Evaluation of Minimally Invasive Indices for Predicting Ascites Susceptibility in Three Successive Hatches of Broilers Exposed to Cool Temperatures

ROBERT F. WIDEMAN JR.,*[,1] TERRY WING,† YVONNE KOCHERA KIRBY,* MARY F. FORMAN,* NATHAN MARSON,* CAYE D. TACKETT,* and CIRO A. RUIZ-FERIA*

Department of Poultry Science, University of Arkansas, Fayetteville, Arkansas 72701, and †Cobb-Vantress, Inc., Rose, Oklahoma 74364

ABSTRACT Broilers from three consecutive hatches were exposed to cool temperatures to amplify the incidence of pulmonary hypertension syndrome (PHS, ascites). The largest apparently healthy individuals on Day 42 were evaluated using minimally invasive diagnostic indices [percentage saturation of hemoglobin with oxygen, hematocrit (HCT), heart rate, electrocardiogram (ECG) Lead II, body weight), then they were subjected to the ongoing pressures of fast growth and cool temperatures to determine which of these indices are predictive of the subsequent onset of PHS. Approximately 20% of the males and females evaluated on Day 42 subsequently developed PHS by Day 51. When data for all hatches were pooled and broilers that subsequently developed ascites were compared with those that did not (nonascitic), body weights, heart rates, and percentage saturation of hemoglobin with oxygen were lower on Day 42 for ascitic than for nonascitic males, and HCT was higher in ascitic males and females than in nonascitic males and females, respectively. Comparisons of the ECG Lead II wave amplitudes for all hatches pooled indicated that RS-wave amplitude was larger in ascitic than in nonascitic males, and that S-wave amplitude was more negative in ascitic males and females than in nonascitic males and females. Necropsies conducted on Day 51 revealed higher right:total ventricular weight ratios in ascitic than in nonascitic broilers, whereas normalizing the left ventricle plus septum weight for differences in body weight generated similar values for ascitic and nonascitic males and females, respectively. These results support a primary role for pulmonary hypertension but not cardiomyopathy in the pathogenesis of ascites triggered by cool temperatures. Values obtained for minimally invasive diagnostic indices on Day 42 also establish predictive thresholds that can be used to evaluate the PHS susceptibility of large and apparently healthy male and female broilers.

(*Key words*: pulmonary hypertension, heart, broiler, electrocardiography, oxygen)

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INTRODUCTION

Pulmonary hypertension syndrome (PHS, ascites) is initiated in susceptible broilers when the right ventricle (RV) is forced to develop an elevated pulmonary arterial pressure to propel the cardiac output through the pulmonary vasculature (Julian, 1993; Odom, 1993; Wideman and Bottje, 1993; Wideman and Kirby, 1995a). Extensive research has been conducted to characterize the pathophysiological progression leading from the onset of pulmonary hypertension to the development of terminal ascites. Concurrent efforts have focused on identifying diagnostic indices that reliably predict the susceptibility of broilers to PHS (Rhoads *et al.*, 1995; Roush *et al.*, 1996, 1997; Kirby *et al.*, 1997; Wideman *et al.*, 1997). These indices are categorized as terminally invasive when birds must be euthanatized for histopathologic or gross necropsy evaluations. For example, histopathologic evaluations consistently reveal hypertrophy within the medial smooth muscle layers of pulmonary arterioles in birds developing PHS (Cueva *et al.*, 1974; Sillau and Montalvo, 1982; Huchzermeyer, 1985; Hernandez, 1987; Peacock *et al.*, 1989; Maxwell, 1991; Enkvetchakul *et al.*, 1995). Specific hypertrophy of the RV, as reflected by an elevated right:total ventricular (RV:TV) weight ratio, provides definitive but terminally fatal evidence that the RV has performed additional work to maintain an elevated pulmonary arterial

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[1]To whom correspondence should be addressed: rwideman@comp.uark.edu

Abbreviation Key: ECG = electrocardiogram; HCT = hematocrit; LVS = left ventricle plus septum; PHS = pulmonary hypertension syndrome; RV = right ventricle; RV:TV = right:total ventricular weight ratio; TV = total ventricle.

pressure (Burton *et al.*, 1968; Cueva *et al.*, 1974; Huchzermeyer and DeRuyck, 1986; Hernandez, 1987; Peacock *et al.*, 1989; Julian, 1993; Odom, 1993; Wideman and Bottje, 1993; Lubritz *et al.*, 1995). Parameters correlated with total lung capacity, such as lung length, lung volume, and the inter-rib distances bracketing the intercostal zone of lung insertion, also must be quantified using terminally invasive procedures (Julian, 1989; Owen *et al.*, 1995a,b).

Minimally invasive diagnostic indices are acquired without substantially altering the viability or production characteristics of subject birds. The pulmonary arterial pressure can be directly measured by carefully advancing a catheter through a peripheral venous site of insertion; however this technique is impractical for evaluating large numbers of broilers (Guthrie *et al.*, 1987; Owen *et al.*, 1995c; Wideman *et al.*, 1996a,b). Elevated serum levels of troponin T, a cardiac-specific protein, have been detected during the onset of PHS in broilers, but proprietary components of this assay are not commercially available for veterinary use in the U.S. (Maxwell *et al.*, 1994, 1995). Other minimally invasive techniques, such as electrocardiography, oximetry, hematology, and measures of growth, can be used to routinely evaluate the susceptibility of broilers to PHS. For example, broilers developing PHS under a wide variety of conditions develop increasingly negative electrocardiogram (ECG) Lead II S-wave amplitudes, which are predictive of elevated RV:TV ratios (Owen *et al.*, 1990, 1995a,b,c; Odom *et al.*, 1991, 1992; Wideman and Kirby, 1995a, 1996; Wideman *et al.*, 1997). A reduction in the oxygen content of arterial blood (hypoxemia) develops early during the pathophysiological progression leading to PHS, apparently because the rate of pulmonary blood flow can exceed the pulmonary gas diffusing capacity in susceptible broilers (Wideman and Kirby, 1995a,b; Wideman *et al.*, 1996a,b). The onset of hypoxemia can be quantified using a pulse oximeter to directly measure the saturation of hemoglobin with oxygen (Peacock *et al.*, 1990; Julian and Mirsalimi, 1992; Wideman and Kirby, 1995a,b). Sustained hypoxemia also triggers an adaptive increase in the hematocrit (HCT), which has been successfully used as a genetic predictor of PHS susceptibility (Burton and Smith, 1967; Maxwell, 1991; Mirsalimi and Julian, 1991; Yersin *et al.*, 1992; Lubritz and McPherson, 1994; Shlosberg *et al.*, 1996; Fedde and Wideman, 1996). Heart rates obtained during pulse oximetry or ECG recordings are lower for broilers in the terminal stages of PHS than for clinically healthy broilers (Roush *et al.*, 1996, 1997; Kirby *et al.*, 1997; Olkowski *et al.*, 1997). Finally, growth tends to decelerate subsequent to the onset of hypoxemia, presumably reflecting a cardiopulmonary capacity that is inadequate to deliver oxygen in quantities sufficient to sustain maximal growth (Owen *et al.*, 1990, 1995a,b; Witzel *et al.*, 1990; Yersin *et al.*, 1992; Roush *et al.*, 1994; Wideman *et al.*, 1995a,b; Wideman and Kirby, 1996).

The present study was designed to evaluate minimally invasive diagnostic indices using a protocol that is applicable to commercial genetic selection programs. The broilers were reared under conditions designed to maximize the rate of body weight gain, and were subjected to cool temperatures to further amplify the incidence of PHS. Within the context of large selection programs, a routine exposure to cool temperatures has practical advantages over other techniques for inducing PHS that require surgical intervention or prolonged exposure to hypobaric hypoxia (Lubritz and McPherson, 1994; Rhoads *et al.*, 1995; Wideman *et al.*, 1995a,b; 1997; Shlosberg *et al.*, 1996). In commercial selection programs, all birds exhibiting symptoms of PHS are culled through the day of final selection, after which the selected individuals are subjected to feed and light restriction programs to optimize reproductive performance. Management programs that reduce the ongoing cardiopulmonary challenge associated with fast growth potentially may help conceal subclinical carriers of PHS susceptibility (Reeves *et al.*, 1991; Shlosberg *et al.*, 1991, 1992; Arce *et al.*, 1992; Acar *et al.*, 1995; Tottori *et al.*, 1997). Therefore, in the present study, we evaluated the reliability of minimally invasive diagnostic indices for predicting which of the largest and apparently healthiest individuals on Day 42 would subsequently develop PHS when subjected to the ongoing pressures of fast growth and cool temperatures through Day 51.

MATERIALS AND METHODS

To amplify the diversity within a small research population, breeder parents were selected from families tending to exhibit characteristics indicative of susceptibility to PHS, including cyanosis of the comb, narrow inter-rib distances bracketing the intercostal zone of lung insertion, and elevated RV:TV ratios. Eggs collected from these breeders were set on a weekly basis in a commercial hatchery to produce three successive hatches of 201, 180, and 169 chicks, respectively. Chicks were wing-banded and transported to the University of Arkansas Poultry Environmental Research Laboratory on the day of hatch (Day 1), where they were placed on fresh wood shavings litter in environmental chambers (8 m^2 floor space). They were brooded at 32 and 30 C during Weeks 1 and 2, respectively, and after Day 14 the temperature was maintained between 13 and 16 C. The daily photoperiod was 24 h of light on Days 1 to 5, and 23 h light:1 h dark thereafter. Feed and water were provided for *ad libitum* consumption. Water was provided in Plasson waterers based on previous experience that the use of nipple waterers markedly reduces the incidence of PHS (Wideman, unpublished observations). A corn-soybean meal-based broiler ration formulated to meet or exceed the minimum NRC (1984) standards for all ingredients, including 22.7% CP, 3,059 kcal ME/kg, 1.5% arginine, and 1.43% lysine, was provided as crumbles during Week 1, and the same ration was provided as pellets thereafter.

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The Day 1 and 14 body weights were recorded for all chicks. At 42 d of age, approximately 50 of the largest apparently healthy birds surviving from each hatch were weighed and evaluated. Most of the survivors on Day 42, including by necessity many of the selected individuals, exhibited a visible cyanosis of the comb that is typical of large full-fed birds grown under cool temperature conditions (Wideman, personal observations). Electrocardiograms were recorded as described previously for assessments of Lead II R-, RS-, and S-wave amplitudes (Wideman and Kirby, 1996; Wideman *et al*., 1997). A universal "C" sensor[2] attached to a Vet/Ox™4403 pulse oximeter[2] was positioned on the wing to illuminate the tissue between the radius and ulna for measurements of heart rate and percentage saturation of hemoglobin with oxygen (Peacock *et al*., 1990; Julian and Mirsalimi, 1992; Wideman and Kirby, 1995a). Blood was obtained by venipuncture for duplicate HCT determinations using heparinized capillary tubes and a microhematocrit centrifuge. Final body weights were recorded and necropsies were conducted on all mortality occurring after Day 21, or on Day 51 for survivors. Birds were euthanatized with CO_2 gas, the sex of each bird was verified, and the heart was removed, dissected, and weighed for calculation of RV:TV ratio as an index of pulmonary hypertension (Burton *et al*., 1968; Cueva *et al*., 1974; Sillau *et al*., 1980; Huchzermeyer *et al*., 1988; Peacock *et al*., 1989). Ascites was diagnosed if abdominal fluid accumulation was evident or if a plasma clot adhered to the surface of the liver. Birds were removed from the data set if they were culled due to leg problems or poor Day 1 to 21 performance, or if they died from causes other than ascites.

Statistical Analysis

The incidences of ascites were analyzed by sex and hatch using a G-test of independence with the Williams correction factor (Sokal and Rohlf, 1981). For other parameters, data were analyzed using general linear models and ANOVA (SAS Institute, 1982). Where appropriate, mean separation tests were conducted using Tukey's Studentized range test (HSD) and Ryan-Einot-Gabriel-Welsch multiple range test or contrasts. The SigmaStat® (Jandel Scientific, 1994) linear regression procedure was used to evaluate relationships between diagnostic indices and RV:TV ratios.

RESULTS AND DISCUSSION

Cumulative Data from all Birds

The challenges of fast growth and cool temperatures triggered uniformly high cumulative incidences of ascites in all three hatches of progeny from breeder parents selected for characteristics indicative of PHS susceptibility (Table 1). Males had a higher incidence of ascites than females in Hatch 1; otherwise, the incidence of ascites was similar for males and females. Previously, sex did not influence the incidence of ascites when pulmonary hypertension was initiated by surgically occluding a pulmonary artery or an extrapulmonary primary bronchus (Wideman *et al*., 1997). Body weights on Days 1 and 14, prior to the start of the cool temperature challenge, were not predictive of the subsequent development of ascites; however, in all three hatches, the final body weights of ascitic males and females were lighter than for nonascitic males and females, respectively (Table 2). The markedly lower final body weights for ascitic broilers reflect the inclusion of data from young birds succumbing to PHS throughout the Day 21 to 51 age range. Consequently the potential influence of hypoxemic growth suppression cannot be estimated from these cumulative body weight comparisons. As shown in Table 3, the RV:TV ratios of ascitic males and females were consistently higher than for nonascitic males and females in all three hatches, thereby confirming the role of pulmonary hypertension in the pathogenesis of ascites triggered by cool temperatures. Individual RV, left ventricle plus septum (LVS), and total ventricle (TV) weights varied widely within the ascitic male and female groups due to large differences in age at the time of necropsy (data not shown).

TABLE 1. Cumulative incidence of ascites for male and female broilers from three consecutive hatches through 51 d of age

	Sex					
Hatch	Male		Female		Male + Female	
Hatch 1	51/93	(55%)^{ab}	32/97	(33%)^{c}	83/190	(44%)^{bc}
Hatch 2	40/72	(56%)^{ab}	54/91	(59%)^{a}	94/163	(58%)^{a}
Hatch 3	46/76	(61%)^{a}	46/82	(56%)^{ab}	92/158	(58%)^{a}
All hatches	137/241	(57%)	132/270	(49%)	269/511	(53%)

$^{a–c}$Incidences with no common superscript differ significantly for all comparisons among separate hatches ($P \leq 0.05$).

Data from Birds Selected on Day 42

Of the males and females identified as the largest apparently healthy individuals on Day 42, an average of 20% across all three hatches subsequently developed ascites by Day 51 (Table 4). For all hatches pooled, the body weights on Days 1 and 14 were not predictive of the subsequent onset of ascites; however, Day 42 body weights of ascitic males were lighter than for nonascitic males, and the final body weights for ascitic males and females were lighter than for nonascitic males and females, respectively (Table 5). These differences in final body weights were minimally influenced by age; consequently, the data in Table 5 support previous observations that growth rates decelerate subsequent to the onset of hypoxemia in broilers developing PHS (Owen *et al*., 1990, 1995a,b; Witzel *et al*., 1990; Yersin *et al*., 1992; Roush *et al*., 1994; Wideman *et al*., 1995a,b; Wideman and Kirby, 1996).

[2]Sensor Devices, Inc., Waukesha, WI 53188.

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TABLE 2. Day 1, Day 14, and final body weights for male and female broilers from three consecutive hatches that subsequently did (ascitic) or did not (nonascitic) develop ascites by 51 d of age[1]

Age	Hatch	Nonascitic males	Ascitic males	Nonascitic females	Ascitic females
		(g)			
Day 1	Hatch 1	41 ± 0.7^{bc}	42 ± 0.5^{abc}	41 ± 0.5^{bc}	40 ± 0.7^{c}
	Hatch 2	43 ± 0.8^{abc}	43 ± 0.6^{abc}	45 ± 0.7^{a}	42 ± 0.5^{abc}
	Hatch 3	43 ± 0.9^{ab}	43 ± 0.7^{ab}	42 ± 0.8^{ab}	43 ± 0.5^{abc}
	All hatches	42 ± 0.5^{x}	43 ± 0.3^{x}	42 ± 0.4^{x}	42 ± 0.3^{x}
Day 14	Hatch 1	341 ± 8^{abc}	361 ± 7^{a}	331 ± 5^{bc}	342 ± 9^{abc}
	Hatch 2	343 ± 8^{abc}	357 ± 7^{ab}	333 ± 7^{abc}	325 ± 7^{bcd}
	Hatch 3	313 ± 10^{cd}	326 ± 7^{bcd}	294 ± 8^{d}	334 ± 7^{abc}
	All hatches	335 ± 5^{xy}	348 ± 4^{x}	322 ± 4^{y}	332 ± 4^{y}
Final	Hatch 1	3,003 ± 118^{a}	1,683 ± 93^{c}	2,680 ± 66^{ab}	1,494 ± 96^{c}
	Hatch 2	3,085 ± 144^{a}	1,649 ± 108^{c}	2,814 ± 75^{ab}	1,792 ± 101^{c}
	Hatch 3	2,631 ± 164^{ab}	1,597 ± 94^{c}	2,411 ± 115^{b}	1,601 ± 83^{c}
	All hatches	2,916 ± 83^{x}	1,644 ± 56^{z}	2,633 ± 50^{y}	1,653 ± 56^{z}

[a–d]Means with no common superscript differ significantly for all comparisons among separate hatches ($P \leq 0.05$).

[x–z]Means with no common superscript differ significantly for comparisons of all hatches within an age ($P \leq 0.05$).

[1]Data are means ± SEM; number of birds per category shown in Table 1.

Comparisons of heart rate, HCT, and percentage saturation of hemoglobin with oxygen are shown in Table 6. Within the separate hatches, HCT and percentage saturation of hemoglobin with oxygen differed for females only in Hatch 2, and the heart rate and HCT differed between ascitic and nonascitic males only in Hatch 3. For all hatches pooled, the heart rate and percentage saturation of hemoglobin with oxygen were lower in ascitic than in nonascitic males, and HCT was higher in ascitic males and females than in nonascitic males and females, respectively. The combined oximetry data for nonascitic males and females averaged 75% saturation of hemoglobin with oxygen, thereby confirming the hypoxemia extant in otherwise healthy birds under the conditions of the present study. Similar ranges for heart rate, HCT, and oximetry previously were reported when ascitic and nonascitic broilers from a different genetic line were compared after surgical occlusion of a pulmonary artery or exposure to cool temperatures (Kirby *et al.*, 1997). Clinically healthy broilers reared under less challenging conditions exhibit substantially higher oximetry values and lower HCT values (Peacock *et al.*, 1990; Julian and Mirsalimi, 1992; Wideman and Kirby, 1995a,b; Wideman *et al.*, 1996a,b).

Comparisons of the ECG Lead II R-, RS-, and S-wave amplitudes are shown in Table 7. In Hatches 2 and 3, the S-wave amplitude was more negative for ascitic than for nonascitic males, whereas the RS-wave amplitude differed between ascitic and nonascitic males only in Hatch 2. For all hatches pooled, the R-wave amplitude was not lower in ascitic than nonascitic males or females, the RS-wave amplitude was larger in ascitic than in nonascitic males, and the S-wave amplitude was more negative in ascitic males and females than in nonascitic males and females. These results confirm previous reports that an increasingly negative Lead II S-wave amplitude serves as a primary ECG index for the onset of PHS (Owen *et al.*, 1990, 1995a,b,c; Odom *et al.*, 1991, 1992; Wideman and Kirby, 1995a, 1996; Wideman *et al.*, 1997).

TABLE 3. Right ventricular weight to total ventricular weight ratio (RV:TV) for male and female broilers from three consecutive hatches that did (ascitic) or did not (nonascitic) develop ascites by 51 d of age[1]

Hatch	Nonascitic males	Ascitic males	Nonascitic females	Ascitic females
Hatch 1	0.27 ± 0.01^{b}	0.37 ± 0.01^{a}	0.27 ± 0.01^{b}	0.38 ± 0.02^{a}
Hatch 2	0.26 ± 0.01^{b}	0.39 ± 0.01^{a}	0.26 ± 0.01^{b}	0.37 ± 0.01^{a}
Hatch 3	0.27 ± 0.01^{b}	0.38 ± 0.01^{a}	0.28 ± 0.01^{b}	0.37 ± 0.01^{a}
All hatches	0.27 ± 0.01^{y}	0.38 ± 0.01^{x}	0.27 ± 0.01^{y}	0.37 ± 0.01^{x}

[a,b]Means with no common superscript differ significantly for all comparisons among separate hatches ($P \leq 0.05$).

[x,y]Means with no common superscript differ significantly for comparisons of all hatches pooled ($P \leq 0.05$).

[1]Data are means ± SEM; number of birds per category shown in Table 1.

TABLE 4. Incidence of ascites between 42 and 51 d of age for male and female broilers selected on Day 42 as the largest individuals remaining from three consecutive hatches[1]

Hatch	Male	Female	Male + Female
Hatch 1	3/27 (11%)^{ab}	0/23 (0%)^{c}	3/50 (6%)^{bc}
Hatch 2	5/29 (17%)^{ab}	7/20 (35%)^{a}	12/49 (25%)^{a}
Hatch 3	9/27 (33%)^{a}	6/25 (24%)^{a}	15/52 (29%)^{a}
All hatches	17/83 (21%)	13/68 (19%)	30/151 (20%)

[a–c]Incidences with no common superscript differ significantly ($P \leq 0.05$).

[1]Data are number with ascites/total available (percentage).

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TABLE 5. Day 1, Day 14, Day 42 and final body weights for male and female broilers selected on Day 42 as the largest individuals remaining from three consecutive hatches, and that subsequently did (ascitic) or did not (nonascitic) develop ascites by 51 d of age[1]

Age	Hatch	Nonascitic males	Ascitic males	Nonascitic females	Ascitic females
		(g)			
Day 1	Hatch 1	41 ± 1^{a}	40 ± 2^{a}	42 ± 1^{a}	. . .
	Hatch 2	45 ± 1^{a}	46 ± 2^{a}	45 ± 1^{a}	44 ± 2^{a}
	Hatch 3	43 ± 1^{a}	43 ± 2^{a}	42 ± 1^{a}	45 ± 2^{a}
	All hatches	43 ± 1^{x}	44 ± 1^{x}	43 ± 1^{x}	44 ± 1^{x}
Day 14	Hatch 1	353 ± 10^{ab}	370 ± 5^{a}	344 ± 8^{ab}	. . .
	Hatch 2	358 ± 8^{ab}	360 ± 8^{a}	344 ± 11^{ab}	353 ± 13^{ab}
	Hatch 3	325 ± 11^{ab}	333 ± 11^{ab}	304 ± 8^{b}	332 ± 24^{ab}
	All hatches	347 ± 6^{x}	348 ± 7^{x}	330 ± 6^{x}	344 ± 13^{x}
Day 42	Hatch 1	2,552 ± 49^{a}	2,400 ± 105^{abc}	2,215 ± 35^{bc}	. . .
	Hatch 2	2,568 ± 67^{a}	2,325 ± 135^{abc}	2,365 ± 38^{abc}	2,277 ± 49^{abc}
	Hatch 3	2,440 ± 49^{ab}	2,209 ± 116^{bc}	2,143 ± 53^{c}	2,135 ± 86^{bc}
	All hatches	2,527 ± 33^{x}	2,277 ± 74^{y}	2,225 ± 27^{y}	2,211 ± 50^{y}
Final	Hatch 1	3,408 ± 71^{a}	2,592 ± 315^{bcd}	2,916 ± 40^{bcd}	. . .
	Hatch 2	3,387 ± 110^{a}	2,704 ± 223^{bcd}	3,047 ± 51^{abc}	2,576 ± 138^{cd}
	Hatch 3	3,187 ± 66^{ab}	2,483 ± 144^{d}	2,804 ± 75^{bcd}	2,452 ± 92^{d}
	All hatches	3,340 ± 51^{x}	2,567 ± 109^{z}	2,908 ± 27^{y}	2,519 ± 84^{z}

$^{a–d}$Means with no common superscript differ significantly for comparisons among three separable hatches ($P \leq 0.05$).

$^{x–z}$Means with no common superscript differ significantly for comparisons for all hatches combined within an age ($P \leq 0.05$).

[1]Data are means ± SEM; number of birds per category shown in Table 4.

For all three hatches pooled, male ascitic broilers had heavier RV, lighter LVS, lighter TV, and higher RV:TV ratios than male nonascitic broilers (Table 8). Similar comparisons reveal a higher RV:TV ratio in ascitic than in nonascitic females; otherwise, RV was not higher nor was LVS weight lower in ascitic than in nonascitic females. The mass of the RV primarily reflects the work performed to propel blood flow through the pulmonary vasculature, whereas the left ventricular mass increases in proportion to body weight and thereby reflects the work performed to deliver a cardiac output sufficient to supply oxygen and nutrients in support of whole body requirements for maintenance, activity, and growth (Burton and Smith, 1967; Burton *et al*., 1968). In this context, the ventricular weight data for all broilers combined, and for the heaviest apparently healthy broilers on Day 42, were normalized for final body weights (Table 9). Dividing LVS weight by body weight eliminated all differences between ascitic and nonascitic males or females, respectively. In contrast, dividing RV weight by body weight further amplified differences between ascitic and nonascitic broilers. The relative increase in RV weight for ascitic broilers ac-

TABLE 6. Heart rate (beats per min BPM), hematocrit (percentage packed red blood cell volume), and percentage saturation of hemoglobin with oxygen (Hb O_2 SAT) measured on Day 42 in male and female broilers selected on Day 42 as the largest individuals remaining from three consecutive hatches, and that subsequently did (ascitic) or did not (nonascitic) develop ascites by 51 d of age[1]

Parameter	Hatch	Nonascitic males	Ascitic males	Nonascitic females	Ascitic females
Heart rate, BPM	Hatch 1	355 ± 8^{bc}	337 ± 17^{abc}	360 ± 6^{abc}	. . .
	Hatch 2	371 ± 6^{abc}	348 ± 11^{abc}	393 ± 8^{a}	357 ± 7^{abc}
	Hatch 3	381 ± 5^{ab}	337 ± 9^{c}	385 ± 7^{a}	362 ± 7^{abc}
	All hatches	368 ± 4^{x}	340 ± 6^{y}	377 ± 4^{x}	360 ± 5^{xy}
Hematocrit, %	Hatch 1	35.8 ± 0.6^{c}	37.0 ± 1.9^{abc}	36.4 ± 1.0^{bc}	. . .
	Hatch 2	36.4 ± 0.8^{bc}	40.7 ± 2.5^{abc}	35.6 ± 0.6^{bc}	42.3 ± 2.2^{a}
	Hatch 3	35.0 ± 0.9^{c}	41.2 ± 1.4^{ab}	38.2 ± 0.8^{abc}	40.1 ± 2.1^{abc}
	All hatches	35.8 ± 0.4^{y}	40.3 ± 1.1^{x}	36.8 ± 0.5^{y}	41.3 ± 1.5^{x}
Hb O_2 SAT, %	Hatch 1	76.6 ± 2.3^{ab}	56.7 ± 4.3^{abcd}	73.8 ± 2.7^{abc}	. . .
	Hatch 2	73.2 ± 1.9^{abc}	59.8 ± 4.2^{bcd}	75.5 ± 2.6^{abc}	56.9 ± 3.0^{d}
	Hatch 3	78.4 ± 1.8^{a}	67.3 ± 3.7^{abcd}	73.7 ± 2.2^{abc}	69.0 ± 5.3^{abcd}
	All hatches	75.9 ± 1.2^{x}	63.2 ± 2.6^{y}	74.2 ± 1.5^{x}	62.5 ± 3.3^{y}

$^{a–d}$Means with no common superscript differ significantly for comparisons among three separate hatches ($P \leq 0.05$).

^{x,y}Means with no common superscript differ significantly for comparisons of all hatches combined within a parameter ($P \leq 0.05$).

[1]Data are means ± SEM; number of birds per category shown in Table 4.

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TABLE 7. Electrocardiogram lead II R-wave amplitude, RS-wave amplitude, and S-wave amplitude measured on Day 42 in male and female broilers selected on Day 42 as the largest individuals remaining from three consecutive hatches, and that subsequently did (ascitic) or did not (nonascitic) develop ascites by 51 d of age[1]

Parameter	Hatch	Nonascitic males	Ascitic males	Nonascitic females	Ascitic females
Lead II R, mV	Hatch 1	0.040 ± 0.007^{a}	0.049 ± 0.017^{a}	0.062 ± 0.014^{a}	. . .
	Hatch 2	0.068 ± 0.009^{a}	0.030 ± 0.008^{a}	0.053 ± 0.014^{a}	0.031 ± 0.006^{a}
	Hatch 3	0.069 ± 0.014^{a}	0.049 ± 0.008^{a}	0.053 ± 0.009^{a}	0.048 ± 0.006^{a}
	All hatches	0.058 ± 0.006^{x}	0.043 ± 0.006^{x}	0.057 ± 0.007^{x}	0.039 ± 0.005^{x}
Lead II RS, mV	Hatch 1	0.097 ± 0.007^{c}	0.129 ± 0.019^{abc}	0.122 ± 0.014^{c}	. . .
	Hatch 2	0.136 ± 0.011^{c}	0.243 ± 0.060^{a}	0.129 ± 0.016^{c}	0.150 ± 0.020^{abc}
	Hatch 3	0.146 ± 0.163^{ab}	0.234 ± 0.024^{ab}	0.119 ± 0.011^{c}	0.187 ± 0.029^{abc}
	All hatches	0.124 ± 0.007^{y}	0.218 ± 0.023^{x}	0.122 ± 0.008^{y}	0.167 ± 0.017^{xy}
Lead II S, mV	Hatch 1	–0.056 ± 0.008^{a}	–0.080 ± 0.032^{ab}	–0.060 ± 0.008^{a}	. . .
	Hatch 2	–0.068 ± 0.014^{a}	–0.213 ± 0.066^{b}	–0.076 ± 0.013^{a}	–0.118 ± 0.021^{ab}
	Hatch 3	–0.077 ± 0.017^{a}	–0.185 ± 0.018^{b}	–0.065 ± 0.011^{a}	–0.138 ± 0.027^{ab}
	All hatches	–0.066 ± 0.007^{x}	–0.175 ± 0.024^{y}	–0.066 ± 0.006^{x}	–0.128 ± 0.016^{y}

$^{a–d}$Means with no common superscript differ significantly for comparisons among three separate hatches ($P \leq 0.05$).

^{x,y}Means with no common superscript differ significantly for comparisons of all hatches combined within a parameter ($P \leq 0.05$).

[1]Data are means ± SEM; number of birds per category shown in Table 4.

TABLE 8. Right ventricular weight (RV), left ventricular plus septum weight (LVS), total ventricular weight (TV), and right ventricular weight to total ventricular weight ratio (RV:TV) for male and female broilers selected on Day 42 as the largest individuals remaining from three consecutive hatches, and that subsequently did (ascitic) or did not (nonascitic) develop ascites by 51 d of age[1]

Parameter	Hatch	Nonascitic males	Ascitic males	Nonascitic females	Ascitic females
RV, g	Hatch 1	3.29 ± 0.19^{abcd}	4.04 ± 1.03^{abcd}	2.66 ± 0.19^{d}	. . .
	Hatch 2	3.78 ± 0.28^{abc}	4.62 ± 0.44^{a}	2.71 ± 0.21^{bcd}	3.43 ± 0.30^{abcd}
	Hatch 3	3.62 ± 0.21^{abcd}	4.40 ± 0.20^{a}	3.18 ± 0.18^{abcd}	3.69 ± 0.27^{abcd}
	All hatches	3.56 ± 0.14^{y}	4.40 ± 0.23^{x}	2.85 ± 0.11^{y}	3.55 ± 0.20^{xy}
LVS, g	Hatch 1	9.58 ± 0.22^{ab}	6.71 ± 0.65^{c}	8.26 ± 0.26^{bc}	. . .
	Hatch 2	10.56 ± 0.39^{a}	6.90 ± 0.52^{c}	8.42 ± 0.25^{bc}	7.13 ± 0.46^{c}
	Hatch 3	10.69 ± 0.45^{a}	7.72 ± 0.53^{bc}	8.43 ± 0.29^{bc}	7.37 ± 0.59^{bc}
	All hatches	10.24 ± 0.21^{x}	7.30 ± 0.34^{y}	8.36 ± 0.16^{y}	7.24 ± 0.35^{y}
TV, g	Hatch 1	12.86 ± 0.28^{ab}	10.75 ± 1.68^{abc}	10.92 ± 0.37^{c}	. . .
	Hatch 2	14.33 ± 0.55^{a}	11.52 ± 0.76^{abc}	11.13 ± 0.37^{bc}	10.56 ± 0.54^{bc}
	Hatch 3	14.31 ± 0.50^{a}	12.12 ± 0.66^{abc}	11.61 ± 0.40^{bc}	11.06 ± 0.61^{bc}
	All hatches	13.79 ± 0.27^{x}	11.70 ± 0.48^{y}	11.21 ± 0.23^{y}	10.79 ± 0.40^{y}
RV:TV ratio	Hatch 1	0.25 ± 0.01^{bcd}	0.36 ± 0.05^{ab}	0.24 ± 0.01^{d}	. . .
	Hatch 2	0.26 ± 0.01^{bcd}	0.40 ± 0.02^{a}	0.24 ± 0.01^{d}	0.33 ± 0.02^{abc}
	Hatch 3	0.25 ± 0.01^{bcd}	0.37 ± 0.01^{a}	0.27 ± 0.01^{bcd}	0.34 ± 0.02^{ab}
	All hatches	0.26 ± 0.01^{y}	0.38 ± 0.01^{x}	0.25 ± 0.01^{y}	0.33 ± 0.02^{x}

$^{a–d}$Means with no common superscript differ significantly for comparisons among three separate hatches ($P \leq 0.05$).

^{x,y}Means with no common superscript differ significantly for comparisons of all hatches combined within a parameter ($P \leq 0.05$).

[1]Data are means ± SEM; number of birds per category shown in Table 4.

TABLE 9. Right ventricle (RV), left ventricle plus septum (LVS), and total ventricular (TV) weights normalized by the final body weight for all male and female broilers (ALL broilers) or for the largest individuals on Day 42 (Day 42 Largest) with all birds from three consecutive hatches pooled[1]

Category	Hatch	n	Parameter	Nonascitic males	Ascitic males	Nonascitic females	Ascitic females
ALL Broilers	ALL	511	RV/BW	0.0012 ± 0.00004^{c}	0.0020 ± 0.00004^{a}	0.0011 ± 0.00003^{c}	0.0018 ± 0.00004^{b}
	ALL	511	LVS/BW	0.0032 ± 0.00006^{ab}	0.0033 ± 0.00006^{a}	0.0029 ± 0.00004^{c}	0.0031 ± 0.00006^{bc}
	ALL	511	TV/BW	0.0044 ± 0.00009^{c}	0.0053 ± 0.00008^{a}	0.0040 ± 0.00006^{d}	0.0049 ± 0.00009^{b}
Day 42 Largest	ALL	151	RV/BW	0.0011 ± 0.00004^{b}	0.0017 ± 0.00008^{a}	0.0010 ± 0.00004^{b}	0.0014 ± 0.00010^{a}
	ALL	151	LVS/BW	0.0031 ± 0.00007^{a}	0.0029 ± 0.00012^{a}	0.0029 ± 0.00004^{a}	0.0029 ± 0.00011^{a}
	ALL	151	TV/BW	0.0042 ± 0.00009^{ab}	0.0046 ± 0.00016^{a}	0.0039 ± 0.00007^{b}	0.0043 ± 0.00017^{ab}

$^{a–d}$Means with no common superscript differ significantly for comparisons across a single row ($P \leq 0.05$).

[1]Data are means ± SEM.

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TABLE 10. Linear regression equations, Pearson correlation coefficients (r), and probability (*P*) values for relationships between right:total ventricular weight ratios (RV:TV) on Day 51 vs minimally invasive diagnostic indices measured for the largest apparently healthy males and females on Day 42

RT:TV vs Indices	Sex[1]	Equation	r	*P*
RV:TV vs final body weight (BWF)	F	RV:TV = –0.00009 BWF + 0.052	0.436	0.0002
RV:TV vs BWF	M	RV:TV = –0.00005 BWF + 0.441	0.357	0.0009
RV:TV vs heart rate (HR)	F	RV:TV = –0.00054 HR + 0.469	0.275	0.0243
RV:TV vs HR	M	RV:TV = –0.0084 HR + 0.585	0.380	0.0004
RV:TV vs hematocrit (HCT)	F	RV:TV = +0.0102 HCT – 0.116	0.742	0.0001
RV:TV vs HCT	M	RV:TV = +0.0100 HCT – 0.087	0.563	0.0001
RV:TV vs saturation of hemoglobin with O_2 (HbO_2)	F	RV:TV = –0.00312 HbO_2 + 0.490	0.597	0.0001
RV:TV vs HbO_2	M	RV:TV = –0.00390 HbO_2 + 0.566	0.588	0.0001
RV:TV vs electrocardiogram Lead II RS-wave (RS)	F	RV:TV = +0.258 RS + 0.233	0.254	0.0379
RV:TV vs RS	M	RV:TV = +0.320 RS + 0.235	0.327	0.0025
RV:TV vs electrocardiogram Lead II S-wave (S)	F	RV:TV = –0.491 S + 0.229	0.415	0.0005
RV:TV vs S	M	RV:TV = –0.323 S + 0.252	0.354	0.0010

[1]n = 67 for females (F), 83 for males (M).

counted for corresponding increases in the values for TV divided by body weight in ascitic compared with nonascitic males and females.

When combined, the data shown in Table 9 provide no support for a primary involvement of cardiomyopathy in the pathogenesis of PHS. Instead, these data indicate the two major factors contributing to differences in ventricular mass between ascitic and nonascitic broilers are the magnitude of the pulmonary arterial pressure developed by the RV, and the body mass being supplied with cardiac output by the left ventricle. The consistently elevated RV:TV ratio for ascitic males and females (Tables 3 and 8) supports a primary role of pulmonary hypertension in the pathogenesis of ascites triggered by cool temperatures (Cueva *et al*., 1974; Huchzermeyer and DeRuyck, 1986; Hernandez, 1987; Peacock *et al*., 1989; Julian, 1993; Odom, 1993; Wideman and Bottje, 1993; Lubritz *et al*., 1995).

None of the minimally invasive diagnostic indices consistently differentiated ascitic from nonascitic males and females within each of the separate hatches (Tables 5 to 7), suggesting the predictive value of these indices may vary when applied to individual birds of different sex. Linear regression analysis was used to evaluate the correlations between diagnostic indices measured in the largest apparently healthy broilers on Day 42 and the corresponding RV:TV ratios on Day 51. The RV:TV ratios were not ($P \geq 0.05$) correlated with body weights on Days 1, 14, or 42, or with the ECG Lead II R-wave amplitude (data not shown). The RV:TV ratios were positively correlated with HCT and the ECG Lead II RS-wave amplitude, and negatively correlated with the final body weight, heart rate, percentage saturation of hemoglobin with oxygen, and the ECG Lead II S-wave amplitude (Table 10). The relatively low Pearson correlation coefficients for these comparisons may partially reflect the discontinuity between the age at which the diagnostic indices were measured and the subsequent 9-d period during which the RV:TV ratios had an appreciable opportunity to increase in birds developing PHS. To address this dilemma within a commercial selection program, multiple diagnostic indices (HCT or oximetry coupled with the ECG Lead II S-wave amplitude) can be evaluated, and the data for each bird can be compared with a series of threshold values known to be predictive for the genotype, sex, and environmental conditions (Tables 6 and 7). For example, under the conditions of the present study, an aggressive threshold for culling on Day 42 might be set near the mean values for nonascitic birds (36% for HCT, 75% for oximetry, –0.066 mV for ECG Lead II S-wave amplitude), or a more relaxed threshold might be set near the mean values for birds that subsequently developed ascites (40% for HCT, 63% for oximetry, –0.150 mV for ECG Lead II S-wave amplitude). These diagnostic indices only have predictive value when applied to very fast growing broilers that have been rigorously challenged to expose subclinical susceptibility to PHS, with the caveat that the incidence of PHS susceptibility can be viewed as a continuum proportional to the magnitude of the induced challenge (Wideman and Bottje, 1993; Rhoads *et al*., 1995; Wideman *et al*., 1997).

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