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Case Report
A Trial Diagnosis of Ascites Syndrome in Broiler Chickens

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Abstract

Background: Ascites syndrome is currently a serious disease issue for the global chicken industry. Ascites syndrome is a metabolic disorder frequently found in fast growing broilers including abdominal distention and standing fluid collection in chicken abdomen. It is one of the most common nutrition metabolic disorders. **Materials and Methods:** In this study, the clinical diagnosis technology of broiler ascites symptoms mainly included the trial inquiry of feeders and administrators, local observation, detection of farm gas and faeces and pathological autopsy. **Results:** The study investigated the case of broiler ascites syndrome of local commercial broiler chickens at the age of 4-5 weeks to reduce outburst of ascites syndrome in broiler chickens. Through the trial clinical diagnosis of broiler ascites symptoms and pathological autopsy and observation, it came to the definite diagnosis of broiler ascites. Subsequent investigation found that the rearing houses were closed and sealed with poor ventilation and a high breeding density and much ammonia gas. **Conclusion:** Under the comprehensive management and drug treatments, there were 800 chickens found ill and later came back to normal from illness after the treatments, except for the death of 38 sick chickens. The appetite and drink of broiler chicken came to normal gradually.

Key words: Broiler chicken, ascites syndrome, metabolic disorder, chick, clinical diagnosis, pathological autopsy, healthy, ambient temperature, feeding regime, drug treatment, Chinese herbal medicine

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Ascites syndrome is presently a kind of serious metabolic disorder disease for the global chicken industry. Although the incidence of this disease is very low in the world's well-managed commercial flocks, it causes significant economic losses to the poultry breeding industry indeed[1]. Ascites syndrome is actually one of the most serious nutrition metabolic disorders in the poultry industry. It is a metabolic disorder frequently found in fast growing broilers including abdominal distention and standing fluid collection and it can lead to as heart failure or metabolic disorder syndrome in broiler chickens. In recent years, the harm is increasingly serious and the morbidity and mortality of broiler chickens are on the rise. Therefore, the clinical diagnosis and prevention measures for poultry husbandry breeding are the key to keep the hen house good ventilated environment to ensure appropriate temperature of the farm house, reasonable collocation of high-energy feed and conventional energy feed ratio in order to control the rapid growth of broiler chickens. It is regarded that genetic, physiological, environmental and management factors all might interact to produce a cascade of events that culminate in ascites syndrome[2]. The obvious clinical features of ascites syndrome are the abdominal enlargement and difficulty in walking of chicken or chicks.

The occurrence of this disease is due to the association with oxygen and cold weather. Particularly, the previous studies suggested that low temperature and hypoxia stress should be main influencing factors for the chicken pathogenesis of broiler ascites syndrome. As one of the most important factors of ascites syndrome, cold weather can active many metabolic disorders and activities leading to ascites, but the detailed mechanism is complex and may be related to nutrient and genetic and environmental factors. Meanwhile, broiler ascites syndrome is regarded as a crucial metabolic disease with high incidence and mortality rate, which causes serious loses for the global broiler chicken production due to the practical fact that broiler chickens are often intensively selected and raised in high-density for productive traits. Odom *et al.*[3] investigated and identified the ascites syndrome in broiler chickens with electrocardiographic analysis. In their study, a variable ascites-related mortality was observed as 40% and the majority of the chicken deaths occurred between 5 and 8 weeks of age. Arce *et al.*[4] conducted the control experiment of ascites syndrome by feed restriction techniques to reduce broiler ascites with minimizing weight depression. Zhu[5] reported the chicken ascites syndrome caused by furazolidone too. Julian[6] reviewed and investigated the physiological, management and environmental trigger aspects of chicken ascites syndrome. Moghadam *et al.*[7] estimated the genetic parameters for ascites syndrome in broiler chickens. Balog *et al.*[8] evaluated the effect of cold stress on broiler chickens selected for resistance and/or susceptibility to ascites syndrome. Rajkhowa[9] carried out a clinical and histopathological experiment of chicken ascites syndrome in India. Li *et al.*[10] investigated the precise ultrastructural location of Ca^{2+} and Ca^{2+}-ATPase in the right ventricular myocardium of chickens with ascites syndrome induced by low ambient temperature. They found the increased calcium deposits and decreased Ca^{2+}-ATPase in right ventricular myocardium of ascitic broiler chickens[10]. They inferred there was the right ventricular diastolic dysfunction in the ascitic broilers[10]. Wideman *et al.*[11] reported a susceptibility to pulmonary arterial hypertension in broiler chickens. They proposed the following hypothesis that broiler susceptibility to pulmonary hypertension syndrome (i.e., ascites syndrome) was a consequence of anatomically inadequate pulmonary vascular capacity combined with the functional predominance of the vasoconstrictor 5-HT (5-hydroxytryptamine) over the vasodilator nitrogen monoxide (NO) in broiler chickens[11].

Druyan *et al.*[12] developed the ascites-resistant and ascites-susceptible broiler lines. Since then, Druyan *et al.*[12] Druyan and Cahaner[13] and Druyan *et al.*[14-16] conducted multiple evaluations and regarded the growth rate, body weight, heart rate and blood parameters of broiler chickens as potential indicators for selection against susceptibility to the chicken ascites syndrome. They suggested that moderate heritability might serve as an early indicator for selection against ascites syndrome, albeit with a limited efficacy[12-16]. Olkowski[17] studied the pathophysiology and biochemical characteristics of heart failure in broiler chickens. Baghbanzadeh and Decuypere[2] made a glance over broiler ascites syndrome with physiological and nutritional perspectives. They summarized that manipulation of the diet composition and/or feed allocation system could have a major effect on the incidence of ascites and optimization of the house temperature and ventilation in cold weather would be helpful to decrease the incidence of ascites[2]. Later, Ozkan *et al.*[18] investigated the effects of rearing temperature and feed restriction on growth and ascites mortality of broilers chicken at low versus high altitudes, cold versus normal ambient temperatures and three feeding regimens. Pavlidis *et al.*[19] reported the divergent selection for ascites incidence in different chicken lines.

Hassanzadeh *et al.*[20] found evidence for the involvement of anatomical parameters of the cardiopulmonary system in the development of ascites syndrome of broiler chickens. Smit *et al.*[21] detected the effect of non-ventilation during early

incubation on the embryonic development of chicks of two commercial broiler strains differing in ascites susceptibility. They found that non-ventilation during the first 10 days of incubation had a stimulatory effect on embryonic development of the 2 broiler strains with no effect of heart weights but with effects on hormone levels, air cell pressures and hatching parameters[21]. Closter *et al.*[22] studied the genetic and phenotypic relationships between blood gas parameters and ascites-related traits in broilers. They computed and inferred that the heritability for blood gas parameters and the genetic correlations between blood gas parameters for ascites[22]. Hamal *et al.*[23] observed the differential gene expression profile of proinflammatory chemokines and cytokines in lungs of ascites-resistant and ascites-susceptible broiler chickens following intravenous cellulose microparticle injection. Hassanzadeh *et al.*[24] regarded the partial pressure of carbon dioxide in the venous blood of young birds as a predictor of ascites susceptibility in broiler chickens and deduced the function of thyroid hormones should be taken into consideration in further selection procedures for meat-type chickens. Van As *et al.*[25] thought the blood gas parameters could be used to predict ascites susceptibility in juvenile broilers. Kalmar *et al.*[26] found dietary N,N-dimethylglycine supplementation improved nutrient digestibility and attenuates pulmonary hypertension syndrome in broilers.

Wideman *et al.*[27] observed and reported the pulmonary vascular pressure profiles in broilers selected for susceptibility to pulmonary hypertension syndrome compared with pulmonary hypertension syndrome-resistant broilers regarding age and sex. Kai *et al.*[28] found increased calcium deposits and decreased Ca^{2+}-ATPase in erythrocytes of ascitic in broiler chickens. Gupta[29] thought optimization of the house temperature and ventilation in cold weather was helpful in practices to decrease the incidence of ascites. Actually, as to the broiler ascites syndrome, high metabolic rate (i.e., fast growth) is regarded as a major factor contributing to the susceptibility of broiler ascites syndrome and appropriate selection strategies plus feed restriction and/or light restriction to slow down the broiler growth rate were practically viable ways to control chicken ascites syndrome. Tisljar *et al.*[30] studied the impact of L-NAME and L-arginine chronic toxicity induced lesions on the ascites and pulmonary hypertension syndrome development in broiler chickens. Wang *et al.*[31] observed changes of hepatic biochemical parameters and proteomics in broilers with cold-induced ascites. Kalmar *et al.*[32] and Wideman *et al.*[33] reviewed the characteristics of broiler pulmonary arterial hypertension (ascites syndrome) respectively and thought there was a collateral damage from efficient feed to meat conversion. Saki *et al.*[34] observed and analyzed the supplemental arginine administered *in ovo* or in the feed significantly reduced the susceptibility of broilers to pulmonary hypertension syndrome. Zhang *et al.*[35] observed and detected the expression of hypoxia-inducible factor 1α mRNA in hearts and lungs of broiler chickens with ascites syndrome induced by excess salt in drinking water.

Shen *et al.*[36] conducted a study of metabolomics and found that bile acids and phospholipids contribute much to variable responses to low-temperature-induced ascites syndrome. They found there were significant differences in metabolic profiling between the ascites syndrome group and the control group and deemed those differences or changes were related to the perturbations of broiler lipid metabolism[36]. Shi *et al.*[37] reported an integrative analysis of transcriptomics and metabolomics profiling of ascites syndrome in broiler chickens induced by low temperature. They found that two biological pathways (i.e., tryptophan biosynthesis and metabolism and glycerophospholipid metabolism) might contribute to the induction of ascites syndrome in broilers[37]. Varmaghany *et al.*[38] examined the effects of increasing levels of dietary garlic bulb on growth performance, systolic blood pressure, hematology and ascites syndrome in broiler chickens. Their study found that the inclusion of 5 g kg^{-1} garlic bulb in susceptible broiler chicken diets has a systemic anti-hypertensive effect and could decrease ascites incidence without impairing the performance of broiler chicken[38].

Kamely *et al.*[39] counted the incidence of ascites syndrome and related hematological response in short-term feed-restricted broilers raised at low ambient temperature. They observed some key indices of ascites susceptibility would increased when the broiler chickens were exposed to cool temperatures[39]. Hassanpour *et al.*[40] measured the lipid peroxidation, protein oxidation, antioxidant capacity, enzymatic activity and gene expression for some metabolic enzymes in chickens and they found significant results in the evaluation of the chicken oxidant and antioxidant status of their brain (hindbrain, midbrain and forebrain) with cold-induced pulmonary hypertension. Hasanpur *et al.*[41] attempted to explore the suitability of some blood gas and biochemical parameters as early diagnostic indicators of ascites syndrome in broiler chickens to find a reliable indicator trait as a selection criterion against susceptibility to ascites syndrome. Yang *et al.*[42] made the transcriptome analysis and gene identification in the pulmonary artery of broilers with ascites syndrome with chicken pulmonary arteries obtained from two positive ascites syndrome and two normal broilers for RNA sequencing (RNA-seq) analysis and pathological

observation. They found some candidate genes and pathways that involved in pulmonary artery remodeling further contributing to the ascites syndrome progression in broilers[42]. Mohammadalipour *et al.*[43] reported the effect of early feed restriction on physiological responses, performance and ascites incidence in broiler chickens raised in normal or cold environment. They concluded that feed restriction reduces ascites incidence mainly by allowing better development of broiler internal organs to cope with the high metabolic pressure[43]. However, the pathogenesis of this syndrome remains unclear and more studies are needed. The objective of the study was to investigate and report the case of broiler ascites symptom occurred at the age of 4-5 weeks in a local chicken farm. It was aimed to reduce occurrence or outburst of ascites syndrome in commercial broiler chickens.

MATERIALS AND METHODS

Birds and farm management: The trial clinical diagnosis was conducted in a Chinese chicken farm. Before starting the clinical trial, the poultry rearing units were thoroughly cleaned and birds were fed mainly with broiler chick pellet feed plus leafy vegetables and free drinking water in a half of cage-free raising mode.

Equipment and chemical reagents: There were many tools used, such as surgical scissors tweezers, retractors, scalpels, towel forceps, bowel scissors, forceps, enterotomy scissors, normal saline, 3-5% lysol and sodium dihydrogen phosphate solution, etc.

Clinical diagnosis methedology: There were many methods used for the clinical diagnosis of broiler ascites symptom, mainly including the inquiry of feeders and administrators, local observation, detection of farm gas and faeces and pathological autopsy (i.e., ammonia gas).

RESULTS AND DISCUSSION

Description of the case: In total, 2000 broiler chickens raised in a chicken farm were endangered at the age of 27 days and broiler ascites syndrome came to a sudden onset in these chickens. The trial clinical diagnosis and pathological autopsy and subsequent investigation found that the rearing houses were closed and sealed with poor ventilation and a high breeding density and ammonia gas that might caused the broiler ascites symptoms. With the comprehensive management measures and drug treatments, there were finally only 800 broiler chickens found ill and finally came back to normal from illness after the treatments, except for the death of 38 sick chickens.

At first, only a few chicks twitched and suddenly died. Then, the farmer found about one third of these broiler chickens caught diseases and the whole farm was infected as ill. The clinical symptoms of sick chickens were described as follows (Fig. 1). It could be observed and deemed that those sick chickens were depressed with closed eyes and tumbled feathers, moving slowly with two drooping wings and abdominal ptosis, walking abnormality with red crown and purple meat beard and drinking little water and intake reduced feed. Severe sick chicken acted like penguins with accelerated breathing, abdominal enlargement, red skinness and a water bottle belly (Fig. 1). They usually didn't move and squated and finally came to death. Some of the dead chicken had diarrhea phenomenon too.

By clinical observation and local inquiry of the broiler chicken breeder, the inspectors initially inferred this was probably the case of ascites syndrome. In order to find out the cause, the inspectors decided to take samples from those sick and dead chicken died in less than 6 h to the laboratory for further pathologic autopsy. The autopsy symptoms were described as follows (Fig. 1). Through autopsy of the dead chickens, it could be observed that the whole body had obviously extravasated blood, intra-abdominal collections of a large volume of clear ascites or reddish yellow ascites (about 500-600 mL) and the chicken body could be seen in a half of cellophane tape or sample peptone clots (Fig. 1). Those characteristics were frequently observed and reported[2,6-9,18,24,27-29,32,33,38-43]. Furthermore, there were cloudy thickening of pericardium, heart expansion, relaxed ventricular wall with an increased volume and heart cavity hemorrhage, increased pericardial fluid and cardiac enlargement. In fact, the right ventricle hypertrophy expansion was very obvious and there were pulmonary diffuse hyperemia edema and cloudy airbag in the chicken lung. In addition, the liver was in blood swollen stasis in dark purple or red covered with gray and/or yellow hard cellulose membrane and the splenomegaly was color dark. Moreover, there were renal enlargement in moving urate calm, small thin intestine with hemorrhage, chest congestion and subcutaneous edema.

Diagnosis and treatment: Through these trial clinical diagnosis of broiler ascites symptom and pathological autopsy and observation, it came to the definite diagnosis of broiler ascites. Subsequent investigation found that the rearing houses were closed and sealed with poor ventilation and

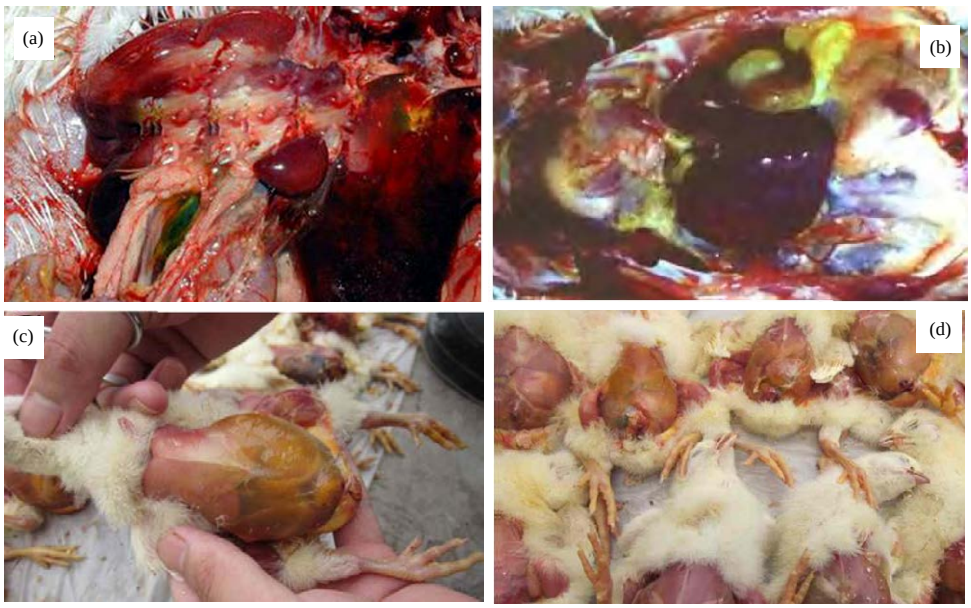

Fig. 1(a-d): Pathological autopsy of sick broiler chickens

a high breeding density and much ammonia gas. There was a very strong smell of ammonia gas in the dry air. By analysis of the farm management of broiler chicken, it was confirmed that those were chicken symptoms of broiler ascites indeed. As the broiler chicken were reared and fed in the winter with low temperature and cold stress and the windows and doors were all shut and ventilated not free in order to maintain the temperature of the farm houses, those all could caused the outburst of broiler ascites syndrome[8,31-33,36-41]. Furthermore, the present chicken breeding density was too high with excess carbon dioxide and too much ammonia gas and dust were shed in the dry air of chicken farming houses, which might cause the seriously lack of fresh air supply and hypoxia. These were all the possible causes of the broiler ascites occurred. The treatment of broiler ascites syndrome was very difficult and there was cold stress caused by relatively low temperatures in the winter. Thus, only a few broiler chickens might recover after growing up, which would bring great economic losses to farmers. In addition, broiler ascites syndrome was also an important cause of other diseases. Therefore, comprehensive measures must be adopted to prevent and control broiler ascites.

Before treatment, sick chickens should be isolated accordingly. Then, corresponding drugs were adopted for chicken treatment[2,32,33,36-41]. Firstly, the large amount of vitamin C (600 mg kg^{-1}) was added to the feed no less than 5-7 days and the usual amount of vitamin C was used again for 1 week after half a month according to the normal application. Secondly, norfloxacin and hydrochlorothiazide were added in the feed and used for three days to prevent secondary disease infection (about 2 times a day and 6-8 mL for one time usage). Thirdly, the feed of sick chickens should be changed immediately to moderate limited feeding or lower energy level feeding. As for the healthy broiler chickens, Chinese herbal medicines and/or their complex soluble powders (main ingredients as *Codonopsis pilosula*, *Astragalus membranaceus*, dried tangerine or orange peel *Citrus reticulata* Blanco, *Salvia miltiorrhiza, Paeonia lactiflora* pall, *Poria cocos*, *Alisma plantago-aquatica* linn and *Magnolia officinalis* rehd, etc.) with vitamin C and/or other vitamins for disease prevention were suggested to be added to the feed. Finally, abdominal puncture could be used for serious sick chickens in the lower abdomen after the tincture of iodine disinfection in order to reduce the influence of abdominal ascites on chicken cardiovascular, liver and kidney.

Then, the broiler chicken feeding regime should be changed at the same time for the broiler chicken in the period of adjusting feed formula with temporarily lower energy level compositions in the diet to reduce the content of fat and grease. Actually, the broiler chicken feeding regime was re-designed to control the growth rate of chicks and it was conducted to reduce future broiler ascites syndrome. Furthermore, tunnel ventilation was carried out in a timely manner to eliminate the harmful gas of inner fecal in time and keep clean air in the farm houses. With these comprehensive management measures and drug treatments, there were only 800 broiler chickens found ill and finally came back to normal from illness after the treatments, except for the death of

38 sick chickens. The appetite and drink of all chickens came to normal gradually and the illness situation was finally under control soon in the farm too.

CONCLUSION

The present study was to investigate and report the case of broiler ascites symptom occurred at the age of 4-5 weeks in a local chicken farm. Through the trial clinical diagnosis of broiler ascites symptoms, pathological autopsy and observation, it came to the definite diagnosis of broiler ascites. Subsequent investigation found that the rearing houses were closed and sealed with poor ventilation and a high breeding density and much ammonia gas. Under comprehensive management, prevention and control measures taken and drug treatments, there were 800 broiler chickens found ill and finally came back to normal after the treatments, except for the death of 38 sick chickens. The appetite and drink of broiler chicken came to normal gradually.

SIGNIFICANT STATEMENTS

In the contemporary society, the intensive selection of broiler chickens for faster growth and better feed efficiency resulted in greater susceptibility to metabolic disorders such as ascites syndrome, which is one of the major causes of mortality and economic loss in broiler industry. Actually, ascites syndrome is currently a serious disease issue for the global chicken industry. Ascites syndrome is frequently found in fast growing broilers including abdominal distention and standing fluid collection in chicken abdomen and it is regarded as one of the most common nutrition metabolic disorders. The study investigated a case of broiler ascites syndrome in local commercial broiler chickens at the age of 4-5 weeks to reduce outburst of ascites syndrome in broiler chickens. Through trial clinical diagnosis of broiler ascites symptoms and pathological autopsy, it came to the definite diagnosis of broiler ascites. Subsequent investigation found that the rearing houses were closed and sealed with poor ventilation and a high breeding density and much ammonia gas. Chinese herbal medicines and/or their complex soluble powders, vitamin C and other drugs were adopted for chicken treatment. Under the comprehensive management and drug treatments, there were 800 ill chickens came back to normal from illness after the treatments, except for the death of 38 sick chickens. The appetite and drink of broiler chicken came to normal gradually.

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REFERENCES

1. Maxwell, M.H. and G.W. Robertson, 1997. 1993 UK broiler ascites survey. World's Poult. Sci. J., 53: 59-60.
2. Baghbanzadeh, A. and E. Decuypere, 2008. Ascites syndrome in broilers: Physiological and nutritional perspectives. Avian Pathol., 37: 117-126.
3. Odom, T.W., B.M. Hargis, C.C. Lopez, M.J. Arce, Y. Ono and G.E. Avila, 1991. Use of electrocardiographic analysis for investigation of ascites syndrome in broiler chickens. Avian Dis., 35: 738-744.
4. Arce, J., M. Berger and C.L. Coello, 1992. Control of ascites syndrome by feed restriction techniques. J. Applied Poult. Res., 1: 1-5.
5. Zhu, L., 1999. Investigation of chicken ascites syndrome caused by furazolidone. China Poult., 21: 16-17.
6. Julian, R.J., 2000. Physiological, management and environmental triggers of the ascites syndrome: A review. Avian Pathol., 29: 519-527.
7. Moghadam, H.K., I. McMillan, J.R. Chambers and R.J. Julian, 2001. Estimation of genetic parameters for ascites syndrome in broiler chickens. Poult. Sci., 80: 844-848.
8. Balog, J.M., B.D. Kidd, W.E. Huff, G.R. Huff, N.C. Rath and N.B. Anthony, 2003. Effect of cold stress on broilers selected for resistance or susceptibility to ascites syndrome. Poult. Sci., 82: 1383-1387.
9. Rajkhowa, T.K., 2004. Clinical and histopathological study of ascites syndrome in chicken from Aizawl, Mizoram. Indian J. Vet. Pathol., 28: 142-143.
10. Li, K., J. Qiao, L. Zhao, S. Dong and D. Ou *et al*., 2006. Increased calcium deposits and decreased Ca^{2+}-ATPase in right ventricular myocardium of ascitic broiler chickens. J. Vet. Med. Ser. A: Physiol. Pathol. Clin. Med., 53: 458-463.
11. Wideman, R.F., M.E. Chapman, K.R. Hamal, O.T. Bowen, A.G. Lorenzoni, G.F. Erf and N.B. Anthony, 2007. An inadequate pulmonary vascular capacity and susceptibility to pulmonary arterial hypertension in broilers. Poult. Sci., 86: 984-998.
12. Druyan, S., A. Ben-David and A. Cahaner, 2007. Development of ascites-resistant and ascites-susceptible broiler lines. Poult. Sci., 86: 811-822.

13. Druyan, S. and A. Cahaner, 2007. Segregation among test-cross progeny suggests that two complementary dominant genes explain the difference between ascites-resistant and ascites-susceptible broiler lines. Poult. Sci., 86: 2295-2300.
14. Druyan, S., A. Shlosberg and A. Cahaner, 2007. Evaluation of growth rate, body weight, heart rate and blood parameters as potential indicators for selection against susceptibility to the ascites syndrome in young broilers. Poult. Sci., 86: 621-629.
15. Druyan, S., Y. Hadad and A. Cahaner, 2008. Growth rate of ascites-resistant versus ascites-susceptible broilers in commercial and experimental lines. Poult. Sci., 87: 904-911.
16. Druyan, S., D. Shinder, A. Shlosberg, A. Cahaner and S. Yahav, 2009. Physiological parameters in broiler lines divergently selected for the incidence of ascites. Poult. Sci., 88: 1984-1990.
17. Olkowski, A.A., 2007. Pathophysiology of heart failure in broiler chickens: Structural, biochemical and molecular characteristics. Poult. Sci., 86: 999-1005.
18. Ozkan, S., C. Takma, S. Yahav, B. Sogut, L. Turkmut, H. Erturun and A. Cahaner, 2010. The effects of feed restriction and ambient temperature on growth and ascites mortality of broilers reared at high altitude. Poult. Sci., 89: 974-985.
19. Pavlidis, H.O., J.M. Balog, L.K. Stamps, J.D. Hughes, Jr., W.E. Huff and N.B. Anthony, 2007. Divergent selection for ascites incidence in chickens. Poult. Sci., 86: 2517-2529.
20. Hassanzadeh, M., J. Buyse and E. Decuypere, 2008. Further evidence for the involvement of anatomical parameters of the cardiopulmonary system in the development of ascites syndrome in broiler chickens. Acta Veterinaria Hungarica, 56: 71-80.
21. Smit, L.D., V. Bruggeman, M. Debonne, J.K. Tona and B. Kamers *et al*., 2008. The effect of nonventilation during early incubation on the embryonic development of chicks of two commercial broiler strains differing in ascites susceptibility. Poult. Sci., 87: 551-560.
22. Closter, A.M., P. van As, M.A.M. Groenen, A.L.J. Vereijken, J.A.M. van Arendonk and H. Bovenhuis, 2009. Genetic and phenotypic relationships between blood gas parameters and ascites-related traits in broilers. Poult. Sci., 88: 483-490.
23. Hamal, K.R., R.F. Wideman, N.B. Anthony and G.F. Erf, 2010. Differential gene expression of proinflammatory chemokines and cytokines in lungs of ascites-resistant and -susceptible broiler chickens following intravenous cellulose microparticle injection. Vet. Immunol. Immunopathol., 133: 250-255.
24. Hassanzadeh, M., M. Maddadi, S. Mirzaie, K. Assasie and H. Moayyedian, 2010. Partial pressure of carbon dioxide in the venous blood of young birds as a predictor of ascites susceptibility in broiler chickens. Acta Veterinaria Hungarica, 58: 221-230.
25. Van As, P., M.G. Elferink, A.M. Closter, A. Vereijken and H. Bovenhuis *et al*., 2010. The use of blood gas parameters to predict ascites susceptibility in juvenile broilers. Poult. Sci., 89: 1684-1691.
26. Kalmar, I.D., A. Cools, J. Buyse, P. Roose and G.P.J. Janssens, 2010. Dietary *N,N*-dimethylglycine supplementation improves nutrient digestibility and attenuates pulmonary hypertension syndrome in broilers. J. Anim. Physiol. Anim. Nutr., 94: e339-e347.
27. Wideman, Jr. R.F., M.L. Eanes, K.R. Hamal and N.B. Anthony, 2010. Pulmonary vascular pressure profiles in broilers selected for susceptibility to pulmonary hypertension syndrome: Age and sex comparisons. Poult. Sci., 89: 1815-1824.
28. Kai, L., L. Zhao, G. Geng, L. Ma and S. Dong *et al*., 2011. Increased calcium deposits and decreased Ca^{2+}-ATPase in erythrocytes of ascitic broiler chickens. Res. Vet. Sci., 90: 468-473.
29. Gupta, A.R., 2011. Ascites syndrome in poultry: A review. World's Poult. Sci. J., 67: 457-468.
30. Tisljar, M., Z. Grabarevic, B. Artukovic, P. Dzaja and S. Cenan *et al*., 2011. The impact of L-NAME and L-arginine chronic toxicity induced lesions on ascites-pulmonary hypertension syndrome development in broiler chickens. Collegium Antropologicum, 35: 547-556.
31. Wang, Y., Y. Guo, N. Dong, Y. Peng and C. Hong *et al*., 2012. Changes of hepatic biochemical parameters and proteomics in broilers with cold-induced ascites. J. Anim. Sci. Biotechnol., Vol. 3. 10.1186/2049-1891-3-41.
32. Kalmar, I.D., D. Vanrompay and G.P.J. Janssens, 2013. Broiler ascites syndrome: Collateral damage from efficient feed to meat conversion. Vet. J., 197: 169-174.
33. Wideman, R.F., D.D. Rhoads, G.F. Erf and N.B. Anthony, 2013. Pulmonary arterial hypertension (ascites syndrome) in broilers: A review. Poult. Sci., 92: 64-83.
34. Saki, A., M. Haghighat and F. Khajali, 2013. Supplemental arginine administered *in ovo* or in the feed reduces the susceptibility of broilers to pulmonary hypertension syndrome. Br. Poult. Sci., 54: 575-580.
35. Zhang, J., X. Feng, L. Zhao, W. Wang, M. Gao, B. Wu and J. Qiao, 2013. Expression of hypoxia-inducible factor 1α mRNA in hearts and lungs of broiler chickens with ascites syndrome induced by excess salt in drinking water. Poult. Sci., 92: 2044-2052.
36. Shen, Y., S. Shi, H. Tong, Y. Guo and J. Zou, 2014. Metabolomics analysis reveals that bile acids and phospholipids contribute to variable responses to low-temperature-induced ascites syndrome. Mol. Biosyst., 10: 1557-1567.
37. Shi, S., Y. Shen, Z. Zhao, Z. Hou and Y. Yang *et al*., 2014. Integrative analysis of transcriptomic and metabolomic profiling of ascites syndrome in broiler chickens induced by low temperature. Mol. Biosyst., 10: 2984-2993.

38. Varmaghany, S., M.A.K. Torshizi, S. Rahimi, H. Lotfollahian and M. Hassanzadeh, 2015. The effects of increasing levels of dietary garlic bulb on growth performance, systolic blood pressure, hematology and ascites syndrome in broiler chickens. Poult. Sci., 94: 1812-1820.
39. Kamely, M., M.A.K. Torshizi and S. Rahimi, 2015. Incidence of ascites syndrome and related hematological response in short-term feed-restricted broilers raised at low ambient temperature. Poult. Sci., 94: 2247-2256.
40. Hassanpour, H., V. Khalaji-Pirbalouty, L. Nasiri, A. Mohebbi and S. Bahadoran, 2015. Oxidant and enzymatic antioxidant status (gene expression and activity) in the brain of chickens with cold-induced pulmonary hypertension. Int. J. Biometeorol., 59: 1615-1621.
41. Hasanpur, K., M.R. Nassiri, G.H. Salekdeh, R.V. Torshizi, A. Pakdel, H. Kermanshahi and M. Naghous, 2016. The suitability of some blood gas and biochemical parameters as diagnostic tools or early indicators of ascites syndrome in broiler sire lines. J. Anim. Physiol. Anim. Nutr., 100: 456-463.
42. Yang, F., H. Cao, Q. Xiao, X. Guo and Y. Zhuang *et al*., 2016. Transcriptome analysis and gene identification in the pulmonary artery of broilers with ascites syndrome. PLoS ONE, Vol. 11. 10.1371/journal.pone.0156045.
43. Mohammadalipour, R., H.R. Rahmani, R. Jahanian, A. Riasi and M.M.N. Nili, 2016. Effect of early feed restriction on physiological responses, performance and ascites incidence in broiler chickens raised in normal or cold environment. Animal, (In Press). 10.1017/S1751731116001555.