

The Role of Oxidative Stress in Development of Congestive Heart Failure (CHF) in Broiler with Pulmonary Hypertension Syndrome (PHS)

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Abstract: The present study examined the possible role of reactive oxygen species in the pathogenesis of heart failure in broilers. The experiment was conducted with 160, 1 day old male broilers (Ross 308) to investigate to clarify the mechanism of cell injury causing the pathogenesis of PHS syndrome. The chickens were divided in 2 groups of 4 replicates and 20 chicks for any replicate. One group of these chickens was raised in Normal Temperature (NT) treatment and the other group was raised in Cold Temperature (CT) treatment for induce pulmonary hypertension syndrome. Mortality was inspected to determine cause of death and diagnose of heart failure. Hematological, biochemical and pathological tests were used to determine the incidence of PHS including total Red Blood Cell (RBC), Hemoglobin (HGB), Hematocrit (HCT), release of Alanine Transaminase (ALT), Aspartate Transaminase (AST), Lactate Dehydrogenase (LDH) and a best indicator of lipid oxidation subsequent to generated oxidative stress was Malondialdehyde (MDA). Sampling of blood and liver tissue were determined at day 21 and 42. At end of the experiment (week 6), 2 chicks from each replicate were randomly selected and slaughtered. The heart was removed; the right ventricle was dissected away from the left ventricle and septum then ratio of Right Ventricle weight to Total Ventricle weight (RV/TV) calculated too. The results of the experiment indicated that there was a significant difference in RBC, HGB at 21 and HGB, RBC, HCT at 42 between groups as CT group had a greater ($p < 0.05$) HGB, RBC at 21 and HGB, RBC, HCT at 42. However, there was no significant difference in ALT, AST and LDH plasma levels between groups at day 21, CT group had greater ($p < 0.05$) levels in AST, ALT and LDH at day 42. The levels malondialdehyde equivalents an indicator of lipid oxidation sub-sequent to generated oxidative stress at plasma and liver tissue was significantly higher ($p < 0.05$) in CT group at day 21 and 42. RV/TV ratio and mortality due to ascites, also were significantly affected by treatments as CT group had greater ($p < 0.05$) RV/TV and mortality due to ascites mortality percentage compared to NT group. In conclusion, the results indicated that the deterioration of heart function in modern fast growing broilers in the experimental model is associated with oxidative stress leading to lipid peroxidation and reactive oxygen species may be involved in the pathogenesis of the pulmonary hypertension syndrome in broilers chickens.

Key words: Oxidative stress, heart failure, ascites, hematological, broiler, Iran

INTRODUCTION

Pulmonary Hypertension Syndrome (PHS) or ascites is a metabolic disorder that mostly occurs in fast-growing broiler chickens. High altitude, hypoxia, poor ventilation, low temperature and fast growth rate are known to be predisposing factors for the incidence of this syndrome (Huchzemeyer and Deruyck, 1986; Maxwell *et al.*, 1986; Wideman *et al.*, 1995; Hassanzadeh *et al.*, 1997; Balog, 2003). Pulmonary hypertension and cardiac dysfunction are the most important features of ascites. Pathological findings indicate that the creation of a cavity on the exterior surface of the right ventricular wall is the 1st sign of damage in pulmonary hypertension. As the injury

progresses, it leads to dilation and hypertrophy of the right ventricle resulting in increased blood viscosity, reduced oxygen supply, Congestive Heart Failure (CHF) and accumulation of fluids in the abdominal cavity (Julian, 1990, 1993; Odum, 1993; Owen *et al.*, 1995).

Oxidative metabolism is a normal process in all tissues. Cardiomyocytes require a constant supply of oxygen for normal cardiac functions. However, oxygen associated metabolism in the myocardium sometimes can contribute to cardiac dysfunction and may ultimately lead to heart failure (Giordano, 2005; Redout *et al.*, 2007). During the normal oxidative metabolic process, various Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) are produced. During this normal

metabolism, 1-2% of oxygen is converted to ROS (Sheeran and Pepe, 2006). ROS are known to be the cause of different disorders including thermal injury, inflammations, sepsis, mutagenesis, carcinoma, autoimmune diseases and ischemia reperfusion injury (Flohe *et al.*, 1985; McCord, 1985; Halliwell, 1989). The role of ROS in the injury induced by ischemia reperfusion has been convincingly shown in different organs including brain, liver, skin, muscle, lung, intestine, kidneys and heart (Halliwell, 1989; Jaeschke, 1991; Diaz-Cruz *et al.*, 1996).

However under some circumstances, increased ROS/RNS production or decreased antioxidant defenses may lead to oxidative stress where the generated reactive species can alter the properties of lipids, proteins and nucleic acids, leading to cellular dysfunctions. Recent research findings from different laboratories suggest that ROS and RNS play a critical role in development of human heart failure (Andreka *et al.*, 2004; Sam *et al.*, 2005; Nediani *et al.*, 2007). Lipid peroxidation can alter the membrane properties of cellular and sub-cellular organelles (mitochondria and sarco-endoplasmic reticulum) crucial for maintenance of normal cardiomyocyte function.

Broilers with Congestive Heart Failure (CHF) show evidence of calcium overload in these sub-cellular components (Maxwell *et al.*, 1993; Li *et al.*, 2006) and evidence of breakdown and release of the protein of contractile apparatus such as myosin and troponin T into the circulation (Maxwell *et al.*, 1994).

The role of oxidative stress has long been debated in the pathogenesis of heart failure in human and animal models of cardiomyopathy. However, limited search has been carried out to investigate the possible involvement of oxidative stress in PHS and CHF in broilers. In order to further understand the physiological and biochemical disturbances leading to PHS and CHF in commercial broilers, the researchers were interested to examine the possible role and molecular mechanisms of oxidative stress in the pathogenesis of these syndromes.

MATERIALS AND METHODS

Birds and diets: About 160, 1 day old male broiler chickens (Ross 308) were used in this experiment. Chickens allocated randomly into 2 treatments groups with 4 replicates each and 20 chicks per replicate (per cage). Two groups including, broilers under Normal (NT) and Cold (CT) environmental temperature. All chicks were fed a basal corn-soybean meal diet to meet the NRC requirement including 22.04% CP and 3,200 kcal kg⁻¹ of ME (1-21 days) or 20.26% CP and 3,200 kcal ME (22-42 days). Feed and water provided *ad libitum*.

Management and measurements: Broilers of NT group were reared under normal temperature until end of experiment. For inducing ascites, the birds of the CT group were raised under 32 and 30°C during week 1 and 2, respectively. The house temperature was decreased to 15°C during week 3 and maintained between 10 and 15 for the rest of the study (Iqbal *et al.*, 2001). Mortality was recorded daily and all of the dead birds inspected for diagnosis of ascites.

Diagnosis of ascites generally depends on observation of the following symptoms; right ventricle hypertrophy, cardiac muscle laxation, swollen and stiff liver, clear, yellowish and colloidal fluid in the abdominal cavity (Geng *et al.*, 2004).

Sampling: At day 21 and 42, one chick from each replicate was randomly chosen and after 3 h starvation, blood sampling from wing vein. After blood sampling, the bird were killed and thorax and abdomen were open sampling from liver tissue for MDA evaluation and inspected for signs of heart failure and ascites. The heart was dissected and removed from the body to determine the ratio of Right Ventricular (RV) weight to Total Ventricular (TV) weight. Bird having RV/TV values, >0.299% were considered to have ventricular hypertrophy. Blood samples were collected in tube with EDTA. Part of each blood sample immediately used for measuring total Red Blood Cell (RBC) count, Hematocrit (HCT) and Hemoglobin (HGB). The remainder was centrifuged and plasma was collected and stored at -80°C until measurement of the other enzymatic and chemical analysis.

Malondialdehyde (MDA): The blood was centrifuged at 1,500×g for 5 min; plasma was collected in labeled tubes and stored at -80°C until analysis. After thawing, 500 µL of plasma was placed in a labeled glass tube and mixed with the reagents of a commercial kit for the measurement of Thiobarbituric Acid Reactive Substances (TBARS) and each tube was covered with a glass marble and incubated at 95°C for 45 min. The tubes were removed from incubation and allowed to cool in an ice bath for 10 min. Once cooled, the tubes were centrifuged at 3000×g for 10 min and the supernatant carefully removed from the tubes for analysis.

The absorbance of the supernatants was measured at 532 nm using a UV/VIS spectrophotometer (Gildford Instrument Laboratories, Inc., Oberlin, OH) and the results were compared against a standard curve made with 100, 50, 25, 12.5 and 0 nmol mL⁻¹ of malondialdehyde dim ethyl acetyl.

Statistical analysis: The data were analyzed based on a completely randomized design using the GLM procedure

of SAS (SAS Institute, 2002). Duncan's multiple range were used to separate the means when treatments means were significant ($p \leq 0.05$) thus a probability level of $p \leq 0.05$ was considered statistically significant. Data were presented as means \pm SD.

RESULTS AND DISCUSSION

Incidence of heart failure: Overall, during the course of this study, the mortality due to PHS between groups was significant different as CT group had higher ($p < 0.05$) mortality than NT group (38 vs. 7.5%). Also, an indicator for incidence CHF, RV/TV ratio was higher ($p < 0.05$) in CT group than NT group (Table 1).

Hematology: The results of hematological values are shown in Table 2, there was a significant difference between NT and CT groups as CT group had higher ($p < 0.05$) RBC, HGB values at day 21 and RBC, HGB, HCT values at day 42 compared NT group.

Enzymes release: The activities of plasma ALT, AST and LDH are shown in Table 3. As these values indicate at day 21 was not significant difference between treatment groups. But at day 42, the CT group had greater ($p < 0.05$) values in ALT, AST and LDH than NT group.

MDA equivalents levels: The MDA equivalents levels in plasma and liver tissue of broiler in two treatment groups are shown in Table 4. As shown the MDA equivalents levels in both plasma and liver at day 21 and 42 were higher ($p < 0.05$) in CT group than NT group.

Hypoxia is thought to be the primary cause in the development of ascites therefore, conditions that impose greater metabolic demand or decreased oxygen consumption increase incidence of PHS (Buys *et al.*, 1999). Hypoxemia initiates a cascade of events that results in PHS and CFH and death (Julian, 1993). Moreover, this hypoxemia leads to some hematological changes such as hematocrit, hemoglobin, red blood cell and blood gas volume changes and some other parameters could be affected as well.

In the study while the increase in the amount of MDA in plasma and liver tissue started from 21 days, risen in enzyme release (ALT, AST and LDH, Table 3) and HCT (Table 2) were observed at day 42. It is suggested that the pathogenesis of ascites syndrome may be initiated by increased production of ROS (OH). As the injury proceeds, it causes dilation and hypertrophy of the right ventricle resulting in the increased PCV, blood

Table 1: RV/TV ration and mortality percentage of broilers under normal (NT) and cold (CT) environmental temperature

Treatments	RV/TV ratio	Total mortality percentage due to ascites (%)
NT	0.02 \pm 0.22 ^b	7.5 \pm 1 ^b
CT	0.31 \pm 0.01 ^a	38 \pm 4 ^a

Data presented as the mean \pm standard error. Means within columns with different superscript letters are significantly different ($p < 0.05$)

Table 2: RBC, HGB and HCT of broilers under normal (NT) and cold (CT) environmental temperature

Treatments	RBC ($10^6 \mu L^{-1}$)	HGB (g dL ⁻¹)	HCT (%)
21 days			
NT	1.71 \pm 0.12 ^b	6.12 \pm 0.31 ^b	29.02 \pm 0.80
CT	2.42 \pm 0.16 ^a	8.57 \pm 0.49 ^a	34.27 \pm 2.04
42 days			
NT	2.00 \pm 0.20 ^b	7.75 \pm 0.35 ^b	32.00 \pm 0.65 ^b
CT	2.80 \pm 0.17 ^a	11.20 \pm 0.35 ^a	39.30 \pm 2.27 ^a

Data presented as the mean \pm standard error. Means within columns with different superscript letters are significantly different ($p < 0.05$)

Table 3: ALT, AST and LDH levels in plasma of broilers under Normal (NT) and Cold (CT) environmental temperature

Treatments	ALT (U L ⁻¹)	AST (U L ⁻¹)	LDH (U L ⁻¹)
21 days			
NT	2.65 \pm 0.57	211.75 \pm 32	2975 \pm 202
CT	4.00 \pm 0.41	223.50 \pm 10	3100 \pm 250
42 days			
NT	3.75 \pm 0.41 ^b	217.50 \pm 10 ^b	3162 \pm 320 ^b
CT	7.37 \pm 0.25 ^a	240.50 \pm 70 ^a	4920 \pm 674 ^a

Data presented as the mean \pm standard error. Means within columns with different superscript letters are significantly different ($p < 0.05$)

Table 4: MDA equivalents levels in plasma and liver tissue of broilers under normal (NT) and cold (CT) environmental temperature

Treatments	MDA in plasma (nm mL ⁻¹)	MDA in liver (nm mL ⁻¹)
21 days		
NT	1.30 \pm 0.31 ^b	0.85 \pm 0.30 ^b
CT	2.50 \pm 0.33 ^a	1.32 \pm 0.23 ^a
42 days		
NT	1.60 \pm 0.20 ^b	1.10 \pm 0.04 ^b
CT	6.27 \pm 0.43 ^a	2.60 \pm 0.25 ^a

Data presented as the mean \pm standard error. Means within columns with different superscript letters are significantly different ($p < 0.05$)

viscosity and the accumulation of fluids in the abdominal cavity due to heart failure (Julian, 1990, 1993; Owen *et al.*, 1995). A transient hypoxia and then reoxygenation followed by frequencies hypoxia can be the major cause of ROS production. The low-flow circulation of blood that occurs in birds predisposed to ascites can induce anoxia in different tissues including the heart. The reoxygenation induced via compensation efforts, may happen continuously in the ischemic tissues, resulting in increased production of ROS (Dawson *et al.*, 1993).

As the results indicate, increased production of ROS was shown at day 21 in the CT chickens. These agents may cause lipid peroxidation in the membrane of the cells resulting in tissue injury in organs, including lung and heart and liver (Arab *et al.*, 2006) (Table 4). The increase in the amount of AST, ALT and LDH at day 42 is an

indicator of a progressive liver cell injury followed by the increased production of ROS resulting in the induction of a chain of oxidative reactions in the liver and other organs (Arab *et al.*, 2006). As the results indicate the amount of ALT, AST and LDH have increased during day 21-42. There is evidence that serum values of ALT and AST are elevated before the clinical signs and symptoms of liver disease appear. As the injury proceeds, the gross damage (heart failure, fluid accumulation (ascites) and death) follows. This process can probably explain the pathophysiology of ascites in broilers.

Nain *et al.* (2008) reported that Morphological changes observed in myocardial mitochondria are consistent with oxidative damage. Notably, mitochondria are the major source of ROS but because of their very high component of membranes they are also a very sensitive target of ROS attack. The membrane lipids are very sensitive to oxidative damage due to the presence of polyunsaturated fatty acids, sub-sequently leading to lipid peroxidation (Halliwell and Gutteridge, 1985). Currently, one of the most common and well recognized approaches to measure the effects of free radicals is by measuring the oxidative damage (i.e., lipid peroxidation) to cellular membranes (Lykkesfeldt and Svendsen, 2007). So, the measurements from the lipid peroxidation in the present study showed that broilers outbreak with PHS suffering by oxidative stress increases as the risk of heart failure increases. The biochemical evidence of oxidative damage (elevated MDA) corresponds well with the observed morphological changes in the mitochondria such as mitochondrial swelling, vacuolization, loss and disintegration of cristae (Nain *et al.*, 2008). The heart is one of the greatest energy consuming organs in the body which requires a constant supply of oxygen to maintain its metabolic functions (Giordano, 2005). In the cardiac tissue, mitochondria comprise 30% of the cardiomyocyte volume (Sheeran and Pepe, 2006). The major sites of ROS formation are at complex 1 and 3 of the electron transport chain located in the inner membrane of mitochondria (Turrens and Boveris, 1980; Turrens *et al.*, 1985). During normal metabolism, 1-2% of oxygen is converted to ROS.

Hence, increased ROS or RNS production or decreased antioxidant defenses leads to oxidative stress. A-ketoglutarate Dehydrogenase (a-KGDH), one of the key rate-limiting enzymes of the tricarboxylic acid cycle is involved in energy synthesis pathways. Studies in rats have demonstrated that a-KGDH is a sensitive target of H₂O₂.

In an anaerobic situation, LDH contributes to energy synthesis by anaerobic glycolysis. An increased production of ROS/RNS occurs during tissue hypoxia

(Chen and Meyrick, 2004) which can negatively affect the activity of energy synthesis and transformation pathways. With hypoxia, activation of LDH enzyme by ROS may work as a force to counter the negative effect of other enzymes on energy synthesis and transformation pathways. Recently, observed higher LDH activity in broilers developing CHF (Nain *et al.*, 2008). Hence, increased activity of LDH in broilers developing CHF is most probably due to generated oxidative stress in the broilers. Insufficiency of creatine phosphate and ATP leads to deterioration in heart pump function in broilers (Nain *et al.*, 2008; Olkowski *et al.*, 2007). This suggests that the observed decline in energy phosphates with deterioration in heart functions might be associated with the decreased activity of these enzymes during oxidative stress.

CONCLUSION

The results in the study show that heart failure in broilers with hypoxia and subsequent PHS can associated with ROS production during oxidative stress. So, oxidative stress due hypoxia is the most initiate the problem with PHS and CFH. As ROS can cause cell injury and increase release enzyme in plasma including ALT, AST and LDH. ROS also can affect deterioration on ATP synthesis in myocardium and subsequent leading to lowered energy reserve in the myocardium.

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