Endothelin-1 Receptor Antagonist BQ123 Prevents Pulmonary Artery Hypertension Induced by Low Ambient Temperature in Broilers

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Evidence has indicated that endothelin-1 is related to the pathogenesis of hypertension. To characterize the role of endothelin-1 (ET-1) in the development of pulmonary hypertension syndrome in broilers, the blockade effect of ETA receptor (ET_A) antagonist, BQ123, on blood pressure in experimental models of pulmonary hypertension was examined. Birds were locally anesthetized and instrumented with venous catheters for pulmonary arterial pressure (PAP) and right ventricular pressure (RVP), followed by packed cell volume (PCV) and Ascites heart index (AHI) measured, after exposed to low ambient temperature for 7 or 14 d. In treated groups, BQ123 (0.4 or 2.0 μg/kg each time, 2 times a day), administered in abdominal cavities for 7 or 14 d during birds kept in low ambient temperature, prevented both PAP and RVP increasing, especially the high dose BQ123 lowered PAP and RVP to normotensive levels as that in control under normal temperature, whereas significant increases ($p<0.05$) were found in the two parameters of broilers in both untreated and saline treated group under low ambient temperature compared with those of birds in control. Furthermore, there was also a reduction in low ambient temperature-induced right ventricular hypertrophy in the groups administered BQ123. The preventive effect of BQ123 suggests that ET-1 is associated with the development of broilers' pulmonary hypertension, which leads to the development of ascites, and BQ123 can prevent the occurrence of pulmonary hypertension.

Key words BQ123; pulmonary artery hypertension; broiler; low ambient temperature

Pulmonary hypertension (PH) may result from numerous clinical entities affecting the pulmonary circulation primarily or secondarily. It is recognized that vascular endothelial dysfunction contributes to the development and perpetuation of PH by destroying the balances between vasodilating and vasoconstrictive forces, and between proliferative and antiproliferative forces. In that context, endothelin-1 (ET-1) and ET receptor (ETR) overproduction was rapidly targeted as a plausible contributor to the pathogenesis of PH.[1)] After binding to the ET_A, one of ETR, ET-1 has potent vasoconstrictive effects and positive inotropic effects on cardiomyocytes,[2,3)] and induces mitogenesis in endothelial cells[4)] and vascular smooth muscle cells in mammals[5)] leading to proliferation of the two types of cells. In addition to its long-lasting pressor actions, it also has been shown to be involved in arrhythmias in mammals. However, the biological function of ET-1 in poultry, especially its pathological role, has not been determined.

The ascites syndrome in broiler chickens, also known as pulmonary hypertension syndrome (PHS), is attributed to metabolic burdening, which is resulted from intensive genetic selection for rapid growth coupled with exposure to extreme environmental conditions, such as low ambient temperature, high altitude and high energy diet. These conditions impose difficulties on the broilers in fulfilling tissue demands for oxygen, namely relative hypoxia, resulting in birds exhibiting a decrease in blood oxygen saturation and high hematocrit values. It is known that PH is the major course in the development of the disease, but the crucial mechanism is still not fully elucidated.[6—8)] Many studies have shown that ET-1 plays a significant role in the human's pulmonary hypertension, which is pathophysiologically similar to PHS in broilers. Recently, increased plasma levels of the ET-1 had been demonstrated PH in broilers in low temperature, which contributes to ascites.[9)] Therefore, an association between ET-1 and pulmonary blood pressure disorders makes it necessary to study the action of ET-1 in the development of PH and ascites in broilers.

The purpose of the study is to understand the actions of ET-1 in the development of PH by BQ123, one of ET_A antagonists, and characterize the preventive effect of this antagonist on hemodynamics and cardiac function of broilers in low ambient temperature. Additionally, it can provide a new idea for preventing the development of PH and right ventricle hypertrophy, which leads to ascites in broilers.

MATERIALS AND METHODS

Animal Preparation and Treatment Day-old male broiler chicks (Arbor Acre, AA) of commercial strain were maintained in a environmental chambers with continuous lighting at a normal temperature of 28—30 °C. They were given free access to water and a commercial chick starter diet (23% crude protein, metabolizable energy=13.4 MJ/kg from 0—15 d) and grower diet (20% crude protein, metabolizable energy=13.4 MJ/kg from 16—30 d). BQ123 was purchased from America Peninsula Lab and kept in −30 °C after dissolved in 0.3% Dimethyl Sulfoxide (DMSO). At 16 d of age, birds were randomly allocated to 6 experimental groups, one control group and one BQ123 (2.0 μg/kg body weight every 12 h, N.BQ123) group in normal ambient temperature (28 °C), four in low ambient temperature (15—18 °C), including low temperature, low temperature+saline (L.S), low temperature+low-dose BQ123 (0.4 μg/kg body

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weight every 12 h, L.BQ123/0.4) and low temperature+high-dose BQ123 (2.0 μg/kg body weight every 12 h, L.BQ123/2.0) group, 60 chicks in each group. Birds were abdominally injected with 0.4 ml of solution containing 0.4 or 2.0 μg/kg BQ123 every 12 h from 16 to 30 d of age, the same volume of 0.9% saline were injected into broiler abdominal cavities at the same time in saline control. Weigh each bird every other day to determine the dosage of BQ123 to be administrated. Twenty birds for time interval sampling were randomly selected from each group at 23 or 30 d of age.

Experimental Protocol At 23 and 30 d old, a modified method based on Guthrie *et al.*,[10] which used a right cardiac catheter was adapted to determine pulmonary artery systolic pressure (PASP), pulmonary artery diastolic pressure (PADP), right ventricle systolic pressure (RVSP), right ventricle diastolic pressure (RVDP) just before killing. Birds were restrained in a dorsal position on the operating-table and locally anesthetized with 5% procaine chloride in the middle of right neck. A polyethylene plastic catheter was inserted into the jugular vein after the jugular vein was separated. The catheter was pushed forward slowly to the right ventricle for RVSP and RVDP, heart rate (HR, beat/min) and then pulmonary artery for PASP and PADP. Pressure signals were sent to the host computer of RM-6000 type Polygraph (Nihon Kohden Ltd., Japan) through a sensor and presented on the monitor. The sensor was placed at the same level as the birds' hearts.

Blood samples were collected from ventrolateral wing veins from the birds whose blood pressure parameters had been acquired, and put into heparinized microhematocrit capillary tubes. After centrifugation, the erythrocyte packed cell volume (PCV) was measured. Ascites heart index (AHI) is the weight of right ventricle proportion percent of the weight of whole ventricle, which is one of the important parameters to determine the right heart hypertrophy.

Statistical Analysis Comparisons between groups at days 23 or 30 were performed using one-way ANOVA followed by the Duncan test if significant variances were found between groups and between the two time points in each group. Differences were considered statistically significant at the level of $p<0.05$, and values are mean±S.E. The statistical analysis was performed with SPSS 11.0 for Windows.

RESULTS

The Effect of BQ123 on Pulmonary Artery Pressure of Broilers in Low Ambient Temperature The effect of BQ123 on pulmonary artery pressure of broilers was shown in Fig. 1. As shown, after being grouped for 7 d (at 23 d old) and 14 d (at 30 d old), the PASP of broilers treated with 2.0 μg/kg BQ123 in normal temperature (2.93±0.251, 3.25±0.328 kPa, respectively) did not show significant differences compared with those in corresponding control (2.90±0.303 and 3.20±0.428 kPa, respectively), indicating that BQ123 with this dose did not change the broilers' PASP in normal temperature. After broilers were exposed to low ambient temperature for 7, the PASP levels of broilers in untreated group and saline treated group (4.68±0.553, 4.20±0.521 kPa, respectively) increased significantly ($p<0.01$ and $p<0.01$ *vs.* control, respectively). Just as expected, the higher levels of PASP induced by low temperature decreased significantly ($p<0.05$ and $p<0.05$ *vs.* low temperature+saline, respectively) in broilers treated with BQ123 at the dose of 2.0 μg/kg (2.99±0.349 kPa) and 0.4 μg/kg (3.27±0.416 kPa), and PASP in the two BQ123 treatments showed no statistical differences compared with that in control. The PASP value of broilers in the high dose treatment was a little lower than that of broilers in low-dose BQ123 treatment group, but the difference between the two groups was not statistically significant ($p>0.05$). Similar effects on PASP were found at 30 d old.

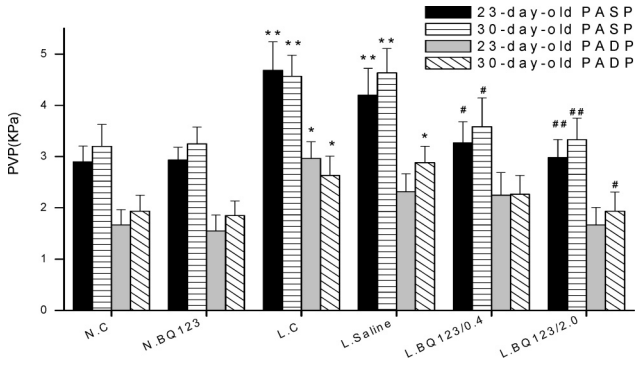

Fig. 1. Changes of Pulmonary Artery Systolic Pressure (PASP), Pulmonary Artery Diastolic Pressure (PADP) at 23 and 30 d Old in Chicks after Being Treated with BQ123 for 7 and 14 d, Respectively

$^{*}p<0.05$, $^{**}p<0.01$ *vs.* corresponding control and $^{\#}p<0.05$, $^{\#\#}p<0.01$ *vs.* corresponding saline group. Values are mean of twenty measurements, and error bars indicate standard errors.

At 23 and 30 d old, PADP of broilers in normal temperature+BQ123 group (1.55±0.310, 1.85±0.288, respectively), and low temperature+BQ123/2.0 group (1.67±0.342, 1.93±0.378, respectively) was at the same level with those in corresponding control (1.67±0.298, 1.93±0.317, respectively). But, low temperature significantly increased PADP of broilers in untreated group to 2.97±0.324 kPa ($p<0.05$ *vs.* control) at 23 d old and 2.63±0.374 kPa ($p<0.05$ *vs.* control) at 30 d old, as well as in saline group to 2.88±0.317 kPa ($p<0.05$ *vs.* control) at 30 d old. High-dose BQ123 significantly decreased PADP at 30 d old to 1.93±0.378 kPa ($p<0.05$ *vs.* saline group) in low temperature. In contrast, PADP of broilers in low dose of BQ123 at both time points (2.25±0.439, 2.27±0.366, respectively) was lower than those in corresponding control and high dose of BQ123 was higher than those in corresponding saline group, differences were not statistically significant.

The Effect of BQ123 on Right Ventricle Function of Broilers in Low Ambient Temperature Figure 2 shows the effect of BQ123 on right ventricle function of broilers in low ambient temperature. RVSP in control (3.18±0.331, 3.07±0.309 kPa, respectively), in normal temperature+BQ123 group (3.07±0.451, 3.17±0.422 kPa, respectively), BQ123/2.0 (3.08±0.572, 2.96±0.441 kPa, respectively) group at 23 and 30 d old and BQ123/0.4 (3.23±0.527 kPa) group at 23 d old was at the same level. Compared with the control, broilers in untreated low temperature group (4.25±0.330, 4.93±0.468 kPa, respectively) and saline treated group (4.32±0.443, 4.75±0.423 kPa, respectively) showed significantly increased RVSP ($p<0.01$, $p<0.01$, respectively) at both ages. Although, two doses of BQ123 significantly decreased RVSP ($p<0.05$, *vs.* corresponding saline group) for 7 d, with the treatment period prolonged (for 14 d),

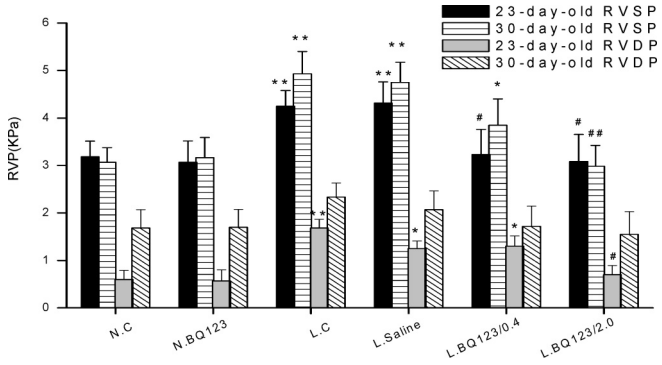

Fig. 2. Changes of Right Ventricle Systolic Pressure (RVSP), Right Ventricle Diastolic Pressure (RVDP) at 23 and 30 d Old in Chicks after Being Treated with BQ123 for 7 and 14 d, Respectively

$*p<0.05$, $**p<0.01$ *vs*. corresponding control and $^{\#}p<0.05$, $^{\#\#}p<0.01$ *vs*. corresponding saline group. Values are mean of twenty measurements, and error bars indicate standard errors.

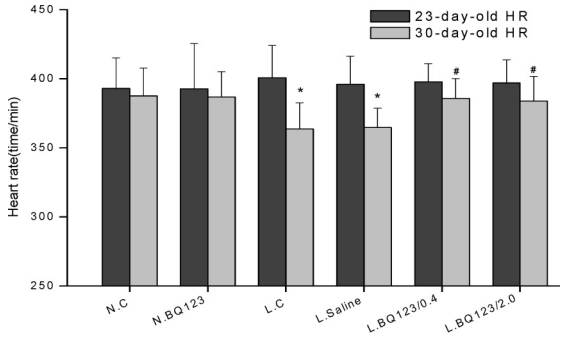

Fig. 3. Changes of Heart Rate (HR) at 23 and 30 d Old in Chicks after Being Treated with BQ123 for 7 and 14 d, Respectively

$*p<0.05$ *vs*. corresponding control and $^{\#}p<0.05$ *vs*. corresponding saline group. Values are mean of twenty measurements, and error bars indicate standard errors.

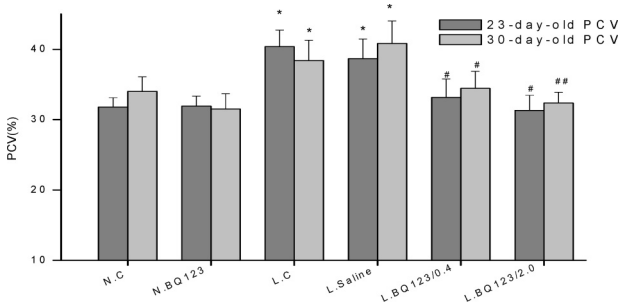

Fig. 4. Changes of Erythrocyte Packed Cell Volume (PCV) at 23 and 30 d Old in Chicks after Being Treated with BQ123 for 7 and 14 d, Respectively

$*p<0.05$ *vs*. corresponding control and $^{\#}p<0.05$, $^{\#\#}p<0.01$ *vs*. corresponding saline group. Values are mean of twenty measurements, and error bars indicate standard errors.

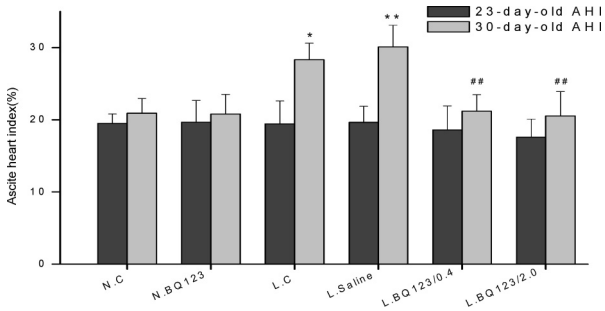

Fig. 5 Changes of Ascites Heart Index (AHI) at 23 and 30 d Old in Chicks after Being Treated with BQ123 for 7 and 14 d, Respectively

$*p<0.05$, $**p<0.01$ *vs*. corresponding control and $^{\#\#}p<0.01$ *vs*. corresponding saline group. Values are mean of twenty measurements, and error bars indicate standard errors.

RVSP value in low dose of BQ123 group (3.85±0.552 kPa,) was significantly higher ($p<0.05$) than that in control, while it had no significant difference compared with that in saline group.

Similar results were found in RVDP. Low temperature significantly increased RVDP of broilers in untreated, saline and low dose of BQ123 groups compared with normal control ($p<0.01$, $p<0.05$, $p<0.05$, respectively) on day 23. BQ123 treatments decreased RVDP significantly compared with corresponding control ($p<0.01$). RVDP of broilers in low temperature untreated, saline treatment was higher than those of other corresponding groups, but differences were not statistically significant.

Figure 3 displays the effect of BQ123 on HR. As shown, on day 23, there were no significant differences between all groups (393±22.1 beat/min in control). At 30 d old, The HR of broiler in untreated low temperature (364±19.0 beat/min, $p<0.05$) and saline (365±14.0 beat/min, $p<0.05$) groups significantly decreased compared with that in control (388±20.2 beat/min). Both BQ123/0.4 and BQ123/2.0 treatments (386±14.5, 384±17.8 beat/min, respectively) in low temperature on day 30 showed no significant difference on HR compared with control, but the two BQ123 treatments had higher HR compared with the saline group ($p<0.05$).

The Effect of BQ123 on Pulmonary Hypertension Syndrome of Broilers in Low Ambient Temperature The effect of BQ123 on PCV of broilers in low ambient temperature was shown in Fig. 4. Compared with control at two ages (31.8±1.34%, 34.0±2.1%, respectively), BQ123 in normal temperature (31.9±1.44%, 31.5±2.16%, respectively) did not affect PCV of broilers. At 23, 30 d old, low temperature significantly increased broilers' PCV in untreated (40.4±2.38%, $p<0.05$, 38.4±2.87%, $p<0.05$, respectively) and saline (38.7±2.79%, $p<0.05$, 40.8±3.19%, $p<0.05$, respectively) groups compared with corresponding control, while both BQ123/0.4 (33.1±2.61%, $p<0.05$, 34.4±2.41%, $p<0.05$, respectively) and BQ123/2.0 (31.3±2.14%, $p<0.05$, 32.3±1.53% $p<0.01$) significantly decreased the PCV compared with corresponding saline group, especially the high dose of BQ123 at 30 d old.

The effect of BQ123 on AHI of broiler in low ambient temperature was shown in Fig. 5. There were no significant differences between all the groups on day 23 (19.5±1.31% in contrl). With the treatment periods prolonged to day 30, AHI of broilers in untreated (28.3±2.30%, $p<0.05$) and saline treatment (30.1±3.01%, $p<0.01$) in low temperature significantly increased compared with corresponding control (20.9±2.03%), whereas high dose (20.5±3.37%, $p<0.01$) and low dose (21.2±2.29%, $p<0.01$) of BQ123 significantly decreased significantly AHI compared with saline treatment.

DISCUSSION

According to the studies upon pulmonary hypertension

and ascites syndrome in broilers, PAP, RVP, AHI, PCV and HR had been determined as the pathophysiologic parameters for assessing the development of the disease in young broilers. It was proved that the PAP, RVP, AHI and PCV of broilers under low ambient temperature were much higher than those in normal controls, inversely, HR was significantly lower in ambient groups.[7,11] In the present work, the significant increases in PAP (including PASP and PADP) and RVP (including RVSP and RVDP) of broilers in untreated and saline groups under low ambient temperature compared with those in control indicated that low temperature did induce the occurrence and development of PH in broilers. Additionally, accompanied with the increases of PCV and AHI respectively because of increased production of erythrocyte and right ventricular hypertrophy, the PH broiler model under low ambient temperature was further confirmed. Interestingly, there were no significant increases in PAP, RVP, PCV and AHI, and no significant decrease in HR of broilers treated with two doses of BQ123 under low ambient temperature compared with those of control birds under normal ambient temperature. Therefore, from these results, it is conceivable that ET-1 and ET_A are critically involved in the pathophysiologic development of PH and ascites in broilers induced by low ambient temperature.

BQ123 Prevented the PH in Broilers Exposed to Low Ambient Temperature Current views suggest that either absolute or relative hypoxia induced by sorts of pathogeny, such as low temperature or lung diseases, could evoke the abnormal release of some cell factors and active media in mammals, such as ET, angiotensin, thromboxane, histamine, catecholamine, nitric oxide (NO), prostaglandins, in addition ET and NO are buffer agents physiologically modulating the basal balance of systemic and pulmonary vascular tone.[12,13] It had already been proved that hypoxia leads abnormal ET-1 or ETR generation to constrict pulmonary vessels.[14,15] Several studies demonstrated increased ET-1 concentrations in children with congenital heart disease associated with increased pulmonary blood flow and PH.[16] In addition, some animal models of pulmonary hypertension demonstrate increases in plasma ET-1 concentrations between the right ventricle and pulmonary vein, suggesting increased local production of ET-1 within the pulmonary circulation.[17,18] Similarly, increased plasma levels of the ET-1 had been demonstrated in broilers with PH.[9] Nevertheless, such evidence remains largely indirect and does not establish an unequivocal cause-effect relationship for ET-1 is one of the local active factor acting by autocrine and paracrine manners, and ET-1 concentration would change according to blood from different positions. Therefore, the abnormality of ET-1 synthesis, release and ETR expression cannot absolutely be interpreted by the ET-1 concentration of blood. It remains to be determined if elevated circulating levels of ET-1 in the setting of disease represents an increase in production and release or a reduction of clearance. From these studies, a role for the ETA receptor has been emphasized for ETA receptor antagonists prevent and may reverse pulmonary hypertension.[19—21] Therefore, in the present study, BQ123 was used to determine if it could prevent the PH in broilers.

BQ-123 is a peptide that selectively antagonizes actions of ETs on ET_A receptors. In a prior study, sustained infusions of BQ123 (0.16—164 nmol/kg/min i.v. for 6 h) in spontaneously hypertensive (SHRs) and normotensive Wistar-Kyoto (WKY) rats produced dose-dependent reductions in mean arterial pressure (approximately 3.9 kPa) in SHRs that were long-lasting (>18 h) and reversible.[22] Another research reported that intraperitoneal perfusion treatment with BQ123 attenuated the hypoxia-induced increase in rats' pulmonary arterial mean pressure and total pulmonary resistance index by 60 and 87% respectively, compared with saline-treated animals.[23] Similarly, pulmonary artery pressure and pulmonary vascular resistance of piglets in the BQ group were significantly lower after 120 or 180 min. given intravenous BQ123 (2 μg/h) than in the saline group.[24] We previously showed that the decreasing action of BQ123 on basal systemic vessel and pulmonary blood pressures of chick implicated that there are ETR on vascular or ventricle wall in poultry.[25] In the present study, results showed that BQ123, especially with the high dose, also prevent broilers' PAP and RVP increasing induced by low ambient temperature. The mechanism of BQ123 preventing PH in broilers is the same case as in mammals, *i.e.*, BQ123 produces its effects by competitively binding to the ETA receptor and preventing the interaction of ET-1 with its receptor, blocking subsequently the intracellular signal pathway, which interdicts the activation of the contractile action on pulmonary vessels,[26,27] resulting in that PAP and RVP did not increase pathologically. The preventive effect of BQ123 suggests that ET-1 is associated with PH in broilers, which leads to the development of ascites, and BQ123 can prevent the occurrence of PH.

BQ123 Prevented the PH Syndrome in Broilers Exposed to Low Ambient Temperature The importance of ET-1 in pulmonary hypertension syndrome (PHS) process has been suggested by mammals' studies in which ET-1 receptor antagonists have not only reduced PH but also resulted in reversal of vascular remodeling and right ventricular hypertrophy.[28] In the current study, BQ123, especially with the high dose, not only prevented the PH but also subsequently protected the hemodynamics and right ventricle function of broilers, preventing the occurrence of syndrome in broilers for PH, such as AHI and PCV significantly increased in control and saline control under low ambient temperature, whereas there were no significant increases in the two parameters in the two BQ123 groups compared with normal control. It was suggested that endogenous production of ET-1 stimulates hypertrophy in rat cardiomyocytes,[29] whereas long-term therapy with specific ETA receptor antagonists reduced the development of PH, PCV and right ventricular hypertrophy in hypoxic rats.[20—22] Our findings suggest that BQ123 would be of benefit not only in the pulmonary vasodilation observed in these studies but also in modulating remodeling. Our results also provide a rationale for at least part of the beneficial actions of BQ123 in the prevention of PHS. A higher PCV indicates higher blood viscosity, which was said to be one of the symptoms of PH.[6] Factors such as cold increased broiler's body demand for oxygen to enhance heat producing for maintaining normal body temperature, which lead to a relative hypoxia status, and that hypoxia is conformed as an important pathophysiologic stimulus for erythrocytes number increment accompanied by PH. Additionally, with the long-term hypoxia and the development of PH which result in pulmonary resistance increase greatly, the right ventricular compensatory hypertrophy

forms, which can explain the increase in the AHI, weight ratio of the right ventricle of the whole ventricle.[30)]

The major findings of this study were that ET-1 was associated in the development of pulmonary hypertension and BQ123 prevented broilers from this process and pulmonary hypertension syndrome. After being raised in low ambient temperature for 2 weeks, BQ123 prevented the occurrence of PH in broilers, whereas untreated birds developed exaggerated PH that was characterized by elevated PAP, RVP, PCV and AHI.

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