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The Incidence of Plexiform Lesion Formation in Lines Divergently Selected for Ascites

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ABSTRACT

Complex Vascular Lesions, (CVL) are a common vascular change associated with idiopathic pulmonary arterial hypertension (IPAH). CVL have been characterized in studies using animal models involving, but not limited to, Sprague-Dawley rats and poultry (Abe et al., 2010; Wideman et al., 2011). IPAH has been documented in poultry although no connection has been made with CVLs. The current study characterizes CVL in the lungs from broilers derived from lines divergently selected for 15 generations for ascites susceptibility under conditions of simulated high altitude. The ascites RES and SUS lines were sampled for CVL incidence over time. Both lines were reared in a common environment and provided feed and water *ad libitum*. At ages 2, 4, 6, 8, 10, and 12 weeks post hatch, five males and five females from each line were sampled and lung tissue fixed, sectioned and stained for microscopy. Lung sections were then scored for CVL incidence by two independent researchers. CVL data were analyzed based on age post-hatch, gender, line, body weight, lung volume and CVL location in the lung. Findings indicate the SUS line exhibited a generally higher CVL incidence than the RES line at all ages when reared at local altitude. Also the RES line had a greater lung volume to body weight ratio, throughout the latter part of the study (56 ≥ days of age). This may suggest that improved lung volume to body weight ratio, may result in a decrease in CVL incidence. These broiler lines are potentially valuable in future animal model research.

This thesis is approved for recommendation
to the Graduate Council.

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INTRODUCTION

The term idiopathic pulmonary arterial hypertension (IPAH) represents a complex group of pulmonary vasculature disorders (Humbert et al., 2004). Symptoms of IPAH include sustained pulmonary vasoconstriction (Reeves et al., 1986), progressive fixed vascular remodeling (Reeves et al., 1986), elevated pulmonary vasculature resistance (Wideman et al., 2011), elevated pulmonary arterial pressure (Wideman et al., 2011), and formation of complex vascular lesions (CVLs) (Wideman et al., 2011). Currently, the cause of IPAH is not understood.

Plexiform lesions or CVLs are often found in lung tissue of human patients whose deaths are attributable to IPAH. Pathological evaluations often indicated that early pulmonary vascular remodeling progressively evolves into the obstructive vascular pathology observed in patients confirmed to have advanced IPAH (Wideman et al., 2011). Plexiform lesions in broilers form immediately downstream from branching points in small muscular pre- and intra-acinar arteries (Wideman et al., 2011). Plexiform lesions are typically spherical or ovoid in shape (Wideman et al., 2011) and around 120 to 200 µm in diameter (Wideman et al., 2011). They are typically found in medial and posterior inter-rib lung divisions (Wideman et al., 2011).

Plexiform lesions have been described as dynamic angiogenic lesions driven by disorded or neoplastic-like endothelial proliferation and myofibroblast infiltration (Tuder et al., 2001). Thrombi and platelet aggregates are commonly found within established plexiform lesions, an anticipated response to endothelial changes that create thrombogenic surfaces (Hoeper et al., 2006). Inflammatory cells consisting of macrophages, T and B cells (mononuclear cells) infiltrate the perivascular region of affected pulmonary arterioles and plexiform lesions (Nicolls et al., 2005). Dorfmüller et al., 2003, concluded that inflammatory mechanisms can play an important role in the pathogenesis or progression of PAH as well as the development of CVLs. Thus, learning more about CVLs will contribute to our understanding of IPAH overall.

IPAH may also be referred to as “pulmonary hypertension syndrome”, which is frequently used interchangeably with “ascites syndrome” (Wideman, 2000). Ascites syndrome in broilers is a cascade of events that result in cardiac anomalies including an enlarged, flaccid heart and right ventricular

hypertrophy, as well as an accumulation of fluid in the abdominal cavity (Balog, 2003). Attempts to better understand the pathogenesis of obstructive vascular pathology in humans with severe IPAH have been hampered by a deficiency of animal models in which plexiform lesions develop spontaneously (Wideman et al., 2011). Breeding broilers for rapid growth leads to high oxygen demand, and thus a high cardiac output, which increases pulmonary arterial pressure and the workload of the heart (Balog, 2003). These factors result in pulmonary hypertension and increased vascular pressure, which is the most common pathology associated with ascites syndrome in broilers (Balog, 2003). The University of Arkansas currently has two lines of broilers that have been divergently selected for IPAH susceptibility. When exposed to hypobaric hypoxia, the susceptible line (SUS) exhibits 98% ascites mortality over six weeks, compared to the resistant line (RES) at roughly 6% ascites mortality over the same time span (Anthony, Unpublished Results). This divergence in ascites mortality has occurred through the use of hypobaric hypoxia to induce ascites mortality over 15 generations (Anthony, Unpublished Results). Wideman (2011) found spontaneous CVL formation in lung tissue of SUS floor-raised broilers. CVL formation is thought to be due to SUS broilers exhibiting pulmonary arterial pressures consistent with pulmonary hypertension (Wideman et al., 2007). RES line broilers should exhibit lower pulmonary arterial pressures, due to breeding for ascites (IPAH) resistance. Using these two lines, it was proposed to study the incidence of CVL formation over time, with CVL formation being expected more frequently in the SUS line.

PROBLEM STATEMENT

IPAH is a poorly understood vascular disease, which occurs in the absence of identifiable pathogens. CVL's are considered a hallmark of IPAH occurrence. The ascites RES and SUS lines represent valuable research populations for IPAH study, as they too exhibit CVLs. This project seeks to expand the current knowledge of IPAH and develop a reliable animal model for future IPAH research.

STATEMENT OF PURPOSE

Currently, knowledge of IPAH is limited due to a lack of available lung tissue from human patients and a lack of animal models. This study aims to establish the ascites lines as an animal model for future CVL research. The primary hypothesis is that the increased susceptibility to IPAH exhibited by the SUS

line will lead to a significantly greater incidence of lesion formation in comparison with the RES line. The primary objective is to add to current knowledge available on CVL formation.

RESEARCH QUESTIONS

1. Is there a significant correlation between IPAH and CVL formation?
2. Do significant differences in CVL formation exist between ascites SUS and RES lines?
3. What age is most highly associated with CVL incidence?
4. Is there a significant correlation between gender and CVL incidence?
5. Is there a significant relationship between lung volume and CVL incidence?
6. Is there significant interaction between Age, Line or Gender in relation to CVL formation, e.g., A*L, A*G, G*L, or A*G*L.
7. Is there a significant difference in the ratio of lung volume to body weight between the RES and SUS lines?
8. Is there a significant difference between the body weights of broilers from the SUS and RES line when raised at local altitude?

KEY TERMS

1. Ascites syndrome – Ascites syndrome in broilers is a cascade of events that result in cardiac abnormalities including an enlarged, flaccid heart, and right ventricular hypertrophy, as well as an accumulation of fluid in the abdominal cavity (Balog, 2003).
2. Foam-Type Macrophage – A phagocytic immune cell found in the body (Oxford American, 2010). Referred to as foam-type due to appearance under a microscope when present in a CVL.
3. Hypoxia – The physical condition in which the demand for oxygen exceeds an animal's cardiopulmonary capacity (Balog, 2003).
4. Idiopathic – Arising spontaneously or from an obscure or unknown cause (Wideman et al., 2011).

5. Idiopathic pulmonary arterial hypertension – A disease of unknown cause characterized by elevated pulmonary vascular resistance and pulmonary arterial pressure in the absence of other apparent disease (Wideman, et al., 2011).
6. Pulmonary arterial pressure – A measure of the blood pressure found in the pulmonary artery. It is approximately equal to the cardiac output multiplied by the pulmonary vascular resistance (Wideman et al., 2011)
7. Pulmonary arterial hypertension - The physical condition in which pulmonary wedge pressures of ≤15 mmHg are coupled with PAP ≥25 mmHg are specifically attributable to elevated arteriole (precapillary) resistance (Wideman et al., 2011).
8. Pulmonary vascular resistance – The physical condition of increased resistance to blood flow through the pulmonary vasculature (Wideman et al., 2011).
9. Vascular – Of or relating to blood vessels related to the circulatory system (Balog, 2003).
10. Ventricular Hypertrophy – Enlargement of the muscles in the heart ventricles in response to increased workload (Balog, 2003).

ASSUMPTIONS

1. Plexiform lesions arise spontaneously in broilers of the SUS line.
2. Plexiform lesions may arise spontaneously in broilers of the RES line.
3. Plexiform lesions will arise spontaneously through traditional rearing practices without any additional stimulus known to promote CVL formation; (hypoxia, cold stress, microparticle injection etc.)
4. SUS broilers will express pulmonary arterial pressures consistent with severe pulmonary hypertension throughout the experiment when reared at local alttitude.
5. Gender has no effect on the incidence of plexiform lesion formation. Based on findings from Wideman and coworkers, 2011.
6. Dietary regiment is sufficient to promote rapid growth and thus hypoxia needed to induce CVL formation.
7. There is no blocking effect, as broilers were raised in a common environment, through mixed-line, mixed gender brooding with a set airflow and temperature.

8. CVL formation is significantly greater in the SUS line, when compared to CVL formation in the RES line.
9. Long term divergent selection for ascites has affected the incidence of CVL formation.
10. SUS broilers represent an ideal animal model to study the formation of CVLs for use in human health care.
11. Previous studies indicated greatest CVL formation in medial inter-rib lung divisions, thus medial inter-rib lung divisions were selected for slide creation and data collection.
12. Lesions will be identified by the presence of foam-type macrophages (referred to as foam-type due to appearance under magnification).
13. Lesions will be found using the second of the three slides created from each lung lobe, as it represents the central cross section. The other slides will be utilized if further confirmation of a suspected lesion is needed.

LIMITATIONS

1. The technique used for the fixation of lung tissue for slide creation is a complicated and time-consuming process thus limiting the sample to 10 broilers from each line (5 males, 5 females) for each age.
2. Due to the volume of lung tissue collected and the possible number of slides that could have been obtained from the collected tissue, medial inter-rib divisions were selected for slide creation and data collection.
3. Lesions were only determined by two independent investigators due to the time and training involved in scanning lung sections for CVLs.
4. No previous work had been done with the RES line to determine if and how CVLs may arise in the RES line.
5. Due to the limited nature of CVL study prior to this study, it is not known with certainty whether CVLs are a cause of pulmonary hypertension or a symptom thereof. CVLs are potentially unrelated to pulmonary hypertension.

REVIEW OF THE LITERATURE

Idiopathic pulmonary arterial hypertension (IPAH) is a multi-faceted vascular disease that occurs in the absence of identifiable pathogens. IPAH may also be referred to as “pulmonary hypertension syndrome”, which is frequently used interchangeably with “ascites syndrome” (Wideman, 2000). Ascites syndrome in broilers is a cascade of events that result in cardiac abnormalities including an enlarged, flaccid heart and right ventricular hypertrophy, as well as an accumulation of fluid in the abdominal cavity (Balog, 2003).

Plexiform lesions or CVL are often found in lung tissue of human patients whose deaths are attributable to IPAH. Pathological evaluations traditionally indicated that early pulmonary vascular remodeling progressively evolves into the obstructive vascular pathology observed in patients confirmed to have advanced IPAH (Wideman et al., 2011). The University of Arkansas currently has two lines of broilers that have been divergently selected for IPAH susceptibility. When exposed to hypobaric hypoxia the susceptible line (SUS) exhibits 98% ascites mortality over six weeks, compared to the resistant line (RES) at roughly 6% ascites mortality over the same time (Anthony, Unpublished Results). These differences in susceptibility made the divergent lines the logical model to characterize the development of CVL in association with IPAH.

Ascites syndrome (AS) is an often-fatal cascade of events that affects meat-type poultry (Balog, 2003). Any event, which results in increased oxygen requirements can be viewed as a trigger of AS. Ascites mortality can range from 0 to 30% in broiler flocks (Pavlidis et al., 2007). Ascites has been characterized as a metabolic disease with affected broilers exhibiting an enlarged flaccid heart, variable liver changes, and accumulation of fluid in the abdominal cavity (Riddell, 1991). Pavlidis et al., (2007) concluded that ascites is an economically important disease to the broiler industry, as it affects aspects of both live production and processing sectors. Ascites is a multifactorial disease relating to both physiology and environment, and both must be closely monitored to manage the incidence of AS in commercial flocks.

ENVIRONMENTAL TRIGGERS OF ASCITES

ALTITUDE

High altitude has long been considered the primary environmental factor in broilers exhibiting ascites syndrome (Balog, 2003). Julian (2000) notes that, "at sea level, oxygen makes up 20.9% of the atmosphere. The equivalent percentage of oxygen drops approximately 1.0% for every 500 m rise in altitude." This is critical as oxygen availability has often been viewed as the most important trigger of AS in broilers (Balog, 2003; Julian, 2000). The lack of oxygen places a strain on a broiler's cardio-pulmonary system to meet the high O_2 demands associated with rapid growth (Balog, 2003). This is consistent with findings indicating that hypoxia, when a broiler's demand for oxygen exceeds its cardiopulmonary capacity, and the following increase in cardiac output, is a primary cause of AS (Julian, 1987,1988).

Ascites has been effectively studied through the use of a hypobaric chamber to simulate high altitude conditions (Owen et al., 1990, Witzel et al., 1990; Owen et al., 1995a,b; Balog et al., 2000a,b; Balog et al., 2001; Anthony et al., 2001, Pavlidis et al., 2002, 2007). By simulating high altitude a strain can be placed on a broiler's cardio-pulmonary system to supply the necessary oxygen, due to the decrease in environmental oxygen availability. In order to meet O_2 demand, broilers increase red blood cell production, resulting in increased hematocrit levels (Julian, 2000). As demand continues to outpace supply, blood begins increasing in viscosity due to the buildup of RBCs and hematocrit. Increased hematocrit and thus increased oxygen carrying capacity should be beneficial when dealing with hypoxia, but studies have shown the increased blood viscosity associated with increased hematocrit is detrimental to the right ventricle and accelerates complications associated with pulmonary hypertension (Shlosberg et al., 1998).

Ascites at local altitude is a common broiler production related disease (Julian, 2000). Oxygen hemoglobin saturation studies have shown that fast-growing broilers have lower oxygen saturation than slow-growing broilers (Reeves et al., 1991; Julian and Mirsalimi, 1992). Low arterial blood oxygen levels, presumably due to hypoxia, at local altitude (20.9% oxygen) are found in some broiler lines (Reeves et al., 1991; Julian and Mirsalimi 1992). Low oxygenation levels may result from increased blood flow through the capillary bed of the lung (Wideman and Kirby, 1995a,b). This would result in hemoglobin having insufficient time to become fully oxygenated (Wideman and Kirby, 1995a,b).

Inadequate oxygen levels result in increased synthesis of erythropoietin, which accelerates erythrocyte and hemoglobin production, to help meet oxygen demands (Balog, 2003). Increased erythrocyte levels leads to a concomitant increase in hematocrit and blood viscosity (Balog, 2003). Julian (2000) notes, that research into AS has indicated polycythemia, increasing blood viscosity, as a primary cause of AS. Thus explaining the direct correlation between AS and increased elevation.

TEMPERATURE

Cold exposure (60-65º F) has been noted as an important factor resulting in AS in poultry production (Julian et al., 1989; Wideman and Tackett, 2000b). A broiler's ability to respond to cold temperatures requires an increase in metabolic rate to maintain body temperature (Julian, 2000). Cold stressed birds respond to rising oxygen demand by increasing blood oxygen carrying capacity. As described previously, inadequate oxygen levels results in erythropoietin production, couples with erythrocyte and hemoglobin production to help meet oxygen demand (Balog, 2003). This in turn leads to polycythemia, increasing blood viscosity through the increase in percentage of red blood cells, which has been cited as primary cause of AS (Julian, 2000).

Cold temperatures have been utilized as a testing variable in several studies to determine ascites susceptibility (Lubritz et al., 1995; Moghadam et al., 2001; Wideman and French, 1999; Druyan et al., 2007a). The presence of cold temperatures can thus be considered an AS-inducing condition (AIC) (Druyan et al., 2007a). Druyan et al. (2007a) reported 44% mortality in broilers reared using his cold stress method. Broilers were reared in individual cages starting at 19 days post-hatch and exposed to air movements of 3 m/s in addition to an ambient temperature ranging from 18 to 20º C. The caged environment prevented broilers from escaping the AIC environment by obtaining warmth via huddling and warmth provided by microbe fermentation in the litter. The 44% mortality was consistent with findings reported in six commercial lines reared in a hypobaric chamber by Cisar et al. (2003). It was lower, however, than the 66% in a commercial sire line reared in the same hypobaric chamber of a simulated altitude of 2,896 m (Anthony and Balog, 2003). Wideman and Kirby (1995 a,b) reported a 70% AS incidence induced by the pulmonary artery clamp method. This method, while effective, is time consuming and requires considerable surgical skill to implement, making it poorly feasible for industry implementation.

Heat stress is another environmental factor to consider in relation to AS. Heat stress increases the body's need for metabolic oxygen, but at the same time results in decreased growth and activity. This decrease in growth and activity stabilizes oxygen requirements and fails to promote AS.

PHYSIOLOGICAL TRIGGERS OF ASCITES

Ascites, as it is seen in most broiler flocks today, is a product of genetic selection for increased growth. Scheele et al. (1991) theorize that the modern broiler is reaching its metabolic limit for growth in relation to O_2 consumption. This selection was carried out without a concomitant increase in a bird's cardio-pulmonary system (Havenstein et al., 1994). Groves (1997) explains that this selection practice has resulted in broiler populations having the potential to outgrow their cardio-pulmonary capacity.

GENETIC SELECTION

Inadequate oxygen supply (hypoxia) is primarily related to rapid growth and high metabolism (Balog, 2003). This lack of supply is due to little selection pressure being placed on lung and internal organ growth, to match the intense selection pressure for body weight and breast weight (Julian, 2000; Balog 2003). Avian lungs make up a lower percentage of body weight, than mammals and an even lower percentage of body weight in broilers (Julian, 2000). In fact, lungs represent a lower percentage of body weight in broilers than in Leghorns (Julian, 2000). This deviation is accentuated during the broiler growth phase with the ratio between lung volume/body weights declining with age (Julian, 1989).

Pavlidis et al. (2007) described the process of divergent selection for ascites from a line representative of commercial broiler populations available in 1995. Broilers placed in a hypobaric chamber experienced a decreased oxygen partial pressure similar to those observed at an altitude of 2896 m. This decreased partial pressure of oxygen, led to a significant increase in mortality due to ascites. These mortality data were then used to make divergent selections of future breeders.

Divergent selection in simulated high altitude conditions resulted in an increase of ascites mortality of 0.95% per generation in the SUS line (Pavlidis et al., 2007). Ascites mortality decreased by 6.75% in the RES line, and 3.14% in the relaxed line per generation (Pavlidis et al., 2007). This divergence led to ascites mortality extremes of 95.14% in the SUS line and 7.14% in the RES line (Pavlidis et al., 2007). As mortality incidence diverged, so did the livability of the ascites lines. The

livability of the RES line increased by 11.5 days, while SUS birds experienced a decrease of 8 days over 10 generations (Pavlidis et al., 2007). This correlation is to be expected, because as ascites susceptibility increases, early onset ascites mortality becomes more frequent.

Genetic selection for growth may have led to an increase in ascites incidence, but it may also lead to a potential end of AS in the industry. Balog (2003) concluded that breeding ascites RES broilers and identifying ascites susceptibility markers represent the best options for dealing with AS.

BLOOD

Several studies have been done comparing healthy birds to birds suffering from ascites (Shlosberg et al., 1996; Kirby et al., 1997; Olkowoski et al., 1997, 2005; Wideman et al., 1998; de Greef et al., 2001; Luger et al., 2001). In several of these studies, the makeup of a broiler's blood proved to be a significant physiological factor in the development of ascites. While the blood has not been utilized as an early indicator of AS, as of yet, it may prove valuable in AS management programs.

VISCOSITY

Blood viscosity is considered a primary factor in the development of AS at altitude (Julian, 2000). Blood viscosity increases as a direct result of increased oxygen demand by the broiler, otherwise known as hypoxia. As hypoxia worsens, the bird responds by increasing hematocrit levels. This is accomplished by the increased synthesis of erythropoietin, which accelerates erythrocyte and hemoglobin production, to help meet oxygen demands (Balog, 2003). As birds develop AS they begin to exhibit significantly higher hematocrit levels than clinically healthy broilers (Druyan et al., 2007a). While increased oxygen carrying capacity should alleviate AS symptoms, birds dying of AS all show increased hematocrit levels (Shlosberg et al., 1998). This is due to increased hematocrit levels contributing to increased viscosity and increased vascular resistance, thus increasing the right ventricular workload. As the right ventricle begins to hypertrophy, blood viscosity continues to increase to help meet oxygen demand. Unfortunately this only aids in the continued hypertrophy of the right ventricle leading to right ventricular failure and eventual death. Selection against high hematocrit levels may be useful in developing AS resistance (Shlosberg et al., 1996). Selection may be difficult to implement, as birds fail to show increased hematocrit levels until the onset of AS. Still selection for lower hematocrit levels offers a possible solution to AS occurrence.

ERYTHROCYTE SIZE AND DEFORMABILITY

Avian lungs lack the ability to expand as in humans, due to their location along the dorsal ridge of the bird and the lack of a diaphragm to expand capacity. Furthermore, they also lack the ability to use under-perfused capillaries as in humans. This makes the avian lung unable to accommodate increased blood flow, more viscous blood or non-deformable blood cells (Julian, 2000). Compounding this problem is the processing age of broilers, typically 42 days. Erythrocytes survive approximately 40 days and are larger at an early age (Julian, 2000). Birds dealing with AS, therefore are dealing primarily with larger erythrocytes. As avian erythrocytes are nucleated, this decreases their deformability. Reduced deformability leads to more sheer stress in the small capillaries, as large, less deformable erythrocytes try to pass through a narrow cross-section. It has been proposed that this stress helps to induce the formation of plexiform lesions, which are hallmarks of pulmonary hypertension.

OXYGEN AFFINITY

Modern layers have a 33% smaller lung volume/body weight ratio and a 28% thicker blood gas tissue barrier than the red jungle fowl (Vidyadaran et al., 1987,1990). By extrapolation broilers with a similar morphology, but much greater body weight will also exhibit a poor lung volume/body weight ratio. This decrease of space has been shown to be a major cause of AS in broilers (Wideman and Tackett, 2000b). It has been shown to lead to a 25% reduction in diffusing capacity of oxygen in the gas tissue barrier per unit of body weight (Vidyadaran et al., 1990). As hypoxemia worsens, cardiac output increases resulting in an inadequate time for oxygen to attach to hemoglobin. Thus even clinically healthy broilers may be unable to fully oxygenate their blood, guaranteeing broilers dealing with AS are unable to cope with oxygen demands.

PULMONARY HYPERTENSION

Pulmonary hypertension is the most common cause of ascites in broilers, so much so that "pulmonary hypertension syndrome" is often referred to as "ascites syndrome" (Wideman, 2000). The primary cause of PH is a lack of available oxygen. As hypoxemia sets in the broiler compensates by increasing its cardiac output and increasing the oxygen carrying capacity of the blood. Unfortunately, the broiler pulmonary vascular system is minimally compliant to increased blood flow. As the blood flow rate increases through a fixed cross-sectional area, the pulmonary arterial pressure increases accordingly.

In response to the excessive resistance to flow, the right ventricle of the heart has to work harder to pump blood at a higher pressure. The heart doesn't respond by increasing the heart rate, but rather by increasing the stroke volume. To increase the pumping pressure, the heart begins to add muscle, a process known as hypertrophy. As the right ventricle grows in size due to hypertrophy, it begins to diminish the effectiveness of the right atrio-ventricular valve. Valvular failure results in backflow to the right atrium and further expansion of the right ventricle. The right ventricle continues to hypertrophy in response to its diminished efficiency, leading to eventual right ventricular failure (RVF). RVF leads to systemic circulatory congestion, which reaches into the liver resulting in edema, and fluid accumulation throughout the abdominal cavity. Fluid accumulation in the abdominal cavity results in what is commonly referred to as "water-belly" and is considered the hallmark of ascites syndrome.

LUNGS

Avian lungs have a relatively fixed volume and are incapable of dealing with increases in cardiac output. This is due to the rigidity of the lung, as it is located adjacent to the vertebral column, with 25% of the lung tissue being encased between five ribs (Wideman, 2000). The lungs being non-inflating lead to an inability to deal with hemodynamic demands (Wideman, 2001). The pulmonary vasculature of birds has been shown to be engorged with blood at normal cardiac output (Wideman, 2001). Therefore at sea level altitude birds are able to adequately deal with oxygen demands, and it is when the bird is challenged that problems begin to arise.

Avian species show an increase in oxygen consumption in response to exercise, cool temperatures, hypoxemia and feeding (Wideman, 1999). These are common factors faced by broilers in production and ascites testing. To deal with the lack of oxygen caused by these factors birds reflexively increase their cardiac output and thus the blood flow rate through the pulmonary vasculature. This lack of oxygen is shown through the presence of cyanosis. The onset of cyanosis has been shown to be caused by a diffusion inequality, due to the rapid flow of blood (Reeves et al., 1991; Peacock et al., 1990; Peacock et al., 1989; Wideman and Tackett, 2000a,b; Wideman and Kirby, 1995a,b; Wideman et al., 1996a,b; Fedde et al., 1998). Cyanosis is sign that a healthy bird is developing ascites and chronic PH (Reeves et al., 1991; Wideman et al., 1998; Peacock et al., 1990; Peacock et al., 1989; Wideman and Kirby, 1995a,b; Julian and Mirsalimi, 1992).

DIFFUSION LIMITATION

Diffusion limitation has been brought about by domestication of the modern broiler. Modern layers have a 33% smaller lung volume/body weight ratio and a 28% thicker blood gas tissue barrier than the red jungle fowl (Vidyadaran et al., 1987;1990). This has been expounded upon through studies done by Wideman and Bottje (1993) showing that domestic fowl have a poor gas exchange diffusion capacity due to multiple factors such as thicker blood-gas barrier and a decreased gas exchange surface area (Wideman, 2000). Diffusion limitation was confirmed as the cause of hypoxemia when 100% oxygen inhalation was shown to reverse systemic arterial hypoxemia in cold-exposed and pre-ascitic broilers (Wideman and Tackett, 2000a,b).

HEART

The normal avian heart has a thick-walled left ventricle and a thin-walled right ventricle, which is similar to the mammalian heart (Balog, 2003). Therefore the heart weight reflects the work done by the left ventricle to provide the necessary cardiac output in order to meet metabolic oxygen demand (Wideman, 2000). However, as oxygen demand and thus cardiac output increases it is the right ventricle that responds to the increased workload imposed by the marginally compliant pulmonary vasculature. This increased workload leads to right ventricular hypertrophy, which is considered an early indicator of the onset of pulmonary hypertension. Understanding how the heart responds to pulmonary hypertension is important to understanding how pulmonary hypertension works as a whole.

As stated previously, the normal avian heart has a thick-walled left ventricle and a thin-walled right ventricle (Balog, 2003). The right atrioventricular valve is also unique in that it is made up of muscle fibers from the right ventricle (Balog, 2003). This means that any change in the right ventricle will be reflected in the muscle fibers of the right atrioventricular valve. Therefore right ventricular hypertrophy in response to increased oxygen demand results in significant changes to the heart anatomy.

As selection has resulted in broilers that consistently exceed their cardio-pulmonary capacity, the heart is challenged to pump a steadily increasing cardiac output (Wideman, 2000). Wideman (2000) defines cardiac output as, “the volume of blood pumped by one ventricle per minute, and is the product of heart rate and stroke volume”. In other terms, CO = HR * SV. As broilers’ pulmonary vasculature system is relatively non-compliant, increases in cardiac output must cause increased blood flow through the lung

(Wideman, 2001). This results in increased work by the right ventricle and increased pulmonary arterial pressure, as birds lack the mechanisms found in mammals for reducing pulmonary vasculature resistance (Wideman, 2000). Furthermore, heart rate has been shown to decline with growth, indicating that increased cardiac output is caused by increased stroke volume associated with right ventricle hypertrophy (Wideman, 2001). Wideman et al., (1999) theorized that increased pulmonary vascular resistance challenges the ability of the right ventricle to propel venous blood through the lungs. To propel venous blood through the lungs, the right ventricle must develop a pressure sufficient to overcome pulmonary vasculature resistance (Wideman, 2000). Pulmonary hypertension is a consequence of this increased arteriole resistance (Forman and Wideman, 2000; Chapman and Wideman, 2001). This was confirmed through the measurement of pulmonary wedge pressures, which are an averaged value of the pulmonary capillary, venous and left atrial hydrostatic pressures (Chapman and Wideman, 2001). Confirmation of arteriole resistance as a primary factor leading to pulmonary hypertension was obtained, as low wedge pressures cannot exist with elevated downstream resistance associated with left ventricle or mitral valve insufficiency (Dawson and Linehan, 1997; Chapman and Wideman, 2001). The presence of low wedge pressures conclusively showed that pulmonary hypertension is a consequence of elevated arterial resistance.

In order to examine how the right ventricle responds to increasing workload, researchers began looking at the ratio between a broilers' right ventricular weight and its total ventricular weight. The RV:TV ratio is considered a reliable standard as it takes into account differences in cardiac output in relation to environment and body weight. As hypoxemia increases, the right ventricle hypertrophies in order to meet increased oxygen demand, while the left ventricle remains relatively normal. This was demonstrated by the fact that left ventricle mass is shown to be very similar in clinically healthy and ascitic broilers (Wideman et al., 1998; Wideman and French, 1999,2000). Elevated RV:TV ratios have been clearly shown to support the idea that right ventricular work and pulmonary hypertension are key in ascites progression (Wideman, 2000). Clinically healthy broilers exhibit RV:TV ratios ranging from 0.15 to 0.27, while pulmonary hypertension results in RV:TV ratios of 0.28 and above (Cueva et al., 1974; Huchzermeyer and DeRuyck, 1986; Julian, 1993; Odom, 1993; Wideman and Bottje, 1993; Hernandez, 1987; Lubritz et al., 1995; Burton and Smith, 1967; Burton et al., 1968; Peacock et al., 1989; Wideman

and Tackett, 2000a). As the right ventricle continues to hypertrophy, it begins to reduce the effectiveness of the right ventricular valve leading to valvular failure and ascites (Julian, 1987).

PLEXIFORM LESIONS

In 1973, the World Health Organization held a meeting of pathologists, physiologist and cardiologists to expand the understanding of primary pulmonary hypertension (Hatano and Strasser, 1975). The meeting declared "plexogenic pulmonary arteriopathy" or Plexiform lesions to be a hallmark of primary pulmonary hypertension (Wagenvoort and Wagenvoort, 1977, Loyd et al., 1988, Wagenvoort, 1989). This opinion has been confirmed by several researchers (Harris and Heath, 1986). Unfortunately, it is not known whether plexiform lesions are a cause or effect of pulmonary hypertension (Abe et al., 2010). It is for this reason that continued research into the relationship between plexiform lesions and pulmonary hypertension is necessary.

LOCATION

Plexiform lesions have been observed in primary plexogenic pulmonary arteriopathy (PPPA), in addition to secondary pulmonary hypertension associated with congential cardiac malformations and in rare instance cases of hepatic cirrhosis (Jamison and Michel, 1995). These conditions are frequently observed in broilers dealing with AS. Thus, broilers are ideal candidates with which to examine plexiform lesions, also known as complex vascular lesions (CVL) and associated pathogenesis. In broilers as in humans, CVL form in pulmonary arteries (Wagenvoort and Wagenvoort, 1970; Ferencz and Greco, 1970; Harris and Heath, 1986; Yaginuma et al., 1990; Ogata and Iijima, 1993). The lesion typically develops as a "eurysmal dilation of a muscular pulmonary artery branch" around 50 to 300 micrometers in diameter (Tuder et al., 1994). This artery has been referred to as a supernumerary artery and arises at a right angle to the parent vessel (Fishman, 2000). The location can be specifically subdivided into intra-acinar or pre-acinar. Intra-acinar lesions being observed near pulmonary arterial branches paired with muscular bronchioles or pre-acinar lesions being found in muscular arteries unpaired with airways or near a respiratory bronchiole (Jamison and Michel, 1995). In human patients with PPPA, 67% of the lesions were intra-acinar, as compared to 34% pre-acinar lesions (Jamison and Michel, 1995). Despite the location CVL still exhibit the same characteristic formation.

STRUCTURE

CVL are easily identified due to their glomoid structure and the inner core being made up of hyperchromatic, ovoid cells (Pietra et al., 2004; Meyrick, 2001). CVL have often been referenced as plexiform lesions, as the proliferative cellular endothelial tissue often assumes the form of a plexus (Heath and Edwards, 1958). Furthermore, intimal proliferation and capillary channels are characteristic of CVL (Heath and Edwards, 1958; Wagenvoort, 1959). The plexiform lesion is also considered dynamic in that early lesions are cellular, but become increasingly fibrotic with maturity (Wagenvoort and Wagenvoort, 1977). This fibrosis also arises in plexiform lesions in supernumerary arteries, and is referred to as concentric laminar intimal fibrosis (CLIF), (Fishman, 2000). These traits are considered hallmarks of the plexiform lesion or CVL.

PATHOGENESIS

It has yet to be conclusively proven whether CVL are a cause or effect of pulmonary hypertension, but a high PAP and pulmonary vasculature resistance has been shown to be crucial in CVL formation. In human cases of CVL development, patients exhibited such a high, fixed pulmonary vasculature resistance that cardiac output is reduced (Edwards, 1957). These cases make up approximately 28-80% of idiopathic pulmonary hypertension (Wagenvoort and Wagenvoort, 1970; Bjornsson and Edwards, 1985; Pietra and Ruttner, 1987; Yamaki and Wagenvoort, 1985). Patients with plexogenic pulmonary hypertension have increased pulmonary arterial pressures, lower cardiac indices and clinically deteriorate more rapidly than patients with pulmonary hypertension associated with thrombotic lesions (Palevsky et al., 1989).

CVL are thought to be a response to vascular injury caused by flow turbulence, high pressure, and intense vasoconstriction (Yamaki and Wagenvoort, 1985; Wagenvoort and Wagenvoort, 1970; Naeye and Vennart, 1959). This theory has been supported in recent work done by Abe et al. (2010) using Sprague-Dawley rats exposed to hypoxia. Abe et al. (2010) theorized that sustained vasoconstriction and progressive fixed vascular remodeling were major factors in CVL formation. Rat studies showed RV:TV ratios indicative of right ventricle hypertrophy (0.74 ± 0.04), which indicates that vasoconstriction, and increased pulmonary arterial pressures were present (Abe et al., 2010). However, lesions were found after hypoxia had ceased, which suggests that increased blood pressure may be required for CVL

development and CVLs may be an effect of pulmonary hypertension as opposed to a cause of pulmonary hypertension (Abe et al., 2010). In spite of these results, more research will be necessary to determine whether CVL are a cause or effect of idiopathic pulmonary arterial hypertension.

IPAH as it occurs in broilers (AS) begins with a hypoxic challenge, which in turn challenges the cardio-pulmonary system. The stressed cardio-pulmonary system is poorly prepared to deal with the challenge and thus responds by triggering PH. This increase in pulmonary arterial pressure is the most common pathology of AS in broilers (Balog, 2003). After conducting a thorough review of the literature, it is thought that CVL formation will be significantly more frequent in broilers suffering from IPAH. CVL have, to this point, been characterized primarily in patients exhibiting advanced IPAH. These CVL may be forming in relation to shear stress, caused by the increased pulmonary resistance associated with IPAH.

PURPOSE OF THE STUDY

Idiopathic pulmonary arterial hypertension (IPAH) represents a complex group of pulmonary vasculature disorders (Humbert et al., 2004). Plexiform lesions or CVL are often found in lung tissue of patients whose deaths are attributable to IPAH. Due to a lack of available human lung tissue, little is known of CVL formation. This study aims to document the development of CVL in broiler lines divergently selected for ascites. This could lead to the establishment of the ascites lines as an animal model for future CVL and IPAH research.

RESEARCH OBJECTIVES

The primary objective was to advance the current knowledge of IPAH by closely examining CVL incidence, which is associated with IPAH. Furthermore, the study sought to establish the ascites lines as a viable animal model for future CVL/IPAH research. In order to accomplish these goals, several questions were put forth. Included in these questions were: (1) Is there a significant correlation between IPAH and CVL formation? (2) Do significant differences in CVL formation exist between the ascites lines? (3) Do age, gender or lung volume significantly impact CVL formation?

MATERIALS AND METHODS

BROILER POPULATIONS

The populations studied were two research broiler lines at the University of Arkansas poultry farm. These lines were selected based on divergent ascites susceptibility. The lines' incidence of ascites mortality over six weeks in an artificially simulated high altitude environment is vastly divergent. The SUS line experiences approximately 98% ascites mortality, while the RES line exhibits 6% ascites mortality (Anthony, Unpublished Results). This dissimilarity was essential to the study, as ascites is the manifestation of severe late stage IPAH in broilers.

ANIMAL WELFARE

Animal procedures were approved by the University of Arkansas Institutional Animal Care and Use Committee (Protocol #06067). The two lines of broilers used in this study were obtained from Dr. Nicholas Anthony. The lines being utilized for the study being the ascites SUS line and the ascites RES line.

SAMPLE

Birds were sampled at two week increments from two to twelve weeks post-hatch. At each sampling age, 10 birds were randomly selected from each line (five males, five females) to perform lung fixation and extraction. Selections were done randomly from any one of the four environmental rearing chambers.

INSTRUMENTATION

DEVELOPMENT

Previous work with the SUS line characterized CVL incidence as the presence or absence of a lesion in broiler lung tissue (Wideman et al., 2011). Prior literature indicated a significant correlation between IPAH and the formation of CVLs (Wideman et al., 2011). CVL incidence reported has been as high as six lesions in an avian lung sample (Wideman et al., 2011). Therefore, lesion incidence in this study was considered two ways: (1) total number of broilers exhibiting at least one lesion and (2) number of lesions found in lung samples of broilers exhibiting CVLs. Concomitant with individual broiler lesion incidence was the comparison of lesion incidence between the lines, i.e., total number of lesions per line.

Lesion incidence was also studied in relation to age (weeks post-hatch), gender, body weight (g), and lesion location. According to previous studies there are no significant differences in CVL formation associated with gender, but age differences may be present (Wideman et al., 2011). Lesion location was measured by accounting for CVL number per left or right lobe of sampled lungs. Lesion locations were also compared between the lines to determine if there were significantly more CVLs in either the left or right lobe in either line. Finally, lung volumes were obtained to compute the ratio between CVL number and lung volume, as significantly greater lung volume may impact CVL formation.

INSTRUMENT VALIDITY

The lung extraction and fixation procedures used were based on previous research shown to identify CVLs effectively (Wideman et al., 2011).

INSTRUMENT RELIABILITY

Suspected lesions were noted in a template representative of the typical lung section. Two independent researchers reviewed all lung sections and suspected lesions were then reviewed to determine the reliability of findings.

PILOT TEST

This current study is a follow up study to research performed by Wideman and coworkers (2011). The objectives of the previous experiment were to study the formation of plexiform lesions through the use of the SUS line of broilers. Data were collected on the incidence of plexiform lesion formation at 8, 12, 16, 20, 24 and 52 weeks of age. Additionally, data were collected on lesion location (left or right lobe of the lung) and gender. Despite answering many questions regarding plexiform lesions, many questions were available for future research.

DATA COLLECTION PROCEDURES

TRIAL ONE

Birds were hatched at the University of Arkansas – Fayetteville Hatchery on September 02, 2009 and transported to the Poultry Environmental Research Laboratory. Male and female chicks (n = 196 SUS, n = 168 RES) were wing banded on the day of hatch (Day 1), and reared on fresh wood litter in four environmental chambers (8 m^2 floor space). In each chamber, 49 SUS chicks and 42 RES chicks were placed, providing 0.0879 m^2 of floor space per bird. Birds were not segregated by sex during rearing. A

constant ventilation rate of 6 m^3 per minute was maintained in each chamber. Birds were brooded at 32°C from day one to day three, and then the temperature was decreased to 31°C during days four to six. At day seven temperature was reduced to 25°C and held constant until day 11, at day 11 temperature was decreased to 25°C and was reduced to a final temperature of 24°C at day 15 and maintained till the end of the experiment, November 24, 2009 (Day 86).

The photoperiod for the experiment was 23 hours of light with one hour of dark for days 1 to 4. From day five until the end of the experiment the light/dark cycle was 16 hours of light, then eight hours of dark. Chambers were equipped with two rows of nipple waterers, and two tube feeders. During the experiment birds were fed a corn-soybean meal based feed, designed to conform to or exceed the minimum NRC (1994) standards for all ingredients including but not limited to, 22.7% crude protein, 3,059 kcal ME/kg, 1.5% arginine, and 1.43% lysine. Feed and water were provided *ad libitum* for the duration of the experiment.

Beginning at two weeks of age and continuing in two-week increments up to 12 weeks of age, 10 birds were randomly selected from each line (five males, five females) to perform lung fixation and extraction. Birds were anesthetized using intra-muscular injections of allobarbitol (3.0mL, anesthesia 25 mg/mL) and ketamine HCL (1.0 to 2.5 mL of 100 mg/mL). Following anesthetic, body weights were recorded and heparinized saline (1 mL per bird of 200 units/mL ammonium heparin in 0.9% NaCl) was injected intravenously to prevent blood clotting. Birds were then euthanized by exsanguination. Once euthanized, the sternum was retracted to allow access to the heart and chest cavity. The right atrium was clamped using a hemostat and the left atrium was opened to allow for drainage. Then, an opening was made in the right ventricle, polyethylene tubing (2.5 mm I.D.) was inserted and the pulmonary vasculature was flushed with a minimum of 200 mL of 0.9% NaCl at room temperature.

Lung fixation occurred *in situ* by trans-cardiac perfusion with 200 - 400 mL of 4% phosphate-buffered paraformaldehyde at room temperature. The thoracic cavity was then filled with fresh 4% paraformaldehyde and the lungs allowed to fix *in situ* for an additional three hours. Lungs were then harvested, and sliced along the inter-rib divisions, and were then allowed to sit overnight in 4% paraformaldehyde.

After the incubation, lung gravimetric volume displacement was obtained. Then lungs were rinsed in tap water and dehydrated in 25%, 50% and 75% ethyl alcohol for 30 minutes each, and stored in 75% ethyl alcohol. Before storage, central lobes from both the left and right lungs were removed and placed in a whirl pack processed for histology. Additionally, heart ventricles were dissected and weighed to calculate the right-to-total ventricular weight ratios, which are indicative of sustained idiopathic pulmonary arterial hypertension (IPAH) (Cueva et al., 1974; Hernandez, 1987; Huchzermeyer and DeRuyck, 1986; Julian, 1988; Peacock et al., 1989; Wideman, 2000).

TRIAL TWO

Based on the results of trial one a second trial was conducted to provide a more comprehensive coverage of the early post-hatch period of growth. Bird rearing was done in floor pens with warm room brooding and line separation, feed and water were provided *ad libitum*. For trial two birds from the RES and SUS line were sampled at hatch and weekly up to six weeks of age. Twenty birds per line were sampled at each age, except for at hatch where 30 birds per line were sampled. The process of lung fixation was consistent with that described for trial one. Given that no difference was found in relation to gender or body weight, these data were not recorded. Furthermore, lung gravimetric displacement was not obtained for birds in trial II.

LUNG HISTOLOGY

Inter-medial lung lobe divisions were embedded in paraffin wax, sectioned at 5 to 7 μm, and stained with hematoxylin and eosin. Central sections were then scanned using overlapping fields of vision with horizontal traverses at 10X objective magnification. Suspected lesions were noted on a slide template, photographed at 40X objective, and their coordinates recorded using a Micro-Slide Field Finder (Gurley Precision Instruments, Troy NY 12180). Two independent researchers reviewed all lung sections and identified lesions were then reviewed to verify findings.

Study Design and Treatments

Trial One has been designed as a three factor fixed effects completely randomized design. Line and gender are considered fixed effects as the two lines are bred in relation to PAH, thus being the only lines of interest to research and gender has only two options. In addition, age is a fixed effect as the ages sampled were the only ages of interest as previous studies have expounded on later ages. As a three

factor fixed effects completely randomized design, the model would be as follows:

Let Y_{IJKL} = observed lesions on a lung from Lth broiler from Ith line of Jth gender sampled at Kth age. I = S,R J = M, F L = 1,…5,…10 K = 2,4,6,8,10 and 12 weeks

Assume:

$$Y_{IJKL} = \mu + L_I + G_J + A_K + LG_{IJ} + LA_{IK} + AG_{KJ} + LGA_{IJK} + E_{IJKL}$$

DATA ANALYSIS

Data were analyzed via the GLM procedure using SAS 9.2. Information examined included: (1) Body weight over time between the two ascites lines (2) Lung volume over time between the two lines (3) Lung volume to body weight ratio over time between the two lines (4) Lesion incidence over time between the two lines and (5) Percentage of birds with at least one CVL over time between the two lines. An alpha level ≤ 0.05 was chosen prior to analysis to assign significance.

RESULTS AND DISCUSSION

Wideman and coworkers (2011) reported that plexiform lesions were present at frequencies greater than 50 percent for SUS line chickens reared at local altitude conditions. This observation prompted the need for a transitional study to explore how early CVLs appear and how this frequency changes over time. In addition, lines divergently selected for PH would be characterized over time.

In the current study birds were sampled from two to twelve weeks post hatch. CVLs were detected in 35 to 30 percent of the chicks sampled, SUS and RES lines respectively. This result led to Trial II where weekly sampling was performed to further characterize the timing and onset of CVL formation. To our surprise CVLs were detected at hatch in 33 and 20 percent of the birds sampled for the SUS and RES line respectively. This is the earliest documentation of CVLs in broilers and may indicate that CVLs and PH do not share a cause and effect relationship. The presence of CVLs at hatch may indicate that the embryo is undergoing stress during development. This stress has been thought to be due to high egg shell temperatures ≥38.9°C, which decrease body and organ development (Molenaar et al., 2011). CVLs at hatch lend credence to the idea that stress, not PH is giving rise to CVLs.

The accumulation of data from Wideman and coworkers (2011) and trial I and II of the present research accounts for data from approximately 900 birds distirbuted across the birds growth period (Figure 1). It appears that although CVLs are present for both lines at hatch the frequency of incidence is generally higher for SUS to 4 weeks post-hatch. Beyond 4 weeks it appears the RES has the same general frequency of CVL incidence as SUS line birds. Considering the data from hatch to 84 days of age, 41% of SUS and 30% RES line birds had at least one CVL.

As expected, the RES line was generally heavier at an early age, and being surpassed by the SUS line as the trial continued. This pattern of growth is consistent with previous body weight recorded for the SUS and RES lines (Pavlidis, 2007). (Figure 2)

Lung volume did not differ between the lines throughout the course of the trial (Figure 3). Lung volume expressed as a percentage of total body weight did, however, differed between the two lines (Figure 4). The SUS line shows a marked decrease in lung volume as a percentage of body weight, which continued to decline as the bird ages. (Figure 4) Contrary to the SUS line, the RES line exhibits an upward trend, with the percentage of lung volume increasing with age. (Figure 4) It appears that the relative lung volume of RES line broilers, improves in order to keep up with oxygen demand.

At four weeks of age the SUS line had a greater lung volume to body weight ratio than the RES line. At six weeks of age the RES line's lung volume to body weight ratio begins to increase, while the SUS line decreased between weeks four and six. The SUS line continues to express a lower lung volume to body weight ratio in weeks 8 and 12, with there being no difference in week 10. This may have been due to a slight increase in variation in the RES line at week 10.

Lung volume to body weight trends are further broken down in Figure 5 for the SUS line and Figure 6 for the RES line. Figure 5 shows how starting at week six the SUS line's lung volume to body weight ratio is significantly less than the its peak at four weeks of age. Figure 6 exposes an opposite upward trend for the RES line with weeks 10 and 12, being greater than week four, and week 12 being greater than week six in addition.

The CVL incidence for the combined CVL trials is presented in Table 1. The CVL incidence represents the percentage of broilers having at least one CVL. The SUS line exhibited a higher lesion mean than the RES line at 21 days of age in trial II. The 21 day lesion mean of the SUS line was also greater than the SUS line lesion mean at 28 and 35 days of age posthatch. The RES and SUS lines failed to be significantly different in their mean number of lesions per bird and this is illustrated in Figure 7. While this does agree with the null hypothesis, it goes against previous research suggesting a link between CVL formation and IPAH (Wagenvoort and Wagenvoort, 1977, Loyd et al., 1988, Wagenvoort, 1989).

CONCLUSIONS

The observations made in this study are unclear as to the relation between long term divergent selection for ascites and the incidence of plexiform lesion formation. While the observed data are consistent with previous findings on lesion formation in SUS (Wideman et al., 2011), it did not fit the hypothesis that RES would exhibit a significantly lower incidence of plexiform lesion formation. This theory was supported by the RES significantly greater lung volume to body weight ratio, which allows it to deal more effectively with hypoxic challenge. Lesion formation is believed to be associated with pulmonary hypertension (Wagenvoort and Wagenvoort, 1977, Loyd et al., 1988, Wagenvoort, 1989); yet a line breed for resistance to AS i.e “pulmonary hypertension” still expressed plexiform lesions.

This only spurs further debate as to whether plexiform lesions are a cause or effect of pulmonary hypertension. As all broilers examined were clinically healthy, it may be assumed that the lesions are arising from a reason separate of pulmonary hypertension and could possibly be a lesser-understood form of vascular remodeling. In spite of this, the lines still possess a significant value to future CVL research, as their formation closely resembles human CVL pathogenesis. Future research would be valuable in determining what factors are influencing early (0-14 days posthatch) CVL formation. Given the early age it is unlikely that pulmonary hypertension is developing during incubation resulting in CVL formation and if it is, it suggests a much faster rate of CVL development than the 1 week presented by Abe et al. (2010). Continued research will be needed to determine the ultimate cause of plexiform lesions.

FIGURE LEGENDS

FIGURE 1. Combined plexiform lesion incidence (percentage of broilers exhibiting ≥1 plexiform lesion) (n ≥ 10) during the course of trial 1 and trial 2, ranging from 0 – 84 days of age post-hatch in IPAH – Resistant and IPAH – Susceptible broiler chicken lines.

FIGURE 2. Mean body weights ± SEM (n ≥ 10) from IPAH – Resistant and IPAH – Susceptible broiler chicken lines during the course of trial 1. Samples were collected from 28 – 84 days post-hatch in 14-day increments.

FIGURE 3. Mean lung volumes ± SEM (n ≥ 10) for IPAH – Susceptible and IPAH – Resistant broiler chicken lines during the course of trial 1. Samples were collected from 28 – 84 days of age post-hatch in 14-day increments.

FIGURE 4. Mean lung volume to body weight ratio ± SEM (n ≥ 10) coupled with linear relationships for the IPAH – Susceptible and IPAH – Resistant lines over the course of trial 1. Samples were collected from 28 – 84 days of age post-hatch in 14-day increments. Ratios differed in between age groups and are marked with different superscripts (a,b) ($P < 0.05$, Duncan's)

FIGURE 5. Mean lung volume to body weight ratio ± SEM (n ≥ 10) for the IPAH – Susceptible line of broiler chickens over the course trial 1. Samples were collected from 28 – 84 days of age post-hatch in 14-day increments. Differences between age groups are marked with different superscripts (a,b) ($P < 0.05$, Duncan's)

FIGURE 6. Mean lung volume to body weight ratio ± SEM (n ≥ 10) for the IPAH – Resistant line of broiler chickens. Samples were collected from 28 – 84 days of age post-hatch in 14-day increments. Differences between age groups are marked with different superscripts (a,b,c) ($P < 0.05$, Duncan's)

FIGURE 7. Combined plexiform lesion occurrence (Avg. number of plexiform lesions per broiler) ± SEM (n ≥ 10) during the course of trial 1 and trial 2, ranging from 0 – 84 days of age post-hatch in IPAH – SUS and IPAH – RES broiler chicken lines. Differences within age groups are marked with different superscripts (a,b) ($P < 0.05$, Duncan's)

TABLE 1. Plexiform lesion incidences[1] in the lungs[2] of broilers[3] from the IPAH-susceptible (SUS) and IPAH-resistant (RES) lines when evaluated from the day of hatch (week 0) through 364 days of age in three separate trials[4]

	Wideman 2009		Trial I			Trial II			Combined-All Data		
Day	SUS %		SUS %	RES %		SUS %	RES %		SUS %	RES %	
0						33 (10/30)	20 (6/30)		33 (10/30)	20 (6/30)	
7						30 (6/20)	30 (6/20)		30 (6/20)	30 (6/20)	
14			35 (7/20)	30 (6/20)		35 (7/20)	25 (5/20)		35 (14/40)	28 (11/40)	
21						50 (10/20)	25 (5/20)		50 (10/20)	25 (5/20)	
28			46 (10/22)	20 (4/20)		5 (1/20)	10 (2/20)		26 (11/42)	15 (6/40)	
35						20 (4/20)	30 (6/20)		20 (4/20)	30 (6/20)	
42			40 (8/20)	42 (10/24)					40 (8/20)	42 (10/24)	
56	52 (29/56)		50 (10/20)	40 (8/20)					51 (39/76)	40 (8/20)	
70			35 (7/20)	45 (9/20)					35 (7/20)	45 (9/20)	
84	52 (22/42)		45 (9/20)	45 (9/20)					50 (31/62)	45 (9/20)	
112	51 (31/61)								51 (31/61)		
140	40 (18/45)								40 (18/45)		
168	37 (51/138)								37 (51/138)		
364	22 (11/50)								22 (11/50)		
ALL			42 (51/122)	37 (46/124)		29 (38/130)	23 (30/130)		40 (255/644)	30 (76/254)	

[1] (Number of lung sections with $\geq$1 lesion/number of lung sections examined) x 100.
[2] Left and right lungs pooled; one section was evaluated per lung.
[3] Males and females pooled within each age group.
[4] Wideman et al., 2011 in press, The Anatomical Record (only the SUS line was evaluated); Mason et al., 2011 (MS Thesis in preparation);

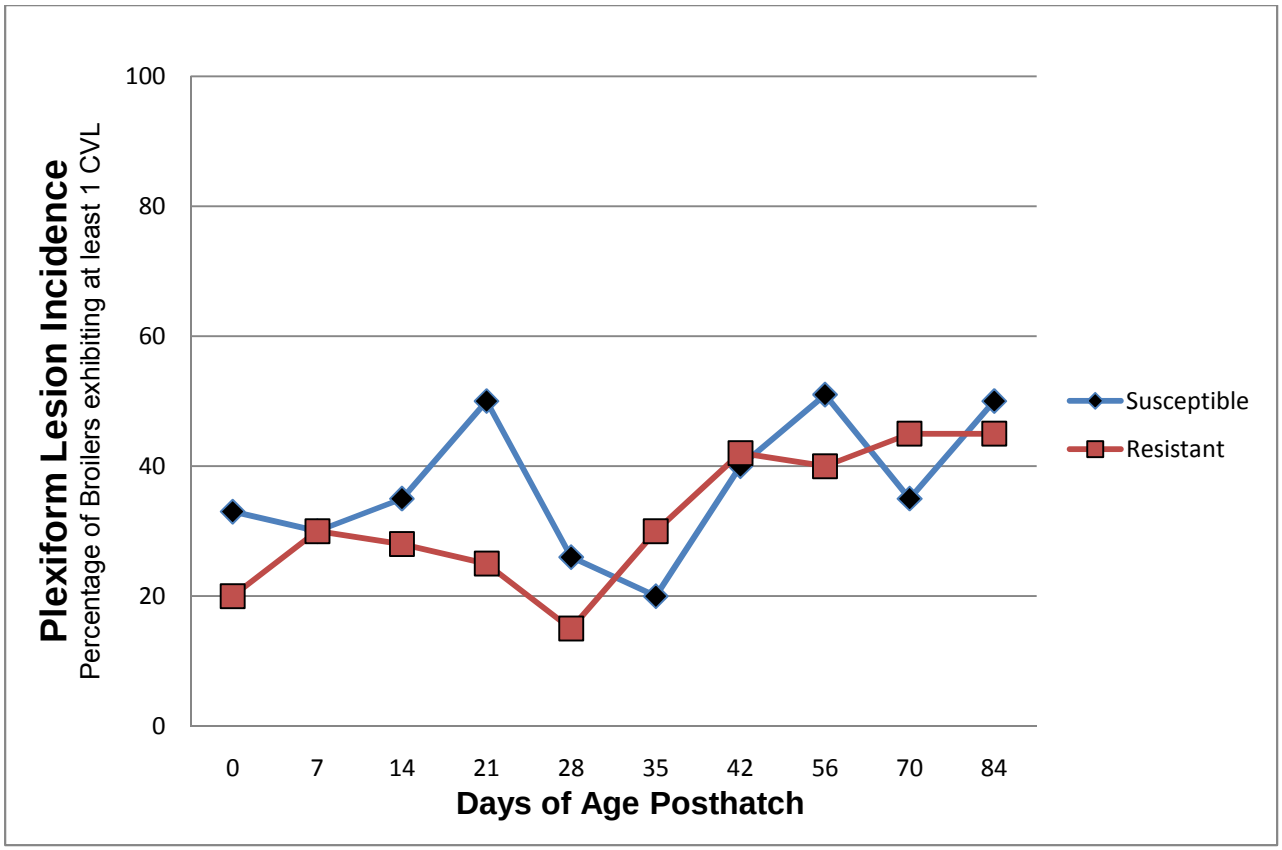

FIGURE 1. Combined plexiform lesion incidence (percentage of broilers exhibiting ≥1 plexiform lesion) (n ≥ 10) during the course of trial 1 and trial 2. Samples collected from 0 – 84 days of age post-hatch in IPAH – Resistant and IPAH – Susceptible broiler chicken lines.

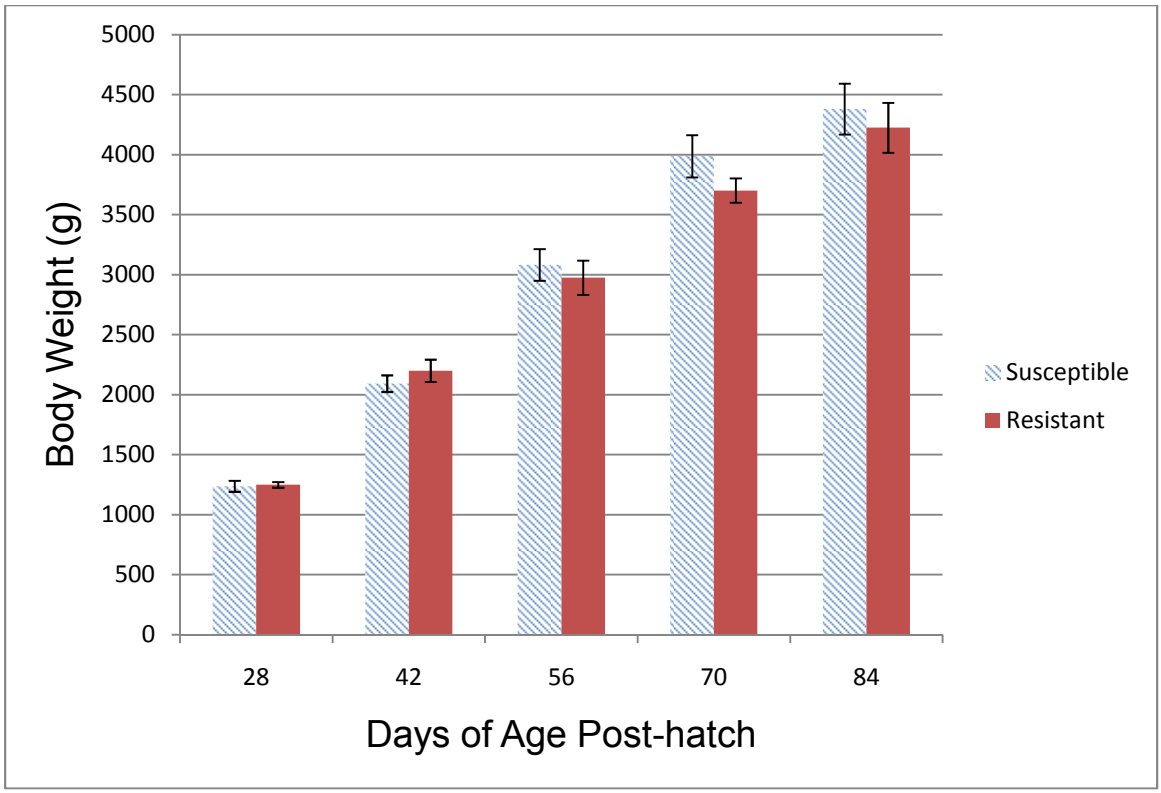

FIGURE 2. Mean body weights ± SEM (n ≥ 10) from IPAH – Resistant and IPAH – Susceptible broiler chicken lines during the course of trial 1. Samples were collected from 28 – 84 days post-hatch in 14-day increments.

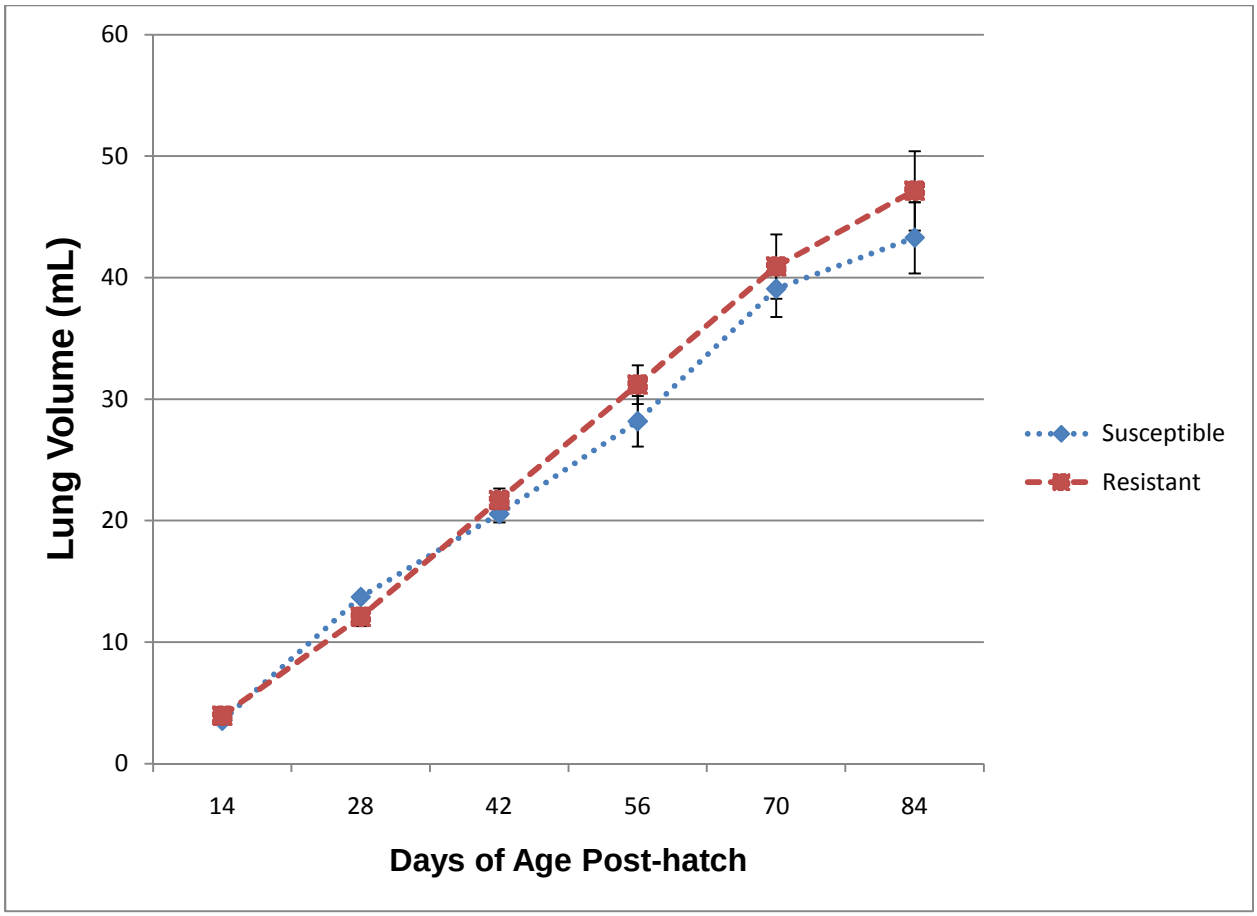

FIGURE 3. Mean lung volumes ± SEM (n ≥ 10) for IPAH – Susceptible and IPAH – Resistant broiler chicken lines during the course of trial 1. Samples were collected from 28 – 84 days of age post-hatch in 14-day increments.

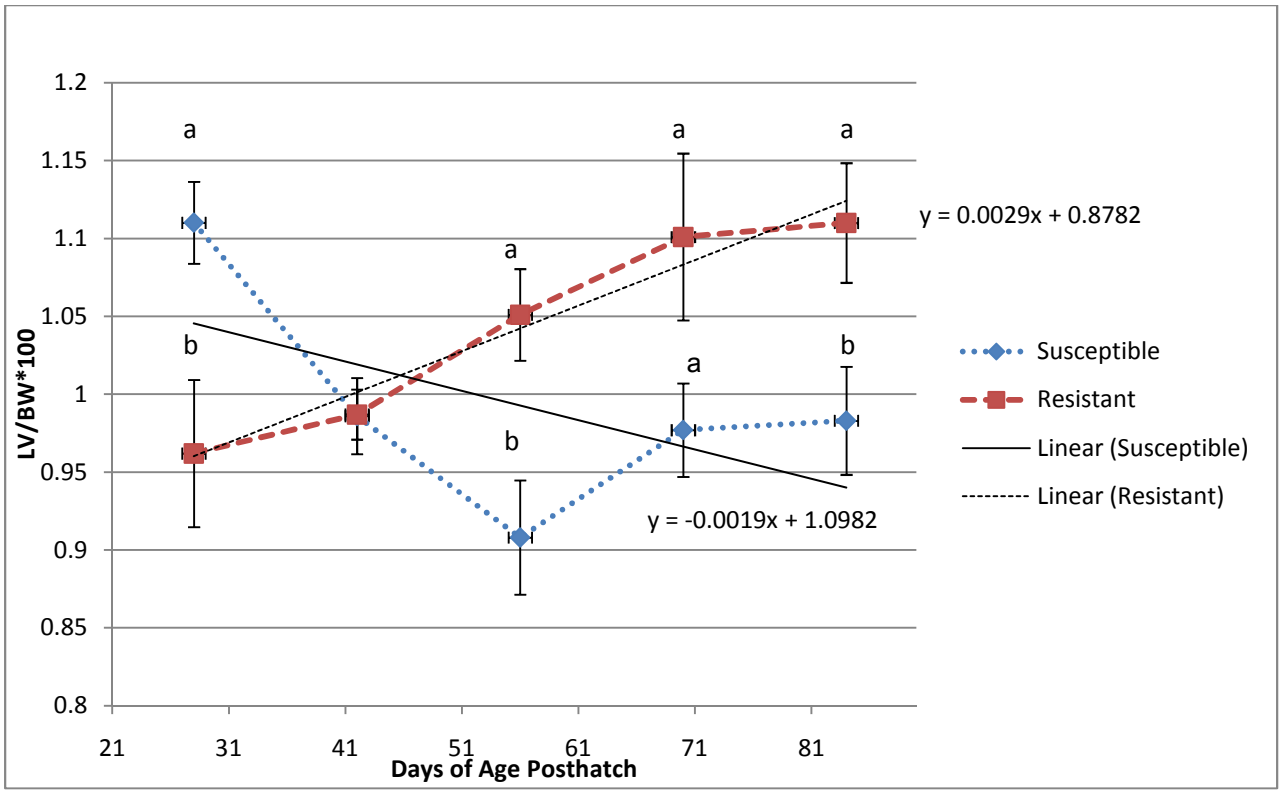

FIGURE 4. Mean lung volume to body weight ratio ± SEM (n ≥ 10) coupled with linear relationships for the IPAH – Susceptible and IPAH – Resistant lines over the course of trial 1. Samples were collected from 28 – 84 days of age post-hatch in 14-day increments. Ratios differed in between age groups and are marked with different superscripts (a,b) ($P < 0.05$, Duncan's)

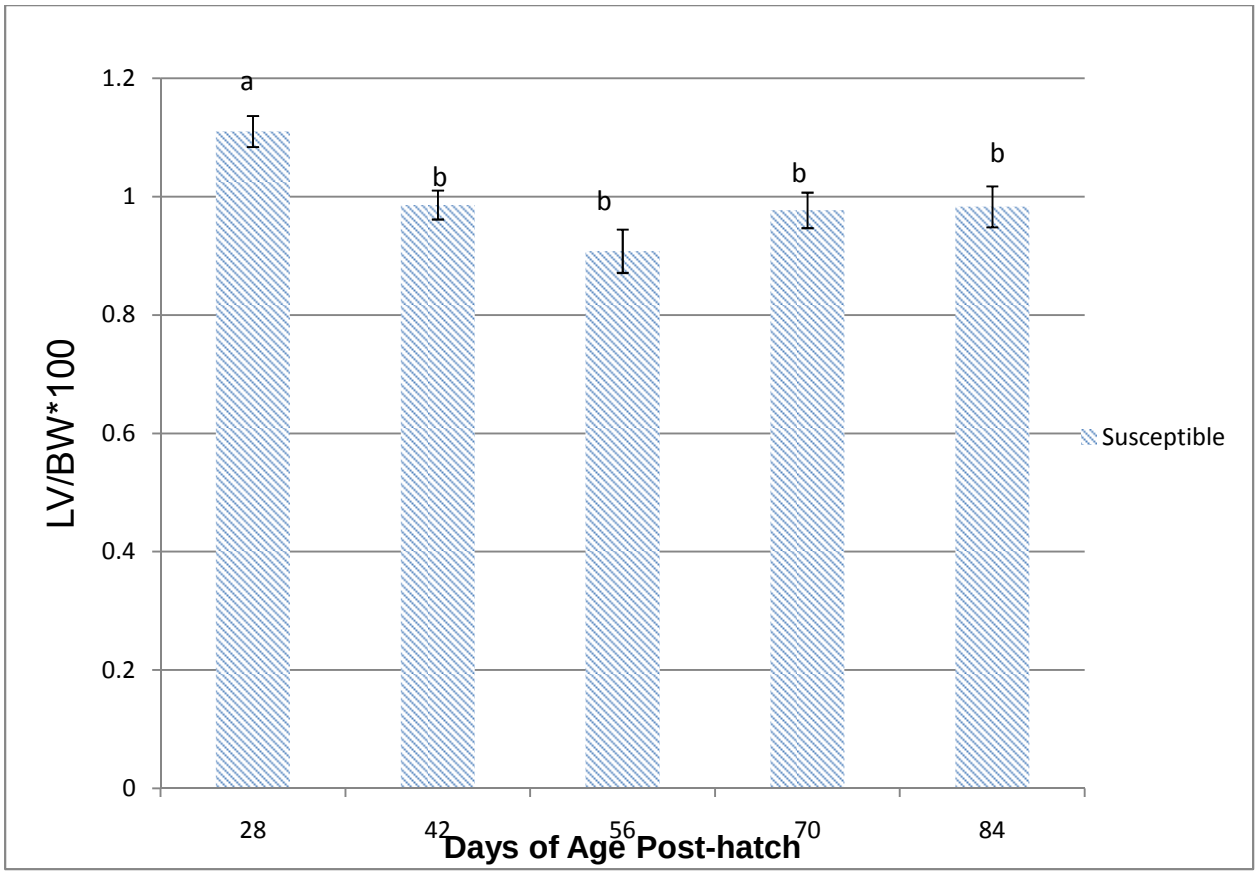

FIGURE 5. Mean lung volume to body weight ratio ± SEM (n ≥ 10) for the IPAH – Susceptible line of broiler chickens over the course trial 1. Samples were collected from 28 – 84 days of age post-hatch in 14-day increments. Differences between age groups are marked with different superscripts (a,b) ($P < 0.05$, Duncan's)

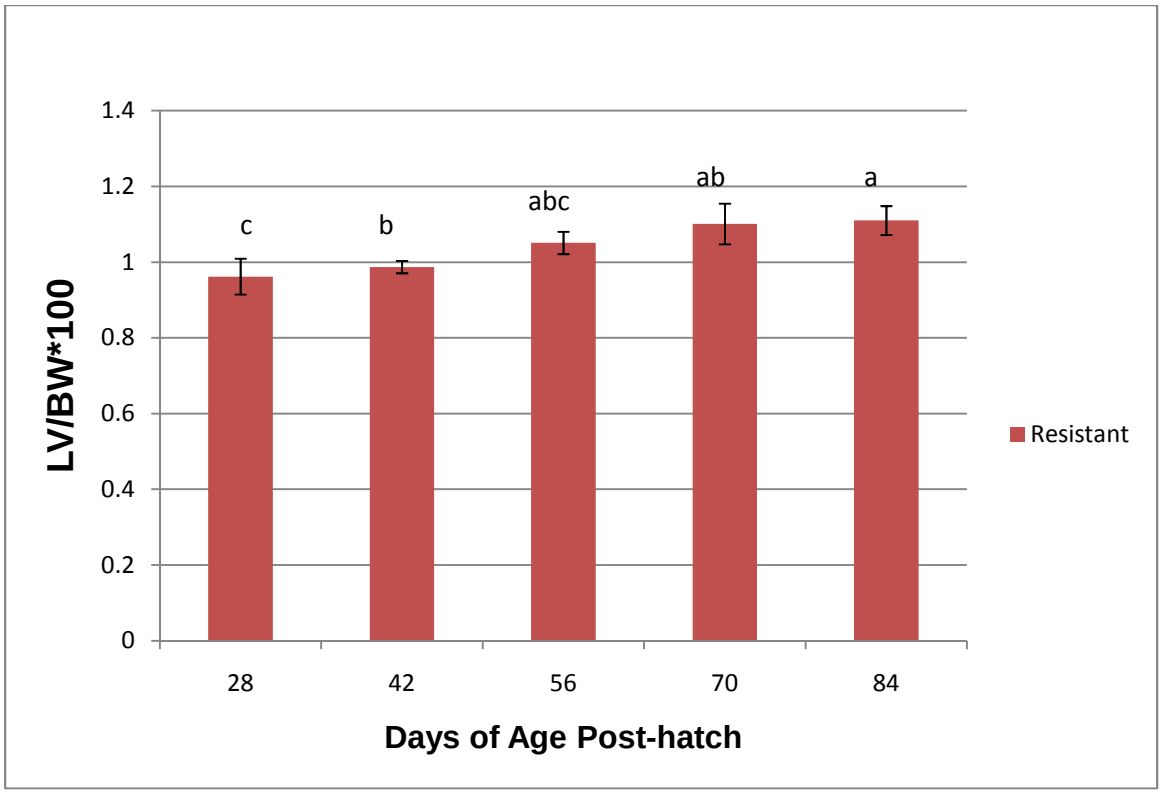

FIGURE 6. Mean lung volume to body weight ratio ± SEM (n ≥ 10) for the IPAH – Resistant line of broiler chickens. Samples were collected from 28 – 84 days of age post-hatch in 14-day increments. Differences between age groups are marked with different superscripts (a,b,c) ($P < 0.05$, Duncan's)

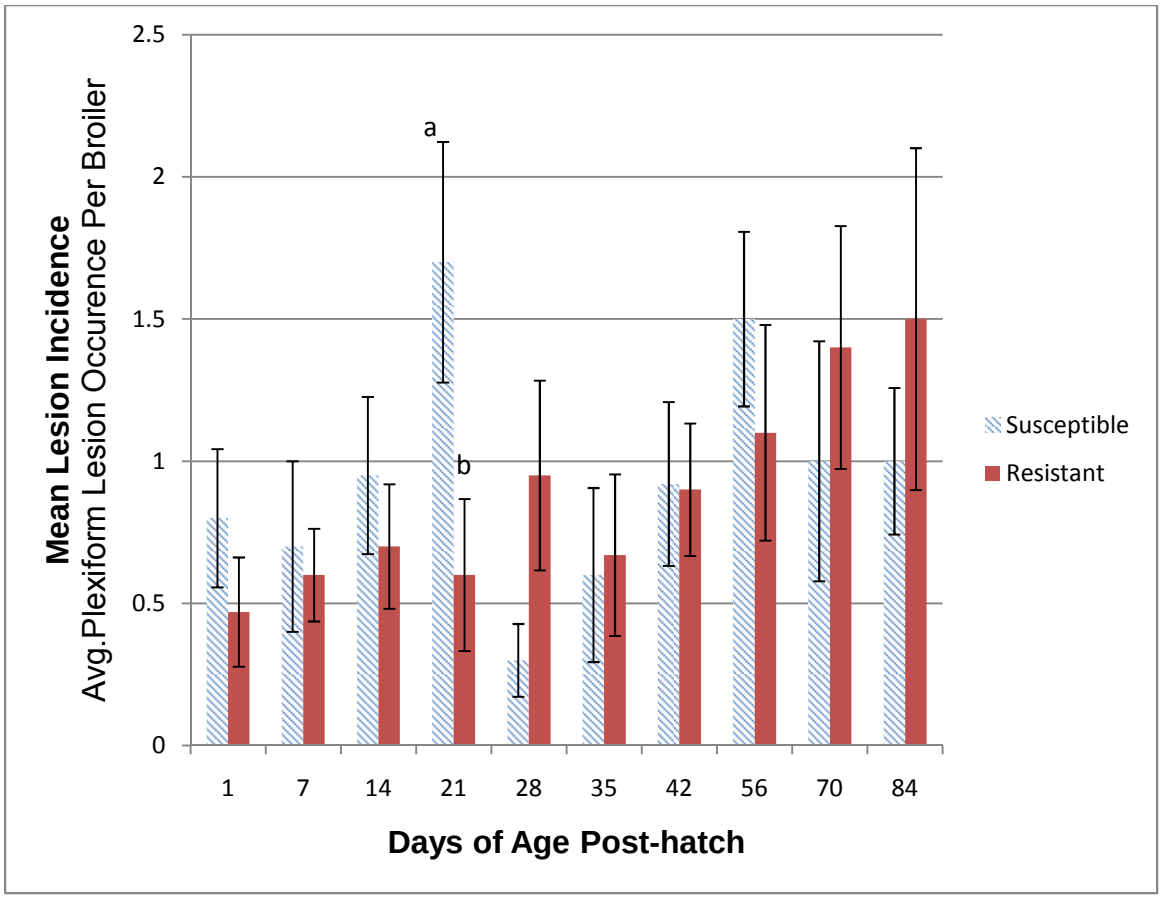

FIGURE 7. Combined plexiform lesion occurrence (Avg. number of plexiform lesions per broiler) ± SEM (n ≥ 10) during the course of trial 1 and trial 2, ranging from 0 – 84 days of age post-hatch in IPAH – SUS and IPAH – RES broiler chicken lines. Differences within age groups are marked with different superscripts (a,b) ($P < 0.05$, Duncan's)

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