Pulmonary vascular remodeling in broiler and Leghorn chickens after unilateral pulmonary artery occlusion

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ABSTRACT Morphological and physiological responses to unilateral pulmonary artery occlusion (PAO) were evaluated in male broiler (B) and Leghorn (L) chickens. All birds were fed a diet containing 3,200 kcal of ME/kg of feed and 23% CP. Broilers (18–21 d old; 507 ± 40 g of BW) and L (61–64 d old, 861 ± 87 g of BW) had surgical PAO (n = 40 each strain) or were sham-operated (SHAM; n = 40 each strain). Hematocrit (%), relative lung weight (wet right + left lung weight/BW × 100), right ventricle to total ventricle weight ratio (RV/TV), and resistance pulmonary arterioles (RPA) thickness were measured in 6 chickens per group one day presurgery, and at 7 and 14 d postsurgery. Data were analyzed using one-way ANOVA. Relative lung weight was higher in L-chickens than in B-chickens at all sampling times. There were no differences in Hc between B and L presurgery, but at d 7 and 14, L-PAO chickens had the highest Hc (35 ± 1.4 and 40 ± 1.9, respectively); the B-SHAM had a lower Hc (28 ± 1.2, and 29 ± 1.0) than the L-SHAM (32 ± 1.2 and 34 ± 1.1) and the B-PAO (32 ± 1.2 and 34 ± 2.0) chickens, with no differences between L-SHAM and B-PAO. The RV/TV ratio was highest in the B-PAO at d 7 and d 14, with no differences among the other groups. The B-PAO chickens had the thickest RPA at 7 d and 14 d postsurgery than the rest of the groups, whereas B had thicker RPA than L at presurgery. Broilers had a lower ventilation capacity than L, and after PAO they developed right ventricular hypertrophy and small arteriole remodeling, whereas the L-PAO showed a higher degree of hypoxemia (high Hct), but without changes in RV/TV ratios or small arterial remodeling, suggesting that L-chickens had a better pulmonary arterial vasodilation even after chronic increases in blood flow through a single lung.

Key words: Leghorn, broiler chicken, right pulmonary artery occlusion, pulmonary hypertension syndrome

INTRODUCTION

Unilateral pulmonary artery occlusion (PAO) is a surgical procedure that places a large workload on the right ventricle of the heart (Wideman and Kirby, 1995a,b). By occluding one pulmonary artery the blood volume perfusing the unobstructed lung (pulmonary artery) is doubled. Thus, a proportionally high cardiac output with a low capacity noncompliant pulmonary vasculature can lead to a ventilation/perfusion mismatch, hypoxaemia, and to a rapid development of pulmonary hypertension syndrome (PHS) in fast-growing broilers (Koyama and Horimoto, 1983; Nagasaki et al., 1984; Reeves and Rubin, 1998). Wideman and Kirby (1995b) reported that PAO induced pulmonary hypertension, with dilated hearts as early as 24 h after PAO. In general, PAO initiates a progression of symptoms typical of those observed in broilers developing PHS spontaneously, under a variety of environmental and commercial conditions, or during exposure to cold or hypobaric hypoxia. Leghorn chickens, however, have been reported to be resistant to PHS and to show superior cardiovascular capacity (Hassanzadeh et al., 2005), better endothelium-dependent vasodilation (Martinez-Lemus et al., 1999), and thinner media layer in pulmonary arterioles (Peacock et al. 1989) under normoxia compared with broiler chickens.

In susceptible fast-growing broiler chickens small diameter arterioles are responsible for the increased resistance to blood flow in the lungs. For example, hypoxic vasoconstriction resides proximal to the capillaries and occurs in resistance arterioles 30 to 300 μm in diameter (Reeves and Rubin, 1998). Endothelial dysfunction and smooth muscle hypertrophy or proliferation (remodeling) are key histopathological changes of PHS in animal models (Wideman et al., 2011) and in idipathic pulmonary arterial hypertension (PAH) in humans (Wagenhofer and Wagenhofer, 1970). The red jungle fowl, ancestor of both broilers and Leghorns, showed a transient increase in pulmonary arterial pressure when subjected to acute PAO (i.e., acutely tightening a snare around the pulmonary artery) followed by...
flow-dependent vasodilation, which brought pulmonary arterial pressure back to basal levels (Wideman et al., 1998). The red jungle fowl has superior cardiovascular capacity to Leghorn chickens, and Leghorns have a better cardiovascular capacity than that of broilers. These differences in cardiovascular capacity probably reflect physiological changes brought about by the selection pressure placed upon broilers and Leghorns for higher production. The remodeling (e.g., increased medial thickness) of resistance pulmonary arterioles (RPA) caused by PAO in the 2 types of chickens, broilers versus Leghorns, has not been documented. In vitro studies have shown that pulmonary arteries from broiler chickens have a reduced endothelium-dependent relaxation compared with those from Leghorn chickens of the same age (Martinez-Lemus et al., 1999; Odom et al., 2004). In addition, in vitro acute hypoxia demonstrated a higher contraction of intrapulmonary arteries from broiler chickens than in those from Leghorns (Zoer et al., 2004). In the present study, male broiler and Leghorn chickens of similar BW and fed a similar diet (a typical high-energy broiler diet) were subjected to PAO, to conduct a comparison of their physiological responses to chronically increased pulmonary vascular resistance (PVR) as a result of the entire cardiac output perfusing a single lung vasculature. We hypothesized that Leghorns will show a similar degree of hypoxemia than broilers chickens but will not become hypertensive and will avoid RPA vessel remodeling.

MATERIALS AND METHODS

Experimental Design

One-day-old male Cobb 500 broiler chicks (B; n = 50) and Leghorn (L; Hy-Line) chicks (n = 50) were used. The chicks were wing-banded and raised in wire battery cages; they were brooded conventionally with temperature starting at 32°C and decreasing 2°C each week until they reached the target BW of 800 g, recommended to perform PAO surgery in broiler chickens (Wideman and Kirby, 1995a). Leghorn chickens were given a 6-wk head start growing period to attain a similar BW as broilers. Throughout the experiment, all birds were fed a corn-soybean meal-based diet (23% CP, 3,200 kcal/kg), formulated to meet or exceed the requirements for broilers specified by the NRC (1994). The BW and hematocrit (% Hc) previously described (Ruiz-Feria et al., 1999). Birds from the PAO groups (broilers and Leghorns) were anesthetized to a surgical plane with intramuscular injections of a 1:1 mixture of ketamine HCl (100 mg/mL, Bioniche Pharma USA LLC, Lake Forest, IL) and xylazine (AnaSed 100 mg/mL, Akorn Inc., Decatur, IL), at a dose of 0.007 to 0.015 mL of the mixture/100 g of BW (Harvey et al., 1985). Anesthetized chickens were fastened in a supine position with the neck extended. Feathers of the thoracic inlet were plucked, and the skin was swabbed with Betadine (Purdue Products L.P., Stamford, CT). Lidocaine (2% s.c. xylocaine, Astra-Zeneca, Wilmington, DE) was infiltrated subcutaneously along the midline of the thoracic inlet as a supplemental local anesthetic. A midline incision was made, the crop and trachea were retracted to the right, and the left thoracic air sac was opened. The left pulmonary artery was located and clamped with a silver vascular clip fashioned from 0.38-mm diameter silver wire (World Precision Instruments, Sarasota, FL). The incision was closed with stainless steel surgical wound clips and sprayed with a topical antibacterial powder. The chicks were placed under a heat lamp for up to 2 h to recover from anesthesia, and then they were returned to their cages. Another group of birds (broilers and Leghorns) were sham operated; they underwent surgery as described above, the pulmonary artery was isolated but was left intact (not clamped).

Determination of Physiological and Morphological Parameters

Physiological and morphological assessments were made at 3 sampling times from at least 6 randomly chosen chickens per treatment: presurgery (1 d before surgery), 7 and 14 d postsurgery. The ventilation capacity was assessed by determining the relative lung weight: total wet lung weight (right lung + left lung) divided by BW and multiplied by 100 (Hassanzadeh et al., 2005). The experimental birds were monitored daily for mortality, the cause of death was determined and the heart was dissected to determine right ventricle weight/total ventricle weight ratio (RV/TV; Burton et al., 1968). The RV/TV ratios were also determined in birds that were humanely killed at the sampling times previously described. The BW and hematocrit (% Hc) were recorded at the sampling times.

Determination of Small Pulmonary Arteriole Hypertrophy

The right lung (perfused) was excised from 6 randomly selected chickens from each experimental group. One transverse section of the paleopulmonic region was collected in tubes containing Trump’s fixative. Ten micrometer-thick sections were cut and subsequently stained with Humberstone and Gomori’s elastin stain for elastic tissue. Measurements of medial thickness of at least 20 small muscular pulmonary arterioles, ex-
ternal diameter 30–200 μm, were analyzed per bird. Medial thickness (MT) was estimated as the mean of 4 measurements for each vessel (MT1 + MT2 + MT3 + MT4), whereas the external diameter (D) was taken as the mean of 2 measurements at right angles to each other (D1 + D2). Medial thickness was expressed as percentage of external diameter (% thickness): MT/D \times 100 (Tucker et al., 1975). Medial thickness, a measure of vascular remodeling, has been used reliably for estimating the amount of vascular smooth muscle in several species (Tucker et al., 1975).

Resistance Pulmonary Arteriole Hypertrophy

The mean diameter of the resistance vessels of less than 100 μm in diameter studied was 70 ± 6 μm, with no differences in mean external diameter among arteriole groups at any of the 3 sampling times (Table 1).

Results on % thickness of resistance pulmonary arteriole (RPA) are presented in Figure 1. Before surgery there was no difference in RPA thickness among birds of the different groups. At 7 d postsurgery, however, B-PAO (21 ± 1 μm) chickens had thicker (P < 0.05) RPA than those from chickens in the other groups, with no differences among these groups. At 14 d postsurgery, the B-PAO chickens (21.6 ± 0.7 μm; P < 0.05) had thicker RPA than the B-SHAM chickens (20.1 ± 0.7 μm). In turn, the B-SHAM group had thicker RPA than both the L-SHAM (17.6 ± 0.8 μm) and the L-PAO (17.4 ± 0.8 μm) groups, with no differences between the 2 Leghorn groups.

The mean diameter of the RPA between the diameter of 100 to 200 μm studied was 138 ± 6 μm. There were no differences in mean external diameter among arteriole groups at any of the 3 sampling times (Table 1).

Results on % thickness of pulmonary arterioles of 100 to 200 μm are presented in Figure 2. Before surgery, broilers (16.5 ± 0.7 μm) had thicker RPA than Leghorns (14.0 ± 0.7 μm). At 7 d postsurgery, the B-SHAM group (17.1 ± 0.9 μm) had thicker RPA compared with birds in the other groups, but with no differences among B-PAO, L-SHAM, and L-PAO groups of arterioles. At d 14 postsurgery, the B-PAO group had (P < 0.05) thicker RPA than the L-PAO group (14.8 ± 1.0 μm), but not different than those of the B-SAHM or L-SHAM birds (Figure 2).

Physiological and Morphological Parameters

There were no differences in relative lung weight between L-SHAM and L-PAO birds or between B-SHAM and B-UPAO birds and means were separated by the Student-Newman-Keuls method.

Table 1. Average diameter (±SE) of pulmonary arterioles in male broiler chickens subjected to surgery to occlude one pulmonary artery (B-UPAO) and their sham-operated control (B-SHAM) and in Leghorn male chickens with occluded pulmonary artery (L-UPAO) and their sham-operated control (L-UPAO; n = 40)

<table>
<thead>
<tr>
<th>Diameter (μm)</th>
<th>Sampling time</th>
<th>B-SHAM</th>
<th>B-UPAO</th>
<th>L-SHAM</th>
<th>L-UPAO</th>
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<tr>
<td>&lt;100</td>
<td>Presurgery</td>
<td>73 ± 5</td>
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<td>7 d postsurgery</td>
<td>66 ± 4</td>
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<td></td>
<td>14 d postsurgery</td>
<td>70 ± 10</td>
<td>71 ± 10</td>
<td>63 ± 11</td>
<td>86 ± 11</td>
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<tr>
<td>100–200</td>
<td>Presurgery</td>
<td>138 ± 6</td>
<td>138 ± 6</td>
<td>131 ± 6</td>
<td>131 ± 6</td>
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<tr>
<td></td>
<td>7 d postsurgery</td>
<td>133 ± 6</td>
<td>133 ± 8</td>
<td>130 ± 7</td>
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<td></td>
<td>14 d postsurgery</td>
<td>138 ± 8</td>
<td>134 ± 8</td>
<td>135 ± 9</td>
<td>151 ± 12</td>
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and B-PAO birds. Therefore, the data on relative lung weight were pooled by strain of bird. Leghorns had a higher ($P < 0.05$) relative lung weight than broilers at all sampling times. Also, the relative lung weight tended to decrease with age in both types of birds (Figure 3).

Results on hematocrit (%, $Hc$) are presented in Figure 4. Before surgery, there was no difference among chickens from the different experimental groups. At 7 d post surgery, the B-PAO group had higher $Hc$ ($32 \pm 1.2$; $P < 0.05$) than the B-SHAM birds ($28.4 \pm 1.2\%$), and the L-PAO group had higher $Hc$ ($34.9 \pm 1.4$; $P < 0.05$) than the L-SHAM birds ($31.9 \pm 1.2$). Also, L-PAO birds had higher $Hc$ than B-PAO birds, whereas L-SHAM and B-PAO birds had similar $Hc$ levels.

At 14 d postsurgery, the B-PAO birds had higher $Hc$ ($33.9 \pm 2.0$; $P < 0.05$) than the B-SHAM birds ($29.3 \pm 1.0$), and the L-PAO birds had higher $Hc$ ($39.7 \pm 1.9$) than the L-SHAM birds ($34.5 \pm 1.1$). Also, L-PAO birds had higher $Hc$ than B-PAO birds, whereas L-SHAM and B-PAO birds had similar $Hc$ values. Results on RV:TV ratio are presented in Figure 5. Before surgery, broiler chickens ($0.16 \pm 0.01$) had lower RV:TV ratios than Leghorns ($0.19 \pm 0.01$; $P < 0.05$). At 7 ($0.31 \pm 0.06$) and 14 d ($0.32 \pm 0.03$) postsurgery, B-PAO chickens had the highest RV:TV ratios, whereas the RV:TV ratios did not differ among the other groups.

The success rates for attempted surgeries in the present study were as follows: 100% (21/21), 100% (20/20), 74% (25/34), and 88% (21/24) for the L-PAO, L-SHAM, B-PAO, and B-SHAM groups respectively. All mortality was recorded within 24 h postsurgery. There were 2 cases (2/20; 10% incidence) of clinical PHS, as evidenced by the presence of ascitic fluid in the abdominal cavity, in the B-PAO chickens, whereas no clinical cases were seen in the other experimental groups. Before surgery, male Leghorns (average $861 \pm 87$ g) were heavier than male broiler chickens (average $507 \pm 40$ g), but at d 7 and 14 postsurgery, broilers were heavier than Leghorns. Within strain, there were no differences in BW between PAO or SHAM birds (Figure 6).

**DISCUSSION**

The present investigation compares resistance pulmonary arteriole remodeling and physiological responses of male Leghorn chickens, resistant to pulmonary

![Figure 2](image1.png)

**Figure 2.** Percentage thickness of pulmonary arterioles with 100 to 200 μm in diameter in male broiler chickens with one surgically occluded extra-pulmonary artery (B-UPAO) and their sham-operated control (B-SHAM) and male Leghorns with occluded pulmonary artery (L-UPAO) and their control (L-SHAM). The thickness of the media (smooth muscle) was divided by the artery diameter and then multiplied by 100. Data are expressed as means ± SE (m = 40 per group and time point). Letters a and b indicate group differences between each time point; means without a common letter are different ($P \leq 0.05$).

![Figure 3](image2.png)

**Figure 3.** Specific lung weight of experimental birds at the different sampling points; the specific lung weight was determined dividing the total lung wet weight by 100 g of BW. Data are expressed as means ± SE (n = 8 per group and time point). Asterisks indicate differences between the 2 bird strains within days after surgery ($P \leq 0.05$).

![Figure 4](image3.png)

**Figure 4.** Hematocrit (%) of male broiler chickens with one surgically occluded extra-pulmonary artery (B-UPAO) and their sham-operated control (B-SHAM) and male Leghorns with occluded pulmonary artery (L-UPAO) and their control (L-SHAM). Data are expressed as means ± SE (n = 8 per group and time point). Letters (a–c) indicate group differences between each time point; means without a common letter are different ($P \leq 0.05$).
hypertension, and male broiler chickens, susceptible to pulmonary hypertension, after the entire cardiac output is directed to one lung by surgically occluding one pulmonary artery.

**RPA Hypertrophy**

Small-diameter arterioles are responsible for the increased resistance to blood flow in the lungs; for instance, hypoxic vasoconstriction resides proximal to the capillaries and occurs in resistance arterioles 30 to 300 μm in diameter (Reeves and Rubin, 1998). Endothelial dysfunction and pulmonary arterial smooth muscle hypertrophy are key histopathological changes that characterize pulmonary hypertension. Medial thickness (% thickness) is a reliable estimate of the amount of smooth muscle in several species (Tucker et al., 1975; Moreno de Sandino and Hernandez, 2006). In the present study, we measured medial thickness in 2 size categories: RPA of less than 100 μm in diameter and RPA between 100 and 200 μm in diameter. The reason for this arbitrary differentiation was to better assess the medial thickness response to the PHS-inducing method. For example, it has been shown that RPA <60 μm in diameter show neomuscularization in the peripheral areas of the lung (Xiang et al., 2002). Thus, studying pulmonary arterioles <100 μm separately was deemed to be a better approach to assess responses by previously nonmuscularized vessels that become muscularized as a response to PAO. Regarding the smaller arterioles (less than 100 μm), broilers and Leghorns had nondifferent arterial thickness before surgery; however, B-PAO chickens had thicker RPA (21 ± 0.69%) than chickens from the other groups at 7 and 14 d post-surgery (Figure 1). At d 14 after surgery, B-PAO had thicker RPA than B-SHAM birds, and B-SHAM birds had thicker RPA than Leghorns. These results are in agreement with those reporting that broiler chickens with PHS consistently exhibit medial hypertrophy or lesions in RPA with a diameter of less than 100 μm. For example, broiler chickens exposed to suboptimal temperature (12–14°C) presented neomuscularization of pulmonary arterioles (<60 μm in diameter) in the hilum (Pan et al., 2005; Tan et al., 2005). Also, broiler chickens exposed to chronic hypoxia had more lesions in the RPA (50–100 μm), evidenced by a reduced nitric oxide synthase expression (source of NO), compared with age-mated birds raised under normoxia (Moreno de Sandino and Hernandez, 2006). Enkvetchakul et al. (1995) found that broiler chickens with cold-induced PHS had thick resistance pulmonary arteries 50 to 100 μm in diameter. Overall, these results indicate that small-diameter pulmonary arterioles are more likely to undergo remodeling once the PVR is increased, as in the case of PAO used in the present investigation to amplify PHS. Also, the fact that PAO did not lead to increased medial thickness in the L-PAO chickens suggests that Leghorns were capable of avoiding sustained increased PAP and endothelial dysfunction.

Larger RPA (100–200 μm) were already thicker in broilers than in Leghorns before surgery (Figure 2). However, the effects of pulmonary artery occlusion were not consistent on this type of arterioles. At d 7, arterial thickness was highest in B-SHAM birds, whereas B-PAO, L-PAO, and L-SHAM birds had similar arterial thicknesses. Conversely, by d 14, B-PAO birds had thicker RPA than L-PAO birds but not different from those of B-SHAM or L-SHAM birds. Moreno de Sandino and Hernandez (2006) reported that hypertensive chickens (RV/TV ratio of 0.44 ± 0.03) developed thicker RPA, regardless of diameter (50–100 μm or 100–200 μm), compared with nonhypertensive chickens (RV/TV ratio of 0.24 ± 0.03). One reason for this difference may be that in the present investigation all experimental hypoxemic broilers (B-PAO) were clinically healthy (RV/
TV ≤0.32), whereas Moreno de Sandino and Hernández (2006) studied very hypertensive, most probably clinically ascitic broilers. These results suggest that the degree of vascular remodeling depends on the size of the pulmonary artery/arteriole affected, the severity of the challenge imposed by the PHS-inducing methods, and the degree of hypertension developed by the birds.

**Physiological and Morphological Parameters**

In the present study, broilers had consistently lower relative lung weight, a parameter of ventilation capacity, than Leghorns throughout the experiment (Figure 3). These results agree with those reported by Hassan-zadeh et al. (2005), who found that Leghorn chickens had a consistently higher lung volume and lung weight as a percentage of BW than broiler chickens. In their investigation, the authors showed that both parameters were highly correlated from 7 d to 42 d of age. A low lung volume reduces the gas exchange area, explaining why in broilers, the developing cardiopulmonary system fails to keep up with growth rate, thus resulting in high susceptibility to PHS, compared with Leghorns under normoxic conditions. Furthermore, this limitation also explains why broilers challenged with factors that increase vascular resistance (e.g., PAO) are more prone to become more hypertensive than their SHAM counterparts in the present investigation.

Unilateral PAO leads to ventilation-perfusion mismatch because the entire cardiac output is forced through the unobstructed pulmonary artery at higher speed with lower time for gas exchange, with a concomitant reduction in arterial partial pressure of oxygen ($P_{O_2}$; Wideman and Kirby, 1995b). In the present investigation, the $P_{O_2}$ in the blood was not measured, but previous research has shown that the $P_{O_2}$ in arterial blood drops from 103 mmHg to 83 mmHg following acute PAO in anesthetized broiler chickens (Wideman and Kirby, 1995b).

In the present study, L-PAO chickens developed higher polycythemia ($P < 0.05$) than L-SHAM birds and broilers (PAO or SHAM), at both sampling times following PAO surgery. In chickens, hypoxic hypoxemia stimulates the production of erythropoietin (EPO), which induces polycythemia (Julian, 2007). Mirsalimi and Julian (1993) reported that age-matched male Leghorns and male broiler chickens exposed to hypobaric hypoxia (simulated 2,054 m above sea level) developed equal levels of polycythemia, which was attributed to equal increases in EPO secretion by the kidney.

Studies in Sprague-Dawley rats have shown that after exposure to chronic hypoxia, circulating levels of EPO and tissue EPO mRNA levels were increased, in both pulmonary hypertension-susceptible (Hilltop) and -resistant (Madison) strains of rats (Ou et al., 1998). However, Hilltop rats developed excessive polycythemia, accentuated hypoxemia, severe pulmonary hypertension, and high mortality, whereas Madison rats developed moderate polycythemia and hypertension with no significant mortality. Rather than strain-specific differences in EPO regulation, a more severe renal tissue hypoxemia in the Hilltop than in the Madison rats seemed to account for the divergent responses observed. The results of these studies suggest that in the hypoxemia-EPO-polycythemia response, the level of hypoxemia is the main determinant of the level of polycythemia rather than strain differences in sensitivity to hypoxemia.

Thus, our results showing that L-PAO chickens were more polycythemic than B-PAO chickens may suggest that Leghorns became more hypoxic than broilers as a result of PAO, suggesting that the lungs of Leghorns were less able to adjust to the ventilation:perfusion mismatch created by forcing the entire cardiac output through a single lung when compared with broilers, regardless of the better pulmonary vasodilation response.

Alternatively, it may be possible that Leghorns were able to release greater amounts of EPO in response to low blood oxygen levels (brought about by pulmonary artery occlusion in the present study), which might explain the higher blood hematocrit in Leghorns than in broilers. Although this is different from what was observed by Mirsalimi and Julian (1993), there could be differences between hypobaric hypoxia (low atmospheric oxygen) and the decrease in blood oxygen delivery to the lungs that occurs with PAO.

The results on success rates for attempted surgeries showed that the B-PAO group of birds had 26% more mortality than the L-PAO one. This may indicate that the most PHS-susceptible broilers from the B-PAO group were very hypoxic following surgery but died before d 7 postsurgery. So at this sampling time, only relatively normoxic broilers were still alive, whereas all Leghorns survived including the most hypoxic ones. However, the B-SHAM group had 12% more mortality than the L-SHAM one and yet the latter had higher Hc at the same sampling time than the B-SHAM group. Therefore, although differences in mortality soon after PAO surgery may reflect differential susceptibilities to hypoxemia and PHS, such differences did not fully explain higher Hc levels recorded in Leghorns than in broilers.

The RV/TV ratio has been used as a reliable indicator of pulmonary hypertension (Wideman, 2001). Clinically healthy domestic fowl with normal pulmonary arterial pressure have RV/TV ratios ranging from 0.15 to 0.27, whereas sustained pulmonary hypertension causes RV/TV ratios above 0.28 (Wideman, 2001). In the present study, the higher susceptibility of broiler chickens to pulmonary hypertension compared with Leghorns was evidenced by the fact that B-PAO developed right ventricular hypertrophy (RV:TV above 0.28 at d 7 and d 14 postsurgery) as a result of the sustained pulmonary hypertension, whereas L-PAO chickens did not show signs of sustained hypertension (RV:TV at or below 0.24; Figure 5). These results confirm that broilers have a low capacity pulmonary vasculature and
lower pulmonary vascular vasodilation capacity than Leghorns. Thus, pulmonary arteries from broilers have a reduced endothelium-dependent relaxation compared with those from Leghorns (Martinez-Lemus et al., 1999; Odom et al., 2004), and acute hypoxia produces higher constriction of intrapulmonary arteries in broilers than in Leghorns (Zoez et al., 2009).

In this study, Leghorns had higher lung capacity than broilers (Figure 3), and this higher ventilation capacity in addition to the morphological and physiological advantages reported above, allowed Leghorns to maintain thin RPA even after the PAO, in contrast with the increase in the RPA thickening seen in broilers at 7 d and 14 d postsurgery (Figure 1). Sustained high pressure (hypertension) in a normally low-pressure pulmonary circulation induces changes in the artery wall including smooth muscle cell proliferation. In this study, increases in PAP elicited thickening of small RPA, but more in broilers than in Leghorns, which indicates that arterial thickening is an integral part of PHS development (Mandegar et al., 2004), given that pulmonary artery thickening (especially in resistant arterioles <100 μm; Figure 1) was observed only in broiler chickens (B-PAO group). Also, broilers have an age-dependent increase of arterial thickening, because at 14 d after surgery, sham-operated broilers had thicker arterioles than L-PAO or L-SHAM (Figure 1). It has been reported that in normoxic broiler chickens, the PAP increases from 18 mmHg at 2 wk of age to 25 mmHg at 3 wk of age and stays high until 6 wk of age (Forman et al., 2000). Such increases in PAP were attributed to fast growth rate, thus placing a burden on the pulmonary vasculature and the right ventricle. This may explain why the broiler chickens showed an age-dependent increase in the RV/TV ratio observed in the present study. Results in the present investigation also showed that, coinciding with the thickening of pulmonary resistance arterioles, there was onset of hypertension, measured by an increased RV/TV ratio (Figure 5), again with a larger effect on broiler chickens than in Leghorns.

The fact that both B-PAO and L-PAO became polycythemic but L-PAO neither developed pulmonary hypertension nor showed PA remodeling supports the notion that polycythemia is not a major contributing factor to PHS in chickens.

Thus, in broiler chickens, the physiological responses brought about by PAO-induced PVR led to a vicious cycle that included pulmonary hypertension and pulmonary arteriole remodeling (thickening of resistance pulmonary arterioles), where a low pulmonary vascular capacity may have played a more determinant role, rather than hypoxemia. These physiological changes were evidenced by a higher degree of right ventricular hypertrophy and susceptible broilers succumbing to PHS. On the other hand, the slow growth rate of Leghorns, associated with a low metabolic rate and a better pulmonary vasculature flow-dependent vasodilation may have played an important role in reducing PVR, pulmonary arteriole remodeling, and pulmonary hyper-tension. Further research is warranted to elucidate the cellular mechanisms that preserve endothelial function in Leghorn chickens after acute arterial pulmonary occlusion and moderate systemic hypoxemia.

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