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Comparison of the Susceptibility of Six Male Broiler Hybrids to Ascites by Using Hematological and Pathological Parameters

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ABSTRACT

This study was conducted to compare susceptibility of six different strains of male broilers to ascites syndrome. In this experiment, 612 one-day-old male broiler chicks from five international hybrids (e.g, Arbor Acres, Cobb 500, Hubbard, Lohmann, and Ross 508) and an Iranian hybrid (Arian) were used. Growth performance, ascites mortality ratio, the ratio of right ventricle to total ventricles weight (RV:TV) in dead and healthy broilers, hematocrit values, serum levels of thyroid hormones (T3, T4) and T3: T4 ratio were studied to evaluate the susceptibility of the birds. The results showed that there were significant differences among the hybrids in ascites susceptibility. Arian and Hubbard were more susceptible, while, Lohmann was more resistant to ascites than the other hybrids. The results of this study showed that RV:TV ratio and the hematocrit value can be sensitive indicators for differentiating hybrids susceptible to ascites from the resistant ones.

Keywords*:* Ascites syndrome, Broiler strains, Mortality, Susceptibility.

INTRODUCTION

Over the last 40 years, genetic improvement for rapid growth and enhancement of feed efficiency has been achieved very effectively in meat-type chickens; but, this progress has faced some problems such as ascites and sudden death syndrome (Hassanzadeh, 2009; Julian, 1998; Olkowski, 2007). Ascites is a metabolic disorder of fast growing meat-type birds, characterized by accumulation of fluid in the abdominal cavity (Balog *et al*., 2003; Julian, 1993). The pathogenesis of ascites is an imbalance between oxygen supply and oxygen need, which causes hypoxemia in chickens (Hassanzadeh, 2009; Luger *et al*., 2001; Olkowski, 2007).

The incidence of ascites is influenced by both environmental and genetic factors (Aftab and Khan, 2005; Balog *et al*., 2003; Decuypere *et al*., 2000). Both of these factors are very important in the occurrence of ascites, but it is very clear that occurrence of ascites is more closely related to genetics characterizations.

There are differences in susceptibility to ascites among various commercial broiler hybrids because each breeding company follows a different selection program (Silversides *et al*., 1997). Madrigal *et al*. (1993) and Buys *et al*. (1993) found no differences between commercial strains in susceptibility to ascites, but Gonzales *et al*. (1998a) did find differences. A strong relationship between thyroid hormones activity and ascites incidence reported

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previously infers that ascites-susceptible broiler chickens have an altered thyroid hormone metabolism (Decuypere *et al*., 2000; Hassanzadeh *et al*., 2000; Scheele *et al*., 2003).

In the current study, differences among six commercial male broiler hybrids with regard to some hematological parameters such as: hematocrit value (Balog *et al*., 2000; Gonzales *et al*., 1999 ; Luger *et al*., 2001; Talebi *et al*., 2005; and Zheng *et al*., 2007), serum levels of thyroid hormones (T3, T4) (Gonzales *et al*., 1999), and pathological parameters (the ratio of the right ventricle to the total ventricles) (Decuypere *et al*., 2000 and Hassanzadeh *et al*., 2004) indicating susceptibility to ascites were investigated.

MATERIALS AND METHODS

Birds and Rearing

The study was performed in the State Animal Science Research Institute in Karaj, Iran, and the Hematological and endocrinological tests were conducted in Endocrine Research Center at Taleghani Hospital in Tehran as well as in Tarbiat Modares University.

The hybrids were reared from 1 to 56 days. One thousand and five hundred 1-day-old broiler chicks from six different hybrids were hatched from eggs of 56-week-old broiler breeders (except Arbor Acres which were 60-week-old). Eggs of each hybrid were collected on the same day, and classified by weight and quality of the egg shell, to exclude the effect of differences in egg weights or shell quality on hatched chick. All the eggs were incubated in the same incubator. After hatching, the chicks were vent-sexed.

Experimental Design

Six hundred and twelve male broiler chicks (102 of each hybrid) were randomly assigned to six treatments (Arian, Arbor Acres, Cobb 500, Hubbard, Lohmann and Ross 508) with six replications of 17 chicks each. Birds were allocated at random to one of the 36 floor pens (3.4×1.01 m) furnished by wood shavings and each pen was equipped with one feeder and a drinker. Feed and water were provided *ad libitum* throughout the experiment. The house temperature was 34ºC initially, and then gradually reduced by 3ºC each week until it reached 21ºC. Lighting schedule during the experimental period provided 23:1 hour of light:dark. All birds received a mash broiler starter diet (2,900 kcal ME kg^{-1}, 20.9% protein) from 1 to 3 weeks. A grower diet (2,900 kcal ME kg^{-1}, 18.2% protein) was fed from 3 to 6 weeks. A finisher diet (2,900 kcal ME kg^{-1}, 16.4% protein) was fed from 6 to 8 weeks. Feed intake and body weight were measured weekly on a pen basis. Feed conversion ratio was calculated from consumed feed and body weight gain.

Hematological and Pathological Measurement

Blood samples were taken at 21, 42, and 56 days from two randomly selected birds per pen. The blood samples were collected from a wing vein. After collecting, the samples were centrifuged (1,000 rpm, 10 minutes) and serum was separated and stored at -20ºC for hormone analyses. Thyroid hormones (T3 and T4) were tested by ELISA. Blood samples from the same birds were taken into heparinized tubes for hematocrit measurement. These birds were weighed and euthanized by cervical dislocation. After necropsy, hearts were removed and weighed, then, the right ventricle was carefully separated from the left ventricle and septum and weighed to calculate the right ventricle to total ventricular ratio (RV:TV) (Gonzales *et al.,* 1998b; Silversides *et al*., 1997).

The average of RV:TV index measured in our experiment was 0.22%, which was far less than the cutoff limits (0.25-0.30 %) reported for ascitic broilers (Julian, 1987;

Cawthon *et al*., 2001) (Tables 4 and 5). Mortality was recorded daily and all dead birds were weighed and necropsied to determine the cause of death. Birds with abdominal ascetic fluid or hydropericardium were diagnosed as having died due to ascites syndrome. The few birds that died from other causes were excluded from the data analyses and, ultimately, the heart of ascitic birds were examined for right ventricular hypertrophy.

Statistical Analysis

Statistical analysis of the data was performed by the general linear model program of SAS using Duncan's multiple rang test to compare treatment means (SAS Institute, 1998). The RV: TV ratio and mortality rate's data were subjected to the Chi square test. The percentage data were subjected to arc-sin transformation prior to analysis of variance.

RESULTS

Performance Parameters

There were significant differences in overall performances among hybrids ($P< 0.05$), except for feed intake measured in grower period. In general, Hubbard hybrid showed the greatest body weight gain and feed intake, while Lohmann had the least weight gain and feed intake (Table 1).

Cobb hybrid showed the greatest body weight gain and feed intake during 1-7 and 1-8 weeks in comparison to the other hybrids. Lohmann hybrid exhibited highest FCR during starter and grower periods indicating poor efficacy of food to gain, and Arian hybrid had the highest FCR during

Table 1. Body weight gain (BWG, g d^{-1}), feed intake (g d^{-1}) and feed conversion ratio (FCR).

	Starter	Grower	Finisher	(1-7) weeks	(1-8) weeks
BWG (g d^{-1})	33.6 ± 2 [1ab]	60.8 ± 4.4 [ab]	68.9 ± 4.9 [b]	51.3± 1.6 [bc]	52.6± 1.4 [bc]
	30.7 ± 3.2 [b]	60.8 ± 4.5 [ab]	66 ± 2.8 [b]	49.2± 2.5 [cd]	50.8± 2.3 [c]
	30.7 ± 0.89 [b]	64.6 ± 5.3 [a]	66.3 ± 3 [b]	51.02± 1.6 [bcd]	52.3± 2.1 [bc]
	31.6 ± 1 [b]	64.2 ± 2.1 [a]	81.4 ± 6.5 [a]	55.2± 2.3 [a]	56.3± 2.04 [a]
	26.6 ± 1.3 [c]	56.3 ± 5.1 [b]	73.2 ± 4.5 [b]	47.4± 2.7 [d]	49.4± 2.3 [c]
	35.7 ± 2.3 [a]	64.3 ± 2.8 [a]	71.1 ± 2.5 [b]	53.9± 2.4 [ab]	55.3± 1.9 [ab]
	**	*	**	**	**
Feed intake (g d^{-1})	44.1 ± 1.7 [ab]	128.3 ± 8.2	189.1 ± 12.5 [c]	83.1± 3.8 [b]	92.5± 4.4 [b]
	41.5 ± 1.9 [bc]	133.7 ± 6.3	205.8 ± 6.2 [bc]	85.7± 7.5 [ab]	98.3± 7.7 [ab]
	41.4 ± 1 [bc]	130.2 ± 6.7	194 ± 14.6 [bc]	85.8± 2.7 [ab]	92.2± 4.3 [b]
	42.4 ± 2 [abc]	128.7 ± 4.2	226.4 ± 10.1 [a]	91.9± 3.4 [a]	100.2± 3.9 [a]
	40.6 ± 3 [c]	131.3 ± 7.6	200.5 ± 11.1 [bc]	81.9± 4.4 [b]	94.8± 2.9 [ab]
	45 ± 2.7 [a]	131.2 ± 6.4	211.3 ± 9.7 [ab]	90.8± 1.6 [a]	99.3± 3.2 [a]
	*	NS	**	**	*
FCR	1.31 ± 0.09 [b]	2.11 ± 0.09 [b]	2.74 ± 0.18 [b]	1.61±0.59	1.75±0.07 [b]
	1.36 ± 0.08 [b]	2.20 ± 0.13 [ab]	3.12 ± 0.15 [a]	1.74±0.18	1.93±0.17 [a]
	1.34 ± 0.04 [b]	2.02 ± 0.15 [b]	2.93 ± 0.20 [ab]	1.68±0.05	1.76±0.04 [b]
	1.34 ± 0.06 [b]	2 ± 0.04 [b]	2.78 ± 0.16 [b]	1.66±0.05	1.77±0.02 [ab]
	1.53 ± 0.09 [a]	2.34 ± 0.20 [a]	2.74 ± 0.24 [b]	1.73±0.11	1.92±0.10[ab]
	1.26 ± 0.06 [b]	2.04 ± 0.07 [b]	2.96 ± 0.09 [ab]	1.68±0.05	1.79±0.03 [ab]
	**	**	**	**	**

[d] Starter= 1 to 3 weeks; Grower= 3 to 6 weeks, Finisher= 6 to 8; 1-7, 1-8 weeks.
[a-c] Means in the same column with no common superscript differ significantly.

finisher period, 1-7 and 1-8 weeks (P< 0.01) (Table 1).

Mortality

The hybrids significantly influenced the mortality rate due to ascites (P< 0.05) (Table 2). The highest mortality rate due to ascites was observed in Arian hybrids (9.80%), while the lowest mortality rate was recorded for Lohmann (0.98 %).

Hematocrit Values

Hematocrit values in male broilers from different hybrids were significantly affected by hybrids at 21, 42, and 56 days (Table 3). The highest and lowest values for hematocrit belonged to Hubbard (32.16%) and Arbor Acres broilers (27.41%), respectively (P< 0.05). Arian (33.33%) and Hubbard (32.83%) broilers showed the highest hematocrit value at 42-day, while Lohmann (26.66%) broilers showed the lowest hematocrit value (P< 0.01). At 56-day, Arian (28.50%) and Hubbard (27.58%) broilers showed the highest hematocrit (P< 0.01).

Right Ventricle to Total Ventricular Ratio (RV:TV)

The ratio of right ventricle to total ventricles weight (RV:TV) in different hybrids are shown in Table 4. At 42-day, the hybrids were significantly different in this ratio (P< 0.01). The highest and the lowest values for RV:TV ratios were observed in Hubbard and Lohmann broilers (0.243, 0.207 respectively) at this age. Hubbard and Arian broilers did not show significant differences at 21 and 56 days, although their RV:TV indexes was greater than other hybrids at 21-day. This observation concurs with the higher mortality from Arian and Hubbard broilers compared to Lohmann broilers (Table 2).

Table 2. Total ascites mortality in different hybrids of male broilers during 8 weeks experiment.

Hybrid	Total ascites mortality (%)[a]	(N)[b]
Arbor Acres	2	1.96
Arian	10	9.8
Ross 508	3	2.94
Cobb 500	3	2.94
Lohmann	1	0.98
Hubbard	7	6.86

[a] Percent of ascites mortality, [b] Number of ascites mortality.

The overall ascites incidence showed that Hubbard and Arian male broilers had 28.5 and 20% ascites cases, respectively, recognized by having RV:TV ratio greater than 0.22%. Interestingly, the Lohmann and Cobb hybrids did not show any ascites cases (Table 5).

Thyroid Hormones

Data on serum thyroid hormones at 21, 42, and 56 days from the six hybrids are

Table 3. Hematocrit value in male broilers of different hybrids at 21, 42 and 56 days of age.

Hybrid	21 days	42 days	56 days
Arbor Acres	27.41 ± 2.88 [c]	30.50 ± 2.30 [ab]	22.75 ± 1.75 [b]
Arian	30.67 ± 2.20 [ab]	33.33 ± 2.42 [a]	28.50 ± 3.40 [a]
Ross 508	30.25 ± 2.33 [abc]	29.50 ± 2.09 [bc]	25.91 ± 4.28 [ab]
Cobb 500	29.20 ± 1.91 [bc]	29.20 ± 1.53 [bc]	26.70 ± 2.13 [ab]
Lohmann	28.91 ± 1.39 [bc]	26.66 ± 2.65 [c]	25.41 ± 2.63 [ab]
Hubbard	32.16 ± 2.42 [a]	32.83 ± 2.65 [a]	27.58 ± 3.29 [ab]
Significance	*	**	NS

[a-c] Means in the same column with no common superscript differ significantly.
Means ± SD, * *P*< 0.05; ** *P*< 0.01, NS: Non Significant.

Table 4. Right ventricle to total ventricular Ratio (RV:TV) in male broilers of different hybrids at 21, 42 and 56d of age.

Hybrid	21 days	42 days	56 days
Arbor Acres	0.226 ± 0.01	0.218 ± 0.01 [ab]	0.206 ± 0.01
Arian	0.232 ± 0.01	0.236 ± 0.01 [ab]	0.238 ± 0.04
Ross 508	0.216 ± 0.02	0.212 ± 0.02 [ab]	0.213 ± 0.02
Cobb 500	0.222 ± 0.02	0.212 ± 0.01 [ab]	0.218 ± 0.01
Lohmann	0.209 ± 0.02	0.207 ± 0.01 [b]	0.212 ± 0.01
Hubbard	0.223 ± 0.01	0.243 ± 0.02 [a]	0.235 ± 0.02

[a-b] Means in the same column with no common superscript differ significantly (P<0.01).

Table 5. Comparison of ascites incidence among different hybrids at 21, 42 and 56 days of age.

Hybrid	sample	21 days	42 days	56 days	Total of three different days
Arbor Acres	18	1/5 (20%)	0/6 (0 %)	0/6 (0 %)	1/17 (5.8%)
Arian	18	0/6 (0%)	2/4 (50%)	1/5 (20%)	3/15 (20%)
Ross 508	18	0/6 (0 %)	0/6 (0 %)	1/5 (20%)	1/17 (5.88%)
Cobb 500	18	0/6 (0 %)	0/6 (0 %)	0/6 (0 %)	0/18 (0 %)
Lohmann	18	0/6 (0 %)	0/6 (0 %)	0/6 (0 %)	0/18 (0 %)
Hubbard	18	0/6 (0 %)	2/4 (50%)	2/4 (50%)	4/14 (28.5%)
Total	108	1/35 (2.85%)	4/32 (12.5%)	4/32 (12.5%)	9/99 (9.09%)

Ascitic sample/ Healthy sample

shown in Tables 6 and 7. At 21 and 42 days, Hubbard broilers had the highest serum thyroxin (T4) concentrations and Lohmann broilers exhibited the lowest serum T4 concentrations, which were significantly different from the others (P< 0.01). In addition, Arian broilers showed the higher T4 concentrations at 21 and 42 days compared to Arbor Acres, Ross, Cobb and Lohmann (Table 6). At 21-day, Lohmann and Arbor Acres hybrids had the highest and the lowest serum triiodo-thyronine ($\mathbf{T_3}$) respectively (Table 7). Arbor Acres, Hubbard and Arian hybrids did not show significant differences in T3 value at 21-day, but Arbor Acres broilers showed the lowest serum T3 value (P< 0.05) at 56-day compared to other hybrids (Table 7).

Serum T3:T4 ratios did not differ in 42, and 56 days, but serum T3:T4 ratios were significantly affected by the hybrids at 21-day (P< 0.01): Lohmann broilers had the highest and Arbor Acres broilers had the lowest T3:T4 ratio compared to the other hybrids (Table 8).

DISCUSSION

The aim of this study was to compare the susceptibility of six male broiler hybrids to ascites syndrome by using hematological

Table 6. Serum T_4 (ng/ml) concentration in male broilers of different hybrids at 21, 42 and 56 days of age.

Hybrid	21 days	42 days	56 days
Arbor Acres	9.95 ± 1.26 [a]	9.29 ± 0.91 [a]	9.64 ± 0.89
Arian	10.34 ± 1.26 [a]	10.15 ± 0.59 [a]	8.64 ± 1.25
Ross 508	8.58 ± 1.12 [ab]	10.42 ± 0.50 [a]	9.77 ± 0.93
Cobb 500	9.74 ± 1.24 [a]	10.38 ± 0.48 [a]	8.39 ± 1.18
Lohmann	7.04 ± 2.10 [b]	7.81 ± 1.24 [b]	10.22 ± 0.67
Hubbard	10.38 ± 1.52 [a]	10.66 ± 0.80 [a]	9.80 ± 1.96

[a-b] Means in the same column with no common superscript differ significantly (P< 0.01).
Means ± SD

Table 7. Serum T3 (ng ml^{-1}) concentration in male broiler of different hybrids at 21, 42 and 56 days of age.

Hybrid	21 days	42 days	56 days
Arbor Acres	1.61 ± 0.63 [c]	1.03 ± 0.27	1.27 ± 0.39 [b]
Arian	2.23 ± 0.63 [abc]	1.16 ± 0.21	1.62 ± 0.18 [ab]
Ross 508	2.39 ± 0.77 [ab]	1.33 ± 0.27	1.78 ± 0.31 [a]
Cobb 500	2.38 ± 0.32 [ab]	1.23 ± 0.21	1.61 ± 0.31 [ab]
Lohmann	2.91 ± 0.29 [a]	0.98 ± 0.18	1.62 ± 0.25 [ab]
Hubbard	2.05 ± 0.50 [bc]	1.12 ± 0.29	1.90 ± 0.36 [a]

[a-c] Means in the same column with no common superscript differ significantly (P< 0.05).
Means ± SD

Table 8. T3:T4 ratio in male broilers of different hybrids at 21, 42 and 56 days of age.

Hybrid	21 days	42 days	56 days
Arbor Acres	0.16 ± 0.07 [b]	0.11 ± 0.02	0.13 ± 0.04
Arian	0.22 ± 0.09 [b]	0.11 ± 0.02	0.19 ± 0.04
Ross 508	0.29 ± 0.11 [ab]	0.12 ± 0.02	0.18 ± 0.03
Cobb 500	0.25 ± 0.06 [b]	0.11 ± 0.01	0.22 ± 0.08
Lohmann	0.45 ± 0.16 [a]	0.13 ± 0.04	0.16 ± 0.03
Hubbard	0.21 ± 0.09 [b]	0.10 ± 0.03	0.19 ± 0.03

[a-b] Means in the same column with no common superscript differ significantly (P< 0.01).
Means ± SD

and pathological parameters under normal conditions. The results showed that the hybrids were significantly different in terms of performance parameters for the periods of starter, grower and finisher, ascites mortality ratio, the hematocrit value (21 and 42 days) and RV:TV ratio at 42-day, serum levels of T3 (21- and 56-d), T4 at 42-day and T3:T4 ratio at 21-day of age (P< 0.01, P< 0.05).

The Arian hybrid, in spite of having the lower growth rate in comparison to the other hybrids, was more susceptible to ascites and showed the highest mortality rate on 1 to 56 days (9.80%). Ascites syndrome is well known as a multifactorial disorder. Although high growth rate is recognized as a major predisposing factor, it is not the sole causative factor (Luger *et al*., 2001). Other factors like genetics and physiological status of birds could account for the observed differences among the studied broiler hybrids as well as accelerated growth rate (Silversides *et al*., 1997).

Therefore, the important role of genetics in ascites susceptibility of Arian hybrid should be emphasized. High mortality rates by ascites syndrome was observed mainly for hybrids of high productivity (Hubbard) during this trial. Worldwide average rate of 4.7% mortality due to ascites in different hybrids of male broilers has been reported by Gonzales *et al*. (1998b) and Hassanzadeh *et al*. (2008). This rate is similar to our finding in the present study (4.3%) for all high productive hybrids.

It is well addressed by others that ascites syndrome is accompanied by increase in hematocrit value (Gonzales *et al*., 1999; Talebi *et al*., 2005 and Zheng *et al*., 2007). In the present study, the birds susceptible to ascites also exhibited an increase in hematocrit levels, which is an adaptive response to hypoxia. The body compensates for the hypoxia status via several mechanisms such as increase in the heart rate, the number of erythrocytes, and the hematocrit value (Silversides *et al*., 1997). Increase in RV: TV ratio is known as ascites index (Hassanzadeh *et al*., 2004; Lorenzoni *et al*., 2008; Olkowski, 2007).

In general, it has been accepted that an RV: TV index greater than 0.25 is indicative of right ventricular hypertrophy in dead birds (Gonzales *et al*., 1998; Silversides *et*

al., 1997). The RV: TV ratio of dead birds was high and exceeded 0.25, which indicates the occurrence of right ventricle hypertrophy (Data not shown).

However, it is known that thyroid hormones change with age (Gonzales *et al*., 1999; Hassanzadeh *et al*., 2004). Thyroid hormones regulate the metabolic rate in poultry (Gonzales *et al*., 1999). The T3:T4 ratio seems to be a sensitive criterion of ascites prediction, however, its sensitivity is limited to young birds (i.e. 21 day). In general, in the first half of the production period the higher the T3:T4 ratio, the lower the ascites susceptibility.

A higher metabolic rate is associated with increased secretion of the T4, which is de-iodinated to T3 in the peripheral tissues, mainly in the liver and kidneys (Luger *et al*., 2001). Selection for diverse productive parameters has also induced endocrine changes, more particularly, at the level of thyroid hormones.

These results are in agreement with the previous study of Gonzales *et al*. (1999), who found that T3:T4 ratios were low in ascitic hybrids.

Additionally, as birds develop ascites, they begin to exhibit specific hematological alterations. These changes occur prior to gross physical changes and can be used as early markers that indicate a bird or a group of birds is in the process of developing ascites syndrome (Balog *et al*., 2000).

In conclusion, differences in mortality, right ventricle hypertrophy, thyroid hormones especially serum T3:T4 ratio measured at 21 day, and hematocrit value confirmed that there were differences among the six studied broiler hybrids in susceptibility to ascites. Based on our results, Hubbard and Arian hybrids were recognized as the most susceptible and Lohmann was the most resistant hybrid to ascites, with regards to RV:TV ratio and hematocrit values. Therefore, the possibility of using these parameters to predict development of ascites syndrome in broilers is considered.

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مقایسه حساسیت شش هیبرید جوجه نر گوشتی به سندرم آسیت توسط پارامترهای فیزیولوژیکی و پاتولوژیکی

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چکیده

مطالعه ای برای مقایسه حساسیت شش هیبرید جوجه نر گوشتی به سندرم آسیت انجام شد. در این آزمایش تعداد ۶۱۲ قطعه جوجه نرگوشتی از ۵ هیبرید خارجی (آربورایکرز، کاب۵۰۰، هوبارد، لوهمن و راس ۵۰۸) و ۱ هیبرید ایرانی (آرین) استفاده شد. عملکرد رشد، نسبت تلفات آسیت، نسبت وزن بطن راست به وزن مجموع دو بطن در جوجه های گوشتی تلف شده و سالم ، میزان هماتوکریت خون ، سطوح هورمون های تیروئید سرم خون (T_3 ، T_4) و نسبت T3 به T4 مورد مطالعه قرار گرفت. نتایج نشانگر اختلاف معنی دار بین هیبریدها در میزان حساسیت به آسیت بود. در مقایسه بین هیبریدها آرین و هوبارد مستعد و لوهمن مقاوم به آسیت بودند. بنابراین میزان هماتوکریت خون، نسبت وزن بطن راست به وزن مجموع دو بطن از شاخص های مهم تشخیص در هیبریدهای مستعد نسبت به هیبریدهای مقاوم به آسیت می باشند.