1. Introduction

The growth rate of broiler chicken stocks has drastically risen as a result of consistent selection by poultry breeding companies. As a result, modern chicken consume more feed per unit of time, have better feed efficiency, grow quicker, and have a higher carcass yield than ordinary broiler chicken of 1950s (Havenstein 1994). The contemporary chicken has undergone rigorous selection for faster growth rates, which indirectly leads to faster protein synthesis, the metabolic process requiring more oxygen (Decuypere 2005). Modern strains of broiler chicken are able to achieve market weight in 60% less time than the broiler chicken of 40 years ago. The need for oxygen rises with rapid growth which increases the workload of heart. Thus, fast-growing poultry are more susceptible to ascites development and the degree of ascites and metabolic rate are significantly associated (Al-Zahrani et al. 2019).

However, due to the fact that pulmonary and cardiac capacities of modern broiler chicken strains are quite similar to the old strains, their cardio-pulmonary system is forced to operate very close to its physiological limit (Lorenzoni 2006). The lung capacity is not always sufficient to meet the oxygen demands of 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). If the lungs of the chicken grow less rapidly than the rest of the body, it could result in hypoxia with consequent ascites (Julian 2000). Pulmonary hypertension syndrome (PHS) or ascites is a metabolic disorder that mostly occurs in fast-growing broiler chicken. High altitude, hypoxia, poor ventilation, low temperature, and fast growth rate are known to be predisposing factors for the incidence of this syndrome (Wideman et al. 1995; Hassanzadeh et al. 1997; Balog 2003).

Pathological findings in ascites induced mortality imply...
that the development of a cavity on the exterior surface of the right ventricular wall is the first sign of damage due to pulmonary hypertension. As the injury progresses, it leads to dilation and hypertrophy of the right ventricle resulting in increased blood viscosity, reduced oxygen supply, congestive heart failure, and accumulation of fluids in the abdominal cavity (Biswas 2019; Wideman et al. 2013; Biswas et al. 2012; Ahmed et al. 2013). The two main characteristics of blood that affect its flow in capillaries are plasma viscosity and erythrocyte deformability. And, hypoxia and acidosis significantly impact erythrocyte deformability in a number of clinical situations (Grygorczyk and Orlov 2017). The ability of haemoglobin to bind oxygen is lowered at low blood pH, often known as acidosis. Reduced erythrocyte deformability is a possible contributing factor to pulmonary hypertension and ascites in broiler chickens. The physical characteristics of the blood, primarily haematocrit and plasma viscosity, are important determinants of the rate of oxygen transport in the circulation (Mirsalimi 1991).

According to certain investigations, ascites syndrome may occur as a result of the oxidative stress caused by reactive oxygen species (ROS) (Kalmar et al. 2013). It has been reported that increasing hypoxia caused by insufficient lung function in chickens with ascites is frequently linked to the emergence of PHS (Reeves 1991). The systemic hypoxia may induce cellular hypoxia and increased production of free radicals (Bottje and Wideman 1995). The ROS such as H$_2$O$_2$, superoxide, and hydroxyl ions have potential to induce considerable cell death via lipid peroxidation. They have the ability to oxidize proteins, DNA, and some other tiny molecules inside of a cell, starting a series of negative reactions and harming nearby cells (Halliwell and Gutteridge 1990).

Beyond their role as cellular powerhouses, mitochondria are increasingly being identified as vital players in molecular signal transduction and cell fate determination via reactive oxygen species (ROS). Mitochondria are especially abundant in cardiac tissue; hence, mitochondrial dysregulation and ROS production are thought to contribute significantly to cardiac pathology. Moreover, there is growing appreciation that medical therapies designed to mediate mitochondrial ROS production can be important strategies to ameliorate cardiac disease (Peoples et al. 2019). Recently it has been identified that mitochondrial ability to produce ATP is been diminished by ROS due to the mutations in key genes present in mitochondrial DNA responsible for ATP generation. Depletion of ATP in cardiac myocytes is one of the causes of their inefficiency and eventually failure (Kornfeld et al. 2015).

The four main postmortem findings considered before assigning any mortality due to ascites were water belly with reduced pumping power of heart. This study aimed to assess the role of Ascitox – Q10, a proprietary dietary formulation, in amelioration of ascites and sudden death syndrome in broiler chicken in winter season.

2. Materials and methods

Among different broiler chicken farms located at different locations of same eco-climatic zone in northern India, nine farms showing the cases of ascites were selected for this study. The period of study was from November 2021 to February 2022. Normal winter management practices were followed at all farms and no ascites preventive management was followed at any farm except farm 2, 4, 5, 7, and 8 which were given a dietary proprietary formulation – Ascitox on 2-3-4, 13-14-15, and 23-24-25 day for the amelioration of ascites in birds. The Ascitox was given at the rate of 4 g/L drinking water. Farm 1 to 6 reared and managed Cobb 430Y and farm 7 to 9 reared and managed Skylark Hubbard broiler chicken strains according to the conventional feeding and management practices. Brooding were done in closed house inside brooding guard and under heating source with false ceiling at all the farms (Fig. 1). The mortality from first day to the end of rearing period were recorded. Postmortem of dead birds was conducted regularly on all the farms for tentative diagnosis by the available qualified veterinarians. The feed conversion ratio (FCR) and total feed consumption was recorded to access the tentative economic losses due to the ascites in the birds. For convenience of interpretation total mortality was divided into 2 parts; M1 indicating mortality in first 15 days and M2 indicating mortality after 15 days. It was hypothesized that mortality due to ascites will be seen after 15 days of age when metabolism and oxygen requirements are high. Mortality lesions were specially identified during postmortem for the specific pathology associated with ascites in all the birds. Water belly condition in dead birds was given maximum weightage among all the signs associated with ascites (Fig. 2). The four main postmortem findings considered before declaring any mortality due to ascites were water belly with

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plasma like fluid in abdomen, enlarged liver with blood clots over it (Fig. 3), hypertrophy of right ventricle of the heart, and oxidative stress markings on the breast muscles.

3. Results and discussion

This study assessed the influence of a propriety formulation, Ascitox, on the winter ascites and the consequent livability and growth performance of broiler chicken. The results in Table 1 revealed significantly lower M1 (p<0.05), M2, and overall mortality (p<0.01) in Ascitox supplemented birds compared to control birds. However, the extent of influence of Ascitox was more pronounced on M2 mortality compared to M1 mortality (Fig. 4). There was no strain effect on the mortality pattern of birds across different farms under study.

Ascites is a common problem of modern day fast growing broiler chicken characterised by fluid accumulation in the abdominal cavity. Because of the disproportionate increase in muscle mass with respect to the normal development of circulatory and respiratory system of modern day broiler chicken the incidence of ascites has increased disproportionately as well (Druyan 2012). It is the condition more commonly witnessed in the winter season because of poor ventilation of the farms due the use of curtains along with the burning of wood, charcoal, and gas to maintain the shed temperature in India. Such activities generate the air contaminants such as ammonia, carbon dioxide, carbon monoxide, dust, and humidity (Aviagen 2009) which creates a hypoxic environment for the birds. This hypoxic environment during the brooding stage of the birds damage their air sacs which makes the respiratory system incompetent to withstand the oxygen demand during the rapid growth phase of birds after 4th week and hence leads to ascites with consequent death. On similar pattern M2 mortality (after 15 days) of birds was higher compared to M1 mortality in this study. Concurrent to this observation it has been reported that hypoxia induced during brooding stage retards the development of lungs, which later on hastens the development of ascites (Wuyi Liu 2016).

Furthermore, during the finisher phase of broiler chicken high energy diets are provided to meet the nutritional demands requiring higher oxygen levels for metabolism, and this in turn demands high blood flow through heart and lungs (Dahal 2011). This physiological demand of high blood flow causes hypertension in pulmonary artery which is not handled well by the less flexible blood capillaries of the lungs resulting in effusion of blood fluids in the body cavity.

It has been postulated that broiler chickens with a high metabolic rate may be in a state of metabolic acidosis when fed at full capacity (Julian 1993). Several studies have shown that fast-growing birds have lower blood oxygen concentrations than slow-growing birds; similarly, birds on full feed have lower blood oxygen concentrations than food-deprived birds.
Fedde et al. 1998). Therefore, in this study a feed withdrawal of 4 hours was practiced uniformly as a preventive measure in all the farms. In early grower stage of broiler chicken temporary feed restriction potentially reduces incidence of ascites without compromising the growth performance (Acar et al. 1995; Decuypere et al. 2000). On the other hand, limiting the quantity of feed by 40% has also reduced the ascites induced mortality in broiler chicken (Mohammadalipour et al. 2017). In general the farms under study were characterized by spells of sudden deaths overnight (Fig. 5). Earlier studies have also associated ascites with sudden death in well-nourished broiler chicken preceded by brief flapping of their wings for 1-2 minutes (Saki and Hemati 2011). Fast growing young and healthy broiler chicken have been observed to die suddenly while standing, walking, or feeding (Julian 2005).

This induced hypoxia favors development higher amount of ROS generation in heart muscles and start causing further complications including arrhythmias. Cardiac arrhythmias can occur in broiler chicken as early as 7 days of age, and the incidence rises with age (Olkowski 2007). But nutritional interventions which aimed to correct the metabolic and biochemical disarrangements in the body could reduce the ascites induced mortality in broiler chicken significantly. In this study Ascitox was used as a nutritional intervention whose composition is: CoEnzyme Q\textsubscript{10}, blood buffers, transmembrane antioxidants, herbal diuretics, herbal antioxidants, sodium ascorbate, furosemide, and sodium selenite. The results depicting the effects of ascites and ameliorating effects of Ascitox on the growth performance of birds are given in Table 2. The average weight gain of birds was significantly (P<0.01) higher in Ascitox supplemented birds compared to control birds with no significant effect on their feed intake. However, numerically feed intake of control birds was more than the Ascitox supplemented birds which has led to significantly lower (P<0.01) FCR of Ascitox supplemented birds. Corroborating the results of the present study, modifications in the acid-base balance of the chicken feed under potential hypoxic conditions reduces the ascites incidence in birds (Owen et al. 1994). In Ascitox the presence of blood buffers tend to increase the blood pH which increases the oxygen hemoglobin affinity with a consequent increase of the oxygen carrying capacity of blood. The use of furosemide, a carboxylated sulfonamide, as diuretic has shown promising results in controlling ascites induced mortality by inhibiting electrolyte reabsorption in ascending loop of Henle (Wideman et al. 1995). High sodium diets of drinking water hastens the

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Farm strength</th>
<th>M1 (%)</th>
<th>Farm strength</th>
<th>M2 (%)</th>
<th>Overall mortality (%)</th>
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</thead>
<tbody>
<tr>
<td>Control</td>
<td>3068</td>
<td>99</td>
<td>2969</td>
<td>123</td>
<td>4.15</td>
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<tr>
<td>Ascitox</td>
<td>2580</td>
<td>64</td>
<td>2516</td>
<td>55</td>
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<tr>
<td>SEM</td>
<td>27</td>
<td>3.5</td>
<td>27</td>
<td>2.9</td>
<td>0.16</td>
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<tr>
<td>Significance</td>
<td>-</td>
<td>-</td>
<td>P&lt;0.05</td>
<td>-</td>
<td>P&lt;0.01</td>
</tr>
</tbody>
</table>

**Strain Effects**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Farm strength</th>
<th>Total mortality</th>
<th>Average weight gain (kg)</th>
<th>Average feed intake (kg)</th>
<th>FCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cobb 430Y</td>
<td>2778</td>
<td>2700</td>
<td>2700</td>
<td>3.13</td>
<td>5.90</td>
</tr>
<tr>
<td>Skylark Hubbard</td>
<td>2833</td>
<td>2752</td>
<td>2752</td>
<td>3.16</td>
<td>5.93</td>
</tr>
<tr>
<td>Significance</td>
<td>-</td>
<td>-</td>
<td>P&gt;0.05</td>
<td>-</td>
<td>P&gt;0.05</td>
</tr>
</tbody>
</table>

M1: Mortality upto 15 days; M2: Mortality after 15 days; SEM: Standard error of mean
development of ascites in fast growing broiler chicken (Julian et al. 1990) and use of diuretics reduces sodium and fluid retention which reduces ascites induces mortality.

The supplementation of vitamins, trace minerals, amino acids, and antioxidants like CoEnzyme Q10 have been reported reducing ascites induced mortalities in broiler chicken (Roch 2000; Xiang 2002; Geng et al. 2004) and Ascitox -Q10 used in this study is a balanced mixture of all these nutrients. Reduced pulmonary arterial pressure and reduced ascites index was observed in broiler chicken supplemented with vitamin C and E (Ruiz-Feria 2009). However, supplementing vitamin C and E individually was found to be ineffective in controlling ascites induced mortality of broiler chicken (Khajali and Fahimi 2010; Villar-Patino et al. 2002). This indicates that antioxidants work in a coordinated fashion to provide protection against ROS. The selenium supplementation in the range of 0.15 to 0.30 mg/kg diet have resulted in significant improvement in ascites-related variables in broiler chicken (Ozkam et al. 2007) and nano-selenium at the level of 0.30 mg/kg diet was observed successfully preventing the right ventricular hypertrophy in broiler chicken (Moghaddam et al. 2017). The dietary CoQ10 exerts a strong antioxidant effect and prevents development of ascites syndrome. Significant reduction in incidence of ascites (Faraji et al. 2019; Sharifi et al. 2016) and ascites induced mortality have been reported by CoQ10 supplementation in diet of broiler chicken (Geng et al., 2004).

On similar lines the herbal supplements containing naturally occurring phytochemicals exhibit a potential for modulating metabolic disorders such as ascites syndrome. The rich pool of bioactive compounds in wild celery (Kelussia odoratissima) have been shown to exert strong suppression on pulmonary hypertension and ascites in broiler chicken (Ahmadipour et al. 2015). The ethanol extract of elecampane (Inula helenium L.) rhizome, rich in sesquiterpenes, and root extract of Prosopis farcta, rich in flavonoids, have prevented ascites in broiler chicken (Abolfathi et al. 2021). The supplementation of purslane (Portulaca oleracea L.) powder, rich in phenolic alkaloids and alpha-linolenic acid, has reduced the incidence of ascites in broiler chicken with improved antioxidant status (Habibian et al. 2017; Shirzadi et al. 2020).

5. Conclusions
The propriety dietary formulation, Ascitox Q10, has significantly reduced the mortality of broiler chicken and thus can be valued as a fruitful nutritional intervention in controlling ascites induced mortality in broiler chicken with improved growth performance at commercial farms.

Declarations

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Conflict of interest: The author declares no conflict of interest

Ethics approval: Not applicable

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Winter ascites in broiler chicken


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