Relationship between venous blood gas parameters, thyroid hormone levels and ascites syndrome in broiler chickens exposed to cold temperature

Moayyedian, H.[1]; Asasi, K.[2*]; Nazifi, S.[2]; Hassanzadeh, M.[3] and Ansari-Lari, M.[4]

[1]Ph.D. Student in Avian Medicine, Department of Clinical Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran; [2]Department of Clinical Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran; [3]Department of Clinical Sciences, School of Veterinary Medicine, University of Tehran, Tehran, Iran; [4]Department of Food Hygiene and Public Health, School of Veterinary Medicine, Shiraz University, Shiraz, Iran

[*]**Correspondence:** K. Asasi, Department of Clinical Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran. E-mail: asasi@shirazu.ac.ir

(Received 14 Apr 2010; revised version 9 Oct 2010; accepted 11 Oct 2010)

Summary

The objective of this study was to find a relationship between blood gas parameters and thyroid hormone activity with ascites syndrome in broiler chickens exposed to cold temperature and receiving a high quality diet. One hundred and sixty one-day-old broiler chicks were randomly divided in two equal groups. To increase the incidence of ascites, chicks of group 1 were fed *ad libitum* with a higher condensed diet and reared under a lower ambient temperature. Weekly, blood gas parameters and thyroid hormone levels were measured and growth performance was recorded. The hearts of dead and slaughtered birds were examined for determination of arterial pressure index (API) values. Ascites incidence was clearly higher in cold-exposing chickens compared with normal rearing chickens. The mean values of carbon dioxide tension of group 1 chickens were significantly higher compared with group 2 chickens at the 4th and 5th weeks of age, while the phenomenon of oxygen was reversed in these two experimental groups. The function of thyroid hormone levels were changed at week 4 of age, as observed for blood gas parameters. In conclusion, the present study showed a significant association between thyroid hormones functions, the levels of venous blood carbon dioxide and oxygen pressures in the two groups of chickens.

Key words: Ascites, Blood gases, Broiler chicken, Thyroid hormones

Introduction

Ascites is an important metabolic disorder in the broiler industry (De Smith *et al*., 2005). Ascites syndrome, or water belly, is an increase in the amount of lymph normally found in the peritoneal space (Julian, 1993). This syndrome is a serious economic concern because it results in a loss of broilers and has a negative impact on animal welfare (Pakdel *et al*., 2002).

The etiology of ascites has not been determined yet, but is generally believed to be multifactorial (Shlosberg *et al*., 1992). Decreased oxygen tension or increased oxygen requirements can create hypoxic conditions in tissue, and this has a major impact on pulmonary vasomotor tone which is supposed to lead to pulmonary hypertension (Wideman and French, 2000). The high metabolic demand, together with decreased availability of oxygen, may lead to hypoxia, hypoxemia and anoxia (Scheele *et al*., 1992; Julian, 1993).

The increase in ascites, both at high and low altitude, has been noted to be more marked in cold weather (Huchzermeyer and De Ruyck, 1986; Fitz-Coy and Hartner-Dennis, 1988; Julian, 1993; Julian, 2005). Recent researches show that the increased metabolic rate induced by cold causes a marked increase in oxygen requirement and cardiac output, resulting in pulmonary hypertension (Balog, 2003; Julian, 2005; Hassanzadeh *et al*., 2008).

Thyroid hormone activity for regulating metabolism could become more apparent at different environmental conditions e.g. low ambient temperature (Scheele *et al*., 1992; Decuypere *et al*., 2000; Hassanzadeh *et al*., 2004), at high altitude in broiler chickens (Hassanzadeh *et al*., 2004; Hassanzadeh *et al*., 2005a), and different diet consumption (Decuypere *et al*., 2000; Hassanzadeh *et al*., 2000).

Previous findings showing that ascites coincided with high venous blood carbon dioxide tensions (pCO_2) values in venous blood (Olkowski *et al*., 1999), and because of the causal relationship between pCO_2 in venous blood and the lung ventilation rate (Fedde, 1986). Oxygen is a critical component in energy metabolism. Broilers use energy for thermoregulation, activity and growth, and any form of energy utilization creates a demand for oxygen. Differences in the oxygen requirements between fast-growing and slow-growing lines are associated with the more pronounced occurrence of right ventricular hypertrophy (RVH) and ascites in the fast growing line (Peacock *et al*., 1990). Buys *et al*. (1998) reported that chickens of an ascites sensitive line consumed more oxygen due to high metabolic activity, and hence showed significantly higher pCO_2 and lower oxygen tension (pO_2) in venous blood, compared with an ascites resistant line of birds. Wideman *et al*. (1998) showed that high carbon dioxide tensions in the venous blood of broiler chickens can increase pulmonary arterial pressure. High carbon dioxide tensions in the venous blood of juvenile domestic chickens are a predisposing factor for the development of ascites (Scheele *et al*., 2005).

The purpose of this study was to examine the relationship between the blood gas parameters and thyroid hormones values of broiler chickens and the occurrence of ascites syndrome. The arterial pressure index (API value) as an accurate and distinguishing criterion was used to classify the severity of the ascites syndrome in chickens.

Materials and Methods

Chickens

One hundred and sixty one-day-old (Cobb 500) chicks were obtained from a commercial hatchery. They were wing-banded and divided randomly into two equal groups. Each group was reared on wood shavings litter in separate rooms. All experiments were conducted after institutional approval of the animal use committee of Shiraz University.

Diets and temperature

The birds were housed in a climate-controlled room. A rapidly decreasing ambient temperature regimen was applied in order to increase the incidence of ascites for group 1. Birds of group 1 were fed *ad libitum* with a more condensed ration compared with group 2 (Table 1). At the first day of age, ambient temperature was 33°C for both groups. In group 1, the primary temperature was reduced by 3°C after day 1 and then declined 1°C daily, until it reached 15°C at day 17. In group 2, the primary temperature was reduced according to the standard program up to 21°C and was kept constant until the end of the experiment.

Lightening

Light regime of 24 h of light and darkness was applied for the first 2 days of age. Then, it was changed to 1 h darkness and 23 h of light for the rest of the period. All other rearing conditions were the same for the two experimental groups.

Sampling and tests

Blood samples were collected weekly from 40 birds per group at certain times of the day. Birds were bled from the basilica vein using sterile disposable plastic syringes and needles (21 gauge). Venous blood carbon dioxide tensions (pCO_2) and oxygen tensions (pO_2) were measured as described before (Scheele *et al*., 2005) in all samples with a blood gas analyzer (ABL 5; Radiometer system, Copenhagen, Denmark). Extra blood was used for the determination of thyroid hormones (T_3 and T_4) concentrations. Blood was centrifuged; the plasma was collected and stored at -20°C until they were needed for testing. The triiodothyronine (T_3) and thyroxine (T_4) concentrations were measured by Radio-immunoassay (Decuypere *et al.*, 1994; Hassanzadeh *et al.*, 2004).

Performance parameters and ascites index

Feed intake and body weight gain of birds in both groups were recorded weekly. Dead birds were recorded and necropsied daily. At the end of the experiment all survived chickens were slaughtered and necropsied. The hearts of dead and slaughtered birds were removed and the atria, major vessels and fat were trimmed off. The right ventricle/total ventricle (RV/TV) ratio was determined and classified as reported earlier (Julian, 1987; Hassanzadeh *et al.*, 2000) for determination of arterial pressure index (API) values.

Statistical analysis

Thyroid hormones and blood gas parameters were analyzed by proc-mixed procedure SAS software (SAS, 1998). Multiple comparisons were made by Tukey's multiple range test. For comparison of performance parameters (FCR and FI), their cumulative values at the end of the sixth week were compared using nonparametric Mann-Whitney U test. P-value less than 0.05 was considered statistically significant.

Results

A few birds in group 1 showed ruffled feathers, reluctance to move, and pale shrunken comb at the end of the second week of age. These signs to some extent were accompanied by cyanosis and abdominal distention in severe cases, more in group 1, and continued to develop till the end of the rearing period. Dead affected birds in group 1, in necropsy, showed accumulation of straw-colored fluids, partly with fibrin clot, in abdominal cavities as seen in Fig. 1. Other gross lesions were round and enlarged heart with right side dilatation filled with blood clot and variable liver fatty changes. The cardiac enlargement was due to dilatation of the right atrium, sinus venosus, caudal vena cava, and right ventricle as shown in Fig. 2. Growth curves of the two experimental groups are shown in Fig. 3.

Total ascites mortality and RV/TV ratios

Fig. 1: Abdominal distension with straw-colored ascetic fluid accumulation in an affected five week-old bird in group 1, experienced step down cold ambient temperature and received a condensed ration

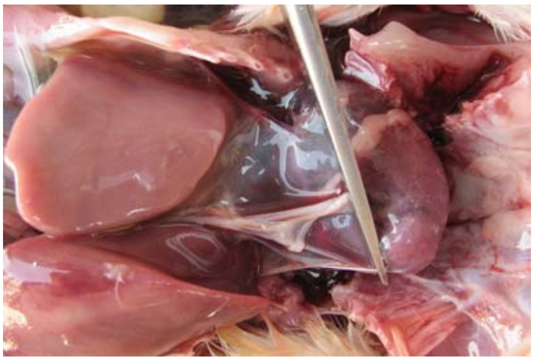

Fig. 2: Gross lesions of a six week-old clinical ascites case in group 1 showing enlarged heart pulled up by pointer to find out distention of vena cava, and progressed fatty changed liver

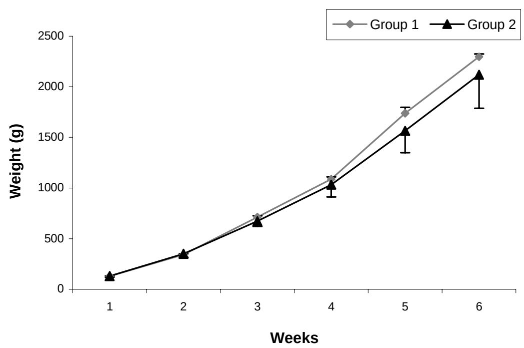

Fig. 3: Comparison of growth curve (means ± SEM) between two experimental groups

of survived birds slaughtered and autopsied at the 6th week of study, are presented in Table 2. Ascites mortality numerically was higher in the cold group compared to birds reared in normal condition (group 2). RV/TV ratio of the survived birds was significantly higher in group 1 compared with group 2 chickens.

Least square means of venous blood gas values in two groups of chickens are shown in Table 3. Values for pCO_2 increased and for pO_2 decreased with increasing of age, especially in the cold group birds. The birds in group 1 showed higher pCO_2 and lower pO_2 values in comparison with those in group 2, but the values were significantly different at the 4th and 5th weeks of age. Table 4 shows weekly mean plasma T_3 and T_4 concentrations. The levels of T_4 were significantly different between the two groups of chickens at the 4th week of age and the levels of T_3 tended to be significant only at the 4th week of age.

Mean body weight, feed intake and feed conversion ratio (FCR) from the 1st to 6th week are shown in Table 5. The difference observed in the mean body weight of the two groups of chickens was significant from week 4 onwards. However, the cumulative feed intake and FCR were not significantly different between the two groups of chickens.

Table 1: Metabolizable energy (ME) [kcal/kg] and crude protein (CP) [%] values in rations of two experimental group chickens

Group	Starter		Grower		Finisher	
	ME	CP	ME	CP	ME	CP
1	3000	20.5	3130	18.7	3200	17.4
2	2860	20.9	2900	19.4	2950	17.7

Table 2: Weekly ascites mortality and right ventricular/total ventricular (RV/TV) ratios of two experimental group chickens that were slaughtered at the end of experiment

Groups/Age	Weekly ascites mortality					Total	RV/TV
	wk 2	wk 3	wk 4	wk 5	wk 6		
1	0	3	4	5	5	17	0.268 + 0.01[a]
2	0	0	0	3	6	9	0.225 + 0.01[b]

*Different superscript letters in a column indicate significant differences at P<0.05. ♦RV/TV = Right ventricle to total ventricles (both ventricles + septum) ratios

Table 3: Least square means of venous blood gas values (mmHg) of two experimental group chickens

Age (week)	pCO_2		pO_2	
	Group 1	Group 2	Group 1	Group 2
1st	44.6 ± 2.05	48 ± 2.27	72.9 ± 2.87	73.1 ± 3.17
2nd	51.1 ± 1.56	53.2 ± 1.61	71.7 ± 2.19	65.2 ± 2.25
3rd	53.9 ± 1.53	53.5 ± 1.51	61.3 ± 2.13	67.8 ± 2.10
4th	58.9 ± 1.56[a]	53.6 ± 1.54[b]	56.4 ± 2.19[a]	64.7 ± 2.16[b]
5th	59.1 ± 1.56[a]	53.6 ± 1.58[b]	53.7 ± 2.19[a]	64.9 ± 2.21[b]

*Different superscript letters in a row of pCO_2 or pO_2 indicate significant differences at P<0.05. ◊Values are means ± SEM

Table 4: Plasma T3 (ng/ml) and T4 (ng/ml) levels of two experimental group chickens

Age (week)	T_3		T_4	
	Group 1	Group 2	Group 1	Group 2
1st	3.67 ± 0.3	3.36 ± 0.4	7.96 ± 1.7	5.78 ± 2.6
2nd	3.08 ± 0.2	2.69 ± 0.8	8.4 ± 1[a]	17.6 ± 5.7[b]
3rd	2.61 ± 0.2	2.81 ± 0.2	8.57 ± 1	7.14 ± 1
4th	2.12 ± 0.2	1.66 ± 0.1	9.11 ± 1[a]	18.4 ± 0.9[b]
5th	1.81 ± 0.1	1.78 ± 0.2	10.9 ± 0.9	6.67 ± 1
6th	1.45 ± 0.2	1.44 ± 0.2	7.6 ± 1.5	6.92 ± 1.6

*Different superscript letters in a row of T_3, T_4, fT_3 or fT_4 indicate significant differences at P<0.05. ◊Values are means ± SEM

Table 5: Body weight (g), feed intake (g) and feed conversion ratio (FCR) of two experimental group chickens

Age (week)	Body weight (g)		Feed Intake (g)		FCR	
	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
1st	130 ± 26.14	130 ± 27.90	88	82	0.98	0.91
2nd	350 ± 25.05	342 ± 25.01	278	255	1.26	1.2
3rd	672 ± 24.94	714 ± 25.01	462	512	1.43	1.38
4th	1032 ± 29.18	1088 ± 28.43	662	675	1.84	1.8
5th	1566 ± 34.06[a]	1738 ± 34.09[b]	1030	1130	1.93	1.74
6th	2050 ± 21.64[a]	2295 ± 23.33[b]	830	990	1.71	1.78
Cumulative	-	-	3350	3644	2.08	1.76

[*]Different superscript letters in a row of body weight or feed intake indicate significant differences at $P<0.05$. [◊]Values are means ± SEM. SEM of feed intake in group 1 and 2 was 143.3 and 166.7, respectively. SEM of FCR in group 1 and 2 were 0.15

Discussion

Ascites syndrome is well-characterized from the epidemiological, clinical, and anatomopathological points of view (Díaz-Cruz *et al*., 2003). Modern broilers have been selected for rapid growth and have experienced an improved growth rate of about 5% a year over 30 years (Julian, 1998). As the presence of ascites can only be confirmed by post-mortem examination (except for severe cases), selection against this disorder has been performed using family information. In recent years, a variety of physiological and anatomical measures have been evaluated as indicators and/or symptom of ascites susceptibility (Hassanzadeh *et al*., 2004, 2005b, 2008; Julian, 2005; Navarro *et al*., 2006).

Genetically, the modern broilers seem to be more prone to develop ascites, which is probably due to selection for growth rate or feed conversion ratio, which puts high demands on metabolic processes and on the oxygen demand (Decuypere *et al*., 2000). Selection of broiler chickens for a low FCR, in fact, could mean a selection for lower lung weights, which may lead to elevated carbon dioxide (CO_2) in blood (Malan *et al*., 2003). It has been shown that the increased metabolic rate induced by cold causes a marked increase in oxygen requirement and cardiac output resulting in pulmonary arterial pressure and a pressure overload on the right ventricle (Julian, 1993). Scheele *et al*. (2003, 2005) studied adaptive metabolic responses to a low ambient temperature, demanding a higher metabolic rate and an increased heat production in populations of chickens differing in growth rate and FCR compared to those at a normal temperature. They found significantly higher values of heart weight and a higher incidence of ascites in broiler chickens which were selected for a high rate of body weight gain and a low FCR, and exposed to low environmental temperature. The authors concluded that exposing birds to a changed environment, demanding a higher metabolic rate and oxygen consumption, may induce cardio pulmonary disturbances, heart failure and ascites.

In the present study, exposing birds to a lower ambient temperature during the post hatching, and imposing a different temperature step down program, seriously influence ascites mortality. So the pellet diet could increase 10-15% metabolism energy alone. The incidence of ascites mortality may be due to receiving a more condensed pellet diet in these groups of chickens.

The unique feature of the present study lies in the possibility of determining the coincident changing in key parameters, which is associated with the development of ascites in fast growing broiler chickens. A strong relationship between the partial pressure of carbon dioxide and ascites susceptibility was previously investigated (Hassanzadeh *et al*., 1997; Buys *et al*., 1998; Wideman *et al*., 1998; Scheele *et al*., 2003; Scheele *et al*., 2005). The present study revealed that cold-exposing chickens, being extremely prone to right ventricular hypertrophy and ascites, were also characterized by the higher pCO_2 values, while in normal conditioning chickens that were supplied a relatively low condensed diet, have a correspondingly lower demand for oxygen and hence exhibited lower venous blood pCO_2 levels, are less involved with ascites, which was confirmed in earlier studies (Scheele *et al*., 2003). This indicates higher oxygen consumption in cold exposing chickens. However, few differences on mean and the variation of pCO_2 values were observed between this study and previous studies, which could be due to the different experimental designs and therefore interaction of environmental factors such as ambient temperature and diet contents.

Age related changes in circulating T_4 and T_3 levels confirm earlier findings (Decuypere *et al*., 1994). The decreased plasma T_4 levels can be explained by a negative feed back of T_3 on the hypothalamus resulting in a decreased thyroid releasing hormone secretion. Scheele *et al*. (1992) pointed out that the birds which were subjected to low environmental temperature showed lower plasma T_4 levels and higher plasma T_3 levels compared with the birds subjected to normal temperature. This was confirmed in our finding; however, the higher tendency for significant effect only appeared at week 4 of age. The lower plasma T_4 and increasing of T_3 at the same time indicate an increased shift in T_4 to T_3 converting, in cold exposing chickens, as most of the biological effects can be attributed to T_3 (Decuypere *et al*., 2000; Hassanzadeh *et al*., 2004).

The negative effect of low environmental temperature on growth rate and FCR has already been reported by Scheele and Frankenhuis (1989). This can be due to the higher metabolic rate and hence higher heat production requiring energy to maintain body temperature in the cold weather (Wideman, 1998). In our study the differences of final growth curves between the cold conditioning and control birds could also be related to a higher incidence of ascites in the cold group compared with the control chickens because ascites cause a significant deterioration in the growth performance of broiler chickens (Julian, 1993; Hassanzadeh *et al*., 2002; Hassanzadeh *et al*., 2004).

In the present study, we were surprised to find a marked association between thyroid hormones functions and the levels of venous blood carbon dioxide and oxygen pressures in the two groups of chickens that are differing in ascites susceptibility. In conclusion, our data indicated that a low pCO_2 and high pO_2 in the venous blood might be a useful symptom for earlier diagnosis of ascites and selection program for broiler chickens. Due to concomitant changing of thyroid hormones activity with blood gas parameters, the function of these hormones should be considered in further selection procedures for meat-type chickens.

Acknowledgement

This research was funded by the Research Committee, School of Veterinary Medicine of Shiraz University, Shiraz, Iran.

References

Balog, JM (2003). Ascites syndrome (pulmonary hypertension syndrome) in broiler chickens: are we seeing the light at the end of the tunnel? Avian Poult. Biol. Rev., 14: 99-126.

Buys, N; Dewil, E; Gonzales, E and Decuypere, E (1998). Different CO_2 levels during incubation interact with hatching time and ascites susceptibility in two broiler lines selected for different growth rate. Avian Pathol., 27: 605-612.

Decuypere, E; Buyse, J and Buys, N (2000). Ascites in broiler chickens: exogenous and endogenous structural and functional causal factors. World's Poult. Sci. J., 56: 367-377.

Decuypere, E; Vega, C; Bartha, T; Buyse, J; Zoons, J and Albers, GAA (1994). Increased sensitivity to triiodothyronine (T_3) of broiler lines with a high susceptibility for ascites. Br. Poult. Sci., 35: 287-297.

De Smith, L; Tona, K; Bruggeman, V; Onagbesan, O; Hassanzadeh, M; Arckens, L and Decuypere, E (2005). Comparison of three lines of broilers differing in ascites susceptibility or growth rate. 2. Egg weight loss, gas pressures, embryonic heat production, and physiological hormone levels. Poult. Sci., 84: 1446-1452.

Díaz-Cruz, A; Serret, M; Ramírez, G; Ávila, E; Guinzberg, R and Piña, E (2003). Prophylactic action of lipoic acid on oxidative stress and growth performance in broilers at risk of developing ascites syndrome. Avian Pathol., 32: 645-653.

Fedde, MR (1986). Respiration. In: Sturkie, PD (Ed). *Avian physiol*. (4th Edn.), Herdelberg, Berlin, Springer-Verlag. PP: 191-221.

Fitz-Coy, SH and Hartner-Dennis, JM (1988). Incidence of ascites in broiler and roaster chickens. Poult. Sci., 67: 87.

Hassanzadeh, M; Bozorgmeri, FMH; Akbari, AR; Buyse, J and Decuypere, E (2000). Effect of intermittent lighting schedules during the natural scotoperiod on T_3-induced ascites in broiler chickens. Avian Pathol., 29: 433-439.

Hassanzadeh, M; Bozorgmeri, FMH; Buyse, J; Bruggeman, V and Decuypere, E (2004). Effect of chronic hypoxia during the embryonic development on the physiological functioning and on hatching parameters related to ascites syndrome in broiler chickens. Avian Pathol., 33: 558-564.

Hassanzadeh, M; Buys, N; Dewil, E; Rahimi, G and Decuypere, E (1997). The prophylactic effect of vitamin C supplementation on broiler ascites incidence and plasma thyroid hormone concentration. Avian Pathol., 26: 33-44.

Hassanzadeh, M; Buyse, J and Decuypere, E (2002). Further evidence for the involvement of cardiac β-adrenergic receptors in right ventricle hypertrophy and ascites in broiler chickens. Avian Pathol., 31: 177-181.

Hassanzadeh, M; Buyse, J and Decuypere, E (2008). Further evidence for the involvement of anatomical parameters of the cardiopulmonary system in the development of ascites syndrome in broiler chickens. Acta Vet. Hungrica. 56: 71-80.

Hassanzadeh, M; Gilanpour, H; Charkkar, S; Buyse, J and Decuypere, E (2005b). Anatomical parameters of cardiopulmonary system in three different lines of chickens: further evidence for involvement in ascites syndrome. Avian. Pathol., 34: 1-6.

Hassanzadeh, M; Shojadoost, B; Feyzih, A; Buyse, J and Decuypere, E (2005a). Effect of intermittent lighting schedules at the young age of broiler chickens on the incidence of ascites and metabolic parameters. Archiv. Für. Geflügelkunde., 69: 57-61.

Huchzermeyer, FW and De Ruyck, AM (1986). Pulmonary hypertension syndrome associated with ascites in broilers. Vet. Rec., 119: 94.

Julian, RJ (1987). The effect of increased sodium in the drinking water on right ventricular hypertrophy, right ventricular failure and ascites in broiler chickens. Avian Pathol., 16: 61-71.

Julian, RJ (1993). Ascites in poultry - Review article. Avian Pathol., 22: 419-454.

Julian, RJ (1998). Physiological, management and environmental triggers of the ascites syndrome. Poult. Int., PP: 28-33.

Julian, RJ (2005). Production and growth related disorders and other metabolic disease of poultry – a review. Vet. J., 169: 350-369.

Malan, DD; Scheele, CW; Buyse, J; Kwakernaak, C; Siebrits, FK; Van Der Klis, JD and Decuypere, E (2003). Metabolic rate and its relationship with ascites in chicken genotypes. Br. Poult. Sci., 44: 309-315.

Navarro, P; Visscher, PM; Chatziplis, D; Koerhuis, ANM and Haley, CS (2006). Genetic parameters for blood oxygen saturation, body weight and breast conformation in 4 meat-type chicken lines. Br. Poult. Sci., 47: 659-670.

Olkowski, AA; Korver, D; Rathgeber, B and Classen, HL (1999). Cardiac index, oxygen delivery, and tissue oxygen extraction in slow and fast growing chickens, and in chickens with heart failure and ascites: a comparative study. Avian Pathol., 28: 137-146.

Pakdel, A; Van Arendonk, JA; Vereijken, AL and Bovenhuis, H (2002). Direct and maternal genetic effects for ascites-related traits in broilers. Poult. Sci., 81: 1273-1279.

Peacock, AJ; Pickettm, CK; Morris, KG and Reeves, JT (1990). Spontaneous hypoxemia and right ventricular hypertrophy in fast growing broiler chickens reared at sea level. Comp. Biochem. Physiol. A., 97: 537-547.

SAS (1998). *Language Guide for Personal Computers*. Version 6. SAS Institute, Inc., Cary, NC.

Scheele, CW; Decuypere, E; Vereijken, PFG and Schreurs, FJ (1992). Ascites in broilers. 2. Disturbances in the hormonal regulation of metabolic rate and fat metabolism. Poult. Sci., 71: 1971-1984.

Scheele, CW and Frankenhuis, MT (1989). Stimulation of the metabolic rate in broilers and the occurrence of metabolic disorders. In: Van der Honing, Y and Close, WH (Eds.), Energy metabolism of farm animals. (Pudoc. Wageningen, The Netherlands). Eur. Assoc. Anim. Prod., 43: 251-255.

Scheele, CW; Van Der Klis, JD; Kwakernaak, C; Buys, N and Decuypere, E (2003). Haematological characteristics predicting susceptibility for ascites. 1. High carbon dioxide tensions in juvenile chickens. Br. Poult. Sci., 44: 476-483.

Scheele, CW; Van Der Klis, JD; Kwakernaak, C; Dekker, RA; Van Middelkoop, JH; Buyse, J and Decuypere, E (2005). Ascites and venous carbon dioxide tension in juvenile chickens of highly selected genotypes and native strains. World's Poult. Sci. J., 61: 113-129.

Shlosberg, A; Zadikov, I; Bendheim, U; Handji, V and Berman, E (1992). The effects of poor ventilation, low temperatures, type of feed and sex of bird on the development of ascites in broilers. Physiopathological Factors. Avian Pathol., 21: 369-382.

Wideman, RFJr (1998). Ascites in poultry. Monsanto Update. 6: 1-7.

Wideman, RFJr and French, H (2000). Ascites resistance of progeny from broiler breeders selected for two generations using chronic unilateral pulmonary artery occlusion. Poult. Sci., 79: 396-401.

Wideman, RFJr; Wing, T; Kirby, YK; Forman, MF; Marson, N; Tackett, CD and Ruiz-Feria, CA (1998). Evaluation of minimally invasive indices for predicting ascites susceptibility in three successive hatches of broilers exposed to cool temperatures. Poult. Sci., 77: 1565-1573.