Sodium Chloride-Induced Acute and Chronic Pulmonary Hypertension Syndrome in Broiler Chickens

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ABSTRACT Two hundred forty 1-d-old Arbor Acres commercial broiler chicks were divided into control and experimental (T_1 and T_2) groups that, between 8 and 42 d of age, were provided drinking water containing 0, 600, or 1,200 mg/L sodium from sodium chloride, respectively. The pulmonary hypertension syndrome (PHS) incidence and the right to total ventricle weight ratio (RV/TV) were calculated weekly, and blood samples and lung tissues were collected weekly from 10 birds per group to evaluate the structural and hemodynamic characteristics of pulmonary vessels. Saline drinking water significantly increased the incidence of PHS and RV/TV ratios. In the T_2 group the PHS mortality exhibited 2 peaks, including an acute peak from 14 to 21 d of age and a chronic peak from 35 to 42 d of age. During the acute peak of PHS mortality the blood volume (BV), filtration index (FI), and packed cell volume (PCV) increased in groups T_1 and T_2 when compared with the control group. During the acute peak there were no differences among groups in the ratio of wall to total area (WA/TA), medial thickness of pulmonary arteriole walls (mMTPA), the percentage of thick-walled peripheral lung vessels (%TWPV), the percentage of muscular arterioles (%MA), or the percentage of nonmuscular arterioles (%NMA) in pulmonary arterioles. During the chronic peak of PHS mortality, group T_2 exhibited the highest values for %TWPV, %MA, WA/TA, and mMTPA and the lowest values for %NMA when compared with the T_1 and control groups. Also during the chronic peak the groups did not differ in BV or FI, whereas PCV remained elevated above control values in groups T_1 and T_2. These observations indicate that hemodynamic changes related to viscous resistance to blood flow (BV, FI, PCV) predominated throughout the acute peak of PHS mortality, whereas, during the chronic stages of PHS mortality, increased vascular resistance to blood flow also was imposed by remodeling of the pulmonary vasculature.

(*Key words*: muscular arteriole, pulmonary hypertension, sodium, vascular resistance)

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INTRODUCTION

A survey in the United States showed that pulmonary hypertension syndrome (PHS, ascites) accounts for over 25% of annual broiler losses (Odom, 1993). An incidence of 5 to 10% PHS was reported in China (Shi, 1993). A global incidence of 4 to 5% PHS has been reported, and this mortality represents a cost to the industry of about US $1 billion annually (Maxwell and Robertson, 1997). Previous studies have demonstrated that excessive sodium chloride intake induces PHS in broilers (Julian, 1987; Mirsalimi et al., 1992a,b). Scrivner (1946) demonstrated that turkey poults develop edema and ascites when provided excessive sodium from a variety of sources, including sodium citrate, sodium iodide, sodium sulfate, sodium hydroxide, sodium bicarbonate, and sodium chloride. The symptoms of right ventricular failure resulting from sodium overdose are similar to those observed in naturally occurring pulmonary hypertension-induced ascites in broilers (Scrivner, 1946; Julian, 1987; Wilson et al., 1988; Julian et al., 1992; Mirsalimi et al., 1992a,b). In coastal areas near the eastern sea in southeastern China, excess sodium in the drinking water from wells induces a high incidence of PHS (Zhang et al., 1996). A previous study indicated that 2 peaks of sodium-induced PHS mortality occur, with the first peak observed from 14 to 21 d of age and the second peak occurring from 35 to 42 d of age (Zhang et al., 1998). The mechanism responsible for triggering these acute and chronic peaks of sodium-induced PHS mortality in broilers was unclear. For field cases of PHS induced by hypoxia, remodeling and muscularization of pulmonary arterioles in broilers with PHS were not observed from 14 to 21 d of age, but these changes

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Abbreviation Key: BV = blood volume; FI = filtration index; %MA = percentage of muscular arterioles; mMTPA = medial thickness of pulmonary arteriole walls; %NMA = percentage of nonmuscular arterioles; PCV = packed cell volume; PHS = pulmonary hypertension syndrome; RV = right ventricle; TV = total ventricle weight; T_1 and T_2 = experimental groups; %TWPV = percentage of thick-walled peripheral lung vessels; WA/TA = ratio of wall to total area.

were observed in broilers developing PHS after 35 d of age (Li et al., 1999; Xiang et al., 2001). Therefore, the objectives of the present study were to evaluate the structural changes of pulmonary vessels and the hemodynamic changes occurring over the course of PHS induced by the use of high levels of sodium chloride.

MATERIALS AND METHODS

Two hundred forty l-d-old Arbor Acres commercial chicks were divided randomly into control and experimental (T_1 and T_2) groups. The chicks were placed in one large floor pen per group (n = 80 per group) on new straw litter. Each large floor pen was subdivided into 3 smaller pens using plastic net barriers, with the smaller pens containing 25, 25, and 30 birds, respectively. Chinese standard corn-soybean-based broiler starter (210 g of protein/kg, 3.08 Mcal of ME/kg, 10 g of calcium/kg, 4.5 g of phosphate/kg, and 3 g of sodium/kg) and grower (195 g of protein/kg, 3.11 Mcal of ME/kg, 8.9 g of calcium/kg, 4.2 g of phosphate/kg, 3 g of sodium/kg) diets were provided ad libitum. All broilers were provided tap water until they reached 7 d of age, after which they were provided with 0, 600, and 1,200 mg/L sodium from sodium chloride in their drinking water (control, T_1 and T_2 groups, respectively) from 8 to 42 d of age.

Throughout the study, mortality was recorded daily, and the PHS incidences were calculated weekly. According to previously described methods (Julian et al, 1989; Monge and Loon-Velarde, 1991), the heart was removed, and the atria, major vessels, and fat were trimmed off. The right ventricle (RV) was cut away from the left ventricle and septum, the RV and total ventricle (TV) weights were recorded, and the RV/TV ratio was calculated. Birds having a RV/TV ratio >0.299 were classified as suffering from right ventricular failure, and RV/TV ratios >0.250 were considered indicative that pulmonary hypertension was present (Julian, 1987; Julian et al., 1989).

Beginning on d 9, blood and lung samples were collected weekly from 10 randomly selected chicks per group, including 3 or 4 chicks from each of the smaller pens. The blood volume (BV), filtration index (FI), and packed cell volume (PCV) were measured as described previously (Mirsalimi et al., 1992a,b). Birds were anesthetized with ether then euthanized by cervical dislocation. The chest and abdomen were opened, the lungs were fixed in situ by instilling buffered 10% formalin through a tracheal cannula for 5 min, and then the lungs were removed and immersed in 10% buffered formalin. Complete transverse sections of the left lung were cut across the hilum, and these sections were stained with Humberstone and Gomori's elastin stain for elastic tissue. Similar populations of vessels in consistent anatomical sites were examined in control and PHS broilers. Vessels in 1 or 2 complete transverse sections of lung were counted (Leach et al., 1977), and the histopathological alterations of intima, media, and adventitia in different types of pulmonary arterioles in control broilers and broilers with PHS were observed and measured under the microscope. Referring to classification methods for pulmonary vascular muscularity for mammals (Widimsky and Herget, 1990; Song et al., 1993), the broiler pulmonary arterioles with complete internal and external elastic lamina were identified as muscular arterioles (MA); those with a partial internal elastic lamina and a complete external elastic lamina were identified as partially muscular arterioles (PMA), and those having a single elastic lamina were categorized as nonmuscular arterioles (NMA). A double elastic lamina was recorded when 2 distinct laminae separated by a space were present for at least half the circumference, or length if the vessel was cut longitudinally. The inner elastic lamina was sometimes interrupted rather than continuous. The wall area (between external and internal elastic laminas), total area (within external elastic lamina), medial thickness (between external and internal elastic laminas), and diameter of external elastic lamina of small vessels up to 60 μm in diameter were measured using a microscopic image analysis program. Next, the ratio of wall area to total area and the mean medial thickness in pulmonary arterioles (the ratio of medial thickness to diameter of external elastic lamina) were calculated (Li et al., 1999). The percentage of muscular arterioles (%MA), percentage of partially muscular arterioles (%PMA), and percentage of nonmuscular arterioles (%NMA) were calculated (Xiang et al., 2001). The percentage of thick-walled peripheral lung vessels (%TWPV) was calculated based on the percentage of small vessels up to 60 μm in diameter and having a double elastic lamina per each histological section.

Highly detailed, labor-intensive microscopic measurements were required to assess the pulmonary vascular muscularity of each individual broiler. By design, the individual broiler was considered an independent replicated unit, and average values for each of the smaller pens were not calculated. Accordingly, within each weekly sampling period, the 10 individual birds from each group were compared among groups by one-way ANOVA (SAS Institute, 1994). Differences between pairs of groups were confirmed by a *t*- test. $P < 0.05$ was considered significant. All results are presented as the mean ± SEM.

RESULTS

As shown in Table 1, between 9 and 42 d of age, 33 of the 160 broilers consuming saline drinking water developed PHS. Group T_2 had the highest PHS incidence, and the PHS incidence for group T_1 was higher ($P < 0.05$) than that of the control group. Acute (d 14 to 21) and chronic (d 35 to 42) peaks of PHS mortality are evident in group T_2, which consumed the highest level of sodium in the drinking water. All broilers diagnosed as having PHS exhibited right ventricular hypertrophy and dilation, and had moderate to severe ascites (100 to 400 mL), with clots of fibrin floating in the fluid and covering the swollen or shrunken liver.

Hemodynamic variables (BV, FI, RV/TV ratios) are shown in Table 2. The RV/TV ratios did not differ between groups at 9 d of age; however, the RV/TV ratios

TABLE 1. The incidence of sodium-induced pulmonary hypertension syndrome (PHS) in broilers consuming water that from 8 to 42 d of age contained supplemental sodium from sodium chloride at 0 mg/L (control group, C), 600 mg/L (group T_1), or 1,200 mg/L (group T_2)

Group	n	Age (g)						Total PHS	Incidence (%)
		9	14	21	28	35	42		
C	80	0	0	0	0	0	0	0	0[c]
T_1	80	0	1	1	0	3	1	6	7.5[b]
T_2	80	0	5	10	1	7	4	27	33.75[a]

[a–c]Means lacking a common superscript within a column are significantly different at $P < 0.05$.

for group T_2 were higher than those of the control group at 14, 21, 35, and 42 d of age. Also at 14 and 28 d of age the RV/TV ratios for group T_1 were higher than those of the control group. During d 9 to 21 the BV was higher for group T_1 than for T_2, and both saline groups had higher BV values than the control group. Thereafter (d 28 to 42), BV did not differ among groups. The FI was higher in groups T_1 and T_2 than in the control group from 9 to 21 d of age, and the FI of group T_2 remained elevated when compared with groups T_1 and the control at 28 d of age. Thereafter the FI values did not differ among groups. As shown in Figure 1, the PCV values did not differ between groups at 9 d of age; however, at 14, 21, 35, and 42 d of age the PCV values for saline groups T_1 and T_2 were higher than those of the control group.

The structural changes of pulmonary arterioles in broilers with pulmonary hypertension were distinctly identifiable under the light microscope, including increases in the thickness and area of arteriole walls and increased numbers of pulmonary arterioles up to 60 μm in diameter having internal and external elastic laminas. Normal broilers typically had more pulmonary arterioles with single elastic lamina. For randomly selected broilers, the groups did not differ in the values for WA/TA and mMTPA through 28 d of age (Table 3), or in the values of %MA, %PMA, %NMA, and %TWPV through 21 d of age (Table 3). However, by d 28 the values in groups T_1 and T_2 were higher for %MA and %TWPV and lower for %NMA when compared with the control group ($P < 0.01$). There were no differences for the values of %MA, %NMA, and %TWPV between groups T_1 and T_2 through d 28 ($P > 0.05$, Table 3). At 35 to 42 d of age the values of WA/TA, mMTPA, %MA, %PMA, and %TWPV were highest in group T_2, intermediate in group T_1, and lowest in the control group. The inverse relationship among groups pertained for %NMA on d 35 and 42 ($P < 0.05$, Table 3).

DISCUSSION

Increases in pulmonary blood flow (cardiac output), increases in the resistance to blood flow (pulmonary vascular resistance, blood viscosity), or both are the primary causes of pulmonary hypertension syndrome in broilers (Wideman and Bottje, 1993; Wideman, 2001). Factors that increase blood flow or increase resistance to flow are additive. Increased blood viscosity caused by the polycythemia of hypoxia, increased erythrocyte rigidity (Mirsalimi and Julian, 1991; Julian, 1993), increased caloric metabolism of thyroid hormone, or muscularization of pulmonary arterioles (Xiang et al., 2001) are all more likely to produce PHS in fast-growing birds than in slow-growing birds.

The results of the present study show that excess sodium in the drinking water successfully produced pulmonary hypertension as measured by the RV/TV ratio in broiler chickens. Evidence of 2 peaks of PHS mortality in the group consuming the highest level of sodium in the drinking water was also revealed. In that group (T_2), 15 of 80 broilers developed PHS from 14 to 21 d of age, mortality was minimal on d 28, then 11 of 80 developed

TABLE 2. The right ventricle to total ventricle (RV/TV) weight ratio and values for blood volume (BV) and filtration index (FI) in broilers consuming water that from 8 to 42 d of age contained supplemental sodium from sodium chloride at 0 mg/L (control group, C), 600 mg/L (group T_1), and 1,200 mg/L (group T_2)

Parameter and group	Age (d)					
	9	14	21	28	35	42
RV/TV						
C	0.218 ± 0.012	0.216 ± 0.015[c]	0.214 ± 0.031[b]	0.217 ± 0.030[b]	0.218 ± 0.035[b]	0.230 ± 0.050[b]
T_1	0.219 ± 0.014	0.234 ± 0.020[b]	0.246 ± 0.042[ab]	0.252 ± 0.022[a]	0.253 ± 0.060[ab]	0.250 ± 0.038[ab]
T_2	0.219 ± 0.030	0.297 ± 0.023[a]	0.273 ± 0.050[a]	0.272 ± 0.033[a]	0.285 ± 0.062[a]	0.280 ± 0.040[a]
BV						
C	4.82 ± 0.19[c]	7.63 ± 0.31[c]	9.17 ± 0.79[c]	9.28 ± 0.38	8.42 ± 0.13	8.66 ± 0.12
T_1	5.95 ± 0.14[b]	8.42 ± 0.29[b]	10.99 ± 0.17[b]	9.59 ± 0.74	8.27 ± 0.23	8.76 ± 0.42
T_2	6.67 ± 0.17[a]	9.55 ± 0.14[a]	11.74 ± 0.13[a]	9.25 ± 0.35	8.67 ± 0.28	8.47 ± 0.10
FI						
C	12.50 ± 1.10[c]	11.91 ± 1.34[c]	11.93 ± 0.73[c]	11.28 ± 1.04[b]	11.75 ± 0.46	10.29 ± 0.84
T_1	14.45 ± 1.34[b]	14.60 ± 0.73[b]	12.50 ± 0.43[b]	11.92 ± 0.63[b]	11.43 ± 0.37	10.66 ± 1.46
T_2	17.23 ± 2.76[a]	19.60 ± 0.89[a]	14.70 ± 0.54[a]	13.30 ± 1.30[a]	12.05 ± 0.99	10.15 ± 1.22

[a–c]Means lacking a common superscript within a column are significantly different at $P < 0.05$.

PHS from 35 to 42 d of age. During the first peak of PHS mortality, the pulmonary arteriole values for %MA, %PMA, %TWPV, %WA/TA, and %mMTPA were not elevated, providing no indication that vascular remodeling (increased pulmonary arteriolar muscularization and thickness) had been triggered by pulmonary hypertension. However, BV had increased in groups T_1 and T_2 when compared with the control group, and it is possible that this added volume might increase the workload on the heart by increasing the venous filling pressure and therefore venous return (Mirsalimi et al., 1992b). Contemporaneous increases in FI in groups T_1 and T_2 indicate that the excess sodium intake led to a reduction of erythrocyte deformability. The reduction of deformability induced with salt treatment in broiler chickens is partly attributed to an increase in mean cell volume (Mirsalimi et al., 1992a). Decreased erythrocyte deformability combined with an increased PCV should increase the blood viscosity and thus increase the resistance to blood flow (Chien et al., 1967). Broilers may be particularly susceptible to increases in blood viscosity because their noninflatable lungs are restricted by the size of the thoracic cavity, and their blood capillaries are small and relatively noncompliant. Low capillary expandibility and reduced erythrocyte deformability may be important in the development of cardiac overload and failure. Therefore, excess sodium caused increases in BV and PCV, and reductions in erythrocyte deformability, which together increased the resistance to pulmonary blood flow through an inadequate pulmonary vasculature and caused acute pulmonary hypertension during the first peak of PHS mortality.

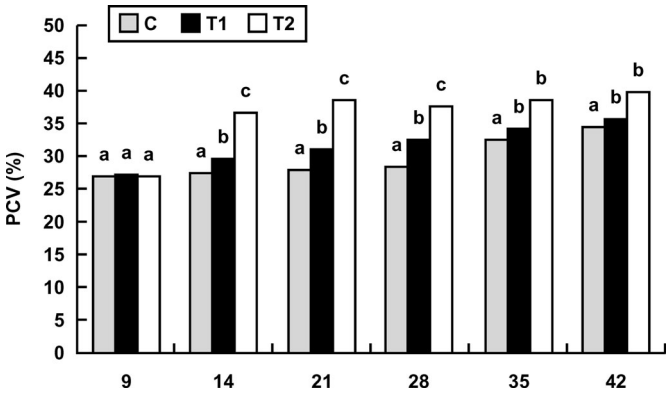

FIGURE 1. The packed cell volume (PCV) of broilers consuming water that from 8 to 42 d of age contained supplemental sodium from sodium chloride at 0 mg/L (control group, C), 600 mg/L (group T_1), and 1,200 mg/L (group T_2). [a–c]Means with the different indices within a column are significantly different at $P < 0.05$.

During the second peak of PHS mortality (d 35 to 42) there was no evidence that sodium caused a sustained increase in BV or FI, but the pulmonary arteriole values for %MA, %PMA, %TWPV, %WA/TA and %mMTPA increased markedly in proportion to sodium intake. These results indicate that during the second peak of PHS, excess sodium intake continued to trigger increases in pulmonary arterial pressure (elevated by RV/TV ratio). The pathogenesis from d 35 to 42 reflects tissue remodeling through the proliferation and hypertrophy of pulmonary arterial cells (Widimsky and Herget, 1990), leading to pulmonary arteriolar muscularization and increased

TABLE 3. The percentage of vessel wall area to total area (WA/TA), mean medial thickness of pulmonary arterioles (mMTPA), muscular arterioles (MA), partially muscular arterioles (PMA), nonmuscular arterioles (NMA), and thick-walled peripheral lung vessels (TWPV) in broilers consuming water that from 8 to 42 d of age contained supplemental sodium from sodium chloride at 0 mg/L (control group C), 600 mg/L (group T_1), or 1,200 mg/L (group T_2)

Parameter and group	Age (d)					
	9	14	21	28	35	42
WA/TA(%)						
C	0.54 ± 0.07	0.58 ± 0.04	0.52 ± 0.05	0.681 ± 0.04	0.660 ± 0.04[c]	0.675 ± 0.04[c]
T_1	0.57 ± 0.05	0.61 ± 0.08	0.50 ± 0.06	0.710 ± 0.08	0.745 ± 0.05[b]	0.725 ± 0.05[b]
T_2	0.55 ± 0.06	0.56 ± 0.05	0.54 ± 0.04	0.708 ± 0.05	0.798 ± 0.03[a]	0.791 ± 0.08[a]
mMTPA (%)						
C	0.32 ± 0.07	0.31 ± 0.06	0.33 ± 0.04	0.44 ± 0.04	0.401 ± 0.04[c]	0.421 ± 0.04[c]
T_1	0.30 ± 0.06	0.32 ± 0.08	0.31 ± 0.05	0.465 ± 0.10	0.495 ± 0.05[b]	0.552 ± 0.05[b]
T_2	0.31 ± 0.04	0.29 ± 0.05	0.34 ± 0.04	0.451 ± 0.06	0.547 ± 0.10[a]	0.620 ± 0.08[a]
MA (%)						
C	6.90 ± 1.07	7.20 ± 1.54	8.60 ± 3.05	10.3 ± 2.54[b]	11.5 ± 2.24[c]	14.4 ± 2.44[c]
T_1	6.20 ± 1.65	7.70 ± 2.28	8.60 ± 2.36	12.7 ± 1.10[a]	17.6 ± 2.05[b]	22.4 ± 1.95[b]
T_2	7.50 ± 2.06	7.40 ± 2.15	9.80 ± 1.84	13.4 ± 2.15[a]	21.4 ± 1.73[a]	28.7 ± 3.18[a]
PMA (%)						
C	5.60 ± 1.57	4.10 ± 2.16	5.80 ± 2.34	7.20 ± 2.14	8.60 ± 1.84[c]	9.30 ± 3.04[c]
T_1	5.70 ± 1.86	5.10 ± 1.08	6.80 ± 1.65	7.90 ± 2.70	11.2 ± 2.35[b]	12.4 ± 2.95[b]
T_2	4.90 ± 2.04	4.80 ± 1.35	6.20 ± 2.44	8.30 ± 3.26	14.1 ± 1.55[a]	15.7 ± 2.58[a]
NMA (%)						
C	87.5 ± 3.47	88.7 ± 2.35	85.6 ± 3.21	82.5 ± 2.47[a]	79.9 ± 3.42[a]	76.3 ± 2.55[a]
T_1	88.1 ± 3.76	87.3 ± 2.65	84.6 ± 2.53	79.4 ± 1.96[b]	71.2 ± 2.13[b]	65.2 ± 3.54[b]
T_2	87.6 ± 2.64	86.2 ± 2.54	84.0 ± 3.42	78.3 ± 4.04[b]	64.5 ± 3.85[c]	55.6 ± 5.12[c]
TWPV (%)						
C	12.5 ± 1.21	11.3 ± 1.47	14.4 ± 2.65	17.5 ± 2.23[b]	20.1 ± 3.11[c]	23.7 ± 2.66[c]
T_1	11.9 ± 1.76	12.7 ± 2.67	15.4 ± 2.16	20.6 ± 2.43[a]	28.8 ± 2.76[b]	34.8 ± 3.81[b]
T_2	12.4 ± 2.05	12.2 ± 1.45	16.0 ± 1.89	21.7 ± 1.88[a]	35.5 ± 3.14[a]	44.4 ± 4.11[a]

[a–c]Means lacking a common superscript within a column are significantly different at $P < 0.05$.

thickness. The muscularized pulmonary arterioles in turn increased the resistance to blood flow and further accelerated the occurrence of PHS during the second peak, which was defined to be a chronic phase of pulmonary hypertension syndrome in broilers.

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