Endogenous and environmental factors interactions that contribute to the development of ascites in broiler chickens: a review

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Abstract
The increasing susceptibility of broiler chickens to ascites syndrome has coincided with a continuing genetic and nutritional improvement in their feed efficiency and growth rate. Ascites is multifactorial and interactions between environmental and endogenous factors play a particularly important role. The selection of fast growing broilers for high feed efficiency, which is characterized by hypothyroidism, can consequently lead to a mismatch between oxygen ($O_2$) delivery and $O_2$ demands. This results in anoxia, hypoxemia and hypoxia. Such selection processes have also resulted in the development of birds with changed anatomical, physiological, metabolic and hematological parameters, including thyroid hormone activity, partial pressure of $O_2$, 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 indicate that the structural or endocrine changes that are often linked with ascites susceptibility may be influenced during the early stages of development, embryogenesis and incubation. In this review, particular attention will be paid to the interactions between endogenous and exogenous factors as predisposing factors for the development of ascites syndrome. Additionally, preventive management procedures will be highlighted, such as different lighting schedules, feed restriction protocols and the supplementation of feed with antioxidants, which reduce the incidence of ascites by altering the metabolic load of broiler chickens amongst other factors.

Introduction
Since the early 1990s, ascites has been a major concern for the broiler industry worldwide. Mortality rates due to ascites may reach 25% in flocks that are reared under natural conditions (Balog, 2003; Julian, 2005), and these concerns are even more prevalent if chickens are reared with exposure to cold or at high altitude (Hassanzadeh et al., 2004, 2008; Bahadoran et al., 2009a). A mortality rate of even 1% due to ascites causes significant economic losses to the broiler industry, mainly because death usually occurs towards the end of the growing period (Maxwell and Robertson, 1997).

Ascites is characterized by a cascade of events that begins when the oxygen ($O_2$) demands for maintenance and growth are not fully met by the cardiovascular system of broiler chickens (Balog, 2003; Hassanzadeh et al., 2008, 2009). Wideman, (2000) argued that decreased oxygen tension or increased $O_2$ requirements can create hypoxic conditions within tissues, and this has a major impact on pulmonary vasomotor tone, which is a potential pathogenetic route for pulmonary hypertension. The imbalance of $O_2$ requirements and supply may be caused by hypoxia. This imbalance can be caused by exogenous as well as by endogenous factors, and both sets of factors can potentiate each other by acting in an additive or even in synergistic way, which leads to an increased susceptibility to ascites (Decuypere et al., 2000).

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 (Hassanzadeh et al., 1999, 2000, 2001; Scheele et al., 2005). Scheele et al. (2005) also illustrated that broilers from stocks that differ with regards to growth rates and feed efficiency differ in their susceptibility to ascites. Peacock et al. (1990) found a positive relationship between hypoxemia, pulmonary hypertension and right
ventricular hypertrophy, and the latter is widely accepted as the preliminary stage in the ascites (Decuypere et al., 2000, 2005).

Changes in hematocrit values, partial pressure of O₂ and carbon dioxide (CO₂) and endocrine function, such as thyroid hormone activity, are also important regulatory mechanisms of the metabolic rate during embryonic development and growth (Buyse et al., 1996; Buys et al., 1998; Decuypere et al., 2000, 2005; Teshfam et al., 2005; Arab et al., 2006; Hassanzadeh et al., 2002, 2004, 2005a, 2005b, 2008; Bahadoran et al., 2009a, 2009b). The role of thyroid hormone in the regulation of metabolism could become more apparent in different genotypes of chickens (Decuypere et al., 2000; Lugér et al., 2001, Hassanzadeh et al., 2002, 2004, 2009) and environmental conditions, such as a low ambient temperature (Scheele et al., 1992), different lighting programs (Buyse et al., 1994; Buys et al., 1998; Hassanzadeh and BozorgmehriFard, 2001; Hassanzadeh, 2002) and even at high altitudes (Hassanzadeh et al., 2002, 2003, 2004, 2005a, 2005b, 2008). A decrease in the activity of thyroid hormone leads to a mismatch between O₂ delivery and O₂ demand, which results in anoxia, hypoxemia and hypoxia (Decuypere et al., 2000; Lugér et al., 2001). This may lead to insufficient development of the anatomy of the lungs or the pulmonary blood vessels in broiler chicken lines that may form the basis of increased susceptibility to ascites (Hassanzadeh et al., 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 more short-term solution, there is no single treatment or preventive system for the control of ascites in broiler chickens. 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).

In this review, particular attention will be paid to the endogenous and environmental factors that interact with other predisposing factors for the development of 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 vitamin supplementation, will be reviewed.

**Predisposing factors for the development of ascites syndrome**

a. Endogenous and environmental factors that contributed to the development of ascites

The increasing susceptibility of broiler chickens to ascites coincided with a continuing genetic and nutritional improvement in their food efficiency and growth rate (Julian, 1993, 2005). Moreover, ascites is a metabolic disorder that is no longer restricted to high altitude, over the last few decades, similar signs in broiler chickens were observed at sea level (Peacock et al., 1990; Hassanzadeh and BozorgmehriFard, 2001; Hassanzadeh et al., 1999, 2001), but until now, there has been no consensus about the origin of the ascites syndrome in sea level broiler chickens. At high altitudes, chronic hypoxia induces a marked increase in pulmonary arterial pressure and right ventricular hypertrophy in chickens followed by the development of ascites (Sillau et al., 1980; Julian, 1993; Hassanzadeh, 2002; Hassanzadeh et al., 2002, 2004). Powell (2000) suggested that the increased growth rate in modern fast-growing broiler chickens at sea level requires a higher metabolic rate and therefore, an increase in cardiac output causes pulmonary hypertension and ascites. Experimental results obtained in other species point at the important role of hypercapnia in causing vasoconstriction and high blood pressure at sea level in broiler chicken populations that are susceptible to ascites (Buys et al., 1998, 1999; Scheele et al., 2003, 2005, Hassanzadeh et al., 2009). 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 (Figure 1).

Balog, (2003) noticed that in the past, much of the blame for the current ascites problem has been placed on genetic selection of chickens for rapid growth and better feed efficiency. The experimental results of Scheele et al. (2005) clearly indicate that there was not always a relationship between the growth rate and susceptibility to ascites. Therefore, it is doubtful whether genetic selection for rapid growth is responsible.

In acute hypoxia, the increase in cardiac blood flow may contribute to the delivery of O₂ to the various tissues (Nesarajah et al., 1983). Olkowski and Classen, (1999) found that the ascites susceptibility of fast-growing broiler chickens at sea level was associated with bradycardia, which is the opposite of the tachycardia that is found in chickens at high altitude. They argued that a low cardiac output could be the key hemodynamic problem that leads to cardiovascular failure in fast-growing broilers. These indicate that a fast growth rate of chickens that are kept at sea level cannot be solely responsible for the effect that is provoked by hypoxia at high altitudes.

Many authors have reported that a low thyroid hormone activity was accompanied in chickens selected for rapid growth rate and improved feed efficiency (Decuypere et al., 2000; Malan et al., 2003), which could also be related to ascites susceptibility in broiler chickens (Scheele et al., 1992, 2003; Decuypere et al., 2000; Lugér et al., 2001; Malan et al., 2003, 2005).
A decrease in the circulating level of thyroid hormone will play a significant role in the inability of chickens to maintain adequate O₂ delivery, which leads to anoxia, heart failure and ascites (Decuypere et al., 2000). Hassanzadeh et al. (2009) argued that a thyroid hormone insufficiency was one of the causal factors of increased feed efficiency and is responsible for impaired O₂ supply when trying to sustain a fast growth rate; this may lead to a shortage of O₂ and hence hypoxemia. This will occur in particular under circumstances where the O₂ requirements of birds are increased, such as in a cold environment, and may lead to metabolic disorders, such as ascites (Decuypere et al., 2000; Hassanzadeh et al., 2009).

A higher incidence of ascites in fast-growing chickens with a high feed efficiency was concomitant with decreased arterial O₂ tension, an increased pCO₂, and a thyroid hormone deficiency, in comparison with the slower-growing lines (Malan et al., 2003). They concluded that there was a relationship between susceptibility for ascites and high feed efficiency accompanied with hypothyroidism, and this was responsible for the insufficient supply of O₂, which resulted in anoxia, hypoxemia and hypoxia (Decuypere et al., 2000).

The relationship between the activity of thyroid hormone and anatomical parameters was discussed by the study of Malan (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. (2005b, 2008), who documented a direct correlation between the lung development of post-hatch broiler chickens with the embryonic activity of thyroid hormone. These investigations showed that post-hatched chickens had higher lung volumes in relation to their body size when higher thyroid hormone levels were recorded during embryonic development. Consequently, such chickens with higher lung volumes ad a lower susceptibility to ascites when exposed to lower temperatures and chronic hypoxia at high altitude.

O₂ is a critical component in energy metabolism. Broilers use energy for thermoregulation, activity and growth; any form of energy utilization creates a demand for O₂. Wideman et al. (1999) showed that a moderate increase in the CO₂ concentration of arterial blood of broiler chickens, accompanied by a decreased pH, clearly increased pulmonary arterial pressure. This confirmed the contribution of variable blood gas tension to the development ascites. Differences in the O₂ requirements between fast-growing and slow-growing lines are associated with the more pronounced occurrence of right ventricular hypertrophy and ascites in the fast-growing line (Peacock et al., 1990). Buys et al. (1999) reported that chickens of a line of chickens that was sensitive to ascites consumed more O₂ due to higher metabolic activity, and hence showed significantly higher CO₂ tensions and lower O₂ tension.
in venous blood, compared to a line that was resistant to ascites. However, no differences were found in the pO2 and pCO2 in the arterial blood between these lines. High CO2 tensions in the venous blood of juvenile domestic chickens are a predisposing factor for the development of ascites (Scheele et al., 2003, 2005; Hassanzadeh et al., 1997a, 2009), since it can increase pulmonary arterial pressure (Wideman et al., 1999).

Previous results have indicated that cardiopulmonary parameters are extremely unfavorable to broiler chickens and suggest that a reduction in the area of gas exchange in broilers therefore causes a higher susceptibility to pulmonary hypertension and ascites (Hassanzadeh et al., 2005b, 2008). Vidyadaran et al. (1987) showed that the respiratory system of the adult domestic fowl was far less well-adapted anatomically for gas exchange compared to the non-galliform species. Additionally, at a low ambient temperature, a high pCO2 in the venous blood of young broiler chickens was a reliable predictor for susceptibility to ascites at later ages (Scheele et al., 2003, 2005; Hassanzadeh et al., 2009; Bahadoran et al., 2009b). 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 and development of ascites syndrome in fast-growth broiler chickens (data not published). In that study, a large fall was observed in the volume of the thoracic cavity, both before and after the removal of the heart and lung tissues, in the fast-growing chickens compared with the slow-growing broiler chickens. This was accompanied by a significant increase of the pCO2 and a decrease of the pO2 in the venous blood of rapid growth compared with the slow-growth chickens. This observation could be due to high metabolical activity and a higher O2 demand (Buys et al., 1998, 1999). However, it might also be caused by the much larger muscle mass, low gas exchange area and O2 saturation, in fast-growing broiler chickens compared to slow-growing chickens as discussed previously (Jones, 1995; Julian, 1989; Decuyper et al., 2000; Hassanzadeh et al., 2005b, 2008).

The modern broiler selection strategy has indirectly affected the partition of energy that can be metabolized between that required for maintenance and for production. More of the energy is deposited in tissue (mainly lean meat) and less energy is used for maintenance purposes. As a further consequence, the proportion growth rate of demand tissues (muscle and fat) is accelerated at the expense of delivery tissues in the case of the cardiovascular and pulmonary system. The reduction in maintenance requirements has also induced a status of functional hypothyroidism (Scheele et al., 1992; Decuyper et al., 2000; Luger et al., 2001; Malan et al., 2003). Such susceptibility to ascites in fast-growing broiler chickens is not attributed to the high O2 requirement per se, but rather to an imbalance between O2 demands and O2 supply, resulting in anoxia, hypoxemia and hypoxia.

The imbalance of O2 requirements and supply may be caused by either an extremely high metabolic demand of the tissues (anoxia) or by an insufficient supply of O2 (hypoxia), or both. This imbalance on both sides can be caused by exogenous as well as by endogenous factors, and both sets of factors can potentiate the other, acting in an additive or even in synergistic way (Decuyper et al., 2000; Hassanzadeh et al., 2004).

b. Myocardial β-adrenergic receptors and ascites susceptibility

Investigations in humans and other mammals have confirmed that β-adrenergic receptors play an important role in the regulation of cardiovascular performance, such as tachycardia. β-adrenergic 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 the developing rat ventricular myocardium (Novotny et al., 1999). In mammals, hypoxia regulates the density of β-adrenergic receptors. In broiler chickens, Hassanzadeh et al. (1997b) demonstrated that the density of β-adrenergic receptors was higher in cardiac cells of ascites-sensitive birds compared to the chickens that were ascites-resistant. Moreover, the characteristics of β-adrenoceptors were found to be different in the cardiac cells of birds with right ventricular hypertrophy and heart failure compared with healthy birds (Hassanzadeh et al., 2001). A similar difference was also observed between the ventricular cells of high-altitude hypoxic birds and low-altitude normoxic birds (Hassanzadeh et al., 2002). Together with the recent observation that chick embryos incubated at high CO2 levels downregulated the expression of myocardial β-adrenergic receptors (Hassanzadeh et al., 2002), this observation indicates that the decrease in receptor density reflects a regulatory phenomenon and is not due merely to right ventricular hypertrophy, as has been observed in mammals (Mardron et al., 1998). These data point to the involvement of 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, and it also points to a possible cardiogenic origin for the susceptibility to ascites on a genetic basis.

c. Hatching parameters and ascites susceptibility

Following selection for fast and efficient growth,
the focus has changed to ensuring that the eggs of commercial broilers are adequately incubated over the last decade. 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 (De Smit et al., 2005; Hassanzadeh et al., 2004, 2005b, 2008). The peak mortality due to ascites occurs at the end of the growth period, but it is now thought that the etiology of this disease might exist 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) discovered that a high CO₂ concentration in the air chamber is trigger for hatching. The findings of Chineme et al. (1995) indicate that the length and/or severity of prenatal hypoxia may influence postnatal characteristics that are related to ascites. Recent studies have focused on the CO₂ concentration in the incubator. 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 ascites susceptibility. In these studies, it was showed that eggs that were 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) hatched earlier than in an environment with normal CO₂ levels. Furthermore, embryos that were incubated at high altitude, such as 2000 m above sea level, for first 10 d or 21 d of incubation had an earlier hatching time than those incubated at low altitude (Hassanzadeh et al., 2004, 2005b; Bahadoran et al., 2009b). In these studies, those chicken 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 that the embryo experiences hypoxia (Buys et al., 1998; Hassanzadeh et al., 2002, 2004; 2005; Bahadoran et al., 2009b; De Smit et al., 2006).

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 these 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 this hypoxic period and, therefore, to the pipping and hatching time.

Blacker et al. (2004) provided evidence for the role of hypoxia during embryonic development with regards to the control of pulmonary surfactant. 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 results in the physiological heterokary 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 that occur 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. This may be a predisposing factor for the development of heart failure and ascites syndrome in later life.

Hypoxic conditions during embryonic development may initiate structural changes in the cardiovascular and pulmonary systems, as was observed in our recent study of chickens at high altitude (Hassanzadeh et al., 2005b, 2008). In these studies, one-day-old high altitude-hatched chicks had a significantly higher proportion of their heart to body weight (1.23 0.08 versus 0.87 0.07) and lung (2.02 0.07 versus 1.24 0.13) compared to 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 low altitude-hatched birds (Hassanzadeh et al., 2004, 2008). Additionally, changes in the anatomy of these chickens were also accompanied with increased levels of thyroid hormone and corticosterone in embryos, which demonstrated a critical role of certain endogenous parameters on the maturation of the pulmonary system. 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).

Besides the environmental incubation conditions,
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 trajectories (Decuyper et al., 2000). Studies that compared broilers from lines that differ in their ascites susceptibility (Dewil et al., 1996; Buys et al., 1998; De Smit et al., 2005) showed that these chicks also differed in certain embryonic characteristics. Chicks of ascites-sensitive lines hatched later, had lower thyroid hormone concentrations, and a lower pCO₂ and a higher pO₂ in the air cell during the late stages of embryonic development.

Management techniques for the control of ascites

a. Lighting

Broilers are usually grown on a near continuous lighting (CL) schedule in order to maximize the feed consumption and growth rate. Early studies in photoperiod manipulation reported a decreased growth rate for broilers raised with a step-down lighting program (Classen et al., 1991). It was hypothesized that limiting the number of hours of light would slow the growth rate slightly and reduce activity that requires additional O₂, which may improve feed efficiency (Julian, 1993; 2005). Subsequent studies on the effect of longer dark periods or intermittent lighting (IL) indicate that manipulating the photoperiod can decrease the incidence of ascites syndrome in a similar manner to feed restriction (Julian, 1993; Buys et al., 1998; Hassanzadeh et al., 2000, 2003, 2005).

Fast-growing broiler chickens are characterized by a very high specific metabolic rate at about two weeks of age (Buys et al., 1994). Providing birds with an intermittent lighting program is beneficial because these alternative lighting programs not only improve broiler performance but also reduce the occurrence of metabolic disorders by controlling the rate of early growth of the young chickens.

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 CL to 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 reached the same final body weight by six weeks (Buys et al., 1998) and seven weeks of age (Hassanzadeh et al., 2000). In both studies, the same underlying physiological mechanisms have been argued. These include lower heat production and consequently lower O₂ consumption in IL birds during the dark period of each light:dark cycle (Buys et al., 1996). Additionally, another causative mechanism may be the altered growth pattern between IL and CL chickens. Indeed, changing from CL to IL at a young age slows down the juvenile growth rate, and therefore reduces the high O₂ requirements. In this manner, the predisposition for incidence of ascites is alleviated. The reduction in juvenile growth rate with a concomitant reduction in metabolic rate at a young age to imposing IL was also reflected by several physiological parameters. The hematocrit as well as the plasma concentrations of the biologically active thyroid hormone, triiodothyronine, were also lower in juvenile IL chickens compared to their CL counterparts.

Recent studies have again confirmed the effect of alternative lighting schedules on the incidence of ascites in broiler chickens. When performed at high altitude, 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 (four 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 to reduce the incidence of ascites in broiler chickens (Hassanzadeh et al., 2003). 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 two studies, the reduction of ascites mortality also coincided with a temporary reduction in the growth rate at young ages, with a concomitant reduction of metabolic indicators, such as the values for hematocrit and plasma thyroid hormones concentrations, which both indicate the reduced utilization of O₂ and heat production.

b. Feed restriction

Early growth restriction is still an important management tool for reducing 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). However, the use of this management technique depends on the body weight of the chicken at certain slaughter ages, which is associated with compensatory weight gain, improvements in carcass yield, feed prices, and the incidence of ascites experienced locally (Balog, 2003). There have been conflicting results in catch-up growth and yield data, which illustrates 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).

Food restriction by limiting of the time of *ad libitum* food intake was aimed at reducing the incidence of ascites without compromising body weight (Albers...
et al., 1990; Balog, 2003). Skip-one-day food restriction programs during the early growth period effectively reduced the number of chickens that developed ascites (Julian, 2005). It has been shown experimentally that other factors, such as age at initiation of restriction, duration of restriction, and its severity, determine to a large extent the ability of the chicken to manifest compensatory growth to their full potential (Julian, 2005). Food 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). Other confounding factors in this mechanism are the strain of broiler chicken, lines within strains, and sex of the chicken (Decuypere et al., 2000). The earlier an effective food restriction program can be introduced, the smaller the potential negative effect on the final body weight at market age will be (Julian, 1993).

c. Antioxidants

Wideman et al. (1995) reported that by reducing the pulmonary vascular resistance, it is possible to reduce the pulmonary hypertension that is needed to increase the cardiac output required to match metabolic demands of broiler chickens, and so delay the pathophysiological progression to ascites. Nitric oxide (NO) is a potent endogenous pulmonary vasodilator that acts by increasing the levels of cyclic guanosine monophosphate (cGMP), and thereby reduces intracellular calcium ion levels in vascular smooth muscle cells (Forstermann et al., 1986). Nitric oxide is produced in the pulmonary endothelium where the enzyme, endothelial NO synthase (eNOS), converts L-arginine to L-citrulline (McQueston et al., 1993). Arginine is an essential amino acid in birds, and must be supplemented in the diet. Certain evidence shows that arginine levels that support the maximal growth rate are not adequate to support maximal NO production (McQueston et al., 1993). Experimental supplementation of arginine has been used successfully to reduce ascites mortality induced by cool temperatures; however, its effects have not been consistent (Wideman et al., 1995).

Oxidative stress is also involved in the pathophysiological progression that leads to ascites (Maxwell and Robertson, 1997). Oxygen-derived free radicals play an important role in the genesis of tissue damage. 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 consequent increased membrane permeability (Maxwell and Robertson, 1997). The superoxide anion causes a loss of NO bioavailability by shortening its half-life and thereby reduces the 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 nitration 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 that is available for the production of NO and maintenance of blood pressure.

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 in reducing the 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 lung and liver, which provides evidence that a compromised antioxidant status is involved in the etiology of pulmonary hypertension syndrome (Enkvetchakul et al., 1993).

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). The antioxidant properties of oxidized vitamin E can also be restored by vitamin C. As vitamin C is able to donate an electron to the tocopherol radical, so generating the reduced antioxidant form of vitamin E, this suggests that a major function of vitamin C is to recycle the vitamin E radical (Bottje and Wideman, 1995).

Supplementation of 500 ppm vitamin C significantly reduced the mortality rate that was due to ascites in broiler chickens with a concomitant reduction in plasma thyroid hormone levels (Hassanzadeh et al., 1997a). This suggests that a role for vitamin C in metabolic activity. Julian (1993) reported that the reduction of ascites in bird that were treated with vitamin C could be a consequence of the reduction of blood flow resistance, especially in the narrow capillaries of the lungs. However, no major effect of vitamin C on the hematocrit values was found in the study by Hassanzadeh et al. (1997a). Xiang et al. (2002) reported a reduction in the incidence of pulmonary hypertension of cold-stressed and T4-fed broiler chickens when vitamin C supplemented their diet. This was due to a reduced muscularization of
pulmonary arterioles of the chickens that received vitamin C dietary supplements.

Conclusions

Different opinions exist in literature as to the primary causal factors that lead to pulmonary hypertension and ascites syndrome. Indeed, in some cases a decrease in thyroid hormone activity, as well as endogenous structural and functional factors, is associated with a higher incidence of ascesis. 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, 2009; Bahadoran et al., 2009b). In this manner, broilers that were selected for rapid growth and high feed efficiency develop a mismatch between the delivery of O2 and O2 demands, resulting in anoxia, hypoxemia and hypoxia.

In some cases, the 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) promote the occurrence of pulmonary hypertension and ascites syndrome. The underlying causative mechanism is the associated increase in metabolic rate. This elevated metabolic rate leads to higher O2 demands, erythropoietin synthesis and stimulated cardiac output. If the higher O2 demands cannot be met adequately by a higher rate of O2 delivery, this results in anoxia, hypoxemia and finally hypoxia. In view of this positive causal relationship between the 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 intermittent lighting, 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 O2 delivery and demand. It is clear that the 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 the nature of the selection pressures or broiler line, certain environmental factors may be differentially important in reflecting genotype versus environmental interactions with regards to the 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, 2005b, 2008; De Smit et al., 2006, Bahadoran et al., 2009b). Therefore, it has been hypothesized that developmental changes that are induced by incubation conditions are another example of genotype versus environmental interactions in ascites susceptibility, which further complicates the etiology of this important syndrome.

References