Increasing susceptibility of broiler chickens to ascites syndrome has coincided with a continuing genetic and nutritional improvement in their feed efficiency and growth rate. Imbalance between oxygen supply and the oxygen required to sustain rapid growth rates and high food efficiencies is believed to be the primary cause of ascites. This imbalance is caused by exogenous and/or endogenous factors.

Selection in fast growth broilers, have also resulted in the development of birds with changed anatomical, physiological, metabolic and hematological parameters including thyroid hormone activity, partial pressure of O₂ and carbon dioxide in venous blood, cardiac β-adrenergic receptors and hematocrit levels that coincide with susceptibility to ascites in broiler chickens. Observations to date have indicated that the structural or endocrine changes are often linked with ascites susceptibility and may be influenced during the early stages of development, embryogenesis and incubation. In this review, particular attention is paid to the interactions between endogenous and exogenous factors as predisposing factors for development of ascites syndrome. Additionally, preventive management procedures like different lighting schedules, feed restriction protocols and supplementation of feed with antioxidants which reduce the incidence of ascites in broiler chickens are highlighted.

Key words: ascites, broilers, endogenous, exogenous, hypothyroidism

Introduction

Ascites is an important metabolic disorder in broiler industry. It is a major noninfectious cause of death among broilers and can account for over 25% of broiler losses (Maxwell and Robertson, 1997; Balog, 2003). Ascites is the result of malfunctioning heart and circulatory system and is caused by an imbalance between oxygen supply and the oxygen required to sustain rapid growth rates and high food efficiencies in broiler chickens (Decuypere et al., 2000). The genetic background involved in this syndrome has been linked to altered metabolic needs of rapidly growing broilers because lines selected for very high growth capacity and low feed conversion are more sensitive to ascites than slower-growing broilers (Chimene et al., 1995; Decuypere et al., 2000; Balog, 2003; De Smit et al., 2005; Hassanzadeh et al., 2010). Ascites is a multifactorial problem mediated by environmental, nutritional and genetic factors (Decuypere et al., 2000; Balog, 2003; Julian, 2005). Ascites syndrome is no longer restricted to birds that are kept at high altitudes, as was initially reported by Cueva et al. (1974). However, it is now found in flocks that are kept at sea level as well (Decuypere et al., 2000; Hassanzadeh et al., 1997a, b, 2000, 2001, 2012; Balog, 2003; Scheele et al., 2005; Al-Mesri and Hassanzadeh, 2010).

Modern strains of broilers are able to achieve market weight in 60% less time than broilers of 40 years ago (Baghbanzadeh and Decuypere, 2008). While, both heart and lung percentages of broiler chickens are tended to be significantly lower in the modern strain at most of the slaughter ages (Havenstein et al., 2003). Havenstein et al. (1994, 2003) investigated on the relative contributions of genetics and nutrition to changes carcass characteristics of broiler chickens. Authors reported that modern diet (e.g. pelleted and crumbles instead of mash) reduced lungs size as a percentage of body weight in comparison to the diet that was used 40 years ago. Again, their data suggest that both heart and lung size as a percentage of live body weight have been decreased with genetic selection for increased growth rate. This, along with the rapid increase in body fat at the later ages on the modern diet may be contributing factors to the increased incidence of late mortality e.g. ascites, in the modern strain (Havenstein et al., 2003). They concluded...
that relative size of the heart and lungs may be contributing factors to the ability of modern broilers to service the respiratory demands of their bodies. Nevertheless, the pulmonary and cardiac capacity of modern broilers is very similar to the old broiler strains which force their cardio-pulmonary system to work very close to its physiological limit (Lorenzoni et al., 2006). The lung capacity does not always meet the oxygen demands necessary for rapid growth. This results in impaired ability to regulate the energy balance under extreme conditions such as low ambient temperature or high altitude (Luger et al., 2003; Hassanzadeh et al., 2003, 2004, 2005a, b, 2008; Ozkan et al., 2010). If the lung of the chicken grows less rapidly than the rest of the body, hypoxia and ascites could result (Hassanzadeh et al., 2005b). Decuyper et al. (2000, 2005) suggested that at low altitude, ascites is not only caused by an increased oxygen requirement in fast growth rate chickens, but also by an impaired oxygen supply to sustain growth rate.

Changes in hematocrit values, partial pressure of oxygen (pO₂) and carbon dioxide (pCO₂) and endocrine function such as thyroid hormone activity are important regulatory mechanisms of the metabolic rate during the embryonic development and growing period (Buyse et al., 1996; Buys et al., 1998; Decuyper et al., 2000, 2005; Luger et al., 2001, 2003; Teshfam et al., 2005; Arab et al., 2006; Hassanzadeh et al., 2002, 2004, 2005a, b, 2008, 2010, 2012; Bahadoran et al., 2010). The role of thyroid hormone in the regulation of metabolism could become more apparent in different genotypes of chickens (Scheele et al., 1992; Scheele, 1996; Decuyper et al., 2000; Luger et al., 2001; Malan et al., 2003; Hassanzadeh et al., 2004, 2010) and under environmental conditions such as at low ambient temperature (Scheele et al., 1992; Hassanzadeh et al., 2003, 2005a, 2010), different lighting programs (Buyse et al., 1994; Buys et al., 1998; Hassanzadeh et al., 2000, 2003, 2005a, 2012) and even at high altitudes (Hassanzadeh et al., 2002, 2003, 2004, 2005a, b, 2008).

It is obvious that genetic selection for a line of broilers that is resistant to ascites would be a permanent solution to the problem of ascites. For a short-term solution, there is no single treatment or preventive system for control of the ascites. The condition is often related to fast growth rate and metabolic activity of birds, and management techniques to reduce early growth rate offer the best short-term preventative scenario (Buyse et al., 1996; Buys et al., 1998; Balog, 2003; Hassanzadeh et al., 2000, 2003, 2005a, 2012).

In this review, particular attention is paid to the endogenous and environmental factors that interact with other predisposing factors for development of the ascites. Furthermore, some of the preventive management procedures that reduce the incidence of ascites in broiler chickens such as different lighting schedules, feed restriction and antioxidant supplementation are also reviewed.

Factors Involvement for Development of Ascites

a. Interaction between endogenous and exogenous factors

The effect of high altitude, either natural or simulated, is a decrease in the partial pressure of the oxygen. When birds are exposed to low atmospheric oxygen levels, pulmonary blood vessels constrict, restrict blood-flow to the lung and increase the work-load on the right ventricle (Julian, 1993). This immediate increase in pulmonary arterial pressure can, over time, cause right ventricular hypertrophy and eventually result in ascites syndrome (Wideman et al., 1998). Additionally, hypoxemia also leads to increase blood viscosity and results in an increase in resistance to blood flow through the pulmonary blood vessels (Julian, 1993; Maxwell and Robertson, 1997).

Powell (2000) suggested that the increased growth rate in modern fast-growing broiler chickens at sea level requires a higher metabolic rate therefore, an increase in cardiac output causes pulmonary hypertension and ascites. According to experimental results, hypercapnia (high CO₂ in the blood) plays important role in activating of vasoconstriction and high blood pressure in broiler chickens susceptible to ascites at sea level (Buys et al., 1999; Decuyper et al., 2000; Scheele et al., 2003, 2005, Hassanzadeh et al., 2010, Al-Mesri and Hassanzadeh, 2010). In addition to cardiac insufficiency, the occurrence of ascites in chickens at sea level can also be caused by malfunctions of the cardiopulmonary system which cause disturbances in the tension of blood gases (Fig. 1).

Although ascites is conventionally associated with the rapid growth rate of broilers, high metabolic rates and high oxygen requirements (Hassanzadeh, 1997; Hassanzadeh et al., 1997a, b, Julian, 1993, 2005; Balog, 2003), recent data indicated that ascites is not always caused by an increased oxygen requirement of fast growth rate per se at low altitude, but by an impaired oxygen supply to sustain the fast growth rate (Decuyper et al., 2000, 2005; Scheele et al., 2005; Malan et al., 2003; Hassanzadeh et al., 2010). Decuyper et al. (2000) mentioned that thyroid function is an important regulatory mechanism of the metabolic rate, it is plausible that early selection for feed conversion (FCR) could have resulted in functional of hypothyroidism, as ascites sensitive birds are believed to be limited in their thyroxin (T₄) production (Scheele et al., 1992; Scheele, 1996). Insufficient thyroid hormone activity to regulate metabolism, related to genetic background, will therefore become especially apparent at low ambient temperature (Scheele et al., 1992). Recent results (Buys et al., 1999; Decuyper et al., 2000; Scheele et al., 1992, 2005; Luger et al., 2001; Hassanzadeh et al., 2010) confirmed those of the Scheele et al. (1992) and indicated that hypothyroidism, observed in lines combining a favourable FCR and fast growth, plays an important part in the reduction of oxygen consumption that lead to anoxia, heart failure and ascites.

Birds that were selected for combination of fast growth and high feed efficiency had a low O₂ and a high CO₂ tensions in venous blood at a low ambient temperature compared with the slower-growing birds (Scheele et al., 2005; Decuyper et al., 2005; Hassanzadeh et al., 2010; Al-Mesri and Hassanzadeh, 2010). Authors argued that a thyroid hormone insufficiency was one of the causal factors
of increased feed efficiency and was responsible for impaired O₂ supply when trying to sustain a fast growth rate. This may develop a mismatch between the delivery of O₂ and O₂ demands, resulting in anoxia, hypoxemia and hypoxia (Decuypere et al., 2000). It will occur in particular under circumstances where the O₂ requirements of birds are increased such as in a cold environment, and may lead to heart failure and ascites (Decuypere et al., 2000; Malan et al., 2003; Bahadoran and Hassanzadeh, 2010; Hassanzadeh et al., 2004, 2008, 2010, 2012).

The relationship between activity of thyroid hormone and anatomical parameters was discussed in the study of Malan et al. (2003). They demonstrated that a deficiency in thyroid hormone could impair lung development and may increase the risk of ascites in chickens with rapid growth. This was confirmed by the recent reports of Hassanzadeh et al. (2004, 2005b, 2008) who documented a direct correlation between the heart and lung development of post-hatch broiler chickens with the embryonic thyroid hormone activity. These data showed that post-hatched chickens had lower

**Fig. 1. Flow chart of the development of ascites syndrome in broiler chickens.**
heart and lungs weights in relation to their body size when plasma thyroid hormone levels were observed lower during the embryonic development. Consequently, such chickens with lower lung capacity had a higher susceptibility to ascites when exposed to low temperatures and chronic hypoxia at high altitude (Hassanzadeh et al., 2008).

To serve as a predictor of broilers susceptibility to ascites, a potential indicator should differ significantly between ascites-susceptible versus ascites-resistant individuals when they are reared together under standard conditions at an early age. Hematocrit was found to be one of the most reliable indicators for detection of ascites (Maxwell et al., 1992; Shlosberg et al., 1996; Wideman et al., 1998). Broilers developing ascites were characterized by lower heart rate as well (Olkowski et al., 2005; Wideman et al., 1998) and higher right ventricle weight to total ventricle weight ratio (Wideman and Kirby, 1995, Druyan et al., 2007). However, in all these studies, differences were found only between broilers that had already developed some degree of ascites when compared to their healthy counterparts. Thus, these differences were most probably secondary to the manifestations of ascites (Druyan et al., 2009), hence, can serve as diagnostic tools but not for early prediction. Such an indicator is essential for indirect phenotypic selection against ascites susceptibility, therefore, all birds should be healthy. Reeves et al. (1991) noticed that ascites-susceptible chickens suffer from relative hypoventilation at an early age, which may cause an elevated partial pressure of CO2 in blood. Later studies by Scheele (1996), Buys et al. (1999), Olkowski et al. (1999), Scheele et al. (2003, 2005), Hassanzadeh et al. (2010), Al-Mesri and Hassanzadeh, (2010) and Van As. et al. (2010) confirmed these findings. They reported that the high partial pressure of CO2 in venous blood of 2-week-old broiler chickens could be as a potential indicator for ascites susceptibility at later age. However, the results of Druyan et al. (2009) suggested that high heart rate, measured by oximeter at 1 wk of age, may serve as an additional or alternative criterion for selection against ascites susceptibility.

Anatomy and physiology of the avian respiratory and cardiovascular systems are important in the susceptibility to pulmonary hypertension syndrome and ascites (Julian, 1989; Decuypere et al., 2000; Hassanzadeh et al., 2005b, 2008). Decuypere et al. (2000) indicated that alteration in the proportional growth as a result of indirect selection for greater muscularity may have had the effect of producing birds with a relatively small respiratory and cardiovascular system. Authors argued that if development of the ascites syndrome is a consequence of structural or morphological/histological alterations, this must result in observable physiological changes. Modern broiler chickens have thicker respiratory exchange area in broilers and therefore a higher susceptibility to pulmonary hypertension and ascites (Hassanzadeh et al., 2005b, 2008).

Recently, we observed a correlation between insufficiencies of the cardiopulmonary system with a rise in pCO2 and a decline in pO2 in the venous blood of birds and the development of ascites syndrome (Al-Mesri and Hassanzadeh, 2010). Indeed, a large fall was observed in the volume of the thoracic cavities, both before and after removal of the heart and lung tissues, in the fast-growing compared with those of the slow-growing broiler chickens. Such phenomenon was accompanied by a significant increase of the pCO2 and a decrease of the pO2 tensions in the venous blood of rapid-growing compared with those of the slow-growing broiler chickens.

b. Mediators contributed to pulmonary arterial hypertension

Anything that increases the pulmonary vascular resistance and/or increases cardiac output can initiate or accelerate the pathophysiological progression leading to pulmonary arterial hypertension and ascites in broiler chickens (Julian, 1993, 2005; Baghbanzadeh and Decuypere, 2008; Wideman et al., 2013). Mediators, like catecholamines (Lefkowitz et al., 1984; Hassanzadeh et al., 1997a, 2001, 2002), endothelin-1 (Villamor et al., 2002; Odom et al., 2004), methylglyoxal (Khajali and Fahimi, 2010; Khajali and Wideman, 2011) and serotonin (Chapman and Wideman, 2002; Kluss et al., 2012) play a major role in accelerating of pulmonary hypertension that may lead to ascites in broiler chickens (Hassanzadeh et al., 1997a, b; Wideman et al., 2013).

Catecholamines such as epinephrine and norepinephrine regulate physiological events by binding to α- and β-adrenergic receptors on the surface of target cells. Nor-
epinephrine released by sympathetic nerve terminals and epinephrine released into the circulation by the adrenal glands in response to stress, may contribute to the increase in pulmonary vascular resistance during exposure to hypoxia and cool temperatures (Lin and Sturkie, 1968; Wideman, 1999). Lefkowitz et al. (1984) and Cohen et al. (1984) reported that the constriction of smooth muscles of the vascular system was a consequence of an adrenergic stimulation due to catecholamine hormones. So, intravenously administered epinephrine elicits immediate pulmonary vasoconstriction accompanied by pulmonary hypertension (Wideman, 1999; Villamor et al., 2002; Lorenzoni and Ruiz-Feria, 2006; Ruiz-Feria, 2009; Bautista-Ortega and Ruiz-Feria, 2010).

β-adrenergic receptors are found on the various organs such as heart tissue, the blood vessels, smooth and skeletal muscles, however cardiac muscle contains predominantly β1 subtypes of the adrenergic receptor. In cardiac tissue epinephrine and norepinephrine appear to be about equally potent to regulate physiological events by binding to β-receptors (Stiles et al., 1984). Investigations in human and other mammals have confirmed that β-adrenergic receptors play an important role in regulation of cardiovascular performance like tachycardia. These receptors are dynamically regulated by a wide variety of pathological and physiological conditions. The physiological levels of thyroid hormone have been suggested to be an important modulator of the normal maturation of the β-adrenergic system in developing rat ventricular myocardium (Novotny et al., 1999). In mammals, hypoxia regulates the density of β-adrenergic receptors. In broiler chickens, Hassanzadeh et al. (1997a) found that the density of β-adrenergic receptors was higher in cardiac cells of ascites-sensitive birds compared to those of the chickens that were ascites-resistant. Moreover, the capacity of β-adrenoreceptors was lowered in the cardiac cells of birds with right ventricular hypertrophy and heart failure compared with those of healthy birds (Hassanzadeh et al., 2001). In the other investigation, the density of myocardial β-adrenergic receptors in non-hypertrophied ventricles of high-altitude hypoxic birds was significantly reduced compared with the low-altitude normoxic birds (Hassanzadeh et al., 2002).

Hypoxia is a potent stimulus to release of catecholamine from sympathetic system and the adrenal medulla into circulation (Cheung, 1989). According to previous works in mammals, increasing activity of the sympathetic nervous system in compensation for the reduced cardiac output seems to be a mechanism of the organism to help the failing heart (Velarde et al., 1996; Browne et al., 1997). Such phenomenon could be characterization of β-adrenergic receptor in heart failure and even in high-altitude hypoxic birds (Hassanzadeh et al., 2001, 2002). It seems the downregulation of β-receptors in these birds could be due to chronically increased receptor occupancy as a result of elevated concentration of circulating and neural released catecholamines as has already been reported in mammals (Voelkel et al., 1981; Mader et al., 1991). Hassanzadeh et al. (2002) reported that chick embryos incubated at high CO2 levels downregulated the expression of myocardial β-adrenergic receptors. Authors argued that the decrease in receptor density reflects a regulatory phenomenon and is not due merely to right ventricular hypertrophy (Mardon et al., 1998). These data point to the involvement of catecholamines together with β-adrenergic receptors in the syndrome of pulmonary hypertension in broiler chickens. In particular, the observation of a differential β-adrenergic receptor density in the cardiac muscle of ascites-sensitive birds may be linked with the slight degree of hypothyroidism that is found in these broilers. Also it points to a possible cardiogenic origin for the susceptibility to ascites on a genetic basis that was already discussed in previous section of this review.

Intimately endothelin-1 is involved in the pathogenesis of pulmonary arterial hypertension in mammals and broiler chickens (Wideman et al., 2013). In broilers, endothelin-1 elicits dose-dependent constriction of pulmonary arteries that can be modulated by nitric oxide (Martinez-Lemus et al., 2003; Villamor et al., 2002; Odom et al., 2004). Repeated intravenous injections of endothelin-1 triggered pulmonary arterial hypertension in broiler chickens (Zhou et al., 2008). Gomez et al. (2007) reported that the lungs of broilers with pulmonary hypertension expressed higher levels of endothelin-1 mRNA compared with lungs from the nonhypertensive broilers. In addition, cardiac expression of endothelin-1 mRNA was higher in the right but not in left ventricle of broilers compared with those of egg-laying chickens, and broilers had higher serum levels of endothelin-1 than those of layers (Hassanpour et al., 2010). Cumulatively these observations reflect a consistent association between excessive endothelin-1 production and pulmonary hypertension (Wideman et al., 2013).

Methylglyoxal is formed from carbohydrates, fatty acids and proteins. It has been shown to damage the vascular endothelium and implicated in vascular remodeling and systemic arterial vasoconstriction and hypertension in mammals. Recently, reports in broiler chickens indicate that intravenous and intramuscular injections of Methylglyoxal both rapidly elicited pulmonary vasoconstriction and pulmonary hypertension (Khajali and Wideman, 2011), which potentially may reveal a mechanistic link between full-feeding, oxidative stress, endothelial damage, and the ascites syndrome (Khajali and Fahimi, 2010).

Serotonin is an extremely potent pulmonary vasoconstrictor that triggers pulmonary hypertension by activating receptors expressed on pulmonary artery smooth muscle cells (Chapman and Wideman, 2002, Kluess et al., 2012). In broiler chickens, plasma serotonin levels normally are quite low, however serotonin can be released from activated thrombocytes and from pulmonary neuroendocrine cells or serotonergic nerves (Chapman et al., 2008). Kluess et al. (2012) pointed that broilers fed diets supplemented with high levels of tryptophan, an essential amino acid and precursor of serotonin, developed higher pulmonary arterial pressures than those of broilers fed diets containing adequate levels of tryptophan. Chapman and Wideman, (2002) mentioned that
serotonin clearly plays a key role in increasing the basal tone (partial state of contracture) of the pulmonary resistance vessels, and potentially can act as a dominant pulmonary vasoconstrictor in broiler chickens. It appears that susceptibility to pulmonary tension in broilers may involve in excessive serotonin biosynthesis, inhibited uptake or enhanced release of serotonin by thrombocytes, enhanced receptor-mediated vasoconstrictive responsiveness to serotonin, or altered internalization of serotonin by a specific transporter associated with vascular remodeling (Wideman and Hamal, 2011).

c. Hatching parameters and ascites susceptibility

Until some years ago, a large part of commercial incubations was performed using the multistage system. Today, the incubation of broiler eggs has become a highly controlled process, during which the temperature, humidity, O₂, and CO₂ concentrations create the optimal environment for embryonic development. The composition of the ambient gaseous environment in the incubator plays an important role during embryonic development, and by manipulating the incubation conditions, the developmental trajectories of the chick embryo have been influenced (Dewil et al., 1996; Hassanzadeh et al., 2002, 2004, 2005b, 2008; De Smit et al., 2005, 2006). The peak mortality due to asces occurs at the end of the growth period, but it is now thought that the etiology of this disease might be existed during embryonic development (Coleman and Coleman, 1991). As the chick embryo consumes 60% more O₂ between the start of pulmonary breathing and hatching compared to earlier stages, it is possible that a shortage of O₂ occurs during this stage. Decuyper et al. (2000) reported that a high CO₂ concentration in the air chamber is a trigger for hatching. The findings of Chineme et al. (1995) indicated that the length and/or severity of prenatal hypoxia may influence postnatal characteristics that were related to asces. Further studies (Buys et al., 1998; Hassanzadeh et al., 2002; De Smit et al., 2006) showed that increased CO₂ concentrations in the incubator by non-ventilation during the first 10 d or the last 7 d of incubation increased the performance of the chick embryo and even had beneficial effects on post-hatch growth and asces susceptibility. In these studies, it was shown that eggs incubated in an environment with a high concentration of CO₂ during the last week (Buys et al., 1998; Hassanzadeh et al., 2002) and/or the first 10 d of their incubations (De Smit et al., 2006) were hatched earlier than in an environment with normal CO₂ levels. Furthermore, embryos that were incubated at high altitude, 2000 m above sea level, for first 10 d of incubation had an earlier hatching time than those incubated at low altitude (Bahadoran et al., 2010). In this study, those chickens which hatched earlier showed a lower incidence of ascites during the growing period, because high concentrations of CO₂ in the incubation environment might actually decrease the length of time and the embryo experiences hypoxia (Buys et al., 1998; Hassanzadeh et al., 2002, 2004; De Smit et al., 2006).

Several explanations have been published for the events. The severity of embryonic hypoxia may be related to the porosity and structure of the egg shell and hence, to the partial pressures of O₂ and CO₂ in the egg and air chamber, especially during the last days of incubation (De Smit et al., 2006). Sadler et al. (1954) explained that the beneficial effects of CO₂ were the result of the reduction of pH of the albumen, which might have retarded the apparent breakdown of the chalaziferous membrane and the thick layer of albumen, leading to reduce the length of incubation. Alternatively, it could be related to the duration of the hypoxic period and, therefore, to the pipping and hatching time.

Blacker et al. (2004) provided evidence for the role of hypoxia during the embryonic development regarding the control of pulmonary surfactant. The authors suggested that the avian pulmonary surfactant system exhibits a high level of plasticity within the early stages of surfactant maturation. A possible explanation for these observations is an interaction between environmental and endogenous physiological factors, such as corticosterone and thyroid hormones, during the critical developmental period of chick embryos (Hassanzadeh et al., 2004, Blacker et al., 2004). These interactions could exploit developmental plasticity, which result in the physiological heterokairy of the surfactant system by altering both the rate and onset of production of surfactant lipids and the earlier commencement of air breathing. These observations indicate that hypoxic conditions occurring in the embryonic period can alter the developmental trajectories of some endogenous parameters in prenatal and postnatal chicks. The development of these important parameters is favorable to the formation of an increased gas exchange area and results in the lower susceptibility of birds to pulmonary hypertension.

Hypoxic conditions during embryonic development may initiate structural changes in cardiovascular and pulmonary systems, as was observed in our recent study of chickens at high altitude (Hassanzadeh et al., 2005b, 2008). In these studies, day-old high altitude-hatched chicks had a significantly higher proportion of their heart (1.23 ± 0.08 versus 0.87 ± 0.07) and lung weights (2.02 ± 0.07 versus 1.23 ± 0.13) to body weight compared to that of low altitude-hatched chicks, while no significant difference was found at the age of slaughter. This coincided with a reduced incidence of ascites and right ventricular hypertrophy in high altitude-hatched birds than those of low altitude-hatched birds (Hassanzadeh et al., 2004, 2008). Additionally, changes in anatomical parameters of high altitude chicks were also accompanied with increased levels of thyroid hormone and corticosterone of their embryos. These early stages of development may be decisive in determining the subsequent adaptive capacity to cope with unfavorable environmental conditions after hatching (Buys et al., 1999; Hassanzadeh et al., 2004, 2008).

In addition to the environmental incubation conditions e.g. high CO₂ levels or altitude, the genetic background of the chick also influences the embryonic developmental pathway. Chickens selected for various post-hatch characteristics also differ with regards to their embryonic developmental tra-
jectories (Decuypere et al., 2000). In comparative studies, broilers from lines that differed in their ascites susceptibility (Dewil et al., 1996; Buys et al., 1998; De Smit et al., 2005, 2006) showed also difference in their certain embryonic characteristics. Therefore, chicks of ascites-sensitive lines hatched later, had lower thyroid hormone concentrations, a lower pCO₂ and a higher pO₂ in the air cell during the late stages of embryonic development.

Management Techniques for Control of Ascites

a. Lighting

It is a common practice to raise broiler chickens in continuous or near continuous illumination and/or is recommended that a short dark period should be included to accustom birds to darkness and to minimize panic in case of power failure (Buys et al., 1996). There is interest in discontinuous lighting patterns such as an increasing photoperiod lighting system (Classen et al., 1991; Hassanzadeh et al., 2003) and/or intermittent lighting schedules (Buys et al., 1998; Hassanzadeh et al., 2000, 2005a) for broilers because these alternative lighting programs could improve broiler performance and reduce the occurrence of metabolic disorders like sudden death and ascites syndromes (Julian, 2005).

Buys et al. (1994) pointed out heat production and hence oxygen requirements per kilogram of metabolic body weight in chickens following a normal growth trajectory at about 2 to 3 weeks of age which is indicative of an amplified metabolic demand. This metabolic demand predisposes chickens to development of ascites. It was hypothesized that limiting the number of hours of light would slow the growth rate slightly, especially at the earlier age, and reduce activity that requires additional oxygen which may improve feed efficiency (Julian, 1993, 2005).

Imposing intermittent lighting schedules (1L: 3D) during the 24 h day (Buys et al., 1998) or during the 12 h of natural scotoperiod, from 8 pm to 8 am (Hassanzadeh et al., 2000) significantly reduced the incidence of right ventricular failure and ascites in broiler chickens. In both studies, the change of continuous lighting (CL) to intermittent lighting (IL) at an early age was followed by initial growth depression as described earlier (Buys et al., 1994, 1996). However, this depression was followed by a period of compensatory growth, so that the birds reared in IL were reached to the same final body weight by six weeks (Buys et al., 1998) and seven weeks of age (Hassanzadeh et al., 2000). The same results were found when the intermittent lighting schedule (1L:3D): repeated six times daily from three to 42 days, or an increasing photoperiod schedule: 4 to 14 days, 6L:18D; 15 to 21 days, 10L:14D; 22 to 28 days, 14L:10D; 29 to 35 days, 18L:6D; 36 to 42 days, 23L:1D, were used at high altitude to reduce the incidence of ascites in broiler chickens (Hassanzadeh et al., 2003, 2005a). The beneficial effect of intermittent lighting on the incidence of ascites was more pronounced when IL was only applied from day 3 to 14 and/or from day 10 to 21 of age (Hassanzadeh et al., 2005a). In these studies, the reduction of ascites mortality also was coincided with a temporary reduction in the growth rate at young ages, with a concomitant reduction of metabolic indicators.

Recently, Hassanzadeh et al. (2012) applied a comparative study on the beneficial effects of four different dark-light schedules on the incidence of ascites and metabolic parameters in fast growing broiler chickens. In this study the rate of ascites mortality was declined when the duration of daily dark period was increased. The reduction of ascites mortality was accompanied with declined pCO₂ tension in venous blood of dark-exposed chickens, strengthening the view that a lower metabolic rate and hence a decrease in oxygen requirement had a beneficial effect on the incidence of ascites (Classen et al., 1991; Buys et al., 1994; Buys et al., 1998; Hassanzadeh et al., 2000, 2003, 2005a, 2012).

b. Feed restriction

Early growth restriction is still an important management tool to reduce the incidence of ascites until an effective selection program is developed and implemented by poultry breeder companies in the future. Growth restriction can be achieved by changes in feeding regimes (Balog, 2003). The hypoxia related to a high metabolic rate can be partially prevented by limiting energy intake via feed restriction in broilers (Julian, 2005; Balog, 2003). Feed restriction reduces growth at a critical time in a broiler chick’s lifecycle when it is the most susceptible to metabolic disease due to its high oxygen demands (Balog et al., 2000; Ozkan et al., 2006, 2010). However, use of this management technique depends on body weight of chicken at certain slaughter ages, which is associated with compensatory weight gain, improvements in carcass yield, feed prices and the incidence of ascites (Balog, 2003). There have been conflicting results in catch-up growth and yield data, illustrating the severity and duration of the growth restriction (Julian, 2005). The distinct advantage of feed restriction is the decrease in mortality (Shlosberg et al., 1991; Balog, 2003). Feed restriction (by reducing ad libitum intake) reduced the incidence of ascites without compromising body weight (Albers et al., 1990; Balog, 2003). Skip-one or two-days a week feed restriction programs during the early growth period effectively reduced the number of chickens that developed ascites (Julian, 2005; Baghbanzadeh and Decuypere, 2008). Experimentally it has been shown that other factors like age at initiation of restriction, duration of restriction and its severity, determine to a large extent the ability of the chickens to manifest compensatory growth to their full potential (Julian, 2005). Feed restriction should not be started too late, for too long and not too severe a regimen should be used otherwise, catch-up growth is not manifested fully (Julian, 2005). Feed restriction can reduce the availability of nutrients and pigmentation precursors, which may have a direct effect on weight gain, muscle mass, and the profit cost relationship. These effects could be more pronounced if the restriction program was not performed correctly (Camacho-Fernandez et al., 2002).

c. Antioxidants

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). Antioxidants play a major role in protecting cells from the actions of ROS by reducing chemical radicals and disrupting the process of lipid peroxidation (Yu, 1994). The low levels of antioxidants in birds with pulmonary hypertension syndrome (PHS) could therefore lead to an inability to control lipid peroxidation (Bottje et al., 1995). The production of free radicals is enhanced by systemic hypoxia, inflammation and thyroid hormones (Bottje and Wideman, 1995) which cause tissue damage through the lipid peroxidation of cell membranes and consequently increased membrane permeability (Maxwell and Robertson, 1997).

L-Arginine is an essential amino acid in the chicken and it has been suggested that the dietary inclusion levels recommended for maximal growth rate do not correspond with those required for maximal nitric oxide (NO) production (Dietert and Austic, 1994). L-Arginine is the substrate of endothelial NO synthase (eNOS), an enzyme that synthesizes NO, a potent vasodilator (Dudzinski and Michel, 2007) and antimitogenic (Tan et al., 2005). Basal levels of endothelium-derived NO signal the pulmonary artery smooth muscle cells to relax, thus maintaining vascular tone (Govers and Rabelink, 2001). Supplementation of Arginine improves cardiovascular performance in birds exposed to cold environments (Ruiz-Feria, 2009) and has been reported to reduce PHS in broiler chickens (Widehan et al., 1995; Tan et al., 2005), although the results have not been consistent.

The superoxide anion causes a loss of NO bioavailability by shortening its half-life and thereby reducing potential for endothelial vasodilation (Lopez-Lopez et al., 2001). Furthermore, the reaction of the superoxide anion with NO leads to the production of peroxynitrite, a potent oxidant agent that is responsible for direct tissue damage by oxidation, peroxidation and nitrination of lipids, proteins, and DNA (Bottje and Wideman, 1995; Julian, 2005). Excessive production of free radicals could cause damage to the pulmonary vascular endothelium, destruction of the cells and therefore, a reduction in the amount of eNOS.

Vitamin E is known to be a powerful lipid-soluble antioxidant that scavenges lipid radicals. It has the ability to react with fatty acid peroxyl radicals, which are the primary products of lipid peroxidation and intercepts the chain reaction, preventing further free radical reactions. During the antioxidant reaction, tocopherol is converted into a stable free radical (Bottje and Wideman, 1995). Therefore, vitamin E could help reduce level of oxidative stress in lung blood vessels and reduce endothelial damage. It has been reported that birds that develop ascites have low levels of tocopherol in their lungs and livers, which provide evidence that a compromised antioxidant status is involved in the etiology of pulmonary hypertension syndrome (Enketchakul et al., 1993). Supplementation of tocopherol in the diet (Villarpitino et al., 2002) and tocopherol as an implant (Bottje et al., 1995) have been used to improve body antioxidant status and prevent ascites incidence in broiler chickens.

Vitamin C, as a water-soluble vitamin, can be synthesized at a sufficient rate to meet the needs under normal conditions of poultry. Dietary vitamin C has been reported to improve resistance to a variety of stressors, including environmental and pathological factors. Vitamin C is also an antioxidant that can react directly with aqueous free radicals, such as hydroxyl radicals, by donating one electron and so reducing their reactivity (Yu, 1994; Bottje and Wideman, 1995, Hassanzadeh et al., 1997b). Moreover, vitamin C has been shown to function in restoring the antioxidant capabilities of oxidized vitamin E or by converting the tocopherol radical back to its reduced state. This suggests that a major role of vitamin C is to recycle the vitamin E radical (Bottje and Wideman, 1995).

Supplemental Arginine improved the pulmonary vascular performance and pulmonary vasodilation of hypoxic broiler chickens and its effects were further improved by the addition of the antioxidant vitamins E and C (Ruiz-Feria, 2009). The authors are making reference to that Arginine and antioxidant vitamins may have played complementary or synergistic roles to increase NO bioavailability and reduce oxidative stress damage, thus improving cardiopulmonary performance.

Dried turmeric rhizome powder used as a spice, food preservative, and a coloring agent, is a rich source of beneficial phenolic compounds: the curcuminoids (Srinivasan, 1953). Three main curcuminoids, curcumin, demethoxy-curcumin and bisdemethoxycurcumin (Balasubramanyam et al., 2005) have been isolated from turmeric. They have strong antioxidant activity (Asai et al., 1999). Many studies have shown the capacity of curcumin, the most active component of turmeric, to prevent lipid peroxidation, a key process in the onset and progression of many diseases (Venkatesan, 1998). Moreover, antioxidant properties of turmeric include protection of haemoglobin from oxidation, inhibition of the generation of ROS, H₂O₂ and nitrite radicals by activated macrophages, the reduction of ROS production in vivo, inhibition of H₂O₂-induced damage in human keratinocytes and fibroblasts (Chattopadhyay et al. 2004). Recently, Daneshyar et al. (2012) reported inclusion of 5.0 g/kg Curcuma longa rhizome (turmeric rhizome) powder, as a strong antioxidant, reduced serum malondialdehyde content and the mortality rate of ascites in broiler chickens. They argued that turmeric rhizome was also sufficient to increase the blood O₂ saturations and bicarbonate concentration of T₃-supplemented chickens which could be related to the lowering of ascsites incidence.

It has been found that decreased deformity of the erythrocytes can increase incidence of ascites in broiler chickens (Mirsalimi et al., 1993). The deformity of erythrocytes is reduced by hypoxiaemia. Studies in human have demonstrated that erythrocyte deformity can be increased by dietary supplementation of omega-3 (n-3) fatty acids from fish oils (Berlin et al., 1992). Archer et al. (1989) found that supplementation with fish oil reduces blood viscosity and right ventricular hypertrophy in rats. However, other studies (Hulan et al., 1989) reported a reduction in growth rate of
birds fed red fish meal as a source of omega-3 fatty acids. This is important, because the incidence of ascites can be reduced by slowing the growth rate of broilers (Julian, 1993). The increased content of unsaturated fatty acids probably increases the fluidity of the erythrocyte membrane and alters membrane function to increase the deformability of the erythrocytes with a potential help to reduce the incidence of ascites (Walton et al., 1999; Baghbanzadeh and Decuypere, 2008). This could explain the reduction in whole blood viscosity under hypobaric conditions with feeding of flux oil. These factors together would decrease the resistance to blood flow and improve the movement of the erythrocytes through the capillaries, thus improving oxygen transport and decreasing ascites (Walton et al., 1999).

Conclusions

Different opinions exist in literature regarding the primary causal factors leading to pulmonary hypertension and ascites syndrome. In some cases decrease in activity of thyroid hormone, is associated with higher incidence of ascites. This may be linked with structural and functional changes in the anatomical and blood gas parameters, as well as correlated with responses to the selection of strains of broiler chickens for improved growth rates and feed efficiencies (Scheele et al., 1992; Buys et al., 1999; Decuypere et al., 2000; Hassanzadeh et al., 2004, 2008, 2010). In this manner, broilers that were selected for rapid growth and high feed efficiency develop a mismatch between the delivery of oxygen and oxygen demands resulting in anoxia, hypoxemia and hypoxia.

In some cases, causative factors for ascites susceptibility are exogenous factors. Indeed, it is well known that environmental factors, such as altitude, cold temperature, continuous lighting and nutritionally related factors (Julian, 2005; Balog, 2003; Hassanzadeh et al., 2000, 2002, 2003, 2008, 2010, 2012) promote occurrence of pulmonary hypertension and ascites syndrome. The underlying causative mechanism is associated with increase in metabolic rate. This elevated metabolic rate leads to higher oxygen needs and stimulated cardiac output. If higher oxygen demands cannot be met adequately by a higher rate of oxygen delivery, it results in anoxia, hypoxemia and finally hypoxia. In view of this positive causal relationship between metabolic rate and ascites incidence, all of the management techniques that act to reduce metabolic rate even temporarily such as mash feeding, temporary feed restriction at young age and discontinuous lighting or increasing of dark period will also alleviate the occurrence of this metabolic disorder. It is also obvious that contemporary broilers are very sensitive to deviations in environmental and nutritional conditions, as they have already been confronted with an imbalance in oxygen delivery and demand. It is clear that relationship between metabolic rate and the pulmonary hypertension syndrome is not simplistic, but it is really “case-dependent” and should be evaluated in such a manner.

Therefore, depending on nature of selection pressures or broiler line, certain environmental factors may be differentially important in reflecting endogenous versus exogenous interactions with regards to susceptibility to ascites. The structural or endocrine changes that are often linked with this susceptibility may be influenced in the early stages of development and even during embryogenesis (Dewil et al., 1996; Buys et al., 1998; Hassanzadeh et al., 2002, 2004, 2005a, b, 2008; De Smit et al., 2005, 2006). Therefore, it has been hypothesized that developmental changes that are induced by incubation conditions are another instance for interactions between endogen and environmental factors in ascites susceptibility, which further complicates the etiology of this important syndrome.

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