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The Effects of Vitamin E and L-Arginine Supplementation on Antioxidant Status and Biochemical Indices of Broiler Chickens with Pulmonary Hypertension Syndrome

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Abstract: An experiment with 320, 1 day male broilers (Ross 308) was performed to evaluate the effects of Arginine (Arg) and Vitamin E (VE) supplementation on antioxidant status, some biochemical parameters and ascites mortality in broiler chickens with cold temperature induced Pulmonary Hypertension Syndrome (PHS). The chickens were distributed between 16 pens of 20 each and each four allocated to each of experimental treatments. Treatment 1: was the birds fed a commercial corn-soybean meal based diet (control), Treatment 2: the birds fed the control diet supplemented with 400 IU VE kg^{-1} of feed (VE), Treatment 3: the birds fed the control diet supplemnetd with 0.3% Arg in drinking water (Arg) and Treatment 4: the birds fed the control diet supplemented with 400 IU VE and 0.3% Arg (Arg-VE). At day 14, temperature was reduced to amplify the incidence of Pulmonary Hypertension. Hematological and oxidants status parameters (in serum and liver) were determined at day 21 and 42 of age. Right/Total Ventricular weight ratio (RV/TV) was recorded at the end of the experiment (week 6). The results of experiment showed that Arg birds at day 42 had the lower ($p \leq 0.05$) RV/TV ratio and ascites mortality yet had improved growth performance parameters. Red Blood Cell (RBC), Hematocrit (Hct) and Hemoglobin (Hgb) was significantly lower in Arg and Arg-VE birds as compared to other birds at day 42 of age. Plasma Malondialdehyde (MDA) wae significantly lower in Arg and Arg-VE birds as compared to other birds at 42. Plasma Glutathione Peroxidase (GPX) activity was greater ($p \leq 0.05$) in both the Arg and Arg-VE birds as compared to other groups at day 42. Plasma and liver Superoxide Dismutase (SOD) activity was not affected by none of the Arg or VE supplementation. In summary level of Arg but not for VE used in this experiment, synchronic, some change effects on MDA and TAS in plasma or hematological parameters had positive effects on cardio pulmonary performance, growth performance and ascites mortality in broiler encounter with low temperature and PHS.

Key words: Ascites, oxidative stress, vitamin E, arginine, broilers, Iran

INTRODUCTION

Pulmonary Hypertension Syndrome (PHS) is a common disorder in broiler chickens that causes considerable mortality (Julian, 1993). High altitude, hypoxia, low temperature and fast growth rate are the predisposing factors for the incidence of this syndrome (Arab *et al*., 2006). PHS can be initiated by an increase in metabolic rate which implies higher oxygen demands (Lorenzoni and Ruiz-Feria, 2006). In gas exchange system working close to its physiological limit, this elevation in metabolic demand can lead to cellular hypoxia (Scheele *at al*., 1991) to avoid cellular hypoxia an increased cardiac output must be propelled through the noncompliant pulmonary vasculature which requires increases in Pulmonary Arterial Pressure (PAP) and Right Ventricle (RV) work. Sustained increases in PAP cause hypertrophy and subsequent dilation of the right ventricle. An elevated ratio of Right to Total Ventricular weight (RV/TV) is thus an anatomical symptom of PHS and has been used as a sensitive index to determine the presence of pulmonary hypertension in broilers (Lorenzoni and Ruiz-Feria, 2006). Broilers with PHS die due to right-sided congestive heart failure which is associated with central venous congestion, pressure induced liver cirrhosis and transudation of ascetic fluid in to the abdominal cavity (Julian, 1993). By reducing the pulmonary vascular resistance, it is possible to reduce the PAP needed to propel the cardiac output required to match broilers' metabolic demands (Wideman *et al*., 1995) and delay the pathophysiologicalprogression leading to ascites. Nitric Oxide (NO) is a potent endogenous pulmonary vasodilator that increases the cyclic Guanosine Monophosphate (cGMP) level and thereby reduces the intracellular Ca^{++} levels in vascular smooth muscle cells (Forstermann *et al*., 1986). Nitric oxide is

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produced in the pulmonary endothelium where the NO Synthase (NOS) enzyme converts L-arginine to L-citrulline (Jorens *et al*., 1993; McQueston *et al*., 1993). Arginine is an essential amino acid for avian species because birds lack the enzyme carbamyl phosphate synthetase which aids in the conversion of ornithine to citrulline and thus Arg (Tamil and Ratner, 1963). It has been suggested that Arg levels normally fed to broiler chickens (National Research Council, 1994) are adequate for growth but may be inadequate to fully support the production of NO by avian macrophages and the pulmonary vascular endothelium (Taylor *et al*., 1992; Dietert *et al*., 1994; Wideman *et al*., 1995, 1996; Martinez-Lemus *et al*., 1999).

Oxidative stress is also involved in the pathophysiological progression leading to ascites (Maxwell *et al*., 1986). Oxidative stress occurs when forces that favor oxidation outweigh antioxidant protection within cells (Yu, 1994). The involvement of oxidative stress in PHS in broilers has now been clearly demonstrated by findings that broilers with PHS exhibit increases in oxidized glutathione (GSSG) or the ratio of oxidized to reduced glutathione (GSSG/GSH) in tissue, increases in plasma lipid peroxides and decreases in major nonenzymatic antioxidants such as GSH, α-tocopherol and ascorbic acid in liver and lung (Enkvetchakul *et al*., 1993; Bottje and Wideman, 1995; Bottje *et al*., 1995, 1997). A major cellular source of oxidative stress in cells occurs within mitochondria due to incomplete reduction of oxygen to Reactive Oxygen Species (ROS) (e.g., superoxide) (Chance *et al*., 1979). Increased mitochondrial ROS production (Maxwell *et al*., 1996; Cawthon *et al*., 2001; Iqbal *et al*., 2001) may be responsible for much of the oxidative stress observed in PHS. The superoxide anion responsible for direct tissue damage by oxidation, peroxidation and nitration of lipids, proteins and DNA (Beckman *et al*., 1990; Szabo, 1996). Vitamin E (VE) is known to be a powerful lipid-soluble antioxidant that scavenges lipid radicals. It has the ability to react with fatty acid peroxyl radicals, the primary products of lipid peroxidation and intercepts the chain reaction preventing further radical reactions. During the antioxidant reaction, α-tocopherol is turned into a stable radical (Burton and Ingold, 1981). Vitamin E could thus help in reducing the oxidative stress in lung vessels and consequently reduce the endothelial damage. It has been reported that birds developing ascites have low levels of α-tocopherol in lung and liver providing evidence that a compromised antioxidant status is involved in the etiology of PHS (Enkvetchakul *et al*., 1993). Nevertheless, studies supporting the use of antioxidants to reduce mortality for ascites have been successful only when the VE is administered as a subcutaneous implant and showing no effects in ascites mortality when VE was given as a feed additive (Bottje *et al*., 1995, 1997; Lorenzoni and Ruiz-Feria, 2006; Ruiz-Feria, 2009). So, objective of this experiment was conducted to investigate the effects of an vasodilator source (Arg) and an antioxidant (VE) in combination on ascites disorder in broiler chickens.

MATERIALS AND METHODS

Birds and diets: The 320 days old male broiler chickens (Ross 308) were used in this experiment. Chuckes were distributed between sixteen cages (replicates) randomly on arrivial and each four were alloctaed to one of the treatments. Four groups including, 1: Control group (Control, basal diet) 2: Vitamin E ($_{DL}$ α-tocopherol acetate) with 400 IU vitamin E kg^{-1} fed (HVE) 3: arginine with 0.3% $_{L}$-Arg (monohydrochloride Canada-Iran) in water (HArg) and 4: containing both compound at same levels from HVE and HArg (HVE-HArg). All chickens were fed a basal corn-soybean diet to meet the 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). The basal diet includes Arg at 1.3% and VE at 40 IU kg^{-1} of feed for both growing periods. From 1-14 days, all chicks were fed the basal diet. From day 15 the chicks received the treatment. Feed and water provided for *ad libitum* consumption.

Management and measurements: Birds reared at 32 and 30°C during week 1and 2, respectively. For inducing ascites, the house temperature was decreased to 15°C during week 3 and temperature was maintained between 10 and 15 for the rest of the study (Iqbal *et al*., 2002). Average body weight gain, average feed intake and average feed conversion ratio were measured and calculated weekly from week 3 and for all experimental period. Mortality was recorded daily and all of the dead birds inspected for diagnosis of ascites. The mortality of ascites was diagnosedby the symptoms of right ventricle hypertrophy, cardiac muscle laxation, swollen and stiff liver and occurrence of clear, yellowish and colloidal fluid in the abdominal activity (Geng *et al*., 2004).

On days 21 and 42 of age, one chick per replicate was randomly selected after a 3 h starvation and used for blood sampling from wing vein. After taking blood samples, the birds were killed and thorax and abdomen were opened and heart was removed and used for determination of RV/TV ratio. Liver tissue for antioxidant enzymatic evaluation and inspected for signs of heart failure and ascites. Bird having RV/TV ratios <0.29% were considered to have ventricular hypertrophy (Julian, 1993). Blood samples were collected in anticoagulant (EDTA) tubes. Part of each blood sample (4 mL) immediately used

for measuring total red cell (RBC) count, Hematocrit (Hct) and Hemoglobin (Hgb). The reminder (6 mL) was centrifuged and plasma was collected and stored at -80°C until measurement of the other enzymatic and chemical analysis.

Antioxidant indices: Antioxidant enzyme activities were determined in whole blood and whole liver tissues. Enzyme activities were detected spectrophotometerically using RANSEL glutathione peroxidase, RANSEL superoxide dismutae and RANDOX total antioxidant status kit.

The activity of GSH-Px was determined according to Paglia and Valentine (1967) with some modifications. Glutathione Peroxidase (GPX) catalyses the oxidation of Glutathione (GSH) by cumene hydroperoxide. In the presence of Glutathione Reductase (GR) and NADPH, the oxidised Glutathione (GSSG) is immediately converted to the reduced form with concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance at 340 nm for 5 min (25°C) is measured. The liver homogenate (50 μL) was incubated with 25 mM potassium phosphate, 0.5 mM EDTA, pH 7.4, 0.5 mM $NaNO_3$, 0.3 mM NADPH, 0.64 U GSH-Rd and 1 mM GSH. The reaction was started with 0.1 mM Cumene Hydroperoxide. Values were corrected for nonenzymatic oxidation of GSH and NADPH by hydrogen peroxide. The result were expressed in unit of GSH-Px activity using a molar extinction coefficient of GPX U L^{-1} of Sample = 8412×ΔA 340 nm min^{-1} (ΔA = deference blank with sample). The unit was defined as U mg^{-1} protein. For evaluation in blood, whole blood (20 μL) was incubated with 0.5 mM potassium phosphate, 4.3 mM EDTA, pH 7.2, 4 mM GSH, 0.5 U GSH-Rd. The reaction was started with 0.18 mM cumene hydroperoxide. The unit was defined as U g^{-1} hemoglobin.

Plasma Superoxide Dismutase (SOD) activity was determined by Xanthine Oxidase (XOD) enzyme. This method employs Xanthine and Xanthine Oxidase (XOD) to generate superoxide radicals which react with 2 (4-iodophenyl) 3-(4-nitrophenol) 5-phenyltetrazolium chloride (I.N.T.) to form a red formazan dye. The superoxide dismutase activity is then measured by the degree of inhibition of this reaction. One unit of SOD is that which causes a 50% inhibition of the rate of reduction of INT under the conditions of the assay. Centrifuge 0.5 mL of whole blood for 10 min at 3000 rpm and then aspirate off the plasma. Then wash erythrocytes 4 times with 3 mL of 0.9% NaCl solution centrifuging for 10 min at 3000 rpm after each wash. The washed centrifuged erythrocytes should then be made up to 2.0 mL with cold redistilled water, mixed and left to stand at +4°C for 15 min. The lysate is diluted with 0.01 mol L^{-1} phosphate buffer pH 7.0 so that the % inhibition falls between 30 and 60%. Then, the lysate incubated for 5 min at 25°C with CAPS 40 mmol L^{-1}, pH 10.2, EDTA 0.94 mmol L^{-1}, Xanthine 0.05 mmol L^{-1}, I.N.T. 0.025 mmol L^{-1}, Xanthine Oxidase 80 U L^{-1}.The results were express as units of activity (U g^{-1} Hemoglobin) (Randox Ransod Superoxide dismutase manual).

Liver Superoxide Dismutase (SOD) activity in liver was determined following thexanthine oxidasmethod described by McCord and Fridovich (1969) with modifications. Briefly, liver homogenate (50 μL) was incubated for 5 min at 25°C with 20 mM potassium phosphate; 1 mM EDTA, pH 7.8; 0.25 mM xanthine and 0.17 mM cytochrome c. The reaction was initiated by adding xanthine oxidase (0.16 U) and was assayed by following the reduction of cytochrome c at 550 nm for 5 min (25°C) in the presence or absence of xanthine oxidase and SOD. The results were expressed as units of activity (U mg^{-1} protein). One unit of the activity was defined as the amount of SOD that inhibited the rate of cytochrome c reduction by 50%.

For plasma Total Antioxidant (TAS) capacity, ABTS (2, 2'-Azino-di-[3-ethylbenzthiazoline sulphonate]) is incubated with a peroxidase (metmyoglobin) and H_2O_2 to produce the radical cation $ABTS^+$. This has a relatively stable blue-green colour which is measured at 600 nm. Antioxidants in the added sample cause suppression of this colour production to a degree which is proportional to their concentration. For evaluation serum (20 μL) was incubated at 37°C with 1 mL Metmyoglobin Chromogen (6.1 mM L^{-1}), ABTS 610 μmol L^{-1}. Then detected absorbance in 600 nm. Then this component incorporate with 250 μmol L Hydrogen peroxide (25% μmol L) and after 3 sec detected aberrance in 600 nm. Reduce amount in cationе $ABTS^+$ result in antioxidant would compared with standard. The results were expressed as units of activity (mmol L^{-1}).

For MDA determination: 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 dimethyl acetyl.

Statistical analysis: The data were analyzed based on a completely randomized design using the GLM procedure of SAS (1996). 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±SD.

RESULTS AND DISCUSSION

The pulmonary arterial relaxation was improved when Arg was supplemented above the National Research Council (1994) requirements and this has been attributed to an increase in NO production (Wideman *et al*., 1995, 1996; Lorenzoni and Ruiz-Feria, 2006). In mammals, inhalation of Nitric Oxide (NO) reduced pulmonary arterial pressure and improved blood oxygenation when pulmonary hypertension was induced with thromboxane analogs or endotoxic shock models (Frostell *et al*., 1991; Weitzberg, 1993). In addition, antioxidants such as VE (Bottje *et al*., 1995) have been shown to reduce PHS mortality. However, the effects of Arg and VE on cardiopulmonary function and ascites incidence have not been consistent when used alone (Ruiz-Feria *et al*., 2001; Lorenzoni and Ruiz-Feria, 2006; Walton *et al*., 2001). We hypothesized that Arg and VE may have complementary effects on cardiopulmonary performance with Arg providing extra substrate for NO production and antioxidant vitamins providing protection against oxidative stress and increasing NO bioavailability. Subsequently, we hypothesize that Arg and VE may have complementary effects in reducing the incidence of ascites with improvement in antioxidant status and protecting the endothelium from oxidative stress damage. Therefore, a major objective of this study was to determine effects of VE and Arg on activities of antioxidant enzymes in plasma and liver tissue obtained from broilers with cold induced PHS. The second objectives of these experiments were to study the effects of Arg and VE in blood and growth parameters and mortality associated with PHS and to investigate if these effects were enhanced by the combination of VE and Arg. Also As antioxidant enzyme activity may be important in combating oxidative stress in blood and liver of broilers with PHS (Iqbal *et al*., 2001), the activities of antioxidant enzymes were measured in plasma (GSH-Px, SOD and TAS) and whole liver homogenate (GSH-Px, SOD).

RV/TV ratio, ascites mortality, hematology and growth performance: As show in Table 1, Arg birds d lower RV/TV ratio and mortality. Arg supplementation causes the pulmonary arterial relaxation which reflect the improved NO synthesis because of higher substrate availability (Taylor *et al*., 1992; Wideman *et al*., 1995, 1996; Lorenzoni and Ruiz-Feria, 2006). Furthermore, it has been reported that additional availability of Arg may support the gigher NO production and consequenly higher vasodilatory capacity. Moreover lower blood Hct was observed for Arg birds. Higher blood hematocrit is associated with sustained hypoxia (Yersin *et al*., 1992) and correlated with ascites susceptibility (Wideman *et al*., 1998). Decreased blood Hct by Arg supplementation results in lower blood viscosity and reduced pulmonary vascular resistance afterwards and as a result reduced Pulmonary Arterial Pressure (PAP). Since, increases in PAP represent a key step in the pathogenesis of ascites, improvements in pulmonary vasorelaxation and reducing in blood viscosity (via reducing pulmonary vascular resistance) should help reduce the mortality caused by ascites (Wideman *et al*., 1995), subsequently induced improving growth performance. Moreover, Wideman *et al*. (1995) reported that birds fed diets supplemented with 1.5% L-Arg HCl had significantly lower right: total ventricle weight ratios than control birds, presumably reflecting a reduction in pulmonary arterial pressure in Arg-supplemented birds. Later, it was reported that supplemental dietary Arg permitted broilers to exhibit flow-dependent pulmonary vasodilatation when a pulmonary artery snare was tightened to force the entire cardiac output through one lung. The reduced pulmonary vascular resistance allowed Arg-supplemented broilers to maintain a low pulmonary arterial pressure at high pulmonary blood flow rates (Wideman *et al*., 1996) which resemblesthe inherent capability of jungle fowl that are known to be highly resistant to PHS (Wideman *et al*., 1998; Kochera-Kirby *et al*., 1999). The reducing in PAP, pulmonary vascular resistance and cardiac output cause decrease hypertrophy and subsequently reducing the RV/TV. The L-Arg-NO mechanism also facilitates vasorelaxation of pulmonary artery rings isolated from domestic fowl (Martinez- Lemus *et al*., 1999).

The efficacy of supplemental Arg for reducing the incidence of PHS in field trials has been highly variable and dose response influences of Arg on PHS have not

Table 1: RV/TV and Ascites mortality in control and other groups

Treatments	Determination time	RV/TV index (%)	Ascites mortality (%)
Control	Day 42	0.32±0.010^{a}	38±4^{a}
HVE		0.30±0.010^{a}	28±2^{a}
Harg		0.26±0.015^{b}	22±1^{b}
HVE-HArg		0.29±0.010^{ab}	26±4^{ab}

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

been demonstrated (Wideman *et al*., 1995). The reasons for this variability are not known but it is possible that under some circumstances supplemental dietary Arg is catabolized by kidney arginase instead of providing additional substrate for NO synthesis especially when increased kidney arginase activity is induced by diets high in Lys or CP (O'Dell and Savage, 1966; Stutz *et al*., 1972). Arginase converts Arg into ornithine and urea. It has been reported that between 40-60% of urea excreted by birds is from Arg metabolism and as birds cannot synthesize ornithine, almost all of the plasma ornithine in the birds is also derived from Arg metabolism (Nesheim, 1968; Austic and Nesheim, 1970; Stutz *et al*., 1972; Chu and Nesheim, 1979). Urea production increases with high levels of Arg and an excess of urea reportedly inhibited the action of nitric oxide synthase in a macrophage cell line (Prabhakar *et al*., 1997).

Antioxidants enzymes activities, TAS and MDA in plasma: Data from antioxidants enzymes activities, TAS and MDA in plasma is show in Table 2. There is no significant difference in these parameters at 21 in plasma. But at day 42 the GPX activity in HArg and HArg-HVE groups was greater than other groups, contemporary HArg and HArg-HVE groups had lower TAS and lower MDA value than the control and HVE. There are no significant differences in SOD activation among groups. Endothelial cells synthesize nitric oxide from L arginine in a reaction catalyzed by the enzyme Nitric Oxide Synthase (NOS). During NO production by NOS, produce amount of superoxide and hydrogen peroxide. So, the antioxidant system should neutralize theses free radicals hence, the lower TAS in HArg and HArg-HVE groups can be justified this lowering. Beside, Arg induce increase GPX activity and amplification of antioxidant system. So, its result reducing in lipid and protein peroxidation and reducing MDA.

Antioxidants enzymes activities and MDA in liver: Contents of Table 3 showing that there are no significant differences in MDA value, GPX and SOD activities in liver among treatment groups at both periods 21 and 42. It seeming that hematological and plasma antioxidant actives changes in response to systemic hypoxemia and oxidative stress may occur more rapidly than liver tissue. The lack of consistency in the efficacy of supplemental Arg on reducing the incidence of PHS may be explained by the multiple independent components that affect PHS susceptibility in addition to the maintenance of an inappropriately elevated vascular tone. These factors include allow anatomical pulmonary vascular capacity, inadequate surface area available for gas exchange and a cardiac output that steadily increases in support of metabolic requirements (Wideman and Kirby, 1995a, b). Additionally, kidney arginase activity may differ among strains of birds or among individual birds within a population (Nesheim, 1968; Chu and Nesheim, 1979). If so, birds with high kidney arginase activity (high rate of Arg degradation) may benefit from supplemental Arg whereas birds with a low kidney arginase activity may not respond to increased levels of Arg. The production of NO is modulated and affected by a wide variety of factors, making it difficult to achieve the predictable results solely by increasing substrate availability *in vivo*.

Theses results supported hypothesis that supplemental Arg improve problems associated with pulmonary hypertension by improve NO synthesis and improving in pulmonary arterial relaxation (Taylor *et al*., 1992; Wideman *et al*., 1995, 1996).

Table 2: MDA, TAS and antioxidant enzymes activities in plasma in control and other groups

Treatments	Determination time	MDA (nmol mL^{-1})	GPX (U mg^{-1} protein)	SOD (U mg^{-1} protein)	TAS (mmol L^{-1})
Control	Day 21	2.50±0.33	39.32±2.80	1371±265	0.71±0.10
HVE		3.60±0.43	40.77±3.50	1800±144	0.74±0.12
Harg		3.10±0.16	43.05±1.50	1527±192	0.97±0.05
HVE-HArg		3.60±0.39	42.86±3.60	1309±334	0.83±0.01
Control	Day 42	6.27±0.43^{a}	30.72±0.82^{b}	913±112	1.36±0.11^{a}
HVE		6.77±1.09^{a}	33.65±2.10^{b}	906±64	1.30±0.23^{ab}
Harg		2.27±0.24^{b}	43.67±1.10^{a}	882±107	0.94±0.20^{b}
HVE-HArg		3.12±0.43^{b}	42.35±2.50^{a}	1061±200	1.01±0.09^{b}

Table 3: MDA and antioxidant enzymes activities in liver tissue in control and experimental groups

Treatments	Determination time	MDA (nmol mL^{-1})	GPX (U mg^{-1} protein)	SOD (U mg^{-1} protein)
Control	Day 21	1.32±0.23	0.25±0.01	8.60±0.48
HVE		1.97±0.27	0.24±0.02	7.55±0.48
Harg		1.45±0.09	0.25±0.01	7.75±0.31
HVE-HArg		1.97±0.11	0.26±0.01	8.25±0.74
Control	Day 42	2.60±0.25	0.25±0.02	8.25±0.75
HVE		1.95±0.39	0.29±0.02	9.45±0.29
Harg		2.10±0.34	0.29±0.01	9.10±0.29
HVE-HArg		2.42±0.21	0.30±0.02	9.07±0.85

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

Table 4: Statistical analysis of RBC, Hgb and Hct in control and other groups

Treatments	Determination time	RBC (10^6 day L^{-1})	Hgb (g dayL^{-1})	Hct (%)
Control	Day 21	2.42±0.16	8.57±0.49	34.27±2.04
HVE		2.39±0.17	8.17±0.64	33.55±1.83
Harg		2.06±0.12	8.12±0.14	32.46±0.94
HVE-HArg		2.28±0.18	7.35±0.66	29.55±0.29
Control	Day 42	2.80±0.17^{b}	11.20±0.35^{a}	39.30±2.27^{ab}
HVE		3.29±0.23^{a}	12.15±0.85^{a}	45.62±3.57^{a}
Harg		2.43±0.11^{b}	8.52±0.14^{b}	33.67±0.65^{b}
HVE-HArg		2.40±0.15^{b}	8.27±0.52^{b}	33.52±1.08^{b}

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

On the other hand we found that VE supplementation did not improve or augment the effects of Arg on hematological, antioxidant enzymes activities, growth performance and problems associated with ascites. It appears that VE supplementation at the levels used in this experiment negated the ability of Arg to improve pulmonary hypertension under cold stress. It was anticipated that vitamin E might improve vasodilation by protecting the endothelium from free radical damage and a healthier endothelium might have more functional cells with higher constitutive levels of NOS which could contribute to a higher NO production. We further anticipated that vitamin E might improve vasodilation by protecting NO from direct free radical attack, increasing its life span and avoiding the formation of peroxynitrite (Beckman and Koppenol, 1996). The levels of MDA in blood were measured as an indicator of lipid peroxidation and oxidative exposure to low temperature. Levels of MDA did differ among treatments at day 42 (Table 4). However, there was a tendency for the birds in the control and HVE groups to have higher MDA values than birds in the other groups ($p = 0.05$). It has been documented that VE may become prooxidative agent under some circumstances. For instance, vitamin E can react with organic peroxides interrupting the chain reaction of lipid peroxidation. This reaction involves the formation of tocopheroxyl radicals which are regenerated by means of hydrogen donors. If this reduction is incomplete, the tocopheroxyl radicals can initiate oxidative processes (Mukay, 1993; Schneider, 2005).

These results agree with recent epidemiological studies in humans in which the use of VE in patients to prevent cardiovascular diseases has had null or even negative effects in high-risk patients (Kris-Etherton *et al*., 2004). There is evidence of chemical differences between the isomers of VE that could be important to consider for future research. Although, γ-tocopherol is considered a less potent chain-breaking antioxidant compared with α- tocopherol, its molecular arrangement appears to make it better to trap lipophilic electrophiles such as reactive nitrogen oxide species (Kamal-Eldin and Appelqvist, 1996). This feature may be important to rapidly decrease molecules like peroxynitrite, a potent oxidant believed to cause direct tissue injury (Beckman and Koppenol, 1996). Furthermore, γ-tocopherol has antiinflammatory properties and a less hydrophobic condition than α-tocopherol (Jiang *et al*., 2000, 2001).

CONCLUSION

In the study, Arg supplementations improved performance and healthy parameters, probably by improving pulmonary hypertension and pulmonary vascular resistance and by increasing NO levels due to the extra substrate availability for NOS production or by reducing in blood viscosity. Also Arg can reduce PAP and induce decreases output cardiac and hypertrophy ant so reduce RV/TV. Beside, Arg can increase the GPX activities and reduce MDA. Subsequently, Arg supplementation can improver mortality and growth performance in broiler associated with cold stress.

Supplementation of VE alone or in combination with Arg did not improve pulmonary hypertension problems after an acute cold challenge. Suggesting that VE at the levels used in these experiments may have detrimental effects on cardiopulmonary system.

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