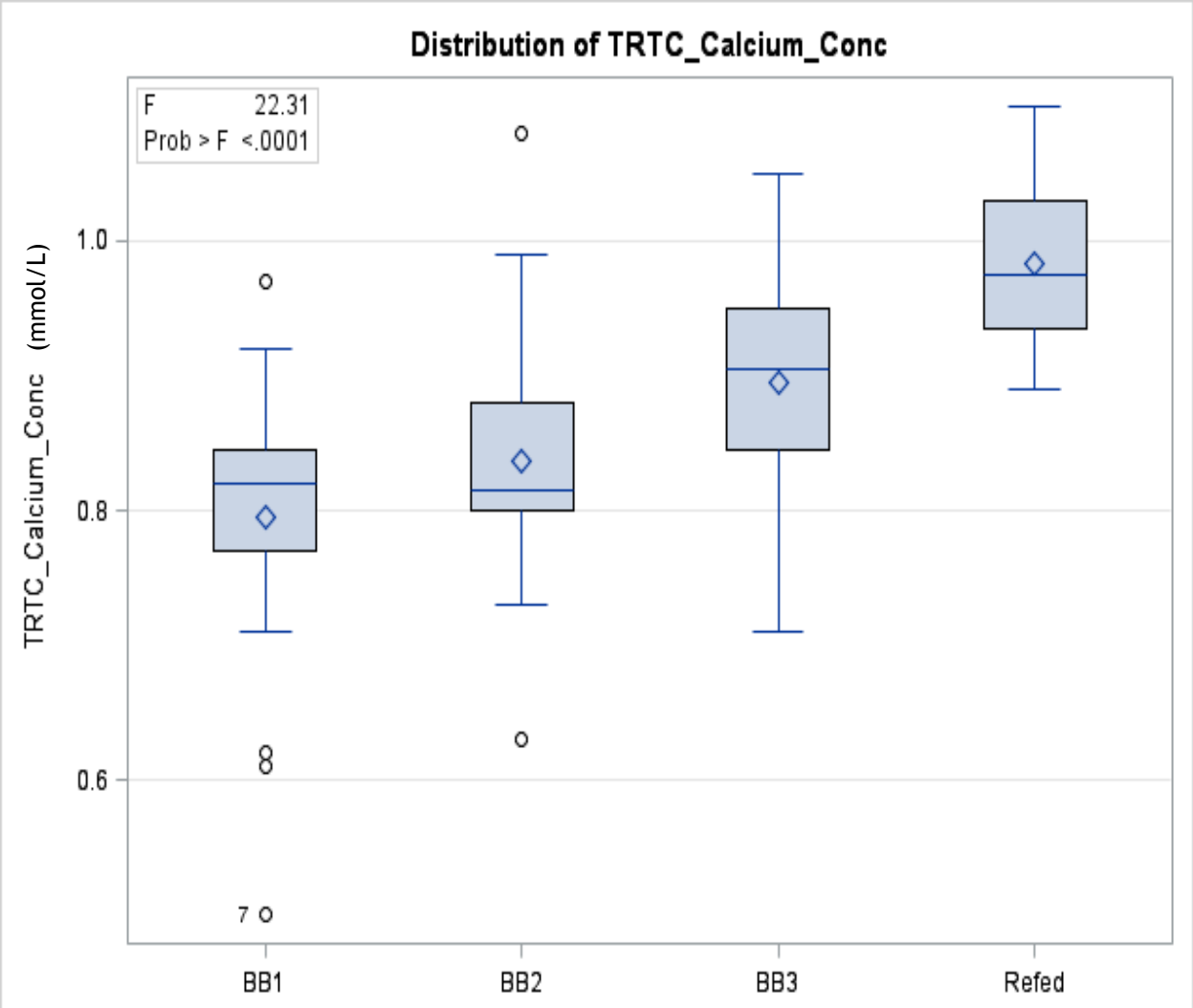

Figure 21. Means (diamonds) and standard deviations (boxes) for calcium plasma concentrations of TRT C

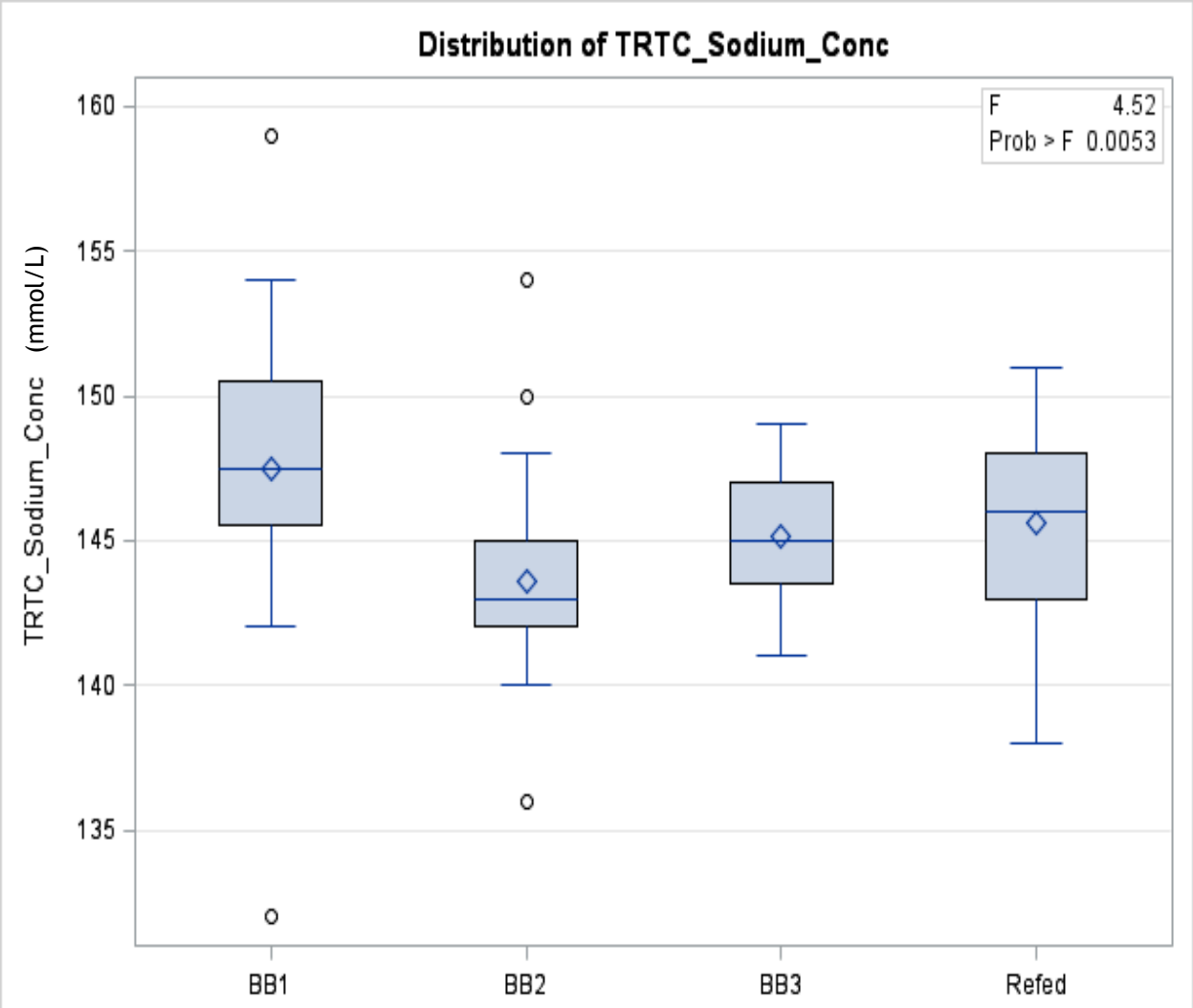

Figure 22. Means (diamonds) and standard deviations (boxes) for sodium plasma concentrations of TRT C

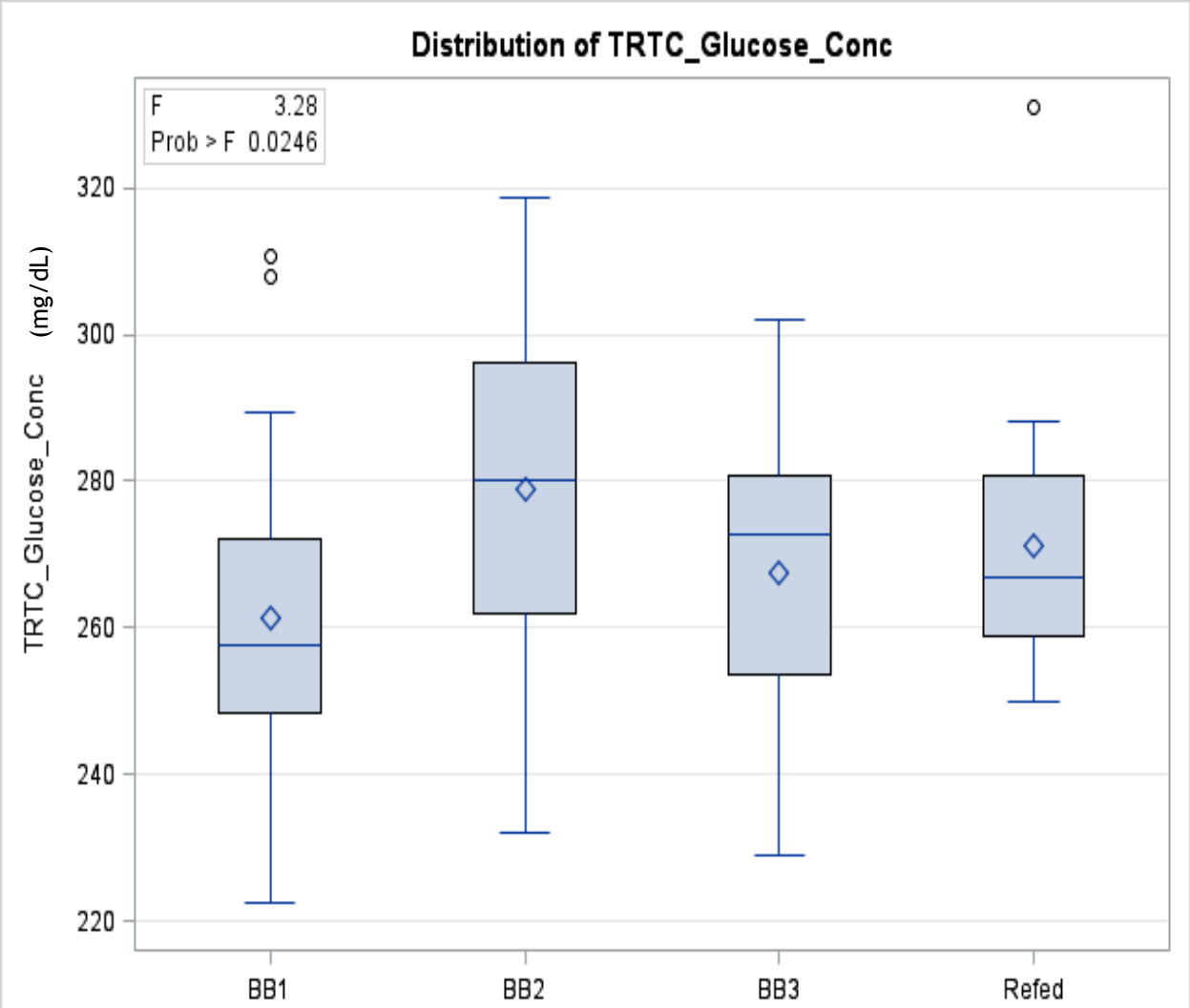

Figure 23. Means (diamonds) and standard deviations (boxes) for glucose plasma concentrations of TRT C

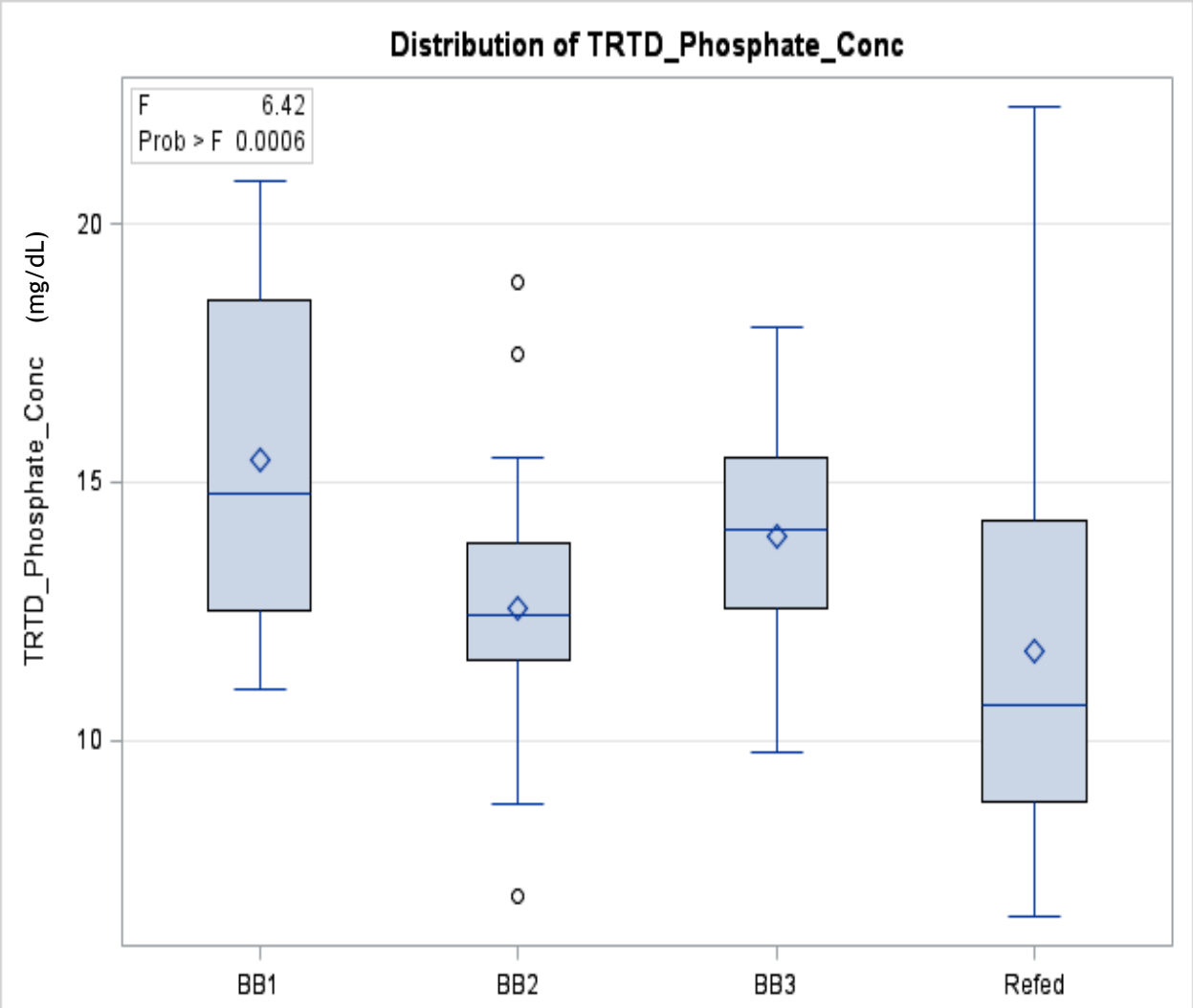

Figure 24. Means (diamonds) and standard deviations (boxes) for phosphate plasma concentrations of TRT D

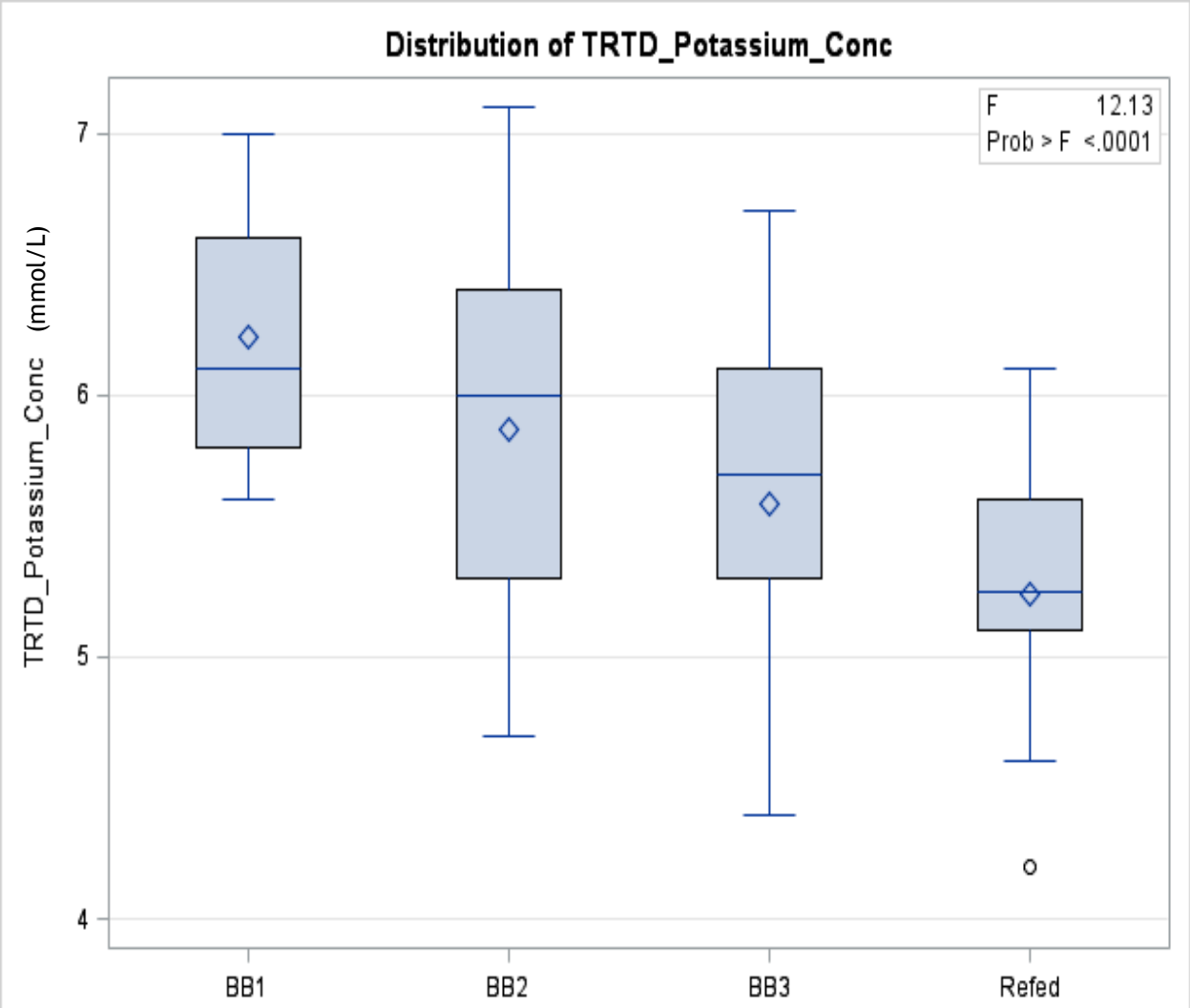

Figure 25. Means (diamonds) and standard deviations (boxes) for potassium plasma concentrations of TRT D

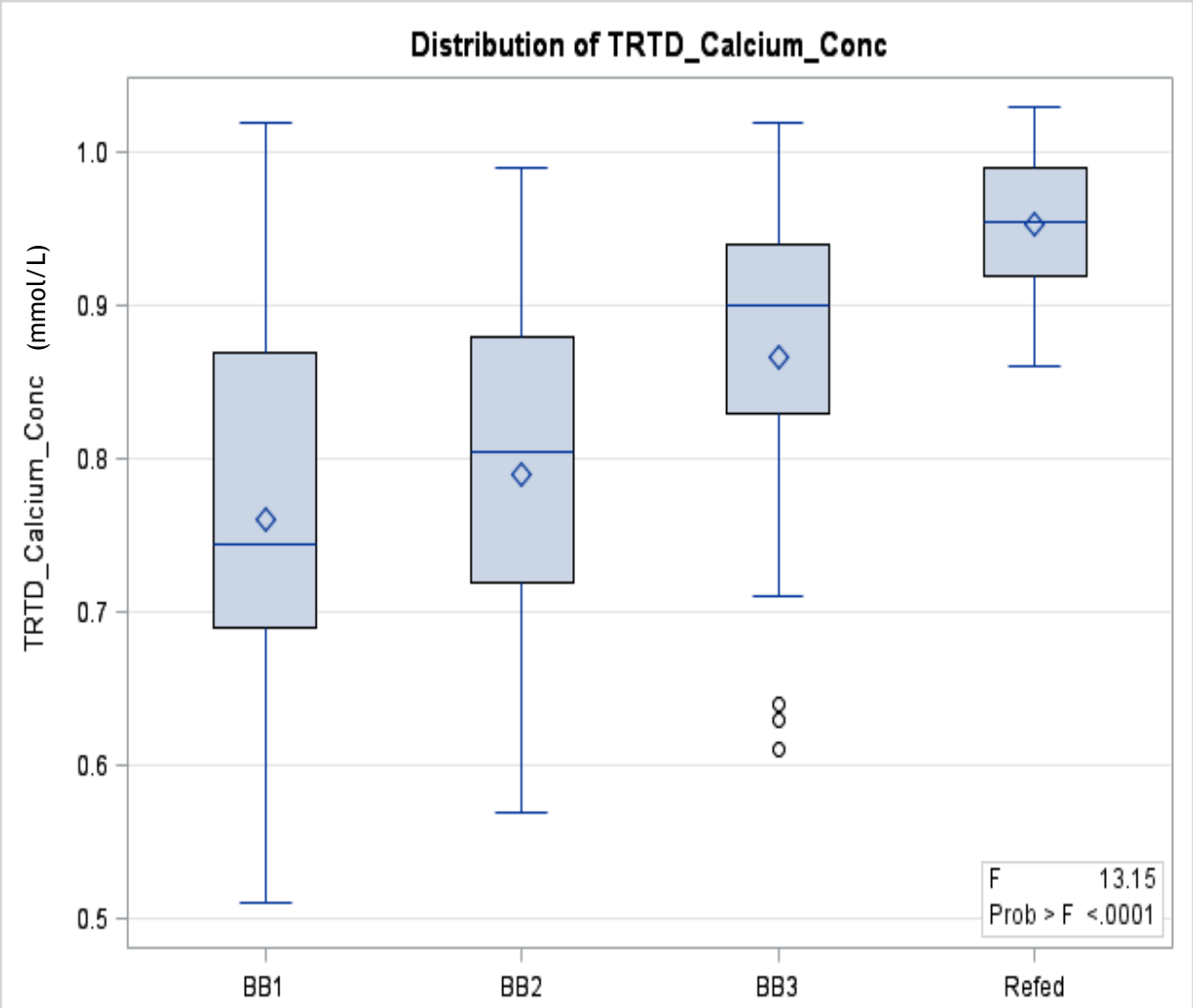

Figure 26. Means (diamonds) and standard deviations (boxes) for calcium plasma concentrations of TRT D

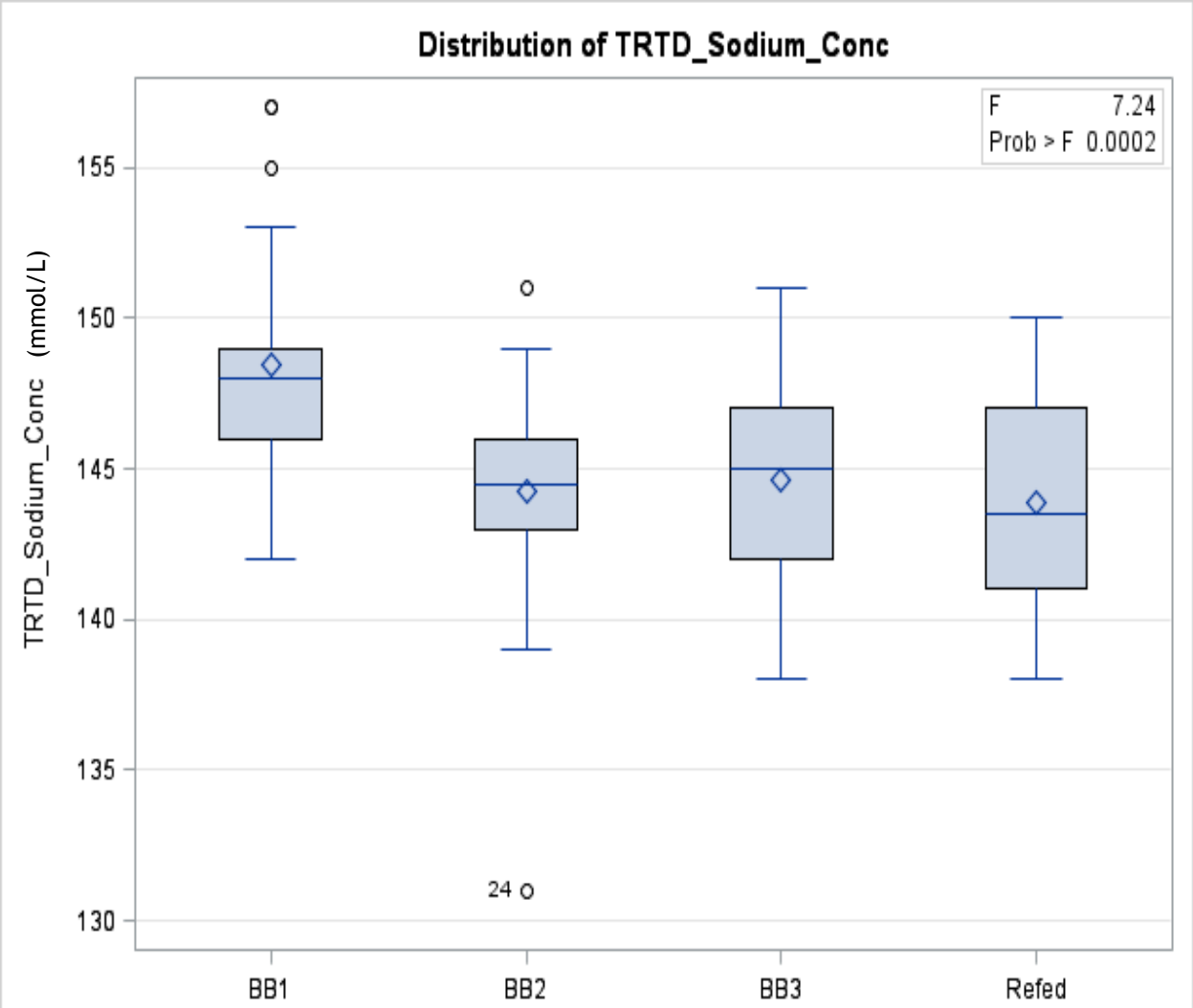

Figure 27. Means (diamonds) and standard deviations (boxes) for sodium plasma concentrations of TRT D

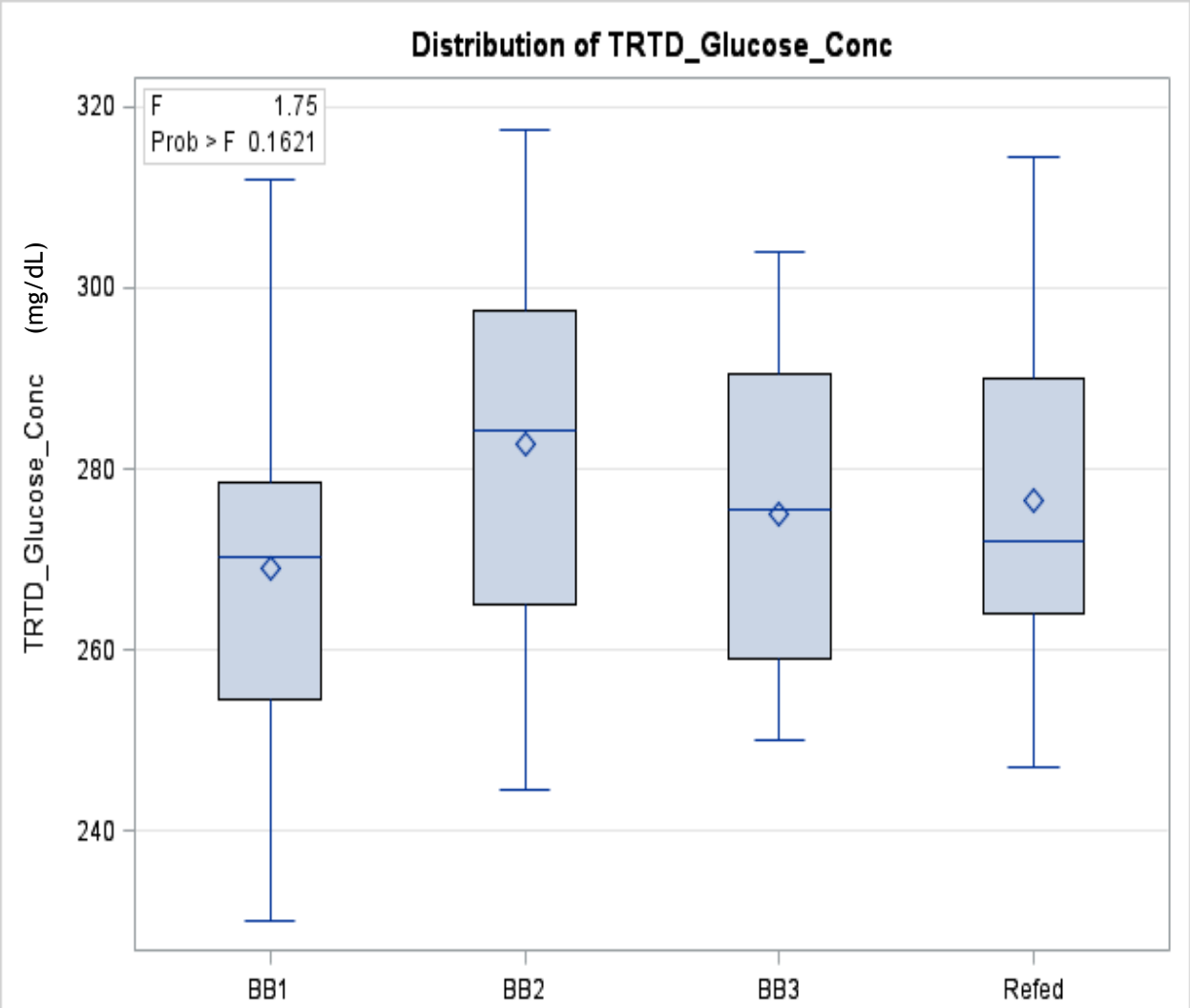

Figure 28. Means (diamonds) and standard deviations (boxes) for glucose plasma concentrations of TRT D

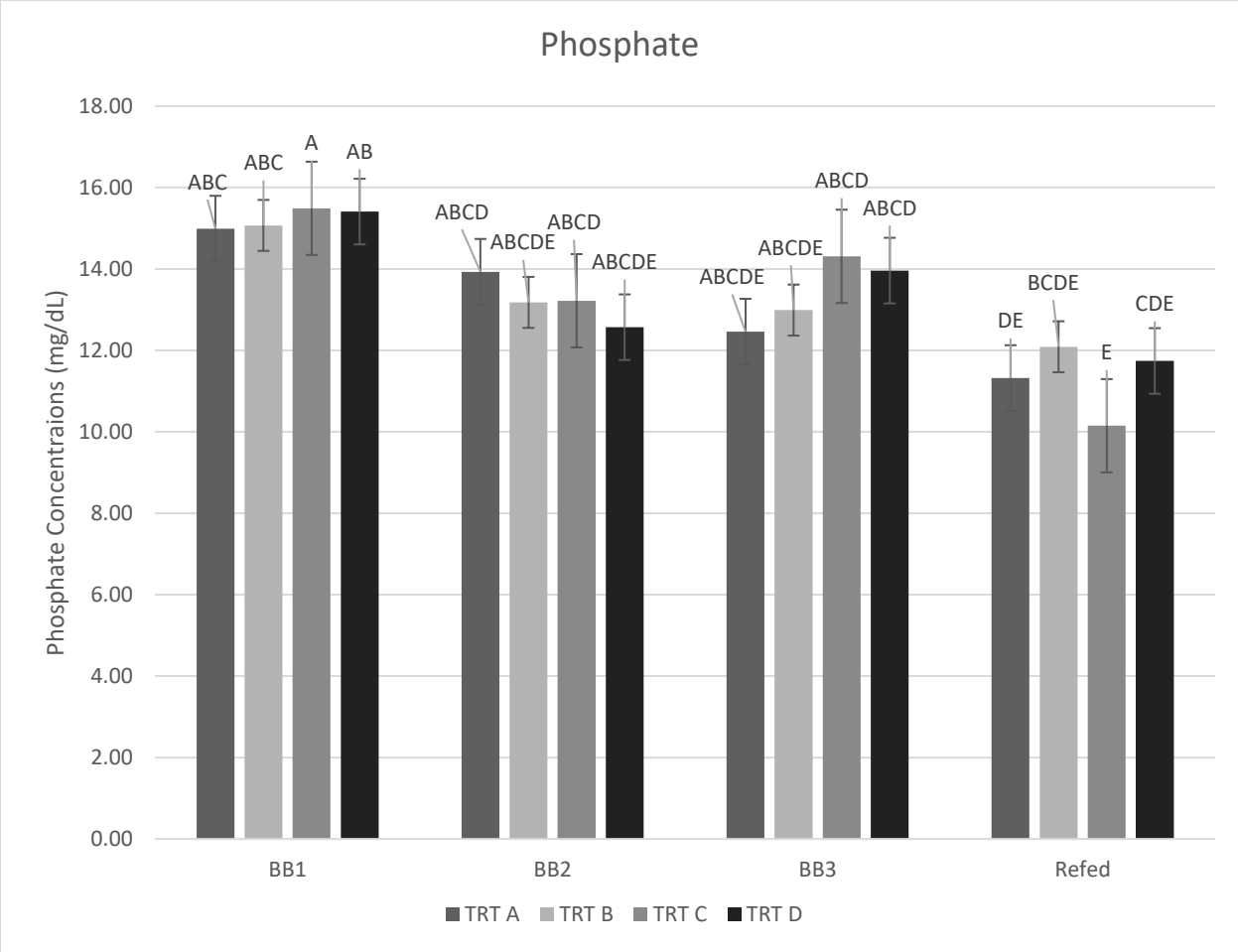

Figure 29. Intertreament phosphate concentration trends.
*Values not followed by same superscripts differ significantly

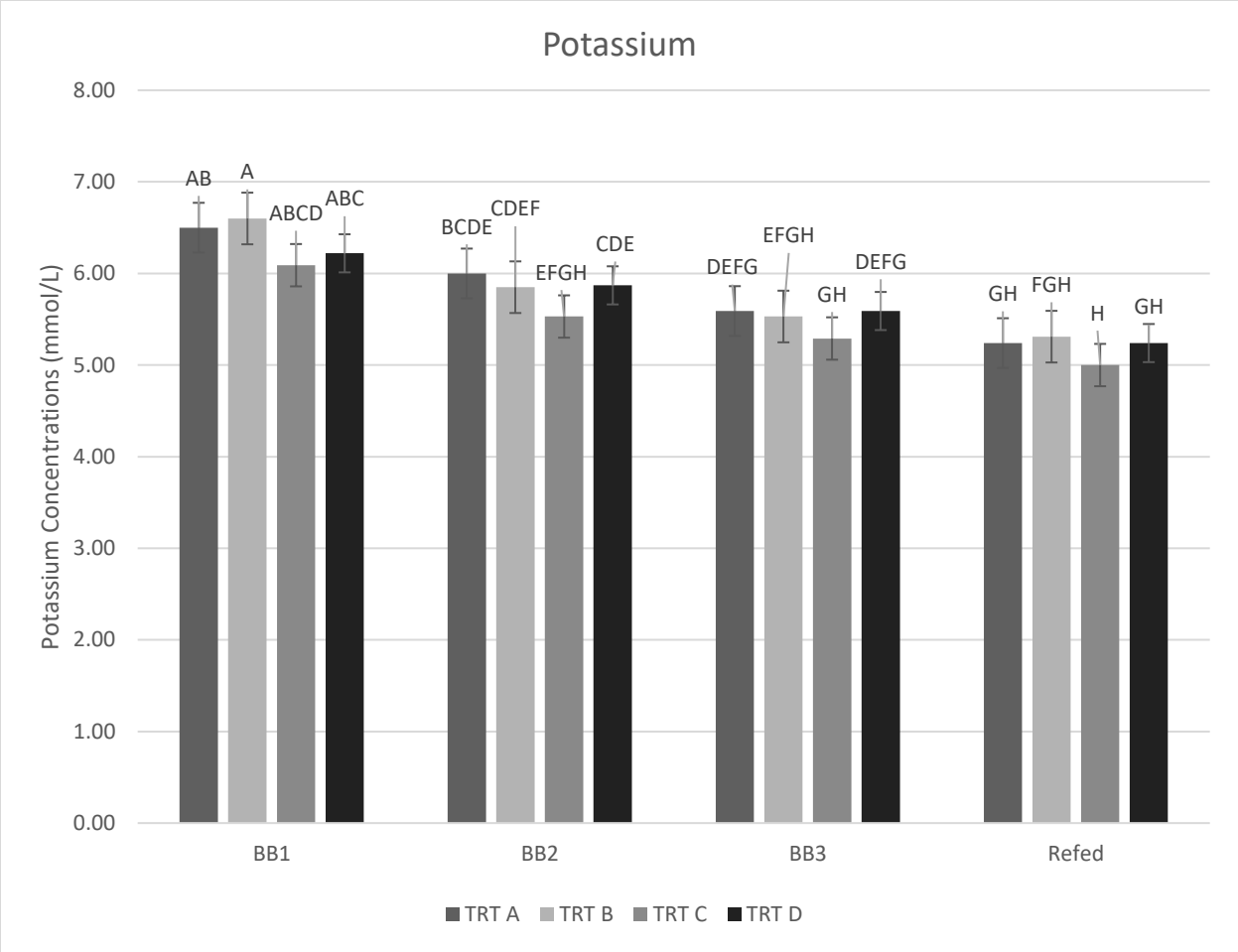

Figure 30. Intertreament potassium concentration trends.
*Values not followed by same superscripts differ significantly

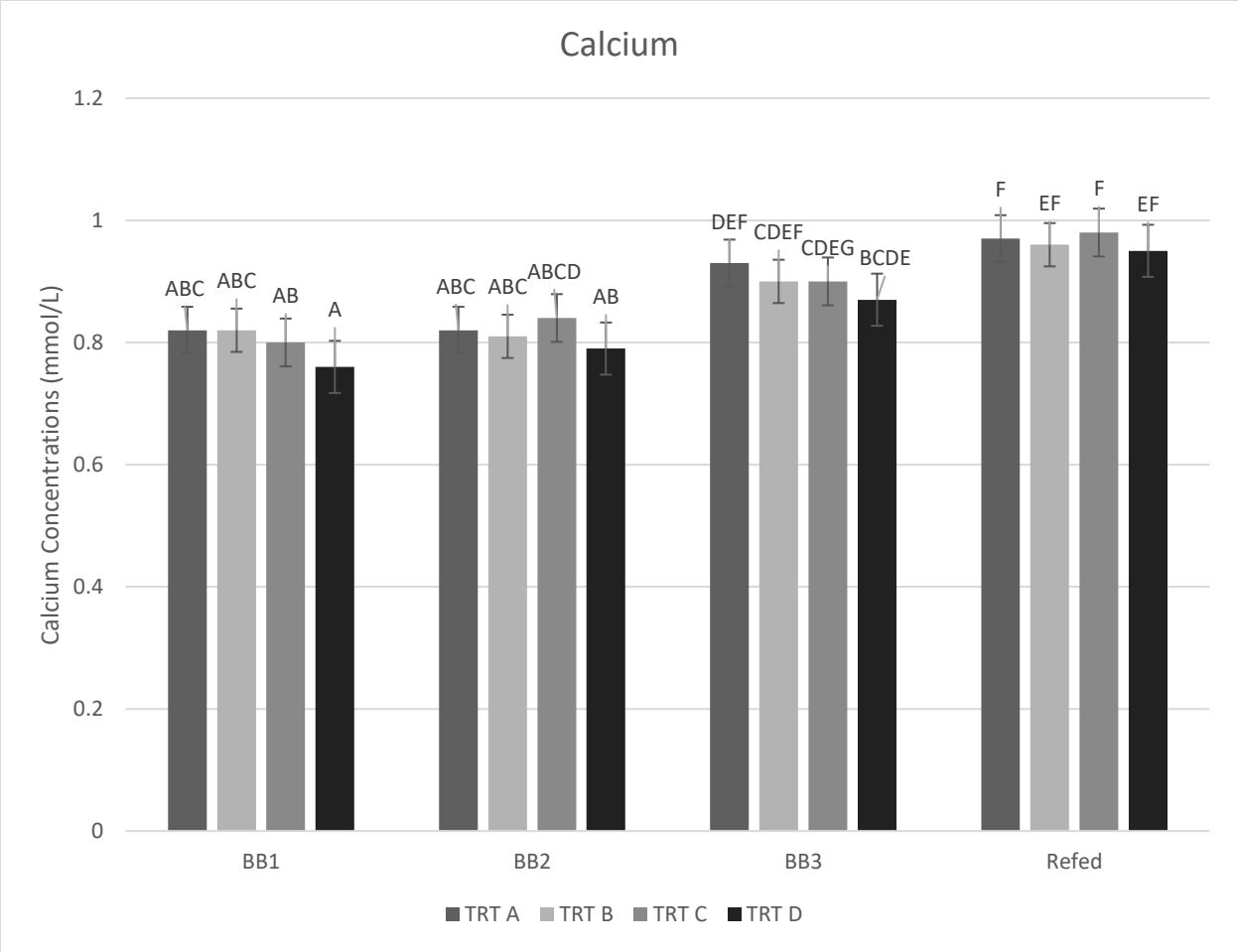

Figure 31. Intertreament calcium concentration trends.
*Values not followed by same superscripts differ significantly

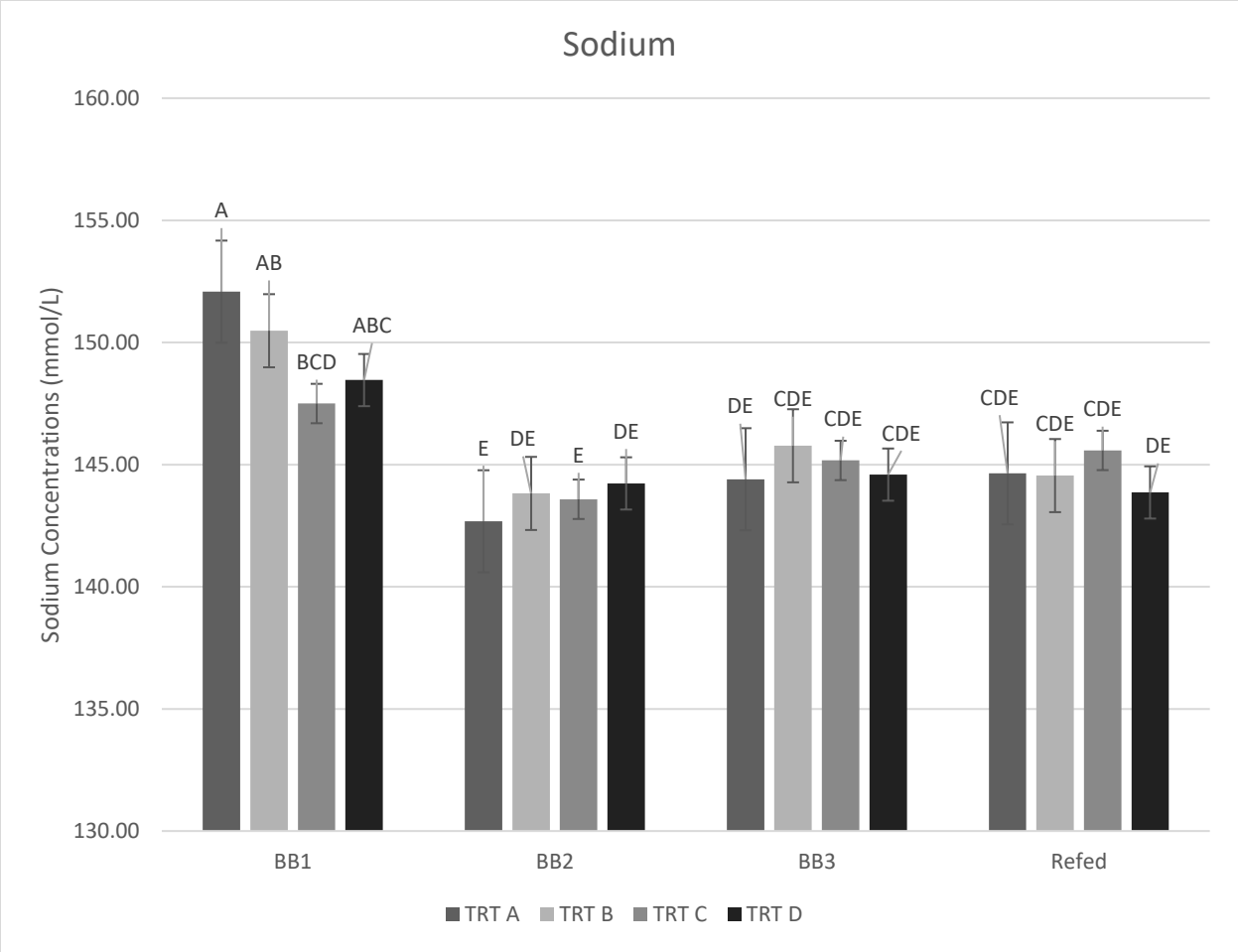

Figure 32. Intertreament sodium concentration trends.
*Values not followed by same superscripts differ significantly

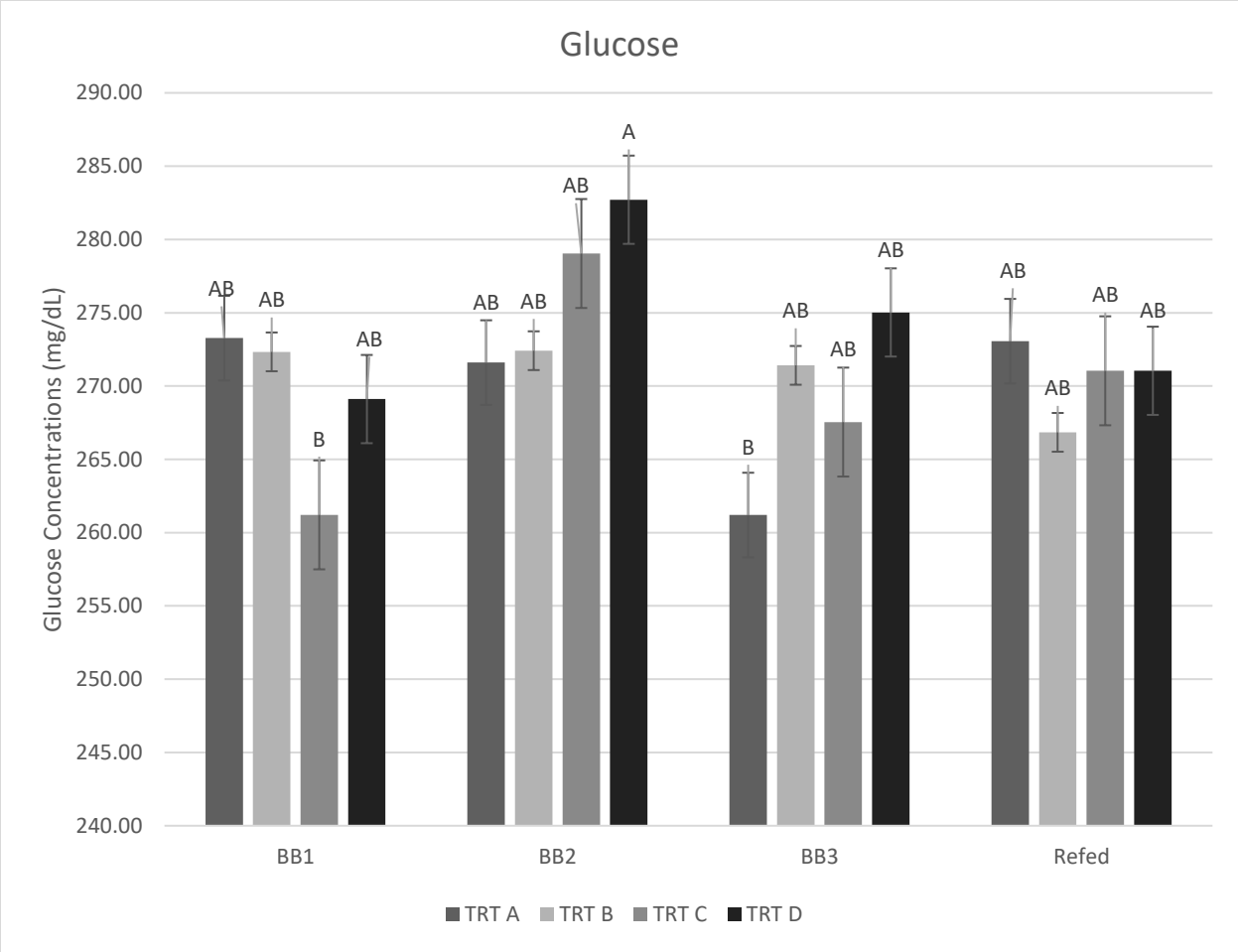

Figure 33. Intertreament glucose concentration trends.
*Values not followed by same superscripts differ significantly

Overall Trends	BB1	BB2	BB3	Refed	F-Value	P-Value
Phosphate (mg/dL)	15.21[a]	13.25[b]	13.42[b]	11.30[c]	21.04	< 0.001
Potassium (mmol/L)	6.35[d]	5.81[e]	5.50[f]	5.20[g]	72.31	< 0.001
Calcium (mmol/L)	0.80[h]	0.81[h]	0.90[k]	0.97[m]	58.84	< 0.001
Sodium (mmol/L)	149.63[n]	143.55[p]	144.97[p]	144.68[p]	43.81	< 0.001
Glucose (mg/dL)	268.87[q]	276.34[r]	268.52[q]	271.87[qr]	3.53	0.02

Table 2. Overall electrolyte and glucose trends for whole breeder flock.
*Values in columns not followed by same superscripts differ significantly

TRT A	BB1	BB2	BB3	Refed	F-Value	P-Value
Phosphate (mg/dL)	14.99[a]	13.93[ab]	12.46[ab]	11.32[b]	4.26	0.01
Potassium (mmol/L)	6.50[c]	6.00[d]	5.59[e]	5.24[e]	27.05	< 0.001
Calcium (mmol/L)	0.82[f]	0.82[f]	0.93[g]	0.97[g]	14.32	< 0.001
Sodium (mmol/L)	152.08[h]	142.68[k]	144.40[k]	144.64[k]	32.06	< 0.001
Glucose (mg/dL)	273.27[m]	271.60[m]	261.20[m]	273.06[m]	2.77	0.05

Table 3. Electrolyte and glucose trends for TRT A breeders.
*Values in columns not followed by same superscripts differ significantly

TRT B	BB1	BB2	BB3	Refed	F-Value	P-Value
Phosphate (mg/dL)	15.07^{a}	13.18^{ab}	12.99^{ab}	12.09^{b}	3.18	0.03
Potassium (mmol/L)	6.60^{c}	5.85^{d}	5.53^{de}	5.31^{e}	27.05	< 0.001
Calcium (mmol/L)	0.82^{f}	0.81^{f}	0.90^{g}	0.96^{g}	11.61	< 0.001
Sodium (mmol/L)	150.48^{h}	143.82^{k}	145.77^{k}	144.55^{k}	11.03	< 0.001
Glucose (mg/dL)	272.33^{m}	272.41^{m}	271.41^{m}	266.84^{m}	0.64	0.59

Table 4. Electrolyte and glucose trends for TRT B breeders.
*Values in columns not followed by same superscripts differ significantly

TRT C	BB1	BB2	BB3	Refed	F-Value	P-Value
Phosphate (mg/dL)	15.49^{a}	13.22^{a}	14.31^{a}	10.15^{b}	11.88	< 0.001
Potassium (mmol/L)	6.09^{c}	5.53^{d}	5.29^{de}	5.00^{e}	17.38	< 0.001
Calcium (mmol/L)	0.80^{f}	0.84^{g}	0.90^{gh}	0.98^{gh}	22.31	< 0.001
Sodium (mmol/L)	147.50^{k}	143.58^{km}	145.17^{km}	145.58^{m}	4.52	0.005
Glucose (mg/dL)	261.21^{n}	279.04^{p}	267.54^{np}	271.04^{np}	3.28	0.03

Table 5. Electrolyte and glucose trends for TRT C breeders.
*Values in columns not followed by same superscripts differ significantly

TRT D	BB1	BB2	BB3	Refed	F-Value	P-Value
Phosphate (mg/dL)	15.41^{a}	12.57^{ab}	13.96^{b}	11.74^{b}	6.42	< 0.001
Potassium (mmol/L)	6.22^{c}	5.87^{cd}	5.59^{de}	5.24^{e}	12.31	< 0.001
Calcium (mmol/L)	0.76^{f}	0.79^{fg}	0.87^{gh}	0.95^{h}	13.15	< 0.001
Sodium (mmol/L)	148.46^{k}	144.23^{m}	144.59^{m}	143.86^{m}	7.24	< 0.001
Glucose (mg/dL)	269.11^{n}	282.71^{n}	275.02^{n}	276.43^{n}	1.75	0.16

Table 6. Electrolyte and glucose trends for TRT D breeders.
*Values in columns not followed by same superscripts differ significantly

Treatment Effect	Phosphate		Potassium		Calcium	
	F-Value	P-value	F-Value	P-value	F-Value	P-value
Room	0.03	0.86	0.13	0.73	1.10	0.30
Feed Regimen	1.16	0.30	1.40	0.25	0.01	0.93
K Supp	3.02	0.10	1.83	0.20	2.04	0.16
Feed Regimen*K Supp	0.34	0.57	0.75	0.40	1.86	0.18

Treatment Effect	Sodium		Glucose	
	F-Value	P-value	F-Value	P-value
Room	0.59	0.45	0.39	0.54
Feed Regimen	0.06	0.81	0.91	0.34
K Supp	1.60	0.21	0.01	0.90
Feed Regimen*K Supp	1.04	0.31	3.04	0.08

Table 7. Factorial analysis for breeder flock for Refed bleed across tested electrolytes and glucose.

References

Abed, J., H. Judeh, E. Abed, M. Kim, H. Arabelo, and R. Gurunathan. 2014. “Fixing a heart”: the game of electrolytes in anorexia nervosa. Nutrition Journal 13:1–5.

Afolayan, M., F. O. Abeke, and A. Atanda. 2016. Ascites versus sudden death syndrome (SDS) in broiler chickens : A review. Journal of Animal Production Research 28:76–87.

Alfonzo, A. V. M., C. Isles, C. Geddes, and C. Deighan. 2006. Potassium disorders—clinical spectrum and emergency management. Resuscitation 70:10–25.

Alonso-Alvarez, C., and M. Ferrer. 2001. A Biochemical Study of Fasting, Subfeeding, and Recovery Processes in Yellow-Legged Gulls. Physiological and Biochemical Zoology: Ecological and Evolutionary Approaches 74:703–713.

As, P. van, M. G. Elferink, A. M. Closter, A. Vereijken, H. Bovenhuis, R. P. M. A. Crooijmans, E. Decuypere, and M. a. M. Groenen. 2010. The use of blood gas parameters to predict ascites susceptibility in juvenile broilers. Poultry Science 89:1684–1691.

Aviagen Breeder Management Handbook. Available at http://en.aviagen.com/brands/ross/ (verified 11 March 2017).

Balog, J. M. 2003. Ascites Syndrome (Pulmonary Hypertension Syndrome) in Broiler Chickens: Are We Seeing the Light at the End of the Tunnel? Avian and Poultry Biology Reviews 14:99–126.

Belo, D. R., and P. S. Rosmos. 1976. Blood metabolites and glucose metabolism in the fed and fasted chicken. The Journal of nutrition 106:1135–43.

Bettinger, J. C., B. Surawicz, J. W. Bryfogle, B. N. Anderson Jr., and S. Bellet. 1956. The effect of intravenous administration of potassium chloride on ectopic rhythms, ectopic beats and disturbances in A-V conduction. The American Journal of Medicine 21:521–533.

Bowes, V. A., and R. J. Julian. 1988. Organ Weights of Normal Broiler Chickens and Those Dying of Sudden Death Syndrome. Can Vet J 29:153–156.

Bowes, V. A., R. J. Julian, S. Leeson, and T. Stirtzinger. 1988. Effect of feed restriction on feed efficiency and incidence of sudden death syndrome in broiler chickens. Poult. Sci. 67:1102–1104.

Bowes, V. A., R. J. Julian, and T. Stirtzinger. 1989. Comparison of serum biochemical profiles of male broilers with female broilers and White Leghorn chickens. Can J Vet Res 53:7–11.

Bustamante, J., and A. Travaini. 1994. Effect of keeping plasma frozen at − 20°C on the concentration of blood metabolites. Comparative Biochemistry and Physiology Part A: Physiology 107:661–664.

Buyse, J., E. Decuypere, V. M. Darras, L. M. Vleurick, E. R. Kuhn, and J. D. Veldhuis. 2000. Food deprivation and feeding of broiler chickens is associated with rapid and interdependent changes in the somatotrophic and thyrotrophic axes. British Poultry Science 41:107–116.

Chamblee, T. N., G. W. Morgan, and C. D. Schultz. 1989. Effect of refeeding following short-term deprivation of feed or water, or both, on selected physiological parameters for broiler chickens. Poult. Sci. 68:1619–1623.

Chung, H. C., W. Guenter, R. G. Rotter, G. H. Crow, and N. E. Stanger. 1993. Effects of dietary fat source on sudden death syndrome and cardiac sarcoplasmic reticular calcium transport in broiler chickens. Poult. Sci. 72:310–316.

Cohen, T. A., R. W. Nelson, P. H. Kass, M. M. Christopher, and E. C. Feldman. 2009. Evaluation of six portable blood glucose meters for measuring blood glucose concentration in dogs. Journal of the American Veterinary Medical Association 235:276–280.

Currie, R. J. W. 1999. Ascites in poultry: Recent investigations. Avian Pathology 28:313–326.

De le Court, C., E. Aguilera, and F. Recio. 1995. Plasma chemistry values of free-living white spoonbills (Platalea leucorodia). Comparative Biochemistry and Physiology Part A: Physiology 112:137–141.

Decuypere, E., J. Buyse, and N. Buys. 2000. Ascites in broiler chickens: exogenous and endogenous structural and functional causal factors. World's Poultry Science Journal 56:367–377.

Dobado-Berrios, P. M., J. L. Tella, O. Ceballos, and J. A. Donázar. 1998. Effects of Age and Captivity on Plasma Chemistry Values of the Egyptian Vulture. The Condor 100:719–725.

Dongilli, R., C. Crivellaro, F. Targa, G. Donazzan, and M. Herrmann. 2016. A Rare Case of Hypokalemia and Hypomagnesemia. Clinical Chemistry 62:436–440.

Druyan, S. 2012. Ascites Syndrome in Broiler Chickens - A Physiological Syndrome Affected by Red Blood Cell.Pages 243–270 in Blood Cell - An Overview of Studies in Hematology. Moschandreou, T., ed. InTech.

Druyan, S., A. Ben-David, and A. Cahaner. 2007a. Development of Ascites-Resistant and Ascites-Susceptible Broiler Lines. Poultry Science 86:811–822.

Druyan, S., and A. Cahaner. 2007. Segregation Among Test-Cross Progeny Suggests That Two Complementary Dominant Genes Explain the Difference Between Ascites-Resistant and Ascites-Susceptible Broiler Lines. Poultry Science 86:2295–2300.

Druyan, S., A. Shlosberg, and A. Cahaner. 2007b. Evaluation of Growth Rate, Body Weight, Heart Rate, and Blood Parameters as Potential Indicators for Selection Against Susceptibility to the Ascites Syndrome in Young Broilers. Poultry Science 86:621–629.

Dunn, J. D., W. W. Benton, E. Orozco-Torrentera, and R. T. Adamson. 2015. The Burden of Hyperkalemia in Patients With Cardiovascular and Renal Disease. The American Journal of Managed Care 21:S307–S315.

E, D., and V. Mw. 1999. Metabolic insights and nutrition of poultry. Tijdschr Diergeneeskd 124:47–51.

Ekmay, R. D., C. Salas, J. England, S. Cerrate, and C. N. Coon. 2012. The effects of pullet body weight, dietary nonpyhtate phosphorus intake, and breeder feeding regimenn on production performance, chick quality, and bone remodeling in broiler breeders. Poultry Science 91:948–964.

Faggioni, M., and B. C. Knollmann. 2015. Arrhythmia Protection in Hypokalemia A Novel Role of Ca2+-Activated K+ Currents in the Ventricle. Circulation 132:1371–1373.

Ferrer, M. 1990. Hematological Studies in Birds. The Condor 92:1085–1086.

Ferrer, M., and P. Dobado-Berrios. 1998. Factors affecting plasma chemistry values of the Spanish Imperial Eagle, Aquila adalberti1. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 120:209–217.

Fiaccadori, E., E. Coffrini, N. Ronda, A. Vezzani, G. Cacciani, C. Fracchia, C. Rampulla, and A. Borghetti. 1990. Hypophosphatemia in Course of Chronic Obstructive Pulmonary Disease: Prevalence, Mechanisms, and Relationships with Skeletal Muscle Phosphorus Content. Chest 97:857–868.

Fisch, C., J. P. Shields, S. A. Ridolfo, and H. Feigenbaum. 1958. Effect of Potassium on Conduction and Ectopic Rhythms in Atrial Fibrillation Treated with Digitalis. Circulation 18:98–106.

Garber, A. K., S. M. Sawyer, N. H. Golden, A. S. Guarda, D. K. Katzman, M. R. Kohn, D. Le Grange, S. Madden, M. Whitelaw, and G. W. Redgrave. 2016. A systematic review of approaches to refeeding in patients with anorexia nervosa. Int. J. Eat. Disord.:1–18.

García-Rodríguez, T., M. Ferrer, J. C. Carrillo, and J. Castroviejo. 1987. Metabolic responses of Buteo buteo to long-term fasting and refeeding. Comparative Biochemistry and Physiology Part A: Physiology 87:381–386.

Gavin, A., and R. M. McDevitt. 1999. Intraspecific variation in muscle and organ growth in three strains of chicken with differential genetic selection for fast growth rate. British Poultry Science 40:19–20.

Gennari, F. J. 1998. Hypokalemia. New England Journal of Medicine 339:451–458.

Gonzales, E., J. Buyse, M. M. Loddi, T. S. Takita, N. Buys, and E. Decuypere. 1998a. Performance, incidence of metabolic disturbances and endocrine variables of food-restricted male broiler chickens. British Poultry Science 39:671–678.

Gonzales, E., J. Buyse, J. R. Sartori, M. M. Loddi, and E. Decuypere. 1999. Metabolic disturbances in male broilers of different strains. 2. Relationship between the thyroid and somatotropic axes with growth rate and mortality. Poultry Science 78:516–521.

Gonzales, E., J. Buyse, T. S. Takita, J. R. Sartori, and E. Decuypere. 1998b. Metabolic disturbances in male broilers of different strains. 1. Performance, mortality, and right ventricular hypertrophy. Poultry Science 77:1646–1653.

Goodwin, M. L., and L. C. Wit. 2012. Biomedical Physiology. 1st ed. Kendall Hunt Publishing.

Grandin, T., and M. J. Deesing. 2013. Genetics and the Behavior of Domestic Animals. Academic Press.

Greenlees, K. J., P. Eyre, J. C. Lee, and C. T. Larsen. 1989. Effect of age and growth rate on myocardial irritability in broiler chickens. Proc. Soc. Exp. Biol. Med. 190:282–285.

Gregory, M. K., M. S. Geier, R. A. Gibson, and M. J. James. 2014. Effect of dietary canola oil on long-chain omega-3 fatty acid content in broiler hearts. Journal of Animal Physiology & Animal Nutrition 98:235–238.

Hall, J. E. 2015. Guyton and Hall Textbook of Medical Physiology. 13th ed. W.B. Saunders Company.

Hassanzadeh, M. 2010. Endogenous and environmental factors interactions that contribute to the development of ascites in broiler chickens: a review. International Journal of Veterinary Research 4:117–126.

Havenstein, G. B., P. R. Ferket, J. R. Grimes, M. A. Qureshi, and K. E. Nestor. 2004. Changes in the performance of turkeys - 1966 to 2003. Proceedings of the 27th Technical Turkey Conference:11–18.

Havenstein, G. B., P. R. Ferket, and M. A. Qureshi. 2003a. Carcass composition and yield of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets. Poultry Science 82:1509–1518.

Havenstein, G. B., P. R. Ferket, and M. A. Qureshi. 2003b. Growth, livability, and feed conversion of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets. Poultry Science 82:1500–1508.

Hocking, P. M., M. H. Maxwell, and M. A. Mitchell. 1993. Welfare assessment of broiler breeder and layer females subjected to food restriction and limited access to water during rearing. British Poultry Science 34:443–458.

Hollmén, T., J. C. Franson, M. Hario, S. Sankari, M. Kilpi, and K. Lindström. 2001. Use of Serum Biochemistry to Evaluate Nutritional Status and Health of Incubating Common Eiders (Somateria mollissima) in Finland. Physiological and Biochemical Zoology 74:333–342.

Hopkinson, W. I. 1991. Reproduction of the sudden death syndrome of broiler breeders: A relative potassium imbalance. Avian Pathology 20:403–408.

Hopkinson, W. I., D. Jessop, D. A. Pass, and D. W. Pethick. 1990. Concentrations of plasma potassium and sodium during the life of a broiler breeder flock. Avian Pathology 19:607–611.

Hopkinson, W. I., W. Williams, G. L. Griffiths, D. Jessop, and S. M. Peters. 1984. Dietary Induction of Sudden Death Syndrome in Broiler Breeders. Avian Diseases 28:352–357.

Huchzermeyer, F. W. 2012. Broiler ascites: a review of the ascites work done at the poultry section of the Onderstepoort Veterinary Institute 1981-1990. World's Poultry Science Journal 68:41–50.

Imaeda, N. 2000. Characterization of lactic acid formation and adenosine triphosphate consumption in calcium-loaded erythrocytes of broiler chickens. Poultry Science 79:1543–1547.

International Symposium on Turkey Diseases, H. M. Hafez, and Deutsche Veterinärmedizinische Gesellschaft (Eds). 2004. 5th International Symposium on Turkey Diseases: Berlin, 16 - 19 June 2004. DVG-Service-GmbH, Giessen.

Jacob, J. P., R. Blair, and E. E. Gardiner. 1990. Effect of dietary lactate and glucose on the incidence of sudden death syndrome in male broiler chickens. Poult. Sci. 69:1529–1532.

Jeffrey, D. A., D. B. Peakall, D. S. Miller, and G. R. Herzberg. 1985. Blood chemistry changes in food-deprived herring gulls. Comparative Biochemistry and Physiology Part A: Physiology 81:911–913.

Jenni-Eiermann, S., and L. Jenni. 1998. What can plasma metabolites tell us about the metabolism, physiological state and condition of individual birds? An overview. Biol Cons Fauna 102:312–319.

Julian, R. J. 1986. The Effect of Increased Mineral Concentrations in the Feed on Leg Weakness and Sudden Death Syndrome in Broiler Chickens. Can Vet J 27:157–160.

Julian, R. J. 1993. Ascites in poultry. Avian Pathology 22:419–454.

Julian, R. J. 1998. Rapid growth problems: ascites and skeletal deformities in broilers. Poultry Science 77:1773–1780.

Julian, R. J., G. W. Friars, H. French, and M. Quinton. 1987. The Relationship of Right Ventricular Hypertrophy, Right Ventricular Failure, and Ascites to Weight Gain in Broiler and Roaster Chickens. Avian Diseases 31:130–135.

Julian, R. J., and E. J. Squires. 1995. Suggestions for reducing ascites in meat-type chickens. Proceedings of 44th Western Poultry Disease Conference 44:19–20.

Kalmar, I. D., D. Vanrompay, and G. P. J. Janssens. 2013. Broiler ascites syndrome: Collateral damage from efficient feed to meat conversion. The Veterinary Journal 197:169–174.

Kempe, K., D. Price, J. Ellison, J. Marhoul, L. Morrow, K. Win, K. Kuschma, and M. Hompesch. 2009. Capillary and Venous Blood Glucose Concentrations Measured During Intravenous Insulin and Glucose Infusion: A Comparison of Steady and Dynamic States. Diabetes Technology & Therapeutics 11:669–674.

Khardori, R. 2005. Editorial Refeeding Syndrome and Hypophosphatemia. J Intensive Care Med 20:174–175.

Kittelsen, K. E., E. G. Granquist, Ø. Kolbjørnsen, O. Nafstad, and R. O. Moe. 2015. A comparison of post-mortem findings in broilers dead-on-farm and broilers dead-on-arrival at the abattoir. Poultry Science 94:2622–2629.

Korbonits, M., D. Blaine, M. Elia, and J. Powell-Tuck. 2007. Metabolic and hormonal changes during the refeeding period of prolonged fasting. Eur J Endocrinol 157:157–166.

Korte, M., A. Sgoifo, W. Ruesink, C. Kwakernaak, S. van Voorst, C. W. Scheele, and H. J. Blokhuis. 1999. High carbon dioxide tension (PCO2) and the incidence of cardiac arrhythmias in rapidly growing broiler chickens. Veterinary Record 145:40–43.

Kumar, P. G. R., M. L. Satyanarayana, and N. Chandrashekhara. 2016. Epidemiology, gross and histopathology of sudden death syndrome in broiler chickens. Journal of Cell and Tissue Research 16:5367–5371.

Lambers, W. M., B. Kraaijenbrink, and C. E. H. Siegert. 2015. The refeeding syndrome. Ned Tijdschr Geneeskd 159:A8610.

Laragh, J. H., and J. E. Sealey. 2001. K+ Depletion and the Progression of Hypertensive Disease or Heart Failure The Pathogenic Role of Diuretic-Induced Aldosterone Secretion. Hypertension 37:806–810.

Leeson, S., and J. D. Summers. 2000. Broiler Breeder Production. University Books.

Lieske, C. L., M. H. Ziccardi, J. A. K. Mazet, S. H. Newman, and I. A. Gardner. 2002. Evaluation of 4 Handheld Blood Glucose Monitors for Use in Seabird Rehabilitation. Journal of Avian Medicine and Surgery 16:277–285.

Machado, J. D. C., V. M. M. Suen, F. B. Chueire, J. F. M. Marchini, and J. S. Marchini. 2009. Refeeding syndrome, an undiagnosed and forgotten potentially fatal condition. BMJ Case Reports 8:521.

Maltsev, V. A., and E. G. Lakatta. 2007. Normal heart rhythm is initiated and regulated by an intracellular Calcium clock within pacemaker cells. Heart Lung Circ 16:335–348.

Marinella, M. A. 2005. Refeeding Syndrome and Hypophosphatemia. J Intensive Care Med 20:155–159.

Mirsalimi, S. M., and R. J. Julian. 1993. Effect of excess sodium bicarbonate on the blood volume and erythrocyte deformability of broiler chickens. Avian Pathology 22:495–507.

Moghadam, H. K., I. Mcmillan, J. R. Chambers, R. J. Julian, and C. C. Tranchant. 2005. Heritability of sudden death syndrome and its associated correlations to ascites and body weight in broilers. British Poultry Science 46:54–57.

Mollison, B., W. Guenter, and B. R. Boycott. 1984. Abdominal fat deposition and sudden death syndrome in broilers: the effects of restricted intake, early life caloric (fat) restriction, and calorie: protein ratio. Poult. Sci. 63:1190–1200.

Nain, S., B. Laarveld, C. Wojnarowicz, and A. A. Olkowski. 2007. Excessive dietary vitamin D supplementation as a risk factor for sudden death syndrome in fast growing commercial broilers. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 148:828–833.

Nazifi, S., N. Mosleh, H. Nili, M. A. Lari, and G. H. Poorghanbari. 2011. Diurnal variations in serum biochemical parameters and their correlations with thyroid hormones in ostrich chicks (Struthio camelus). Comp Clin Pathol 21:1669–1675.

Nestor, K. E., Y. M. Saif, D. A. Emmerson, and N. B. Anthony. 1995. The Influence of Genetic Changes in Body Weight, Egg Production, and Body Conformation on Organ Growth of Turkeys. Poultry Science 74:601–611.

Newberry, R. C., E. E. Gardiner, and J. R. Hunt. 1987. Behavior of Chickens Prior to Death from Sudden Death Syndrome. Poultry Science 66:1446–1450.

Nir, I., Z. Nitsan, Y. Dror, and N. Shapira. 1978. Influence of overfeeding on growth, obesity and intestinal tract in young chicks of light and heavy breeds. British Journal of Nutrition 39:27–35.

North, M. O., and D. D. Bell. 1990. Commercial chicken production manual. 4th ed. Chapman and Hall.

O'Connor, G., and D. Nicholls. 2013. Refeeding Hypophosphatemia in Adolescents With Anorexia Nervosa: A Systematic Review. Nutrition in Clinical Practice 28:358–364.

Olkowski, A. A. 2007. Pathophysiology of Heart Failure in Broiler Chickens: Structural, Biochemical, and Molecular Characteristics. Poultry Science 86:999–1005.

Olkowski, A. A., and H. L. Classen. 1997. Malignant ventricular dysrhythmia in broiler chickens dying of sudden death syndrome. Veterinary Record 140:177–179.

Olkowski, A. A., and H. L. Classen. 1998a. High Incidence of Cardiac Arrhythmias in Broiler Chickens. Journal of Veterinary Medicine Series A 45:83–91.

Olkowski, A. A., and H. L. Classen. 1998b. Progressive bradycardia, a possible factor in the pathogenesis of ascites in fast growing broiler chickens raised at low altitude. British Poultry Science 39:139–146.

Olkowski, A. A., H. L. Classen, C. Riddell, and C. D. Bennett. 1997. A Study of Electrocardiographic Patterns in a Population of Commercial Broiler Chickens. Vet Res Commun 21:51–62.

Olkowski, A. A., C. Wojnarowicz, S. Nain, B. Ling, J. M. Alcorn, and B. Laarveld. 2008. A study on pathogenesis of sudden death syndrome in broiler chickens. Research in Veterinary Science 85:131–140.

Ononiwu, J. C., R. G. Thomson, H. C. Carlson, and R. J. Julian. 1979. Pathological Studies of "Sudden Death Syndrome" in Broiler Chickens. Can Vet J 20:70–73.

Osadchii, O. E. 2010. Mechanisms of hypokalemia-induced ventricular arrhythmogenicity. Fundamental & Clinical Pharmacology 24:547–559.

Owen, J. C., M. K. Sogge, and M. D. Kern. 2005. Habitat and sex differences in physiological condition of breeding southwestern willow flycatchers (empidonax traillliextimus). The Auk 122:1261–1270.

Packer, M. 1990. Potential role of potassium as a determinant of morbidity and mortality in patients with systemic hypertension and congestive heart failure. The American Journal of Cardiology 65:E45–E51.

Pass, D. A. 1983. A cardiomyopathy ("sudden death syndrome") of adult hens. Avian Pathology 12:363–369.

Pérez-Rodríguez, L., C. Alonso-Alvarez, M. Martínez-Haro, and J. Viñuela. 2007. Variation in plasma biochemical parameters in captive adult red-legged partridges (Alectoris rufa) during daylight hours. Eur J Wildl Res 54:21–26.

Raj, K. S., C. Keane-Miller, and N. H. Golden. 2012. Hypomagnesemia in Adolescents With Eating Disorders Hospitalized for Medical Instability. Nutr Clin Pract 27:689–694.

Rajman, M., M. Juráni, D. Lamošová, M. Máčajová, M. Sedlačková, Ľ. Košťál, D. Ježová, and P. Výboh. 2006. The effects of feed restriction on plasma biochemistry in growing meat type chickens (Gallus gallus). Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 145:363–371.

Ricklefs, R. E. 1985. Modification of Growth and Development of Muscles of Poultry. Poultry Science 64:1563–1576.

Robinson, F. E., N. A. Robinson, and T. A. Scott. 1991. Reproductive performance, growth rate and body composition of full-fed versus feed-restricted broiler breeder hens. Can. J. Anim. Sci. 71:549–556.

Rodríguez, P., F. S. Tortosa, and C. Gortázar. 2006. Daily variations of blood biochemical parameters in the red-legged partridge (Alectoris rufa). Eur J Wildl Res 52:277–281.

Rohrer, S., and J. W. Dietrich. 2014. Refeeding syndrome: a review of the literature. Z Gastroenterol 52:593–600.

Ross, J. G., G. Christie, W. G. Halliday, and R. M. Jones. 1976. Determination of haematology and blood chemistry values in healthy six-week old broiler hybrids. Avian Pathology 5:273–281.

Ross, J. G., G. Christie, W. G. Halliday, and R. M. Jones. 1978. Haematological and blood chemistry "comparison values" for clinical pathology in poultry. Vet. Rec. 102:29–31.

Rosso, R., J. M. Kalman, O. Rogowski, S. Diamant, A. Birger, S. Biner, B. Belhassen, and S. Viskin. 2007. Calcium channel blockers and beta-blockers versus beta-blockers alone for preventing exercise-induced arrhythmias in catecholaminergic polymorphic ventricular tachycardia. Heart Rhythm 4:1149–1154.

Scheele, C. W. 1997. Pathological changes in metabolism of poultry related to increasing production concentrations. Veterinary Quarterly 19:127–130.

Scheele, C. W., W. De Wit, M. T. Frankenhuis, and P. F. G. Vereijken. 1991. Ascites in Broilers.: 1. Experimental Factors Evoking Symptoms Related to Ascites. Poultry Science 70:1069–1083.

Scheideler, S. E., D. V. Rives, J. D. Garlich, and P. R. Ferket. 1995. Dietary calcium and phosphorus effects on broiler performance and the incidence of sudden death syndrome mortality. Poult. Sci. 74:2011–2018.

Shimizu, K., H. Ogura, M. Wasa, T. Hirose, T. Shimazu, H. Nagasaka, and K. Hirano. 2014. Refractory hypoglycemia and subsequent cardiogenic shock in starvation and refeeding: Report of three cases. Nutrition 30:1090–1092.

Simon J, and Leclercq B. 1982. Longitudinal study of adiposity in chickens selected for high or low abdominal fat content: further evidence of a glucose-insulin imbalance in the fat line. J Nutr 112:1961–1973.

Skipper, A. 2012. Refeeding Syndrome or Refeeding Hypophosphatemia A Systematic Review of Cases. Nutr Clin Pract 27:34–40.

Sturkie, P. D. 1976. Avian Physiology. 3rd ed. Springer Verlag.

Thandroyen, F. T., A. C. Morris, H. K. Hagler, B. Ziman, L. Pai, J. T. Willerson, and L. M. Buja. 1991. Intracellular calcium transients and arrhythmia in isolated heart cells. Circ Res 69:810–819.

Tona, K., B. Kemps, V. Bruggeman, F. Bamelis, L. D. Smit, O. Onagbesan, J. D. Baerdemaeker, and E. Decuypere. 2005. Comparison of three lines of broiler breeders differing in ascites susceptibility or growth rate. 1. Relationship between acoustic resonance data and embryonic or hatching parameters. Poultry Science 84:1439–1445.

Totzke, U., M. Fenske, O. Hüppop, H. Raabe, and N. Schach. 1999. The Influence of Fasting on Blood and Plasma Composition of Herring Gulls (Larus argentatus). Physiological and Biochemical Zoology 72:426–437.

Unwin, R. J., F. C. Luft, and D. G. Shirley. 2011. Pathophysiology and management of hypokalemia: a clinical perspective. Nature Reviews Nephrology 7:75–84.

Weitgasser, R., A. M. Davalli, and G. C. Weir. 1999. Measurement of glucose concentrations in rats: differences between glucose meter and plasma laboratory results. Diabetologia 42:256–256.

Whittow, G. C. 2000. Sturkie's Avian Physiology. 5th ed. Academic Press.

Wideman, R. F., D. D. Rhoads, G. F. Erf, and N. B. Anthony. 2013. Pulmonary arterial hypertension (ascites syndrome) in broilers: A review. Poultry Science 92:64–83.

Yu, M. W., F. E. Robinson, R. G. Charles, and R. Weingardt. 1992. Effect of Feed Allowance During Rearing and Breeding on Female Broiler Breeders. 2. Ovarian Morphology and Production. Poultry Science 71:1750–1761.

Zhang, W., C. He, J. Qiao, S. S. Dong, S. W. Li, and W. M. Zhang. 2008. Differences in Ventricular Vulnerability, Serum Enzyme Activities, and Electrolyte Concentrations Between Male and Female Broilers. Poultry Science 87:1402–1406.

Appendix 1

Eton Bioscience Glucose Assay Kit 1

Reagent Preparation

1. Glucose Standard
    a. Glucose standards: vial contains 500µL of 800µM glucose standard. Standard must be equilibrated to room temperature.
    b. Dilute 500µL of 800µM glucose standard with 500µL of dH2O to prepare a 400µM glucose standard.
        i. 1mL of diluted standard is enough to make 3 standard curves if assayed in duplicate.
        ii. Store at -80°C when not in use

2. Glucose Assay Solution
    a. Solution contains light sensitive enzymes so it must be stored in dark environments.
    b. Solution must be thawed on ice before use and must be used all at once to prevent thawing and freezing cycles.
        i. Freeze and store assay solution and any aliquots at -80°C

Sample Preparation

1. Serum, plasma, other body fluid, or cell supernatant can be measured directly by a series of dilutions of the sample to ensure readings are within the standard curve.
    a. Dilute samples with dH20
2. Add 50µL of samples to each well of a 96-well microplate. Assay samples in duplicate.

Standard Curve Preparation

1. Assay all standard curves in duplicate. Run a standard curve in each assay.
2. Add 50µL, 40µL, 30µL, 20µL, 10µL, 5µL 1µL, and 0µL of diluted glucose standard to each well. Then, adjust each volume to 50µL/cell with dH2O.

Performing the Assay

1. Add 50µL of glucose assay solution to each well containing glucose standards and test samples.
2. Incubate for 15min at 37°C incubator. *Note: Do not use CO2 incubator
3. Stop reaction by adding 50µL of 0.5M acetic acid per well followed by brief gentle agitation. *Note: Eliminate any air bubbles.
4. Measure absorbance at 490nm using microplate reader.

Calculation

1. Average the OD490nm values of replicate wells of each Glucose standard, test samples, and blank.
    a. To get corrected absorbance, subtract the average OD490nm value of the blank from the averaged OD490nm values from all standards and samples.
2. Make standard curve by plotting OD490nm values from each glucose standards as a function of glucose concentration. This can be done with Excel. Calculate the concentration of glucose in samples using the equation obtained from the linear regression of the standard curve

$$\text{Glucose } (\mu M) = [(\text{Corrected absorbance})-(\text{y-intercept})]/\text{Slope}$$

Appendix 2

Quantichrom Phosphate Assay Kit

Reagent Preparation

1. Store all contents at 4°C.
2. Bring reagent, phosphate standard (0.28mg/dL), and blank control to room temperature before use.

Procedure using a 96-well plate

1. Set up standrds and samples by transferring 50µL distilled water (Blank control), standard, and samples in duplicate wells of a clear bottom 96-well plate.
    a. Dilute sample if necessary.
2. Add 100µL of Reagent and tap lightly to mix.
3. Incubate 30min at room temperature and read optical density at 620nm (600-660nm)

Calculation

1. Phosphate concentration of sample is calculated with the following equation:

$$= [(ODsample - Odblank) / (ODstandard - ODblank)] \times 0.28 \text{ (mg/dL)}$$

2. ODblank, ODsample, and ODstandard are OD620nm values of blank, sample, and standard respectively

Appendix 3

Breeder Grower Diets

Breeder Grower Control	lbs/ton	Diet Percentage
Corn	1131	56.55
SBM	259	12.95
Wheat Midds	500	25.00
Salt	7	0.35
Dical Phosphate	30	1.50
Limestone	26	1.30
DL Methionine	2	0.10
AU Vitamin Premix[1]	1	0.05
Choline	1	0.05
AU Trace Mineral[2] Premix	2	0.10
Poultry Oil	41	2.05

Breeder Grower Supplement	lbs/ton	Diet Percentage
Corn	1122	56.10
SBM	259	12.95
Wheat Midds	500	25.00
Salt	7	0.35
Dical Phosphate	30	1.50
Limestone	26	1.30
DL Methionine	2	0.10
AU Vitamin Premix[1]	1	0.05
Choline	1	0.05
AU Trace Mineral[2] Premix	2	0.10
Poultry Oil	41	2.05
Potassium Chloride	9.3	0.47

1. Vitamin Premix includes per kg diet: Vitamin A, (Vitamin A acetate) 8,000 IU; Vitamin D (cholecalciferol), 2,000 IU; Vitamin E (DL-alpha tocopherol acetate), 8 IU; menadione (menadione sodium bisulfate complex), 2mg; Vitamin B12 (cyanocobalamin), 0.02mg; folacin (folic acid), 0.5mg; D-pantothenic acid (calcium pantothenate), 15mg; riboflavin (riboflavin), 5.4mg; niacin (niacinamide), 45mg; thiamin (thiamin mononitrate), 1mg; D-biotin (biotin), 0.05mg; and pyridoxine (pyridoxine hydrochloride), 2.2mg; choline (choline chloride), 500mg
2. Mineral Premix includes per kg diet: Mn (magnesium sulfate), 120mg; Zn (zinc sulfate), 100mg; Fe (iron sulfate monohydrate), 30mg; Cu (tri-basic copper chloride), 8mg; I (ethlyenediamine dyhydriodide), 1.4mg; Se (sodium selenite), 0.3mg

Appendix 4

Breeder Diets (Post 5% Production)

Breeder Control	lbs/ton	Diet Percentage
Corn	1363	68.15
SBM	451	22.55
Salt	9	0.45
Dical Phosphate	31	1.55
Limestone	140	7.00
DL Methionine	2	0.10
AU Vitamin Premix[1]	1	0.05
Choline	1	0.05
AU Trace Mineral Premix[2]	2	0.10
Poultry Oil	0	0

Breeder Supplement	lbs/ton	Diet Percentage
Corn	1359	67.95
SBM	451	22.55
Salt	9	0.45
Dical Phosphate	31	1.55
Limestone	137	6.85
DL Methionine	2	0.10
AU Vitamin Premix[1]	1	0.05
Choline	1	0.05
AU Trace Mineral Premix[2]	2	0.10
Poultry Oil	1	0.05
Potassium Chloride	5	0.25

1. Vitamin Premix includes per kg diet: Vitamin A, (Vitamin A acetate) 8,000 IU; Vitamin D (cholecalciferol), 2,000 IU; Vitamin E (DL-alpha tocopherol acetate), 8 IU; menadione (menadione sodium bisulfate complex), 2mg; Vitamin B12 (cyanocobalamin), 0.02mg; folacin (folic acid), 0.5mg; D-pantothenic acid (calcium pantothenate), 15mg; riboflavin (riboflavin), 5.4mg; niacin (niacinamide), 45mg; thiamin (thiamin mononitrate), 1mg; D-biotin (biotin), 0.05mg; and pyridoxine (pyridoxine hydrochloride), 2.2mg; choline (choline chloride), 500mg
2. Mineral Premix includes per kg diet: Mn (magnesium sulfate), 120mg; Zn (zinc sulfate), 100mg; Fe (iron sulfate monohydrate), 30mg; Cu (tri-basic copper chloride), 8mg; I (ethlyenediamine dyhydriodide), 1.4mg; Se (sodium selenite), 0.3mg