

# GENETICS AND MOLECULAR BIOLOGY

## Dietary supplementation of 25-hydroxycholecalciferol improves cardiac function and livability in broiler breeder hens—amelioration of blood pressure and vascular remodeling

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**ABSTRACT** A supplement of 69 µg 25-hydroxycholecalciferol (**25-OH-D3**)/kg feed suppressed the mortality in feed-restricted broiler breeder hens and in hens allowed ad libitum feed intake (**Ad-hens**) in a feeding trial from age 26 to 60 wk. Outcomes for the mechanisms found that 25-OH-D3 relieved systemic hypoxia, pathological cardiac remodeling and arrhythmias, and hepatopathology to improve hens' livability. In the study, we further evaluated the effect of 25-OH-D3 on blood pressure and vascular remodeling relative to cardiac pathogenesis of sudden death (**SD**). Ad libitum feed intake increased mechanical loading and contributed to maladaptive cardiac hypertrophy as evidenced by consistently elevated peripheral arterial blood pressure in Ad-hens before SD ( $P < 0.05$ ). In planned longitudinal measurements, Ad-hens also showed higher right ventricle systolic pressure and right ventricle diastolic pressure (**RVDP**) ( $P < 0.05$ ). Supplemental 25-OH-D3 relieved peripheral hypertension and prevented time-dependent

increases of RVDP in Ad-hens through the renin-angiotensin system and circulating nitric oxide availability and by regulating vascular remodeling including elastin/collagen ratio and smooth muscle cell proliferation in the pulmonary artery for improved elasticity/stiffness ( $P < 0.05$ ). The antihypertensive effect via the renin-angiotensin system and nitric oxide regulation in respect to heart rate and arrhythmias by 25-OH-D3 were further confirmed in 51 week-old feed-restricted broiler breeder hens challenged with salt loading for 5 wk. Despite feed restriction, the most feed-efficient hens of feed-restricted groups also exhibited cardiac pathological hypertrophy, in conjunction with higher right ventricle systolic pressure, RVDP, plasma nitric oxide levels, and more dramatic arterial remodeling ( $P < 0.05$ ). These results suggest that peripheral and pulmonary hypertension are the key drivers of SD and that 25-OH-D3 is an effective antihypertensive supplement to alleviate cardiac pathogenesis and improve livability in broiler breeder hens.

**Key words:** broiler breeder hen, 25-hydroxycholecalciferol, cardiac hypertrophy, hypertension, vascular remodeling

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## INTRODUCTION

Most of studies regarding sudden death (**SD**) in broilers were focused on the growing stage in which cardiac and pulmonary capacity is inadequate to support

muscular growth, referred to as ascites (Yu et al., 1992; Griffin and Goddard, 1994; McGovern et al., 1999; Pan et al., 2012). Few studies have examined premature mortality in broiler breeders, particularly in respect to the welfare issues with feed restriction for better egg production vs. chronic hunger that accompanies this management practice.

In a prior short-term feeding trial, we showed a higher mortality rate in broiler breeder hens provided with unlimited access to feed (**Ad-hens**), in association with peripheral hypertension, hyperglycemia, systemic inflammation, and dyslipidemia that triggered cardiac pathological remodeling, metabolic cardiomyopathy, arrhythmic

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electrocardiography (ECG) patterns, and finally heart failure (Chen et al., 2017a; 2017b). To further delineate the cause of SD and improve hens' livability, 69 µg 25-hydroxycholecalciferol (**25-OH-D3**)/kg feed was supplemented into the basal diet to examine its effects on cardiac health throughout the whole egg-laying period. Results showed significantly ameliorated cardiac pathogenesis and functional compromise and improved livability in both Ad-hens (48.2% vs. 29.1%) and feed-restricted broiler breeder hens (**R-hens**) (86.7% vs. 78.9%) (Lin et al., 2019a; 2019b).

Vitamin D deficiency in human subjects is associated with obesity, metabolic syndrome, hypertension, arterial remodeling and dysfunction, and cardiomyopathy (Drincic et al., 2012; Kienreich et al., 2013; Andrukhova et al., 2014). In a variety of animal models and clinical cases, vitamin D improves cardiac health and ameliorates functional failure (Pilz et al., 2010; Chen et al., 2011; Wei et al., 2017) by alleviating systemic cues such as hypoxia, arterial biology and vascular impedance, and type 2 diabetic mellitus (Li et al., 2002; Lee et al., 2015; Tanaka et al., 2017). In the previous reports, we showed that 25-OH-D3 improved breeder hen's livability by ameliorating systemic hypoxia and pathological cardiac hypertrophy in association with reduced hepatopathology (Lin et al., 2019a; 2019b). Based on those reports, this study aimed to determine whether the positive effects of feed supplementation with an additional 69 µg 25-OH-D3/kg feed included those on blood pressure regulation and vascular remodeling.

## MATERIALS AND METHODS

### *Animal Management*

Cobb 500 broiler breeder hens at age of 22 wk were purchased from a local breeder farm. Feed intake up to 26 wk of age followed the breeder company recommendations (105–120 g/day/hen) to achieve a targeted BW with a nutritionally adequate soy and corn-based breeder mash (Lin et al., 2019a). All birds were caged individually in a house, and ambient temperatures were maintained around 24°C to 28°C. Birds were given free access to water throughout the experiment and feed was placed at 8:30 am in conjunction with a 14L:10D (lights on at 5 am) photo schedule. At age of 26 wk, 180 birds were continued with restricted rations (**R-hens**) under weekly adjustment as the recommendation, while another 220 birds were provided with sufficient feed for consumption to appetite (**Ad-hens**). The additional hens were included in the **Ad-hen** groups in anticipation of increased mortality. Within each feed intake treatment, a subgroup of half of the hens was provided with a basal breeder diet containing additional 69 µg/kg feed of 25-OH-D3 (DSM Nutritional Products Ltd., Netherlands). Data relating to feed formulation, feed intake, BW change, livability, and reproductive performance of the flock of hens over the whole laying stage (26–60 wk of age) were described in a previous report (Lin et al., 2019a). All bird husbandry and procedures

were conducted in accordance with an approved animal care protocol by the Institutional Animal Care and Use Committee of National Chung Hsing University, Taiwan.

### *Sampling and Necropsy for Tissue Collection*

At 29 (n = 4), 35 (n = 7), and 47 (n = 7) wk of age, hens were randomly selected for right ventricle pressure (**RVP**) measurement, pathological examination, and tissue collection. Feed was removed the evening before measurements in which hens were physically restrained in dorsal recumbency before isoflurane anesthesia administered via a face mask. Right ventricular pressure was measured before necropsy for tissue collection. After RVP measurement, the heart, liver, abdominal fat, lung, kidney, and main pulmonary artery (from the junction site at the right ventricle to the bifurcation site into the left and right branch) were collected immediately for pathological examination, molecular, biochemical, and histochemical studies.

Totally, 19 and 12 hens in the **R** and **R + 25-OH-D3** groups (n = 90 at 26 wk), and 78 and 57 hens in the **Ad** and **Ad+25-OH-D3** groups (n = 110 at 26 wk) died along the time course (cumulative mortality at 60 wk, 21.1, 13.3, 70.9, and 51.8%, respectively) in which 15, 9, 73, and 52 hens exhibited cardiac pathologies (incidence: 78.9, 75, 93.6, and 91.2%, respectively) (Lin et al., 2019a; 2019b). Cause of mortality was studied within 24 h of death. The heart, liver, and abdominal fat were examined for postmortem indicators of prior cardiac dysfunction. Infectious causes were excluded by veterinarian diagnosis and thus SD was concluded as the major cause of mortalities. Details of mortalities and cardiac pathologies were reported previously (Lin et al., 2019a; 2019b).

Starting at 29 wk of age, an additional 10 hens from each group was randomly selected to participate in longitudinal measurements of peripheral blood pressure, heart, and respiratory rates. Because some hens died suddenly along the time course, the measurements were repeated in the hens surviving to 35, 47, and 60 wk of age. Results were separated to provide outcomes for hens surviving to 60 wk of age (survivors for **R**, **R + 25-OH-D3**, **Ad**, and **Ad+25-OH-D3**, n = 8, 3, and 5, respectively) and those of hens experiencing SD (n = 2, 2, 7, and 5 at 29 wk, n = 2, 2, 5, and 4 at 35 wk, and n = 1, 1, 2, and 2 at 47 wk, respectively).

At age of 51 wk, an additional 4 hens from the **R** and **R + 25-OH-D3** group were challenged with salt loading in drinking water (0.2% NaCl in distilled water) to assess whether hypertension developed when compared with another 4 **R-hens** provided with distilled water as a comparative control group. Peripheral blood pressure, ECG, heart rate measurement, and blood sample collection were made 1, 3, and 5 wk after treatment. Collected blood samples were used for plasma angiotensin II (**Ang II**) and nitric oxide (**NO**) analysis. The sampling scheme along the time course is given in [Supplementary Figure 1](#).

### **Right Ventricle Pressure Measurement**

Right ventricular pressure was measured by cardiac catheterization (Li et al., 2016). In brief, the skin around the right neck was cut open and the jugular vein was separated. Then, a small opening was made on the jugular vein, through which a polyethylene plastic catheter (0.9 mm, external diameter) was inserted and moved slowly into the right ventricle. Pressure signals were recorded using a sensor and displayed by the RM-6000 type Polygraph system (Nihon Kohden Ltd., Tokyo, Japan) on the computer monitor. Recumbency during measurement may influence the actual values of ventricular pressure when compared with a sitting or standing posture (Codd et al., 2005).

### **Peripheral Blood Pressure, Heart, and ECG Analysis**

Blood pressure and heart rate were measured with a portable monitor (SureSigns VM6; Philips India Limited, Kolkata, India), and ECG was carried out using an integrated data recording device and software (Powerlab 15T, T15-0951; ADInstruments, New South Wales, Australia) as described previously (Chen et al., 2017a). QT interval was corrected (QTc) to the period between connective R peaks (RR duration) (Vandenberk et al., 2016).

### **Plasma Ang II and NO Analysis**

Plasma Ang II concentrations were determined using a validated commercial ELISA kit (Shanghai Xinran Industrial Co. Ltd., Shanghai, China) (Hao et al., 2013). Plasma NO levels were analyzed colorimetrically using a commercial kit (Cayman Chemical Company, Ann Arbor, MI) as per the instructions enclosed.

### **Histology Analysis**

Paraffin-embedded sections of the pulmonary artery were used for hematoxylin and eosin and trichrome staining for collagen and smooth muscle cell (SMC) abundance and Elastica van Gieson staining for elastin content (Histology Service of the National Chung Hsing University, Taiwan). The section of the pulmonary artery at the middle (1/2 of the whole length) and 3/4 length above (distal) or below (proximal) the middle line was used for histological studies. Four images per section were used for quantification. The intensity by trichrome and Elastica van Gieson staining was quantified through ImageJ software.

### **Gene Expression by Real-Time PCR Analysis**

Freshly collected tissues were quickly dissected into 1 mm<sup>3</sup> pieces on ice, dumped into RNAlater (Invitrogen, Waltham, MA), and stored at -80°C until use. Total RNA extraction, random priming reverse transcription,

and quantitative real time polymerase chain reaction amplification were conducted as described previously (Pan et al., 2012) using commercial kits (Applied Biosystems, Waltham, MA). Information about the primers is given in Supplementary Table 1. Reactions were conducted in triplicate and the intra-assay CV was less than 10%.

### **Statistics**

Data were analyzed by two-way ANOVA in which feed intake manipulation (Ad or R) and 25-OH-D3 treatment were the classifying variables. Differences between group means were tested using Bonferroni-corrected *t* test when the main effect was significant. If an interaction between feed intake and 25-OH-D3 treatment was found, a mean comparison was performed. Values were expressed as means ± SEM. Mean differences were considered significant at *P* < 0.05. All statistical procedures were carried out using SPSS for Windows 13.0.

## **RESULTS**

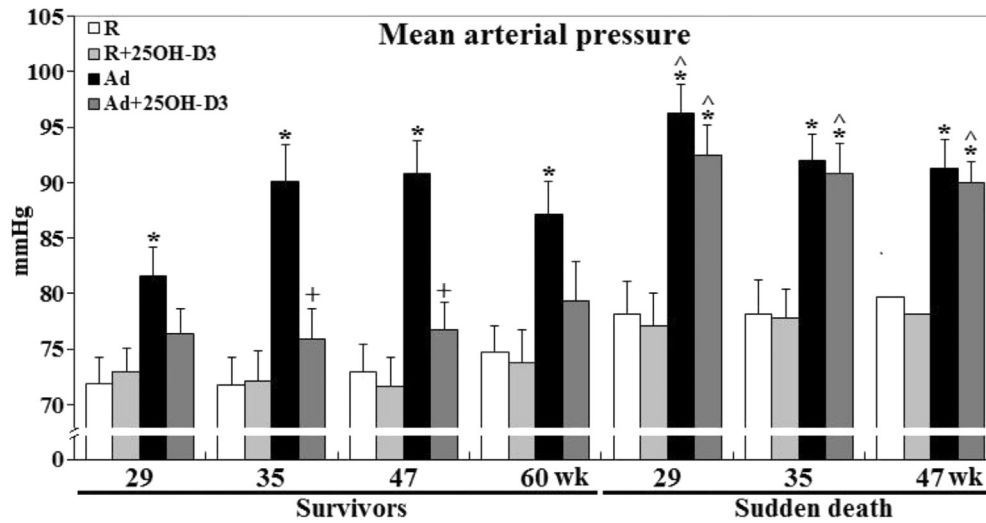
### **Peripheral Blood Pressure and Vasodilation Regulation**

Ad libitum feed intake provoked arterial hypertension that persisted in hens surviving to 60 wk of age (survivors) and in those hens that died before 60 wk of age (SD) (*P* < 0.05, Figure 1). In contrast to R-survivors, SD-hens of R-groups had normal arterial pressure (Figure 1) despite most of the hens developed cardiac pathologies (Lin et al., 2019b). These results suggest that peripheral hypertension persists as a pathological component of cardiac hypertrophy along the time course leading to functional compromise and thus a higher susceptibility of SD in Ad-hens, whereas other cues contribute to cardiac pathologies in R-hens. Supplementation of the basal diet with additional 69 µg/kg feed of 25-OH-D3 alleviated arterial pressure at 35 and 47 wk in Ad-survivors (*P* < 0.05) but had no significant effects on Ad-hens that died suddenly.

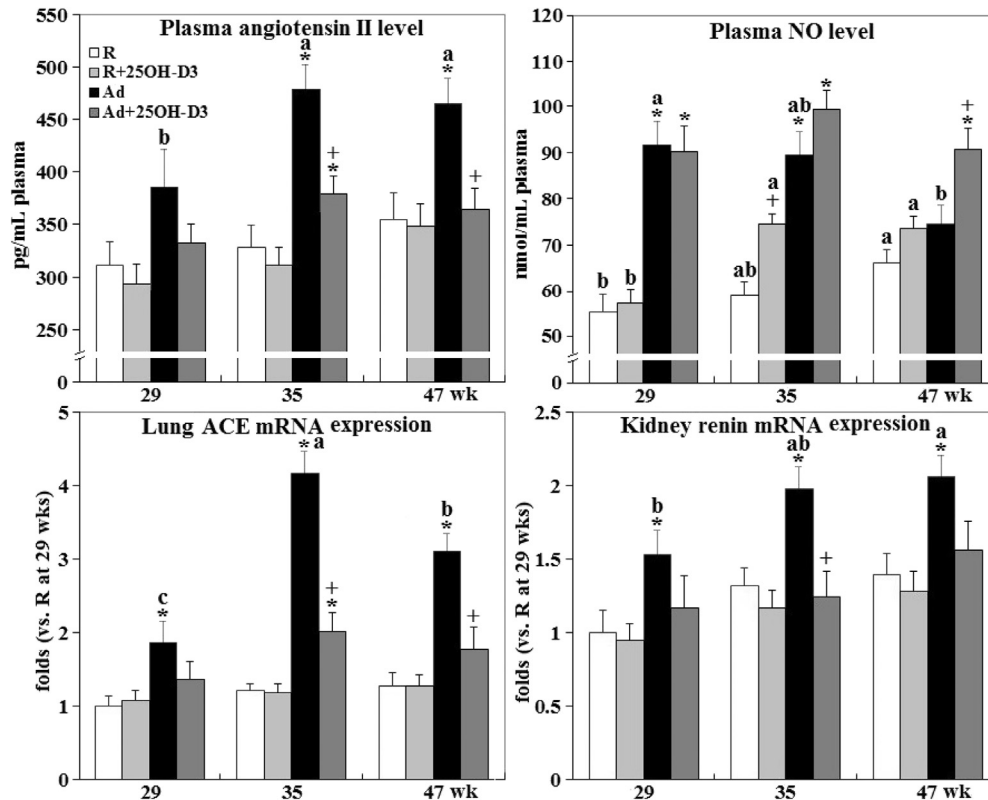
The antihypertensive effect by 25-OH-D3 supplement was further confirmed by a significant blunting of plasma Ang II rise in the presence of elevated plasma NO concentrations and increased mRNA abundance of kidney renin and lung angiotensin-converting enzyme gene expression at 35 and/or 47 wk in Ad-hens (*P* < 0.05, Figure 2). Supplementation of 25-OH-D3 even increased plasma NO levels at 35 wk in R-hens (*P* < 0.05). These results suggested that 25-OH-D3 relieves peripheral blood pressure through renin-angiotensin system (RAS) and NO production.

### **Right Ventricle Pressure and Pulmonary Artery Remodeling**

Ad libitum feed intake increased right ventricle systolic pressure (RVSP) and right ventricle diastolic pressure (RVDP) in hens sampled during along the time the

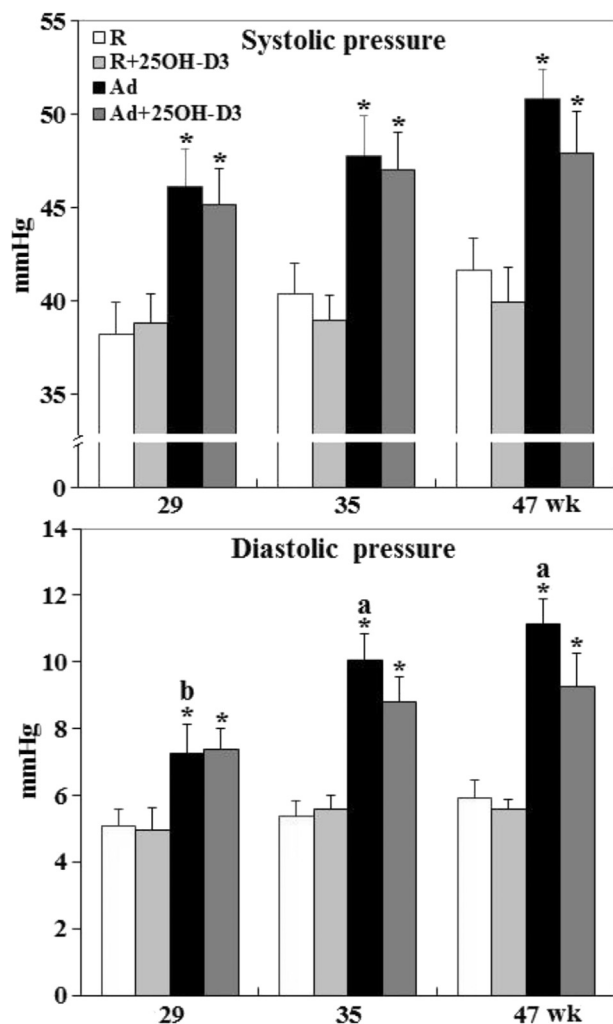


**Figure 1.** Longitudinal effect of dietary 25-OH-D3 supplementation on peripheral blood pressure of broiler breeder hens provided with restricted or ad libitum feed intake. At age of 29 wk, 10 hens from each group were selected for peripheral blood pressure monitoring. The measurements were continued with the selected hens surviving to 35, 47, or 60 wk of age. Results were separated to provide outcomes for hens surviving to 60 wk of age (survivors for R, R + 25-OH-D3, Ad, and Ad+25-OH-D3,  $n = 8, 8, 3,$  and  $5,$  respectively) and those of hens experiencing sudden death ( $n = 2, 2, 7,$  and  $5$  at 29 wk,  $n = 2, 2, 5,$  and  $4$  at 35 wk, and  $n = 1, 1, 2,$  and  $2$  at 47 wk, respectively). \*Significant difference owing to feeding level (vs. corresponding R-hens,  $P < 0.05$ ); ^, significant difference by 25-OH-D3 inclusion (vs. corresponding R- or Ad-hens,  $P < 0.05$ ); +, significant difference vs. hens survived at the same age with the same treatment ( $P < 0.05$ ); R, restriction; Ad, ad libitum; 25-OH-D3, 25-hydroxycholecalciferol.



**Figure 2.** Effect of dietary 25-OH-D3 supplementation on RAAS and plasma nitric oxide level of broiler breeder hens provided with restricted or ad libitum feed intake. When hens were 29, 35, and 47 wk of age, 4, 7, and 7 hens from each group, respectively, were sampled for tissue collection. Angiotensin II and nitric oxide concentrations were measured in plasma, whereas ACE and renin mRNA expression were measured in lungs and kidney, respectively, through qRT-PCR method. Results of qRT-PCR were normalized to  $\beta$ -actin and expressed as ratios relative to R-hens at 29 wk. \*Significant difference by feeding level (vs. corresponding R-hens,  $P < 0.05$ ). ^, ^cMeans with different letter differ significantly among ages within the same treatment ( $P < 0.05$ ). +, significant difference by 25-OH-D3 inclusion (vs. corresponding R- or Ad-hens,  $P < 0.05$ ); R, restriction; Ad, ad libitum; 25-OH-D3, 25-hydroxycholecalciferol; ACE, angiotensin-converting enzyme; qRT-PCR, quantitative real time polymerase chain reaction; RAAS, renin-angiotensin-aldosterone system.





**Figure 3.** Effect of dietary 25-OH-D3 supplementation on right atrial pressure of broiler breeder hens provided with restricted or ad libitum feed intake. When hens were 29, 35, and 47 wk of age, 4, 7, and 7 hens from each group, respectively, were sampled for tissue collection. Immediately before necropsy, selected hens were used for right ventricle pressure measurement. \*Significant difference by feeding level (vs. corresponding R-hens,  $P < 0.05$ ). Means with different letters differ significantly among ages within the same treatment ( $P < 0.05$ ). Abbreviations: R, restriction; Ad, ad libitum; 25-OH-D3, 25-hydroxycholecalciferol.

course ( $P < 0.05$ , Figure 3). The 25-OH-D3 supplement exerted no significant effects on RVSP and RVDP in Ad-hens but blunted time-dependent increases of RVDP ( $P < 0.041$  between age  $\times$  25-OH-D3 interaction) and had no effects on RVSP increases, suggesting a more pronounced effect by 25-OH-D3 on pulmonary vascular impedance of the cardiopulmonary circulation system than on proximal arterial resistance (Bavishi et al., 2016).

Ad libitum feed intake provoked pulmonary artery remodeling including collagen and elastin content and SMC proliferation at 35 and/or 47 wk ( $P < 0.05$ , Figure 4, panel A to C). Improved pulmonary artery elasticity/stiffness by 25-OH-D3 was confirmed as 25-OH-D3 supplementation reduced collagen content and SMC proliferation in both R- and Ad-hens, whereas elastin content was increased by 25-OH-D3 supplementation in R-hens and decreased in Ad-hens leading to

increased elastin/collagen ratios at 35 and 47 wk in both R- and Ad-hens ( $P < 0.05$ , Figure 4, panel A to D). Histology of the pulmonary artery showed a well-arranged structure with neatly stacked layers by 25-OH-D3 in both R- and Ad-hens (Figure 4, panel A).

Ad libitum feed intake had no significant effects on pulmonary artery lumen dimension and wall thickness, but interestingly, 25-OH-D3 supplementation increased arterial lumen dimension of the middle part in Ad-hens and wall thickness along the entire main pulmonary artery (distal, middle, and proximal part) in R-hens ( $P < 0.05$ , Figure 5). These morphological changes by 25-OH-D3 reflect a net outcome of arterial remodeling on the artery per se (Figure 4) and by the RAS regulation (Figure 2) (van Suylen et al., 1998).

### **Right Ventricle Pressure, Plasma NO Concentrations, and Pulmonary Artery Remodeling in R-Hens with Normal or Pathological Cardiac Hypertrophy at Necropsy**

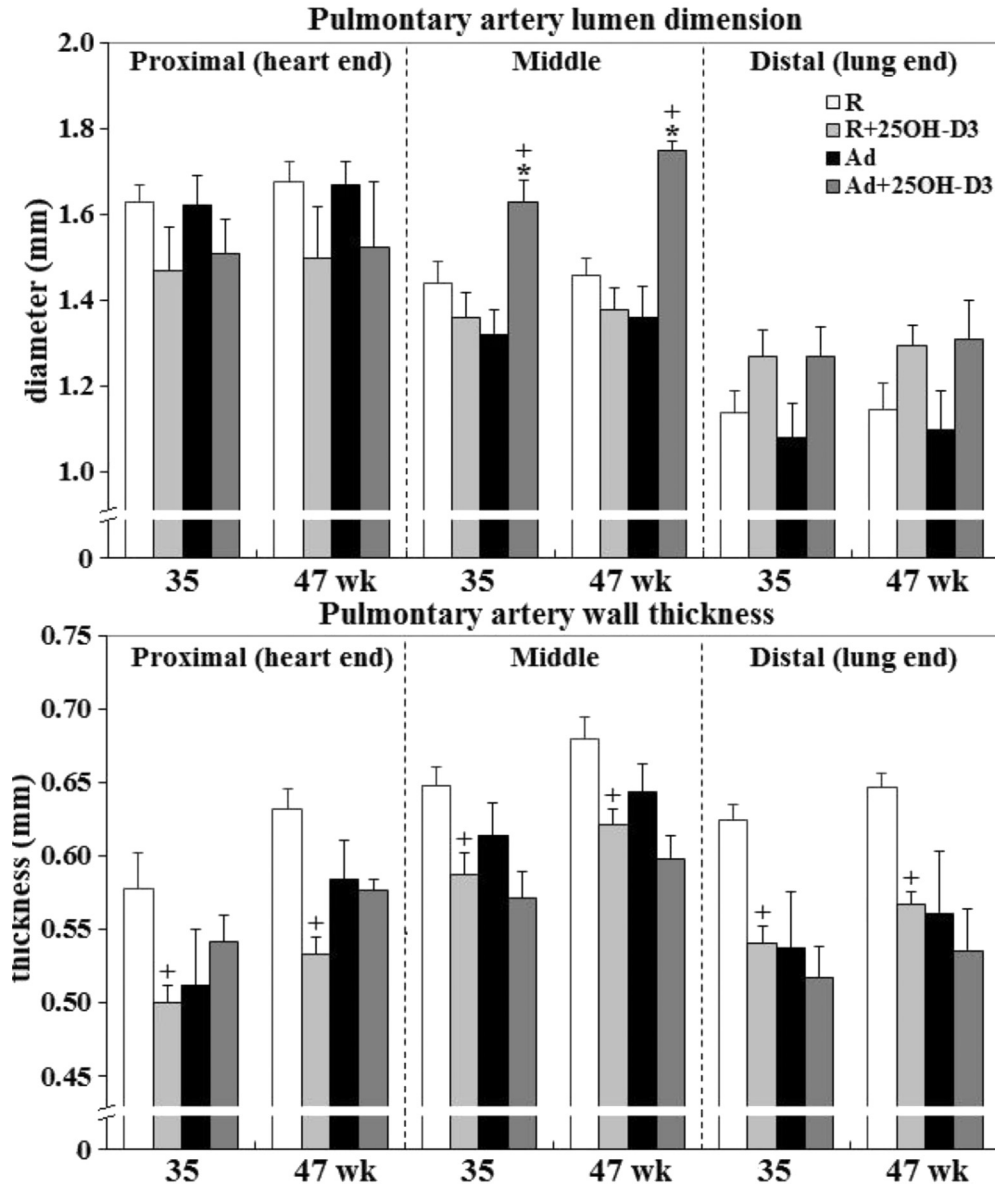
In our previous reports (Lin et al., 2019a; 2019b), regrouping of R-hens as per the presence of cardiac pathologies suggested that the abnormal R-hens ( $n = 4$  at 35 and 47 wks) had better feed efficiency including higher BW, liver and heart fraction in association with hepatic steatosis, hyperlipidemia, irregular ECG patterns, and severe systemic hypoxia and acidosis. Here, we further showed that the abnormal R-hens had higher plasma NO levels, increased RVSP and RVDP, and more dramatic pulmonary artery remodeling including collagen, elastin content, SMC proliferation, and wall thickness ( $P < 0.05$ , Figure 6) but not in lumen dimension, plasma Ang II levels, lung angiotensin-converting enzyme, and renal renin gene expressions (data not shown).

### **Blood Pressure, Cardiac Function, and Plasma Ang II level in R-Hens With Salt-Load Challenge**

R-hens were salt-load challenged through their drinking water to further confirm the mechanism of antihypertensive effects by 25-OH-D3. Supplementation of 25-OH-D3 significantly relieved mean arterial pressure, plasma Ang II levels, and rescued plasma NO levels at 5 wk after high salt loading ( $P < 0.05$ , Figure 7). Improvements associated with 25-OH-D3 supplementation included heart rate, QRS complex amplitude and QTc interval in ECG profile ( $P < 0.05$ , Figure 7; Supplementary Figure 2), suggesting improved cardiac functionality by 25-OH-D3 through vasodilation modulation (Singla et al., 2005; Mangoni and Jarmuzewska, 2018).

## **DISCUSSION**

Previously, we demonstrated that Ad-feed intake caused an elevation of blood pressure in conjunction

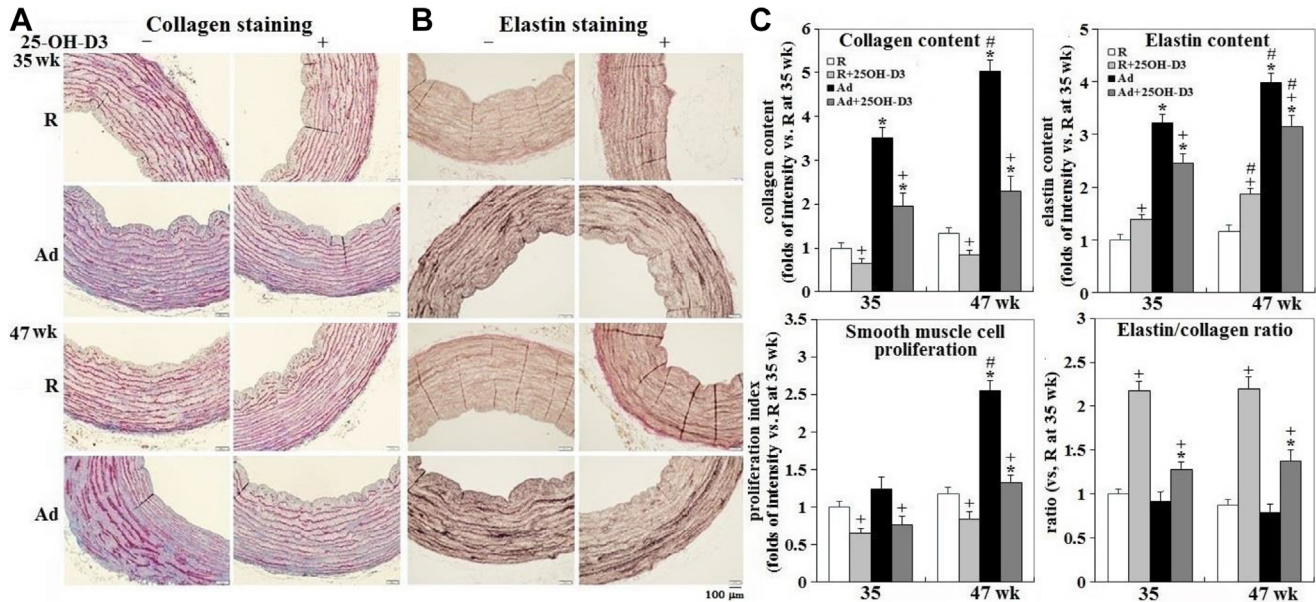


**Figure 4.** Effect of dietary 25-OH-D3 supplementation on arterial remodeling of broiler breeder hens provided with restricted or ad libitum feed intake. When hens were 29, 35, and 47 wk of age, 4, 7, and 7 hens from each group, respectively, were sampled for tissue collection. Pulmonary artery histopathology was determined at 35 and 47 wk. The midsection of the pulmonary artery (1/2 of the whole length) was used for Trichrome Masson and Verhoeff's staining. Connective tissue (collagens) stains blue with fibrosis (panel A), smooth muscle cells stain purple staining (panel A), and elastin stains brown (panel B). In the histology, a well-arranged structure with neatly stacked layers by 25-OH-D3 was noted in both R- and Ad-hens (panel A, left). Quantification of the intensity was made using ImageJ (panel C) and results were expressed as a ratio relative to R-group at 35 wk. Four images per section were used for quantification. \*Significant difference by feeding level (vs. corresponding R-hens,  $P < 0.05$ ). +Significant difference by 25-OH-D3 inclusion (vs. corresponding R- or Ad-hens,  $P < 0.05$ ). #Significant difference vs. corresponding counterparts at 35 wks ( $P < 0.05$ ). Abbreviations: R, restriction; 25-OH-D3, 25-hydroxycholecalciferol.

with the progression of cardiac hypertrophy, which ultimately developed into cardiovascular compromise and a higher incidence of SD (Chen et al., 2017a; 2017b). Then, we showed that 25-OH-D3 supplementation effectively ameliorated cardiac pathogenesis and improved livability in both Ad- and R-hens (Lin et al., 2019a; 2019b). The improvements by 25-OH-D3 operated at systemic hypoxia and cardiac pathological hypertrophy through calcineurin–nuclear factor of activated T-cells, cytoplasmic 4, signaling and myosin heavy chain, cardiac muscle beta, expression in association with reduced hepatic steatosis and fibrosis. The present results with the same flock of hens further confirmed that 25-OH-D3

relieves blood pressure through vasodilation regulation and vascular remodeling to improve hens' heart health.

Consistent with past studies (Li et al., 2002; Lee et al., 2015), an antihypertensive effect of 25-OH-D3 was observed in broiler breeder hens under the stress of either free access to feed or high salt load. In both settings, 25-OH-D3 modulated vasodilators including the RAS and circulating NO availability. In mice, vitamin D deficiency augmented vasoconstriction induced by Ang II and impaired arterial contractility leading to increased systolic blood pressure (Pelham, et al., 2016). Vitamin D, specifically 1,25-OH-D3, is a potent negative



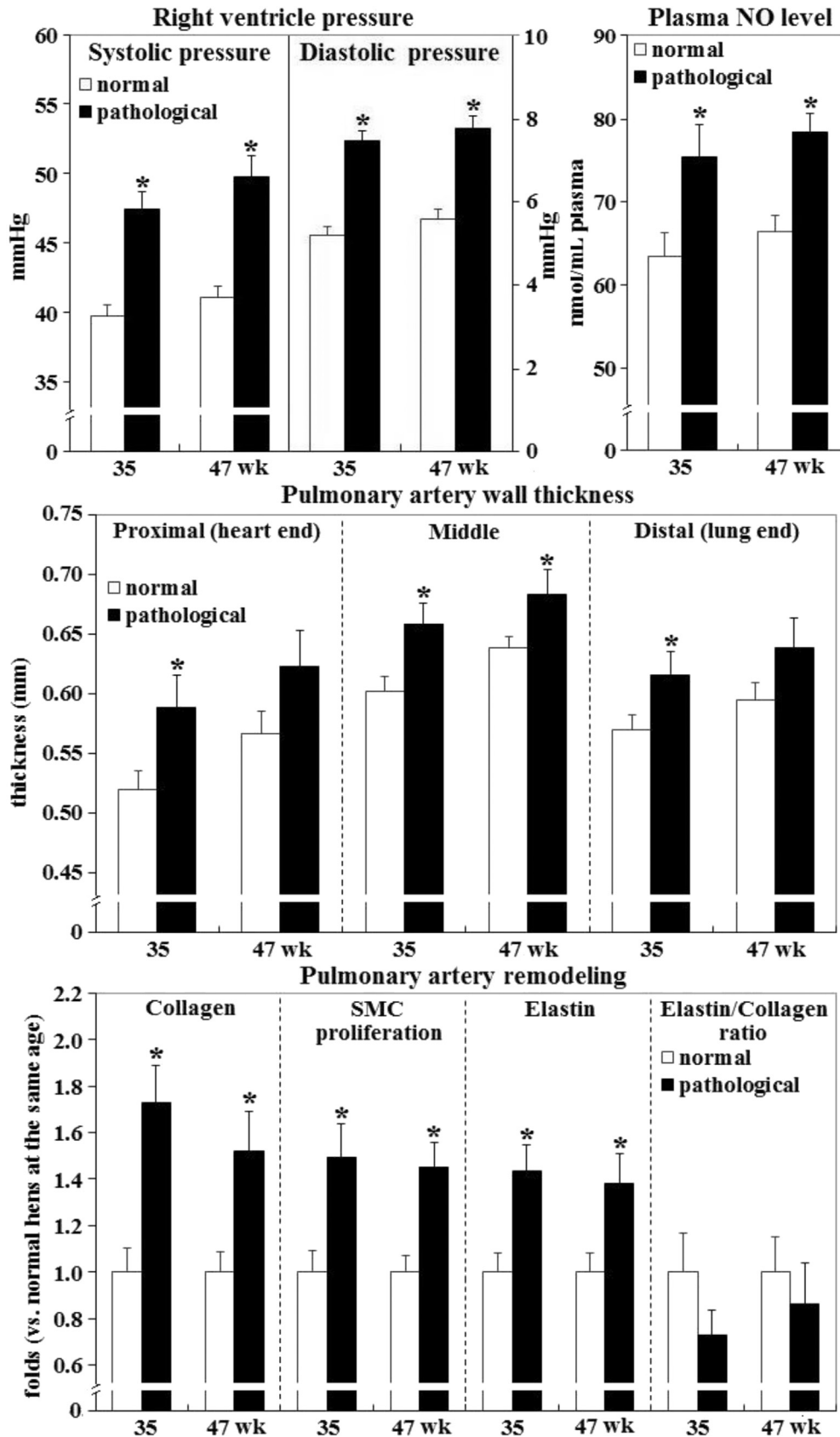
**Figure 5.** Effect of dietary 25-OH-D3 supplementation on pulmonary artery dimension and thickness of broiler breeder hens provided with restricted or ad libitum feed intake. When hens were 29, 35, and 47 wk of age, 4, 7, and 7 hens from each group, respectively, were sampled for tissue collection. Pulmonary artery histology was determined at 35 and 47 wk. The midsection of the pulmonary artery (1/2 of the whole length), 3/4 length above (distal) or below (proximal) the middle line were used for lumen diameter and wall thickness measurements. Results represent an average of 8 measurements at every 45° angle around the circumference of the artery (8 points). \*Significant difference by feeding level (vs. corresponding R-hens,  $P < 0.05$ ). Abbreviations: +, significant difference by 25-OH-D3 inclusion (vs. corresponding R- or Ad-hens,  $P < 0.05$ ); R, restriction; 25-OH-D3, 25-hydroxycholecalciferol.

endocrine regulator of renin gene expression by activating its receptor (vitamin D receptor [VDR]) (Li et al., 2002), which interacts with the cAMP response element-binding protein to hamper the activity of cAMP response elements on the renin gene promoter (Yuan et al., 2007). Moreover, 1,25-OH-D3 reduced endothelium-dependent contraction, modulated vascular tone, and thereby lowered blood pressure (Wong et al., 2010). Parts of the effects can be attributed to activation of VDR, which functions as a direct transcriptional regulator of endothelial NO synthase (Andrukhova et al., 2014).

In addition to RAS regulation, vascular remodeling and structural alterations by Ad-feed intake or in R-hens with higher metabolism for better feed efficiency may impair endothelium integrity, vascular elasticity/stiffness, and contractile function and thus contribute to arterial resistance and pathological progression of cardiac remodeling leading to functional compromise (Lin et al., 2019a; 2019b). Mice with mutant VDR were characterized by higher aortic impedance, arterial remodeling, and stiffness, leading to cardiac hypertrophy and functional compromise, independent of changes in the RAS (Andrukhova et al., 2014). In mouse models, activation of VDR by 1,25-OH-D3 was shown to maintain endothelium integrity and prevent blood-brain barrier disruption induced by hypoxia/reoxygenation (Won et al., 2015) and attenuate vascular remodeling and improve intrarenal arterial lumen dimension leading to reduced resistance to flow and thereby relieved ischemia and kidney fibrosis (Arfian et al., 2018). While we did not examine the kidneys, similar mechanisms by 25-OH-D3 to relieve arterial resistance appear operative

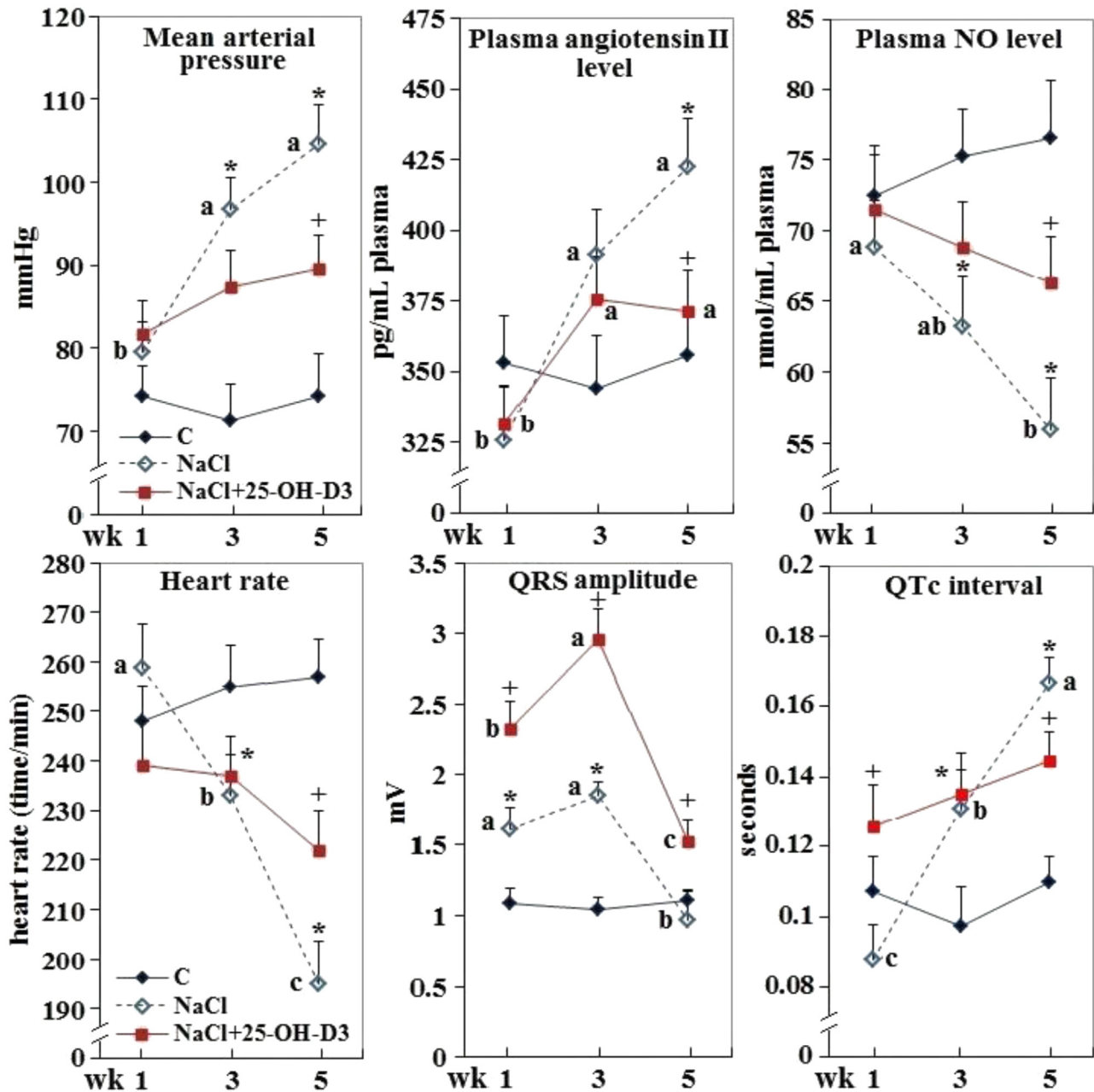
in broiler breeder hens. In addition, 25-OH-D3 supplement relieved hypertension and cardiomyopathy by alleviating inflammation and hypoxia in the perivascular adipose tissue leading to enhanced crosslinks in collagen and elastin, vascular integrity and elasticity, and thereby improved arterial contractile responses (Pelham, et al., 2016). Specific studies are needed to confirm perivascular adipose tissue as a risk factor for SD along rapid BW and fat gains in broiler breeder hens.

The susceptibility of growing young broilers to SD has been attributed to a higher incidence of ascites (also called pulmonary arterial hypertension [PAH] syndrome), which is clinically characterized by hypoxia and mechanical overloads of the cardiopulmonary circulation system leading to right ventricle hypertrophy and cardiomyopathy (Julian, 2002; Wideman et al., 2013). In consistence with the reports, cardiac hypertrophy, higher RVSP, RVDP, and more pronounced pulmonary artery remodeling in conjunction with severer cardiac pathologies, systemic hypoxia, and acidosis by Ad-feed intake were observed, as well as in R-hens with cardiac pathologies (Lin et al., 2019a; 2019b). Because systemic hypertension was not observed in SD R-hens and significantly higher RVSP and RVDP but not RAS regulations were observed between R-hens with normal or abnormal cardiac morphology at necropsy, PAH rather than peripheral hypertension is concluded as one of the pathological factors in cardiac remodeling leading to a higher susceptibility of SD in R-hens with better feed efficiency. In addition, as evidenced by enhanced QRS complex amplitude and relieved QTc interval in R-hens with high salt loading, 25-OH-D3 apparently



**Figure 6.** Right ventricle pressure, plasma nitric oxide level, and arterial remodeling of feed-restricted broiler breeder hens with normal or pathological cardiac hypertrophy at necropsy. At age of 29, 35, and 47 wk, 4, 7, and 7 hens from each group, respectively, were sampled for tissue collection. Immediately before necropsy, selected hens were used for right ventricle pressure measurement. None of the birds in R-groups at age of 29 wk, but 2 hens from each group exhibited pathological cardiac hypertrophy (concentric and eccentric) at 35 and 47 wk. Results thus were regrouped and presented according to normal ( $n = 10$ ) or pathologic cardiac hypertrophy ( $n = 4$ ). \*Significant difference vs. normal hens ( $P < 0.05$ ). Abbreviations: NO, nitric oxide; SMC, smooth muscle cells; 25-OH-D3, 25-hydroxycholecalciferol.





**Figure 7.** Dietary 25-OH-D3 supplementation ameliorates blood pressure, plasma angiotensin II and nitric oxide (NO) levels, and cardiac functional compromise induced by salt loading in broiler breeder hens with high salt loading. At age of 51 wk, additional 4 hens from the R and R + 25-OH-D3 group were challenged with salt loading in drinking water (0.2% NaCl in distilled water) and another 4 R-hens provided with distilled water as a comparative control. Peripheral blood pressure, heart rate, ECG, plasma angiotensin II, and (NO) concentration measurements were made 1, 3, and 5 wks after treatment. \*Significant difference by NaCl loading (vs. control,  $P < 0.05$ ). Abbreviations: +, significant difference by 25-OH-D3 inclusion (vs. NaCl group,  $P < 0.05$ ); R, restriction; 25-OH-D3, 25-hydroxycholecalciferol; ECG, electrocardiography; QTc, interval corrected QT interval in ECG profile.

operates at the heart per se independent of the RAS regulation, to prevent hypertrophic remodeling from pathological progression, and thus decrease incidence of arrhythmia and improve contractility to overcome increased pulmonary vascular resistance (Singla et al., 2005; Chen et al., 2011; Shimoda and Laurie, 2013; Mangoni and Jarmuzewska, 2018). Modulation of ECG by vitamin D has been shown to associate with autonomous nerve activity (Burt et al., 2016).

More than 90% of patients with PAH exhibit serum 25-OH-D3 levels lower than the sufficiency range and show

impaired cardiac output that is inversely correlated with pulmonary artery pressure and vascular resistance (Ryan and Archer, 2014; Tanaka et al., 2017). In rat PAH models, relieved right ventricle hypertrophy, pulmonary arterial pressure, and vascular stiffness by vitamin D were shown to contribute to improved survival rate (Mandell et al., 2015; Tanaka et al., 2017). Replacement therapy of vitamin D also improved right ventricular size and aerobic exercise capacity and endurance in clinical cases of human PAH (Mirdamadi and Moshkdar, 2016). In the present study, a similar

symptomology including right ventricle hypertrophy was observed (Lin et al., 2019b), most commonly in Ad-hens in association with increased RVP indicative of increased vascular resistance of the cardiopulmonary circulation system. However, 25-OH-D3 only prevented time-dependent increases of RVDP in Ad-hens but not RVSP. Because diastolic pressure represents resting cardiac muscle between beats, this discrepancy suggests more pronounced improvements by 25-OH-D3 on distal vascular impedance of the cardiopulmonary circulation system than on arterial elasticity (Bavishi et al., 2016). Alterations of arterial morphology and structure by 25-OH-D3 are a net outcome of arterial collagenesis and SMC proliferation and by vasodilation/vasoconstriction regulation through RAS and NO availability. Thus, RAS modulation in response to a higher cardiac output owing to hyper hemodynamics by Ad-feed intake may account for the differential effects of 25-OH-D3 on lumen dimension and wall thickness between Ad- and R-hens (van Suylen et al., 1998).

Results from the study showed the presence of pathological cardiac hypertrophy and increased arterial pressure as metabolically driven pathogenesis in conjunction with compromised vascular structure and signaling processes in Ad-hens. Both Ad-feed intake and increased feed efficiency independent of feed intake exacerbate the pathogenic progression and increase a risk for SD. Supplemental 25-OH-D3 has several mitigating effects including, as shown here, modulation of vasodilation at the level of RAS and NO availability, and vascular remodeling to relieve arterial pressure and thereby cardiac functional compromise for improved livability in broiler breeder hens.

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## SUPPLEMENTARY DATA

Supplementary data associated with this article can be found in the online version at <https://doi.org/10.1016/j.psj.2020.03.015>.

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