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An Integrated Approach to Evaluating Inherited Predictors of Resistance to Pulmonary Hypertension Syndrome (Ascites) in Fast Growing Broiler Chickens

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BARD – US-2736-96 - Abstract of Final Scientific Report

Background

PHS (pulmonary hypertension syndrome, ascites syndrome) is a serious cause of loss in the broiler industry, and is a prime example of an undesirable side effect of successful genetic development that may be deleteriously manifested by factors in the environment of growing broilers. Basically, continuous and pinpointed selection for rapid growth in broilers has led to higher oxygen demand and consequently to more frequent manifestation of an inherent potential cardiopulmonary incapability to sufficiently oxygenate the arterial blood. The multifaceted causes and modifiers of PHS make research into finding solutions to the syndrome a complex and multithreaded challenge. This research used several directions to better understand the development of PHS and to probe possible means of achieving a goal of monitoring and increasing resistance to the syndrome.

Research Objectives.

(1) To evaluate the growth dynamics of individuals within breeding stocks and their correlation with individual susceptibility or resistance to PHS; (2) To compile data on diagnostic indices found in this work to be predictive for PHS, during exposure to experimental protocols known to trigger PHS; (3) To conduct detailed physiological evaluations of cardiopulmonary function in broilers; (4) To compile data on growth dynamics and other diagnostic indices in existing lines selected for susceptibility or resistance to PHS; (5) To integrate growth dynamics and other diagnostic data within appropriate statistical procedures to provide geneticists with predictive indices that characterize resistance or susceptibility to PHS.

Revisions

In the first year, the US team acquired the costly *Peckcode weigh platform / individual bird I.D. system* that was to provide the continuous (several times each day), automated weighing of birds, for a comprehensive monitoring of growth dynamics. However, data generated were found to be inaccurate and irreproducible, so making its use implausible. Henceforth, weighing was manual, this highly labor intensive work precluding some of the original objectives of using such a strategy of growth dynamics in selection procedures involving thousands of birds.

Major conclusions, solutions, achievements

1. Healthy broilers were found to have greater oscillations in growth velocity and acceleration than PHS susceptible birds. This proved the scientific validity of our original hypothesis that such differences occur.
2. Growth rate in the first week is higher in PHS-susceptible than in PHS-resistant chicks. Artificial neural network accurately distinguished differences between the two groups based on growth patterns in this period.
3. In the US, the unilateral pulmonary occlusion technique was used in collaboration with a major broiler breeding company to create a commercial broiler line that is highly resistant to PHS induced by fast growth and low ambient temperatures.
4. In Israel, lines were obtained by genetic selection on PHS mortality after cold exposure in a dam-line population comprising of 85 sire families. The wide range of PHS incidence per family (0-50%), high heritability (about 0.6), and the results in cold challenged progeny, suggested a highly effective and relatively easy means for selection for PHS resistance
5. The best minimally-invasive diagnostic indices for prediction of PHS resistance were found to be oximetry, hematocrit values, heart rate and electrocardiographic (ECG) lead II waves. Some differences in results were found between the US and Israeli teams, probably reflecting genetic differences in the broiler strains used in the two countries. For instance the US team found the S wave amplitude to predict PHS susceptibility well, whereas the Israeli team found the P wave amplitude to be a better valid predictor.
6. Comprehensive physiological studies further increased knowledge on the development of PHS – cardiopulmonary characteristics of pre-ascitic birds, pulmonary arterial wedge pressures, hypotension/kidney response, pulmonary hemodynamic responses to vasoactive mediators were all examined in depth.

Implications, scientific and agricultural

Substantial progress has been made in understanding the genetic and environmental factors involved in PHS, and their interaction. The two teams each successfully developed different selection programs, by surgical means and by divergent selection under cold challenge. Monitoring of the progress and success of the programs was done by using the in-depth estimations that this research engendered on the reliability and value of non-invasive predictive parameters. These findings helped corroborate the validity of practical means to improve PHS resistance by research-based programs of selection.

DETAILED DESCRIPTION OF THE RESEARCH ACTIVITIES AND RESULTS

Introduction

Experiments conducted

The Israeli team conducted four comprehensive experiments specific to the BARD project in the third year and continued statistical evaluations of some of the data from the second year.

The research concentrated this year on four main topics: -

- Development of two selected genetic lines, resistant and susceptible to the ascites syndrome (AS), and utilizing them to determine physiological parameters associated with the genetic difference between AS-resistant and AS-susceptible broilers.
- Study of blood oxygenation in broilers developing AS.
- Analysis of normal and abnormal electrocardiography (ECG) in broilers prior to and during the progression of AS.
- Examination of blood parameters that may help determine the instigation of the AS, and that could be integrated into an AS prediction protocol.

Manuscripts of works from Year 2 are referred to in the Final Report of the P.I., Dr Wideman, in Sections 18, 19 and 20 on pages 29-34, and the full text appears in the Appendix A to his report.

GENETIC ASPECTS OF THE ASCITES SYNDROME IN BROILERS – SUMMARY AND CONCLUSIONS

There has been some reported evidence for genetic variation in broilers' propensity to develop AS. If specific parameters to predict AS resistance are sought in order to assess their worth as selection criteria in breeding broilers, it is vital to estimate the heritability of these parameters and their genetic correlation to consequent development of AS under ascites-inducing conditions. These genetic parameters were estimated in the second year of the project in two controlled-climate trials. Heritability estimates of all traits in almost all ages were significant, ranging mostly between 0.4 to 0.8. Percentage of AS per family (%AS), combined from the two trials, ranged from 0 to 92%; it was significantly correlated ($r \cong 0.5$) between the first and second trial, indicating the existence of genetic variation. The heritability (0.4 and 0.7) of the RV/TV ratio, a trait highly correlated (0.53 and 0.73) with %AS, also indicated the existence of genetic variation. Significant negative genetic correlations were found between %AS and oxygen saturation of hemoglobin in the arterial blood (SaO_2) and heart rate at most ages ($r = -0.43$ to -0.56). The results suggest that although all traits measured are significantly heritable, only SaO_2 and heart rate are likely to be early predictors for development of AS.

In order to verify the genetic findings and the conclusions of the project's second year, and confirm their practical relevance, a large-scale selection trial under farm conditions was conducted in the third year. The incidence of AS per family (%AS) was used as the selection criterion, rather than the suggested physiological measurements because their potential usefulness is difficult to verify in a trial of this composition and magnitude. A large population of broiler stock dam-lines, comprising 85 sire families, each with about 75 progeny, was exposed to cold conditions on a commercial broiler farm. The observed variation in %AS ranged from 0 to 50%, much wider than could be expected by chance alone. The wide variation, and the high heritability (about 0.6) estimated under these conditions, confirmed the findings of the second year, and suggested that successful selection could be applied.

Being in the project's last year, it was impossible to determine the response to the selection by running a whole selection cycle. Rather, the parents of five families with zero or very low %AS were re-mated to produce a group of AS-resistant (AS-R) broilers. Similarly, the parents of five families with very high or highest %AS were re-mated and produced a

group of AS-susceptible (AS-S) broilers. These matings were repeated twice, and the male progeny were used in two independent trials under AS-inducing conditions (individual cages and medium-to-low ambient temperatures). With regards to %AS, the results fully confirmed the high estimated heritability, with a highly significant difference in %AS between the additional progeny of selected parents. In the AS-S group, 54% died of AS, 15% were ill (seemingly healthy but with necropsy manifestations of AS), and only 31% of the chicks remained healthy by the end of the trials. In contrast, in the AS-R group only 8% died of AS, 14% were ill and about 78% were healthy.

The AS-S and AS-R birds were used also to confirm previous conclusions regarding the physiological measurements. As suggested by the results of the previous year, level of SaO_2 were lower in the AS-S chicks and they exhibited higher heart rates, both measured prior to the cold exposure, but the results were not very consistent. In both trials with the selected groups, the AS-S P wave amplitude was much larger in the AS-S chicks than in the AS-R ones, even at a very early age. However, due to low repeatability of most physiological parameters, and the difficulties associated with their measurement, it is concluded that selection on actual %AS under AS-inducing conditions is the most efficient way to genetically reduce broilers' tendency to develop AS. Moreover, the AS-S and AS-R lines produced in this research, and being maintained by us, will facilitate a more reliable and sensitive research in the future, aimed at a better understanding of the physiological basis behind the established genetic difference in %AS. This research may eventually lead to the identification of a reliable measurement to be used as a criterion for indirect selection against AS. So far, the only such simple measurement is BW gain during the first week, which was significantly higher in the AS-S chicks. However, potential effects of such selection on final BW must be carefully evaluated.

NORMAL AND ABNORMAL BLOOD OXYGENATION IN BROILERS

DEVELOPING THE ASCITES SYNDROME - SUMMARY AND CONCLUSIONS

Upon completion of external respiration, oxygen is transported via the cardiovascular system to the tissues where internal respiration removes oxygen from the hemoglobin (Hb) and transports it to the cells for cellular respiration. A sufficiently high oxygen transport in arterial blood is vital for essential cell and tissue functioning. Birds developing AS gradually develop an hypoxemia that is probably the critical factor initiating a chain of pathophysiological events ultimately leading to the death of the broiler. The body reacts to the hypoxemia by a compensatory rise in hematocrit (HCT) and thence Hb and a consequently greater oxygen carrying capacity of the blood, but these changes are usually not sufficient or not rapid enough to stop the initiation of secondary lethal changes. It is clear that much AS research should utilize oximetry, a non-invasive means of measuring SaO_2 , the oxygen saturation of the Hb in the arterial blood, as an excellent biomarker for the health status of the bird with regard to AS development, and this has been a basis for the present research.

There has been an hypothesis put forward by some AS researchers that susceptibility of individual birds to AS might be manifested in the first few days after hatch due to poor hatchery management. The development of the embryonic lungs could be deleteriously affected, leading to dysfunction in full oxygenation of the blood and consequently AS. Early mortality would be recorded with pathological changes seen with typical AS as noted in much older birds. We examined several hundred birds "cull" birds from 3 random hatches of Anak broilers, the strain used in this research, on day of hatch, by ECG and subsequent necropsy. No abnormalities in ECG amplitudes, duration or arrhythmias were recorded, nor were pathological changes, as reported by others, seen in any birds in any of the hatches. In subsequent trials, we examined the SaO_2 values of hatches, and no reduced levels were found in any birds in the first 2 wks. These data might indicate a lesser sensitivity of Anak broilers to the type of inherited abnormality that might precipitate AS in birds at an early age, but as AS is infrequently recorded before about 25 d of age in Israel in any strain of broiler, it would seem that such early AS mortality, possibly due to poor hatchery husbandry, is not seen in Israel.

Despite the absence of early manifestation of AS in broiler chicks in Israel, it is still clear that changes in the environment of chicks can predispose them to a later development of AS, starting at around the "normal" age of > 21 d; there may also be inherited characteristics that develop phenotypically at an early age, inducing changes that are manifested days or

weeks later. As a reduced SaO_2 value is one of the earliest, and most important, manifestations of incipient AS, it is therefore important to measure this at an early age. Our studies in this research have been the first to measure SaO_2 levels in birds as young as several hours after hatch. The total oxygen content (TOC) of blood is a function of the amount of Hb (measured spectrophotometrically), the oxygen saturation (SaO_2 values) and the oxygen carrying capacity of Hb (a constant for each type of Hb) and is the optimal means of estimating the potential of oxygen transport in the arterial blood, and was used for the first time, in this research, to try and understand the dynamics of AS development..

Previous studies by all AS research groups, including ours, have examined SaO_2 in broilers only aged 21 days and older. A study in Year 2 monitored the TOC of broilers of 150 broilers on Days 1, 10, 16, 25, 35 and just before slaughter at Day 44, in birds kept under normal (NT) and under ascites-inducing cold (CT) conditions. High levels of saturation (means of about 95%) were found at all days up to and including Day 16 in both no-AS and AS birds. Hypoxemia (level of saturation of <85%) was recorded only 3 times up to and including Day 25 (one bird on Day 16 and 25, and another bird on Day 25). However, by Day 35, 25 % of the normal temperature (NT) birds and 47 % of the cold temperature (CT) birds showed such hypoxemia, and by Day 42, 17 % and 42 % of NT and CT birds were hypoxemic. AS was recorded in 4 % and 42 % of NT and CT broilers respectively. Both HCT and Hb levels rose in CT birds, but this was manifested only in the AS CT subgroup of birds. There were no significant changes in total oxygen content (TOC) of the blood between no-AS NT and AS CT groups, whereas no-AS CT had elevated TOC. These results showed that despite the lowering of SaO_2 , compensatory elevations in HCT and Hb effectively restored TOC to normal levels, but that this was still not sufficiently early to prevent AS. It was evident, as we had hypothesized years ago with HCT, that a sub-group of the birds exposed to the severe stress of the cold were not evidently affected at all, as manifested by values of SaO_2 levels, Hb, HCT and body weight no different from NT birds. This resistance to stress might be a phenotype of a genetic ability to counteract environmental stresses including diseases, and these parameters could act as biomarkers for stress resistance in a genetic research plan to produce stress competent birds.

In the final year, 2 trials were initiated to study blood oxygenation in relatively susceptible and resistant populations of the same genetic stock. Significantly higher SaO_2 levels were found in AS-resistant birds compared with AS-susceptible birds, in one trial as early as at 14 d of age, and at 27/28 d and thereafter. The finding in Trial 2 of reduced SaO_2 levels in AS-susceptible birds before cold exposure, albeit also within the range of normal, could indicate a genetic predisposition. HCT values in AS-susceptible birds were generally higher

than in the AS-resistant birds, also at 14 d of age in the only trial (Trial 3) in which it was measured at this age; this could also indicate an inherent influence. Hb levels, as would be suspected from HCT values, were higher in AS-susceptible birds than in the AS-resistant birds in both trials. TOC levels in Trial 2 were similar in the 2 groups at Day 27, indicating successful compensation of the reduced SaO₂ levels. However, in Trial 3 the TOC was lower in AS-susceptible birds compared with the AS-resistant birds, despite being higher at Day 14; here it would seem that compensatory changes were insufficient to correct the oxygen deficit.

The heart rate has been found to be a possible biomarker for developing AS, but it is inconsistent and therefore unreliable in individual birds. A stable, relatively high heart rate has been regarded by us to be a positive parameter for AS resistance. In Trials 2 and 3 the AS-susceptible birds generally had a lower heart rate, consistent with this hypothesis, but most interestingly, they had significantly higher heart rate in both Trials at Day 1; this could be possibly a better predictive parameter for selection of families.

A study was planned (Trial 4) to compare blood biochemical values in cold exposed birds grouped into "high" and "low" levels of oxygen saturation of arterial blood in order to better understand differences between birds starting to develop AS and those apparently resistant. The high saturation group (HSG) did not differ from the low saturation group (LSG) with regard to HCT or respiration rate, but the heart rate in the LSG was generally lower. The RV/TV was considerably higher in the LSG, indicating severe compensatory changes in the heart muscle that were barely manifested clinically. There were no differences in the relative lung weight or volume, nor in the % of fluid in the lungs, suggesting that lung edema was not developed. With regard to the findings of the Critical Care Analyzer for automatic analysis of the blood, it was planned that these results would be critical in evaluation of physiological differences in blood chemistry between the groups. However, as the instrument could not be bought within the framework of the research, it was on loan from the manufacturer, and we could only perform a small scale study, that limited the data for statistical analyses. Such work should be more deeply investigated in future works. No differences between the HSG and the LSG were found in ventricular blood for pH or TOC, whereas pCO₂ was higher in the LSG birds in both ventricles; this is probably a manifestation of both poorer circulation in the tissues and less ridding of CO₂ from the lungs. pO₂ was lower in the LSG birds in only the right ventricle. This was surprising, that by definition the arterial blood should have a lower pO₂ value, indeed the right ventricular value (venous blood) should be dependent upon arterial blood saturation. Most other parameters are calculated from these values. Other values such as HCT and Hb show that the LSG birds were starting to develop AS.

It can be concluded that AS research should continue to utilize oximetry, a non-invasive means of measuring SaO_2 , the oxygen saturation of the Hb in the arterial blood, as an excellent biomarker for the progressive health status of the bird at all ages with regard to AS development. However, SaO_2 levels in young chicks cannot serve as a predictor for AS susceptibility. Bradycardia is frequently associated with birds developing AS, but cannot be reliably predictive; however, the relative tachycardia in AS – susceptible birds on the day after hatch deserves further study. Studies using the Critical Care Analyzer for automatic analysis of physiological parameters of the blood should also be further conducted.

NORMAL AND ABNORMAL ELECTROCARDIOGRAPHY (ECG) IN BROILERS DEVELOPING THE ASCITES SYNDROME - SUMMARY AND CONCLUSIONS

Development of AS in broilers is associated with concomitant biochemical, physiological and pathological changes in the cardiovascular system. As many of these changes are difficult to monitor non-invasively and by methods that themselves will not induce changes, it is impossible to monitor individual birds to follow the exact sequence of development of these changes. Therefore, it is often difficult to prove which manifestations are primary and which are secondary. Electrocardiography (ECG) is a virtually non-invasive, easily performed monitor of electrical activity, and thence a measurement of function, of the heart, a vital part of cardiovascular function. ECG has been used for some years by several AS research groups to monitor and sometimes to predict AS susceptibility.

A slower heart rate (bradycardia) has also been found in birds developing AS. Bradycardia has been recorded previously by this group in broilers developing AS and in birds cold-exposed for very short periods. In the present research, this change was seen as early as 15 d, concomitant with consequent AS mortality at a relatively early age. This bradycardia was confined to AS CT birds, and birds similarly kept at CT but not developing AS had a normal PR wave duration. It would appear that this change is induced by the cold, rather rapidly, indicating that it is largely a primary change.

Of much importance has been the finding that an increased S wave amplitude in standard limb lead II indicates increased susceptibility to AS. Such changes have been found abroad in one trial in the first week of age, purportedly due to poor hatchery conditions. In the present research, S wave amplitude was generally elevated in AS birds, i.e. indicative of birds developing AS, rather than being predictive and is probably an expression of right ventricular hypertrophy. In lead II the P wave represent the depolarization of the atria, causing contraction and ejection of their complement of blood into the ventricles, and is positive in broilers. With right atrial hypertrophy the P wave can become tall and peaked (P pulmonal), and with left atrial hypertrophy, the P wave can become wide (P mitreal); a tall wide P wave is suggestive of bi-atrial enlargement. In this research, AS caused consistent increases in P wave amplitude, possibly indicating right atrial hypertrophy. P wave duration changes were less marked and may be indicative of possible left atrial changes. In 4 large scale trials with many birds in this research it can be seen that the P wave amplitude can give a reliable prediction of AS susceptibility. In one trial in Year 2, the statistically higher P wave

amplitude in birds later developing AS could be seen as early as Day 6 (these birds were later exposed to cold temperatures and began to develop the syndrome after Day 21), and also on 3 subsequent measurements. In Year 3, with an entirely different population, the P wave was significantly higher in the AS - susceptible group than in the AS - resistant group at all 3 measurements, before and after the cold exposure, on Days 1, 7 and 21. In the replicate trial in Year 3, two measurements made before cold exposure, on Days 1 and 14, and one measurement on Day 30, all revealed significantly higher P wave amplitudes in the AS - susceptible group. It can be concluded that the P wave amplitude appears to be an excellent predictor of AS even as early as Day 1.

The P wave duration is a less consistent predictor of AS. In Year 2, a significant difference was observed only on Day 35 when the AS birds had higher values – by that time AS was already well developed in many birds. In contrast, in Year 3, it was noted that the P wave duration was higher in the AS - susceptible group mainly before the cold temperature exposure and development of the syndrome. In one trial in Year 3, the P wave duration in the AS - susceptible group was significantly higher than in the AS - resistant group on Day 7, but not on Days 1 and 21. In the replicate trial in Year 3, this parameter in the AS - susceptible group was significantly higher than in the AS - resistant group Day 1 and 30, but not on Day 14.

These studies show that ECG changes may indicate both ventricular and atrial abnormalities in AS birds, prior to and during AS development, and that a previously unreported change in AS, an elevation in P wave amplitude, can be a practical tool to aid in selection procedures for increased AS resistance in a population.

AS experiments conducted

Trial 1

A large pedigreed population from a fast-growing commercial broiler dam-line was severely cold stressed under farm conditions in order to select families susceptible or resistant to AS.

Trial 2

The parents of the families with lowest or highest incidence of AS in Trial 1 were re-mated and produced, respectively, AS-resistant and AS-susceptible chicks for a detailed physiological study under controlled conditions of AS-inducing cold exposure.

Trial 3

The parents of the chicks studied in Trial 2 were mated again and produced additional batch of AS-resistant and AS-susceptible chicks for a second detailed physiological study under the same controlled conditions of AS-inducing cold exposure.

Trial 4

Commercial male broilers of the same strain were maintained controlled conditions of AS-inducing cold exposure to investigate physiological blood parameters involved in the development of AS.

Trial 5 (from 1999)

Serum samples were examined after the submission of the Annual Report for the second year for levels of T_3 (triiodothyronine) and T_4 (thyroxine) to follow the changes in T_3 and T_4 levels with time, and whether different patterns of change are seen with exposure to different temperatures and with the different parameters associated with AS and the progression to AS.

Trial 1

Materials and methods

A pedigreed population was produced by parents from a commercial dam-line of ANAK Breeders Ltd (Netanya, Israel). Each of 85 males was mated with 15 females to produce half-sib sire families. About 7,000 chicks hatched on February 24, 2000; their number per family ranged from 60 to 110, with an average of 82. At the hatchery, chicks were sexed and individually identified by plastic tags (with both numbers and barcodes) attached to the skin of the neck. These tags facilitated the recording of pedigree and sex of each chick. The trial was conducted on a commercial farm; chicks from all families were reared together on litter in an open-sided house with curtains, under standard management. The standard vaccination regimen used for broilers in Israel was applied: NDV (Newcastle disease virus) and IBV (infectious bronchitis virus) vaccines were sprayed at day of hatch, NDV and IBDV (infectious bursal disease virus) vaccines were injected intramuscularly on Day 12, and NDV vaccination was administered by fogging on Day 20.

The altitude (about 500 m above sea level) of the farm, the type of housing (open-sided house with curtains), and the season (winter), were all chosen on purpose, to assure a cold challenge during the growth period. Indeed, already at brooding time during the first two weeks, ambient temperatures (AT) in the heated house dropped at nights below 30 °C. During most of the 3rd week, AT ranged between 20 to 24 °C. The AT continued to decrease, ranging most of the time between 18 to 22 °C in the fourth week and between 15 to 20 °C in the fifth week and thereafter. The AT data clearly indicate that, as intended, the chicks in Trial 1 were exposed to AS-inducing cold conditions.

Mortality during the first two weeks reflected random variation in chick quality and some vaccination mismanagement, hence dead chicks were recorded only from 2 to 7 wk of age. These were necropsied and diagnosed at the Jerusalem Regional Poultry Disease Laboratory to determine the cause of death i.e., due to ascites or other causes (infectious diseases etc.). All birds with ascitic fluid or hydropericardium were deemed to have died from AS. Birds dying from other causes were excluded from subsequent statistical analyses. Hearts were removed from all the dead birds, the weight of the right ventricle (RV) and of both ventricles (total ventricle, TV) were determined, and consequently the RV/TV ratio was calculated for each bird, as an indicator of right ventricular hypertrophy. Dead birds with $RV/TV > 0.38$ were also deemed to have died from AS. The age and cause of death were recorded for all 2-to-7 wk mortality. After the exclusion of early mortality (0-to-2 wk), the

population under study in this trial consisted of about 6,200 chicks; the average number per family was 75 chicks, with a range of 55 to 105.

A random sample of 12 chicks per family were individually weighed on Day 10. The manual weighing was expedited by identification of the barcode tags with a barcode reader/data logger (Symbol PDT 3100, Symbol Technologies Inc., Bohemia NY, USA) connected to a scientific balance (Sartorius BP 6100, Sartorius AG, Goettingen, Germany); the data from the logger was transferred to JMP (SAS Institute, 2000) software files on a computer for statistical analysis. The weighing was repeated on the same chicks on Days 17, 28 and 48.

At the end of the trial, at 7 wk of age, AS incidence (proportion of birds died of AS) was calculated for each family. In order to verify the presence of genetic variation among families, the hypothesis that differences between families are of random nature had to be rejected. To test this hypothesis, the function of random binomial distribution was applied, using the proportion of AS in the entire population (17.5%), to "mark" each one of the 6200 chicks in the data file as AS-dead or healthy. Then, incidence of this "random AS" was calculate per family, and the distribution and variation of these values, were compared to those of the real incidence of AS per family.

Trial 2

Materials and methods

This was the first of two trials to examine the response of genetically AS-resistant and AS-susceptible broilers to AS-precipitating cold exposure, and to test various physiological parameters for their potential to predict and/or explain AS susceptibility.

Based on the results of Trial 1, the parents of five families with zero or very low incidence of AS were re-mated to produce a group of AS-resistant (AS-R) broilers. Similarly, the parents of five families with very high or highest incidence of AS in Trial 1 were re-mated and produced a group of AS-susceptible (AS-S) broilers. About 400 chicks per group hatched on June 13, 2000, sexed, and females were excluded from the trial due to their lower susceptibility to AS. Male chicks, 170 and 120 from the AS-S and AS-R groups, respectively, were individually identified by plastic tags with both numbers and barcodes attached to the skin of the neck. Standard day-of-hatch vaccination was given. Chicks were taken to the experimental poultry buildings at the Hebrew University's Faculty of Agriculture in Rehovot. All the chicks were reared in a single deep-litter pen from hatch to 19 d of age. During the first 10 days, a standard brooding regime was applied, with AT gradually decreasing from 35

to 30 °C. Starting on Day 11, the temperature was reduced by 1 °C every day, down to 20 °C on Day 19. On that day, 102 and 67 chicks from AS-S and AS-R groups, respectively, were transferred and randomly assigned to individual cages in two temperature-controlled chambers. There, AT was maintained at 20 °C until Day 21, then further reduced to 18 ± 2 °C, and maintained at that level until the termination of the trial, on Day 42. The other chicks, 70 and 50 from AS-S and AS-R groups, respectively, were used as control. They remained on deep litter in the brooding chamber, and reared there to 42 d of age under warm AT (about 28 ± 4 °C). Standard commercial feed was supplied *ad libitum* to all birds; a starter diet (crumbles) was given from hatch to Day 10, a grower diet (pellets) from Day 10 to Day 19, and a finisher diet (pellets) from Day 20 to the termination of the trial.

Birds were individually weighed every 2-3 days (three times a week). The manual weighing was expedited by identification of the barcode tags with a barcode reader/data logger (Symbol PDT 3100, Symbol Technologies Inc., Bohemia NY, USA) connected to a scientific balance (Sartorius BP 6100, Sartorius AG, Goettingen, Germany); the data from the logger was transferred to JMP (SAS Institute, 2000) software files on a computer for statistical analysis. Weighing was started at 8 a.m. on each occasion, but as the birds grew, and it took longer to weigh them, the weighing was instigated earlier in an effort to maintain the same time and avoid any BW change from behaviour or circadian rhythm.

All tests were conducted on birds individually removed from the chambers where they were kept and immediately returned after the few minutes taken to conduct all the tests and sampling. Oximetry, the determination of the oxygen saturation of hemoglobin in the arterial blood (SaO_2), is a real-time, easily performed, non-invasive monitor of hypoxemia, a basic manifestation of AS in broilers. It is not known to what extent various husbandry and environmental factors can also impinge on SaO_2 levels, independent of AS-inducing factors. In addition, pulse rate was measured (by oximetry), as this has been shown to tend to become bradycardic in nature, both with increasing age, and additionally in an early pre-ascitic stage, and is assuming increasing significance in an evaluation of the development of AS. This could provide additional evidence to substantiate the potential of using heart rate, as was proposed in the original research protocol, as a non-invasive marker for ascites sensitivity. In Trial 2, SaO_2 was measured on Days 1, 14, 27 and 34, using a portable veterinary oximeter that can monitor the high pulse rate of birds, the model 8600V (NONIN Medical Inc., Plymouth, MN, USA) with a model 2000T sensor. The sensor was usually placed over the vein of the ventral attitude of the right wing of lightly restrained birds held in lateral recumbency between the examiners knees; SaO_2 and pulse rate were determined simultaneously, usually within 2 min,

always by the same examiner. Up to Day 7 the NONIN lingual sensor model 2000SL was used with the infrared light applied to the medial aspect of the thigh.

The electrocardiograph (ECG) of the birds was measured on Days 1, 7, and 21 using a Biopac MP100 data acquisition system, three ECG100A amplifiers and Acqknowledge software (Biopac Systems, Goleta, CA 93117, USA), and stored on a laptop computer. Unanaesthetized birds were placed in dorsal recumbency with legs and wings restrained with rubber bands fixed to one of 3 wooden boards of varying sizes, depending on body weight. ECG leads ending in crocodile clips were connected to 23 gauge needles inserted through the skin in the left and right ventral parts of the wing close to the heart, and at the medial proximal part of the left leg where it joins the body. In the laboratory, the stored lead II P, R, RS, S and T wave amplitudes and the P, R, S, T wave durations, and the P-R, R-S, T-P intervals of each ECG were measured and noted. Pulse was also measured.

Hematocrit determinations were made on Days 27 and 34. Venous blood was collected by wing vein puncture, on the left wing, with blood taken into heparinized microhematocrit tubes, centrifuged, and the packed cell volume read visually against a calibrated scale. Hemoglobin (Hb) was measured spectrophotometrically in heparinized venous blood taken on Days 27 and 36 using the Sigma-Aldrich kit, procedure number 527.

Total oxygen capacity of the arterial blood (TOC) was calculated on D 27 by the formula used in for humans: $\text{TOC (ml/dl)} = \text{oxygen saturation of hemoglobin} \times 1.34 \times \text{hemoglobin concentration}$.

All birds that died during the trial were necropsied to visually determine whether ascitic fluid or hydropericardium were present and all such birds were deemed to have died from AS. Birds dying from other causes were excluded from the subsequent statistical analyses. On Day 42, after the last weighing, all the surviving birds were killed with CO₂ gas and necropsied to visually determine whether ascites fluid or hydropericardium were present; such birds were also regarded as AS positive. Hearts were removed from all the birds in the trial, the weight of the right ventricle (RV) and of both ventricles (total ventricle, TV) were determined, and consequently the RV/TV ratio was calculated for each bird, as an indicator of right ventricular hypertrophy.

Data of BW and BW gain up to Day 19 were analyzed using a model that included only the genetic group (AS-susceptible and AS-resistant), and the data comprised also the birds that later were left as control. Data of BW and BW gain from Day 19 to Day 42 of the birds kept in cages under low AT were analyzed using a model that included the genetic group (AS-susceptible and AS-resistant) and the two replicated chambers. Data of all the physiological measurements, mortality and RV/TV ratio were obtained only from the

cold-stressed chicks, hence they were also analyzed by the same model. Data of BW of the control birds were analyzed separately, using a model that included only the genetic groups. All statistical analyses were carried out using the JMP software (SAS Institute, 2000) using appropriate linear models or chi-square test (for mortality data).

Trial 3

Materials and methods

This was the second of two trials to examine the response of genetically AS-resistant (AS-R) and AS-susceptible (AS-S) broilers to AS-precipitating cold exposure, and to test various physiological parameters for their potential to predict and/or explain AS susceptibility. The same selected parents, based on the results of Trial 1, were re-mated again to produce the two groups of AS-R and AS-S broilers. About 300 chicks per group hatched on July 11, 2000, sexed, and females were excluded. Male chicks, about 90 and 130 from the AS-R and AS-S groups, respectively, were individually identified by plastic tags as in previous trials. Standard day-of-hatch vaccination was given. Chicks were taken to the experimental poultry buildings at the Hebrew University's Faculty of Agriculture in Rehovot. Brooding procedures and AT regimes were exactly as in Trial 2.

On Day 14 of the trial, thirty 12-d-old commercial male broiler chicks (Ross Breeders, UK; produced and distributed in Israel by Efrat) were added to the trial. Ross broilers have the largest share in the world broiler industry, as well as in Israel, and they are known to exhibit very low incidence of ascites in commercial farms. The Ross chicks were added to the experimental population at the brooding period, and thereafter they were treated and measured on the same days as the AS-S and the AS-S chicks, i.e. they were two days younger at each measurement.

On Day 19, 82 and 122 chicks from AS-R and AS-S groups, respectively, and 28 Ross chicks, were transferred and randomly assigned to individual cages in the two temperature-controlled chambers. There, AT was maintained at 20 °C until Day 21, when it was further reduced to 18 ± 2 °C, and then maintained at that level until the termination of the trial, on Day 47. There were no control chicks (warm AT) in this trial. Standard commercial feed was supplied *ad libitum* to all birds, as in Trial 2. Birds were individually weighed every 2-3 days (three times a week), using the same procedure and equipment as in Trial 2.

All tests were conducted on birds individually removed from the chambers where they were kept and immediately returned after the few minutes taken to conduct all the tests and

sampling. Oximetry was determined in 150 birds randomly selected on Days 1, 14, 28 and 34, as in Trial 2. On Day 1 the NONIN lingual sensor model 2000SL was used, and thereafter the 2000T sensor, as in Trial 2. ECG measurements, as carried out in Trial 2, were made on Days 1, 14, 30 and 34. Hematocrit measurements were made on Days 1, 15, 28 and 34. Hemoglobin (Hb) was measured in heparinized venous blood on Days 14, 28 and 34, as in Trial 2. The TOC was calculated as in Trial 2, for Days 14, 28 and 34.

All birds that died during the trial were necropsied to visually determine whether ascitic fluid or hydropericardium were present and all such birds were deemed to have died from AS. Birds dying from other causes were excluded from the subsequent statistical analyses. On Day 47, after the last weighing, all the surviving birds were killed with CO₂ gas and necropsied to visually determine whether ascites fluid or hydropericardium were present; such birds were also regarded as AS positive. Hearts were removed from all the birds in the trial, the weight of the right ventricle (RV) and of both ventricles (total ventricle, TV) were determined, and consequently the RV/TV ratio was calculated for each bird, as an indicator of right ventricular hypertrophy.

Data up to Day 19 (BW and BW gain, and the physiological measurements) were analyzed using a model that included only the two genetic groups (AS-susceptible and AS-resistant). Data of the physiological measurements taken during the low-AT period (Day 19 to Day 47) and mortality were analyzed using a model that included the three genetic groups (AS-susceptible, AS-resistant, and Ross) and the two replicated chambers. The model used to analyze the data of BW and BW gain during the low-AT period included only the AS-susceptible and AS-resistant groups, because the Ross birds were younger. All statistical analyses were carried out using the JMP software (SAS Institute, 2000) using appropriate linear models or chi-square test (for mortality data).

Trial 4

Materials and methods

About one hundred male broiler chicks from commercial dam lines (ANAK Breeders Ltd., Netanya, Israel) were taken from a commercial broiler operation at age 16 days. After acclimatisation for 3 d at 25 °C, the 50 birds with the highest BW were moved to individual pens in tiered wire pens in a temperature controlled room at the Kimron Veterinary Institute.

The ambient temperature was reduced to 16 °C from Day 21. A standard broiler pelleted feed was given *ad lib*.

All tests were conducted on birds individually removed from the room where they were kept and immediately returned after the few minutes taken to conduct all the tests and sampling. BW was determined on Days 24, 27, 30, 33, 35, 36, 37, 41, and 42. Oximetry was determined in all birds on Days 30, 31, 34, 36, 37, 38, 41 and 42, as in the previous trials. HCT measurements were made on Days 27, 30, 33, 37 and 42, as in the previous trials. The TOC was calculated, as in the previous trials, for blood taken from the left and right ventricles of each birds at the day of its sacrifice. In this trial, the Hb was measured by the Critical Care Analyser mentioned below.

From Day 30, an attempt was made, each day, to classify the birds according to their SaO₂ values, measured by oximetry, whereby HIGH was regarded as > 90% saturation, and LOW was regarded as < 80% saturation. Previous oximetry works by us have defined "normal" saturation as being > 85% saturation. Birds showing on 2 consecutive days SaO₂ values < 80% were considered LOW and were designated for examination of their blood chemistry. Such birds were examined for BW, body temperature, respiration rate and oxygen saturation (SaO₂ values, measured by oximetry). They were then anaesthetized by an intravenous injection of propofol (Diprolol^(Rx), Taro, Israel), at a dosage of 10mg/kg BW; anaesthesia was immediate.

The following procedures were then conducted at maximal speed. The birds' chest was opened surgically, and 0.5-0.8 ml of blood was taken from the right and then the left ventricles into separate heparinized 1 ml syringes. Air was expelled from the syringes, the needles bent to exclude exposure to air, and the syringes were plunged into ice until subsequent examination. For examination, each syringe was upturned several times to ensure blood mixing, the needle removed, the first drop of blood was absorbed onto filter paper, and the syringe was applied to a Critical Care Analyzer (AVL Opti CCA , AVL ROSWELL, Georgia, USA) for consequent automatic analysis of the blood. As soon as the first test was completed, the second syringe was examined.

The heart and lungs were removed. The heart was prepared for RV/TV, and after dividing and weighing, was put into a drying oven at 40 °C overnight, and then reweighed. The volume of the lungs was determined by water displacement, and water content was determined by weighing before and after similar drying at 40 °C.

Birds classified as LOW and treated as above were 17 in number; 7 were treated from Days 30-36 and 10 from Days 37-42. On every day that a LOW bird was examined, a control HIGH bird (birds showing on 2 consecutive days SaO₂ values >90%) was investigated by

exactly the same procedure, immediately thereafter. In all, 18 HIGH birds were examined, 6 from Days 30-36 and 12 from Days 37-42. Some birds had persistent, intermediate SaO_2 values of 82-88 % , and were designated MEDIUM SaO_2 value birds. In all, 11 MEDIUM birds were examined, 3 from Days 30-36 and 8 from Days 37-42.

In order to obtain control values in birds not cold stressed, 11 male birds of the same age, from the same genetic stock, but grown under normal commercial conditions, were similarly successfully examined, all when they were aged 42 d.

Trial 5 (from 1999)

Material and methods

This was a trial whose main aim was to examine the growth pattern of broilers from a specific genetic origin and to test non-invasive parameters for their potential to predict ascites susceptibility in seemingly healthy birds. The part of this trial detailed and discussed here, was to examine the genetic relationship between blood levels of T_3 and T_4 and to follow the changes in T_3 and T_4 levels with time, and whether different patterns of change are seen with exposure to different temperatures and with the different parameters associated with AS and the progression to AS.

A full pedigree population of chicks was produced from parents taken at random from a commercial dam-line of ANAK Breeders Ltd (Netanya, Israel). A total of 27 sires were mated with 5 to 9 dams per sire, and subsequently 2 chicks per dam were kept to form half-sib sire families representing the stock's genetic variation for growth rate and other traits. About 400 chicks (males + females) hatched. Birds were individually identified by plastic tags with both numbers and barcodes attached to the skin of the neck to facilitate recording of the pedigree and sex of each chick. Standard day-of-hatch vaccination was given. All the chicks were floor-brooded at a density of 11 birds / m^2 . Wood shavings were used as litter and were applied to a freshly cleaned and disinfected concrete floor. Birds were brooded at a cold temperature (CT) regimen, i.e., starting with 35 °C on Day 1, 33 °C on Day 4, 31 °C on Day 7, and 29 °C on Day 10. Starting on Day 11, ambient temperature was reduced by 1 °C every day, down to 18 °C on Day 21. On that day, 240 birds (6 to 9 per sire, 1 male or female per dam) were transferred to individual cages in two CT-controlled chambers used in this research. Temperature in these chambers was maintained at 18 °C on Day 22, then further

reduced to 16 °C until the termination of the trial, on Day 44. The remaining 80 chicks (2 to 4 per sire) were kept as a normal temperature (NT) control, and were reared on litter in the brooder house, with ambient temperature maintained at about 23 °C. BW was determined three times a week. T₃ and T₄ levels were measured in venous blood taken on Days 1, 25, and 39. The measurements were made with Canine T₃ C.A.C. and Canine T₄ C.A.C kits (Pharmatrade, DPC, Los Angeles, USA).

Birds dying were necropsied to visually determine whether ascites fluid, hydropericardium or right ventricular hypertrophy were present; all such birds were deemed to have died from AS. Birds dying from other causes were excluded from the subsequent statistical analyses. On Day 44, after the last weighing, birds were killed with CO₂ gas and necropsied to visually determine whether ascites fluid, hydropericardium or right ventricular hypertrophy were present; such birds were regarded as AS positive. Hearts were removed for determination of the weights of the RV, left ventricle + septum and consequently the RV/TV ratio.

Statistical analysis of all data was carried out using the JMP software (SAS Institute, 1994) using appropriate mixed-effect linear models.

Results and discussion by specific trial

Trial 1

In spite of the sub-optimal conditions in the broiler house, mainly due to lower than recommended ambient temperatures (AT), growth rate of the broiler flock in Trial 1 was only slightly lower than that of commercial broilers under standard conditions. On Day 10, the average BW of the entire flock (males and females) was 210 g, increasing to 460 g on Day 17, 1000 g on Day 28, and 2400 g on Day 48, with the heaviest birds reaching BW of well over 3000 g.

Mortality due to AS was first noted on Day 15, and continued until the end of the trial, on Day 48. A total of 1437 birds died during this period, of which 1081 birds were diagnosed as having died from only AS (75 % of total mortality, 17.5% of the entire flock). Infections in the leg joints was diagnosed in 617 dead birds; the majority of these birds (470) exhibited also AS. Infectious bursal disease was the third most frequent cause of death, with a total of 278 birds. The rate of mortality due to AS was relatively low in the 3rd week (39 birds), increased in the 4th, 5th and 6th weeks (139, 173 and 274 per week, respectively) and reached a peak of 461 birds with AS in the 7th week.

As was intended, the incidence of AS mortality (%AS) was a high level, 17.5%, similar to extreme cases of AS mortality observed in commercial flocks in this region. The incidence of AS mortality per family is presented graphically in Figure 1A. The values of %AS ranged from two families with no mortality (0%), to the most susceptible family in which 50% of the birds died from AS. The standard deviation among family levels of %AS mortality was 10.8% and the coefficient of variation (CV = standard deviation divided by the mean) was 0.63.

In order to verify the presence of genetic variation among families, the hypothesis that differences between families are of random nature had to be rejected. To test this hypothesis, the function of random binomial distribution was applied, using the proportion of AS in the entire population (17.5%), to "mark" each one of the 6200 chicks in the data file as AS-dead or healthy. The incidence of this "random AS mortality" was calculated per family; the distribution of these values is presented in Fig. 1B. In a flock where overall AS mortality

reached 17.5%, as in this trial, this would be the expected distribution of %AS mortality per family if differences among them were random only, with no genetic variation. The distribution of the actual % AS mortality per family was much wider than that of the expected random mortality (compare Figs. 1A and 1B), and its standard deviation and CV were much larger than the random distribution (10.8 vs. 4.2% and 0.63 vs. 0.24, respectively). These results clearly indicate that there was a substantial genetic variation in %AS mortality among the families in the dam-line used in Trial 1.

The presence of genetic variation facilitates selection for AS-resistant and AS-susceptible lines. To initiate the selection, the parents of five extremely resistant families and five extremely susceptible families were re-mated. The actual % AS in the selected families are indicated by the white bars (or portions of bars) in Figure 1A. The sires of some families either died or became infertile by the time that Trial 1 was completed and fully analyzed, hence some less-extreme families were used. Among the five families selected to establish the resistant line, one had no AS mortality, and the other four exhibited between 1 to 5% AS mortality. Among the five families selected to establish the susceptible line, one had the highest AS mortality (50%), three families exhibited between 36 to 41% AS mortality, and one family exhibited 30% AS mortality. The AS-resistant (AS-R) and AS-susceptible (AS-S) groups of chicks used in Trial 2 and Trial 3 were the progeny of these re-matings.

The much wider distribution among families of the actual mortality, and the larger variance and standard deviation, clearly indicate the presence of genetic variation between families in their resistance or susceptibility to AS.

1-A. Actual mortality due to AS: distribution of %AS per family, ranging from 0% to 50%.

White bars indicate families selected to establish AS-resistant and AS-susceptible lines.

Chicks obtained by repeated hatches from these families were used in Trials 2 and 3.

1-B. Expected non-genetic distribution of family AS mortality, calculated after each chick was marked at random as healthy or dead based on incidence of AS in the entire population.

Trial 2

AS mortality

Up to Day 19, the chicks were reared together on litter, then 102 from the AS-S group and 67 from the AS-R group were moved to individual cages and kept there under low AT. The remaining birds, 70 and 50 from the AS-S and AS-R groups, served as a control group kept on litter under warm AT. There was no AS mortality among these control birds, whereas under low AT, $63/169 = 37\%$ birds died of AS. Very few birds died from other causes and these were excluded from the data analyses. On Day 42 all surviving birds in which ascites fluid or hydropericardium were consequently recorded at necropsy on the same day were designated as ill-AS, and this supposition was confirmed as their RV/TV was > 0.34 . The birds with no AS manifestations and $RV/TV < 0.34$ were designated as no-AS. The results are presented in Table 2-1, in actual numbers as well as in percentages. In the susceptible group (AS-S), only 31.4% of the birds were no-AS, 14.7% were ill-AS and 53.9% died from AS (dead-AS). The AS mortality in the resistant group (AS-R) was significantly different from that in the AS-S group; 73.2% of the birds were no-AS, 14.9% ill-AS, and only 11.9% were dead-AS (Table 2-1). These results, and the similar ones obtained in the additional hatch from the same parents (Trial 3, Table 2-1) confirm that the differences in % AS between the extreme families in Trial 1 were indeed due to genetic variation for this trait.

Table 2-1. Numbers and percentages of AS birds and of birds diagnosed as ill-AS or no-AS at the end of the trial. Results are presented for the broilers kept under low AT after Day 19, from the AS-susceptible (AS-S) and AS-resistant (AS-R) groups (Trial 2 and Trial 3), and from the Ross broiler stock (Trial 3).

Trial	Group		dead-AS	ill-AS	no-AS	Total
Trial 2	AS-S	n	55	15	32	102
		(%)	53.9	14.7	31.4	
	AS-R	n	8	10	49	67
		(%)	11.9	14.9	73.2	
Trial 3	AS-S	n	66	18	38	122
		(%)	54.1	14.8	31.1	
	AS-R	n	4	11	67	82
		(%)	4.9	13.4	81.7	
	Ross	n	3	6	19	28
		(%)	10.7	21.4	67.9	

Defining AS-resistant (AS-R) and AS-susceptible (AS-S) chicks for physiological comparisons

The divergence between the AS-S and AS-R groups, resulted from only one cycle of selection, hence substantial variation in AS is still expected within groups. Therefore healthy chicks in the AS-S group and non-healthy chicks in the AS-R group were considered outlier genetic segregants, and excluded from the analyses aimed at physiological comparisons between genetically AS-resistant and AS-susceptible broilers. In the following text, figure and table, the terms AS-resistant and AS-susceptible refer only to data of birds that their phenotype confirmed these definitions: 70 AS-affected from the AS-S group (55 + 15), and 49 healthy AS-R birds.

Body weight (BW) and growth rate

The BW of the AS-R and AS-S chicks was determined three times weekly; means are presented in Table 2-2. Up to Day 19, all the chicks were reared together on litter, hence data of all of them was used in the analyses. After Day 19, there was no difference in mean BW between the AS-S and AS-R birds under warm AT, hence a combined mean was calculated from all the control birds at each age. From the birds in cages under low AT, mean BW was calculated only from the 49 healthy AS-R birds, and from the 55 AS-S birds that died from AS and the 15 birds found ill-AS on Day 42.

Table 2-2. Mean body weight (BW) of the AS-resistant (AS-R) and AS-susceptible (AS-S) birds in Trial 2; due to mortality, number is given for each age up to Day 19. Birds were kept together on litter under normal temperatures (AT), then one half was transferred to cages under low AT, and the rest were left on litter as a control group.

Age (d)	Mean BW (g)			P ²
	Control ¹	AS-R	AS-S	
0	--	47 (99) ³	46 (140) ⁴	NS
2	--	68	71	0.0002
5	--	125	130	<0.0001
7	--	177	182	0.017
9	--	237	241	NS
12	--	350	355	NS
14	--	444	451	0.088
16	--	537	542	NS
19	--	673	682	NS
21	825 (50+70)	726 (49) ⁶	729 (70) ⁷	NS
23	906	831	826 (70)	NS
26	1081	1030	1000 (69)	NS
28	1238	1174	1094 (67)	0.0013
30	1357	1337	1159 (64)	<0.0001
33	1602	1561	1272 (49)	<0.0001
35	1739	1707	1370 (39)	<0.0001
37	1900	1849	1442 (33)	<0.0001
40	2096	2104	1499 (18)	<0.0001
42	2289	2224	1626 (13)	<0.0001

¹ Control birds from both groups, kept together on litter under warm-AT after Day 19; ² Significance of difference in BW between the Res. and Sus. groups (under normal AT up to Day 19, and low AT thereafter); ³ Number of informative AS-R birds up to Day 19; ⁴ Number of informative AS-S-birds up to Day 19; ⁵ Numbers of informative AS-R & AS-S control birds, up to Day 42; ⁶ Number of informative AS-R birds that remained healthy under low-AT, up to Day 42; ⁷ Number of informative AS-S birds that exhibited AS under low-AT.

The growth rate of each group was also determined, by calculating the average daily BW gain in each of the 2-day or 3-day intervals, from the first (Day 0 to 2) to the last (Day 40 to 42). These values were calculated separately for the AS-R and AS-S chicks, and plotted against age (Figure 2). The results presented in Table 2-2 and in Fig. 2 indicate that the AS-S chicks exhibited higher BW gain during the first week of life, then the two groups exhibited similar growth (except a single interval between Days 12 to 14) until they were transferred to the individual cages at the low-AT chambers, on Day 19. The BW gain of both lines dropped dramatically during the first two days (Days 19 to 21) until they adjusted to the new conditions, then both returned to the normal growth rate, but only until Day 26. At that time, more and more AS-S chicks apparently started to develop AS and their growth was depressed due to insufficient oxygen supply. The difference in BW gain between the two groups after Day 26 clearly demonstrate the clinical manifestations of AS on broilers, initially growth suppression (Fig. 2 and Table 2-2), followed by high mortality (Tables 2-1 and 2-2).

Fig. 2. Growth of the AS-resistant and AS-susceptible chicks in Trial 2, expressed by average daily BW gain in each of the 2-day or 3-day intervals, from Day 2 (hatch to 2 d of age) to 42 (40 to 42 d of age). The groups differ significantly at all ages except where both lines are marked with the letter "a".

Oxygen saturation in blood (SaO₂)

Of the AS-associated parameters measured, SaO₂ values showed the first response, at Day 14 (Table 2-3) whereby the mean value of all the resistant birds was significantly elevated over the susceptible birds. This difference was also seen at the other measurements, on Days 27 and 34. The reduced SaO₂ values at Day 14 in susceptible birds may indicate an inherently lower SaO₂, as this was before cold exposure. Subsequently lower values in susceptible birds are as expected in birds with a higher AS mortality.

Table 2-3. Oxygen saturation of arterial blood (SaO₂) in broilers, by group and age

Group	Age (d)			
	1	14	27	34
Resistant	95.2 ^a	95.6 ^a	88.1 ^a	86.9 ^a
Susceptible	95.7 ^a	93.0 ^b	81.5 ^b	79.2 ^b

^{a,b} Different superscripts in same column indicate significant (P<0.05) differences in values

Hematocrit (HCT)

HCT values were markedly elevated on Day 34 in the susceptible group (Table 2-4) whereas on Day 27 there was no significant difference between the two groups. Elevated HCT values are usually indicative of developing AS, that was starting on about Day 27, but was well developed by Day 34.

Table 2-4. Hematocrit (%) in broilers, by group and age

Group	Age (d)	
	27	34
Resistant	35.3 ^a	37.0 ^b
Susceptible	36.0 ^a	42.8 ^a

^{a,b} Different superscripts in same column indicate significant (P<0.05) differences in values

Hemoglobin and TOC

The hemoglobin levels were measured in the two groups on Days 27 and 36. No significant difference was observed on Day 27 while on Day 36 the resistant group had significantly higher values (Table 2-5). Hemoglobin values usually parallel HCT values.

No significant difference was observed in TOC on D 27 between the two groups.

Table 2- 5. Hemoglobin levels (g/dl) and TOC (g/dl) in broilers, by group and age

Group	Age (d)		TOC (g/dl)
	Hemoglobin (g/dl)		
	27 calculated	36 calculated	
Resistant	8.9 ^a	8.2 ^b	10.42 ^a
Susceptible	10.0 ^a	10.4 ^a	10.78 ^a

^{a,b} Different superscripts in same column indicate very significant (P<0.01) differences in values

Heart rate

The heart rate of birds had a significant difference on Day 1 where the mean of the susceptible group was higher (Table 2-6). At the two following measurements (Days 14 and 27) no significant difference was recorded between the two groups whereas at the last measurement (Day 34) the heart rate was significantly higher in the resistant group. There is no known interpretation of elevated heart rate at Day 1. At Day 34, the lowered heart rate is typical of AS associated bradycardia.

Table 2-6. Heart rate (beats/min) in broilers, by group and age

Group	Age (d)			
	1	14	27	34
Resistant	416 ^b	436 ^a	388 ^a	389 ^a
Susceptible	438 ^a	445 ^a	375 ^a	352 ^b

^{a,b} Different superscripts in same column indicate significant (P<0.05) differences in values

RV/TV

The mean of the RV/TV ratio of the resistant broilers was significantly lower than that of the susceptible ones (Table 2-7).

Table 2-7. RV/TV ratio by group of broilers

Group	RV/TV ratio
Resistant	0.229 ^b
Susceptible	0.398 ^a

^{a,b} Different superscripts indicate very significant (P<0.01) differences in values

ECG

In Trial 2, ECG was measured on Days 1, 7, and 21.

S wave amplitude

The S wave amplitude (SWA) in broilers is negative in lead II. The measurement of the SWA was made from the baseline of the ECG till the negative peak of the RS complex. The values of the S waves are given in absolute values. A high SWA value can be indicative of a right ventricular hypertrophy and has been shown to be predictive of AS. However, SWA levels in this trial did not show any significant differences in the three measurements (Table 2-8). Our studies have shown that SWA is more indicative of developing AS than predictive, and as these 3 measurements were made before AS developed, no differences were found between the groups. SWA was not predictive of AS.

Table 2-8. S wave amplitude (μ V) in broilers, by group and age

Group	Age (d)		
	1	7	21
Resistant	86 ^a	73 ^a	73 ^a
Susceptible	80 ^a	73 ^a	80 ^a

R wave amplitude

The R wave amplitude (RWA) in the broiler ECG is positive and is measured from the base line of the ECG to the peak of the RS complex.

In this trial, the RWA increased in each of the three measurements in the two groups (Table 2-9); our previous studies also showed that till Day 35 the RWA is constantly increasing. In this trial, on Days 7 and 21 susceptible birds had significantly higher RWA. Our previous studies showed that RWA was higher in no-AS birds from Day 35; the cold exposed birds had higher RWA than normal temperature exposed birds from about Day 36.

Table 2-9. R wave amplitude (μ V) in broilers, by group and age

Group	Age (d)		
	1	7	21
Resistant	17 ^a	27 ^b	77 ^b
Susceptible	27 ^a	60 ^a	142 ^a

^{a,b} Different superscripts in same column indicate significant ($P < 0.05$) differences in values

RS wave complex amplitude

The R and S waves represent the depolarization of the ventricular muscle, which signals the onset of the ventricular systole.

In this Trial (Table 2-10) at Days 7 and 21 the RS wave complex amplitude was significantly higher in the susceptible broiler group. Our previous studies with RS wave complex amplitude showed no differences in the RS wave complex amplitude between AS birds and the no-AS birds in one trial. In another trial in our previous studies it was observed that the cold temperature exposed group had significantly elevated RS wave complex amplitude at Days 36 and 41, manifested in both AS and no-AS birds.

Table 2-10. RS wave amplitude (μ V) in broilers, by group and age

Group	Age (d)		
	1	7	21
Resistant	102 ^a	100 ^b	150 ^b
Susceptible	108 ^a	138 ^a	223 ^a

^{a,b} Different superscripts in same column indicate significant ($P < 0.05$) differences in values

P wave amplitude

The P wave amplitude (PWA) in broilers is positive. In lead II the P wave represent the polarization of the atria, causing contraction and ejection of their complement of blood into the ventricles. With right atrial hypertrophy the P wave becomes tall and peaked (P pulmonal), and with left atrial hypertrophy, the P wave becomes wide – long duration (P mitreal). A tall wide P wave is suggestive of bi-atrial enlargement.

On the three days of examination (1, 7, 21) the PWA was significantly higher in the susceptible broilers (Table 2-11). It seems that the PWA in early age can give a reliable prediction whether the broiler is resistant or susceptible to AS. Our previous studies with PWA showed also that the birds that developed AS had higher PWA than no-AS birds, as seen on Days 6, 21, 26 and 35.

Table 2-11. P wave amplitude (μ V) in broilers, by group and age

Group	Age (d)		
	1	7	21
Resistant	21 ^b	27 ^b	21 ^b
Susceptible	40 ^a	54 ^a	35 ^a

^{a,b} Different superscripts in same column indicate very significant (P<0.01) differences in values

P wave duration

In this Trial, the P wave duration was significantly higher in the susceptible group on Day 7. (Table 2-12). In our previous studies, birds which developed AS had significantly higher PWA on Day 35.

Table 2-12. P wave duration (msec) in broilers, by group and age

Group	Age (d)		
	1	7	21
Resistant	17 ^a	15 ^b	15 ^a
Susceptible	17 ^a	19 ^a	18 ^a

^{a,b} Different superscripts in same column indicate very significant (P<0.01) differences in values

Electrolytes in serum

Sodium (Na⁺)

Sodium is the major cation of extracellular fluid. Its primary functions in the body are to chemically maintain osmotic pressure and acid-base balance and to transmit nerve impulses. Sodium functions at the cell membrane level by creating an electrical potential between different cell membranes causing the transmission of nerve impulses and neuromuscular excitability to be maintained. Sodium is involved in some enzyme catalyzed reactions as a cofactor. The body has a strong tendency to maintain a constant total base content, and only slight changes are found in the blood even under pathological conditions.

Potassium (K⁺)

Potassium is the major cation in the intracellular fluid and functions as the primary buffer within the cell itself. Ninety percent of potassium is concentrated within the cell, and damaged cells release potassium into the blood. Potassium plays an important role in nerve conduction, muscle function, and helps maintain acid-base balance and osmotic pressure. Serum potassium values obtained may be used to monitor electrolyte imbalance in the diagnosis of shock, heart or circulatory insufficiency, acid-base imbalance, kidney problems and other diseases.

Chloride (Cl⁻)

Chloride is an anion that exist predominantly in extracellular spaces. It maintains cellular integrity through its influence on osmotic pressure. Serum chloride levels may have significance in the monitoring of acid-base balance and water balance.

In this Trial these 3 electrolytes were measured in the serum of broilers. No significant differences were observed in the three electrolyte levels on Day 27, a time when AS was starting to develop. The results can be seen in Table 2-13.

Table 2-13. Electrolyte quantity (meq/L) in broilers' blood on Day 27, by group

Group	Electrolyte		
	K ⁺	Na ⁺	Cl ⁻
Resistant	6.9 ^a	146 ^a	104 ^a
Susceptible	7.4 ^a	148 ^a	104 ^a

^{a,b} Different superscripts in same column indicate significant (P<0.05) differences in values

Trial 3

AS mortality

Trial 3 included AS-S and AS-R male chicks from the same parents as in Trial 2, and also a group of commercial Ross male broilers that were two days younger. The experimental protocols were similar, except that no chicks were left as control, i.e. under warm AT. On Day 19, 122 birds from the AS-S group, 82 birds from the AS-R group, and 28 Ross birds were moved to individual cages and kept there under low AT. A total of 73 birds died under these conditions, all from AS. Very few birds died from other causes and these were excluded from the data analyses. On Day 42 all surviving birds in which ascites fluid or hydropericardium were consequently recorded at necropsy on the same day were designated as ill-AS, and this supposition was confirmed as their RV/TV was > 0.34 . The birds with no AS manifestations and RV/TV < 0.34 were designated as no-AS. The mortality results of Trial 3 are presented in Table 2-1, along with the comparable results from Trial 2. In the susceptible group (AS-S), only 31.1% of the birds were no-AS, 14.8% were ill-AS and 54.1% dead-AS. The AS mortality in the AS-R group was significantly different from that in the AS-S group; 81.7% of the birds were no-AS, 13.4% were ill-AS, and only 4.9% were dead-AS (Table 2-1). Almost identical results were obtained in Trial 2, confirming that the AS-R and AS-S groups exhibit remarkable genetic divergence in their response to AS-inducing conditions.

Defining AS-resistant (AS-R) and AS-susceptible (AS-S) chicks for physiological comparisons

As in Trial 2, only healthy chicks in the AS-R group and non-healthy chicks in the AS-S group were used in the analyses aimed at physiological comparisons between AS-resistant and AS-susceptible broilers. In all the following text, figures and tables, the terms AS-resistant and AS-susceptible refer only to the data from birds that their phenotype confirmed these definitions: 84 AS-affected from the AS-S group (66 AS + 18 ill-AS), and 67 healthy (no-AS) AS-R birds. The Ross group was also represented only by the data of its healthy birds.

Body weight (BW) and growth rate

The BW of the AS-R and AS-S chicks was determined three times weekly; means are presented in Table 3-1. Mean BW was calculated only from the 67 no-AS AS-R birds, and

from the 66 AS AS-S birds plus the 18 ill-AS birds that survived to day 47. The BW results of the Ross birds are not presented because they were two days younger.

As in Trail 2, the growth rate of each group was also determined by calculating the average daily BW gain in each of 2-day or 3-day intervals. The results, presented graphically in Fig. 3, and the BW means in Table 3-1, indicate that the AS-S chicks exhibited a more rapid growth up to Day 9, and therefore had higher BW until Day 14. Then, the two groups exhibited similar growth rate until Day 26, as in Trail 2. Also the suppression of growth immediately after the transfer to individual cages at the low-AT chambers was similar in Trail 3 to that observed in Trial 2. From Day 26 onwards, more and more AS-S chicks apparently started to develop AS and their growth was depressed, probably due to insufficient oxygen supply. The difference in BW gain between the two groups after Day 26 clearly demonstrate the clinical manifestations of AS on broilers, initially growth suppression (Figure 3 and Table 3-1), followed by high mortality (Tables 2-1 and 3-1).

In both trials, the AS-susceptible (AS-S) chicks were characterized by a significantly higher BW and BW gain from hatch to Day 7 (Trial 2) or Day 9 (Trial 3). It is possible that broilers with a more rapid growth during the first week of their life are more prone to develop AS, hence they are more susceptible to AS-inducing conditions.

Table 3-1 Mean body weight (BW), 3 times a week, of the AS-resistant (AS-R) and AS-susceptible (AS-S) birds in Trial 3; up to Day 19, they were kept together on litter under normal temperatures (AT), then transferred to cages under low-AT

Age (d)	Mean BW (g)		<i>P</i> ¹
	AS-R	AS-S	
0	47 (67) ²	47 (84) ³	NS
2	65	68	0.0008
5	110	119	<0.0001
7	144	158	<0.0001
9	189	208	<0.0001
12	287	305	0.0038
14	357	371	0.057
16	434	448	NS
19	563	586	NS
21	608	628	NS
23	712	731	NS
26	892	882	NS
28	1022	964 (82) ⁴	0.0073
30	1157	1052 (74)	<0.0001
33	1365	1164 (65)	<0.0001
35	1501	1252 (56)	<0.0001
37	1646	1369 (49)	<0.0001
40	1899	1477 (42)	<0.0001
42	2056	1614 (31)	<0.0001
45	2240	1774 (23)	<0.0001
47	2399	1896 (18)	<0.0001

¹ Significance of difference in BW between the AS-R and AS-S groups (under normal AT up to Day 19, and low AT thereafter); ² Number of informative AS-R birds that remained healthy under low-AT up to Day 47; ³ Number of informative AS-S birds up to Day 26; ⁴ Number of informative AS-S birds that exhibited AS under low-AT after Day 26. Due to mortality, number is given for each age.

Fig. 3. Growth of the AS-resistant and AS-susceptible chicks in Trial 3, expressed by average daily BW gain in each of the 2-day or 3-day intervals, from Day 2 (hatch to 2 d of age) to 47 (45 to 47 d of age). The groups differ significantly at all ages except where both lines are marked with the letter "a".

Oxygen saturation in blood (SaO₂)

Of all the AS-associated parameters measured, and as seen in Trial 2, SaO₂ values showed the first response, at 28 d of age (Table 3-2), whereby the mean value of all the AS-R birds was significantly elevated over the AS-S birds. The significantly lower SaO₂ values seen in AS-S birds at Day 14 in Trial 2 were not seen in this trial on Day 14. The expected, lower SaO₂ values were also seen on Day 34. The Ross group showed SaO₂ values similar to the resistant birds.

Table 3-2. Oxygen saturation of arterial blood (SaO₂) in broilers, by group and age

Group	Age (d)			
	1	14	28	34
Resistant	95.4 ^a	94.1 ^a	90.5 ^a	89.9 ^a
Susceptible	95.9 ^a	93.9 ^a	79.2 ^b	81.9 ^b
Ross		92.6 ^a	90.8 ^a	86.6 ^{ab}

^{a,b} Different superscripts in same column indicate significant (P<0.01) differences in values

Hematocrit values

HCT values were significantly elevated on Days 14 and 34 in the susceptible group compared with the resistant group (Table 3-3). The Ross group showed HCT values similar to the resistant birds.

Table 3-3. Hematocrit (%) in broilers, by group and age

Group	Age (d)			
	1	14	28	34
Resistant	43.7 ^a	32.0 ^b	37.3 ^{ab}	38.2 ^b
Susceptible	41.1 ^a	33.4 ^a	38.8 ^a	44.6 ^a
Ross		32.3 ^{ab}	35.9 ^b	39.8 ^b

^{a,b} Different superscripts in same column indicate significant (P<0.05) differences in values

Hemoglobin levels

On Day 14 the resistant group had significantly lower values than the susceptible group (Table 3-4). The Ross group showed hemoglobin values similar to the resistant birds, except on Day 28, when levels were lower than the other groups. It is difficult to read any physiological significance into these seemingly trendless data.

In this trial, the TOC was significantly higher in the susceptible group than in the resistant group on Days 14 and 28. No significant differences were seen among the three groups on Day 34. On Day 28, the Ross group had also a significantly higher TOC than the resistant group.

Table 3-4. Hemoglobin levels (g/dl) in broilers, by group and age

Group	Age (d)					
	Hemoglobin (g/dl)			TOC (g/dl)		
	14	28	34	14	28	34
Resistant	6.45 ^b	9.00 ^a	7.91 ^{ab}	8.15 ^b	10.87 ^a	9.04 ^a
Susceptible	7.13 ^a	9.29 ^a	8.72 ^a	8.98 ^a	9.81 ^b	9.48 ^a
Ross	6.77 ^{ab}	8.25 ^b	7.63 ^b	8.98 ^{ab}	10.01 ^b	9.02 ^a

^{a,b} Different superscripts in same column indicate significant ($P < 0.05$) differences in values

Heart rate

As seen in Trial 2, the heart rate of the AS-S birds was significantly higher than the resistant group on Day 1 (Table 3-5). At the three following measurements (Days 14, 28 and 34) no significant difference was recorded between the two groups. The heart rate of the Ross broilers was significantly higher than the susceptible group on all three measurements and was also higher than the resistant group on Days 14 and 34. A stable, relatively high heart rate has been regarded by us to be a positive parameter for AS resistance.

Table 3-5. Heart rate (beats/min) in broilers, by group and age

Group	Age (d)			
	1	14	28	34
Resistant	419 ^b	425 ^b	384 ^{ab}	369 ^b
Susceptible	438 ^a	413 ^b	373 ^b	360 ^b
Ross		450 ^a	397 ^a	387 ^a

^{a,b} Different superscripts in same column indicate significant ($P<0.05$) differences in values

RV/TV ratio

The mean RV/TV ratio of the susceptible broilers was significantly lower than that of the resistant and Ross broilers (Table 3-6).

Table 3-6. RV/TV ratio by group of broilers

Group	RV/TV ratio
Resistant	0.245 ^b
Susceptible	0.434 ^a
Ross	0.245 ^b

^{a,b} Different superscripts indicate significant ($P<0.01$) differences in values

ECG wave evaluation

In Trial 3, ECG was measured on Days 1, 14, 30 and 34.

S wave amplitude

S wave amplitude levels were significantly higher in the AS-S birds in comparison with the AS-R birds on Day 30 (Table 3-7). In the three other measurements no significant difference was observed between these two groups. The Ross group was examined only on Days 14, 30 and 34 and in these three measurements the SWA was significantly lower than in the AS-S

birds, and on Day 14 significantly lower than the AS-R birds. There was a definite trend for Ross birds to have lower SWA than the other groups.

Table 3-7. S wave amplitude (μ V) in broilers, by group and age

Group	Age (d)			
	1	14	30	34
Resistant	63 ^a	56 ^a	62 ^b	81 ^{ab}
Susceptible	62 ^a	52 ^a	132 ^a	128 ^a
Ross		35 ^b	50 ^b	36 ^b

^{a,b} Different superscripts in same column indicate significant ($P < 0.05$) differences in values

R wave amplitude

The R wave amplitude did not show any significant differences between the susceptible and resistant groups in the four measurements (Table 3-8). The Ross broilers had significantly higher RWA on Days 14 and 30 than the two other groups whereas on Day 34 the difference was significant only compared with the AS-S birds. In Trial 2, the AS-S birds had elevated RWA.

Table 3-8. R wave amplitude (μ V) in broilers, by group and age

Group	Age (d)			
	1	14	30	34
Resistant	10 ^a	47 ^b	83 ^b	101 ^{ab}
Susceptible	15 ^a	49 ^b	88 ^b	70 ^b
Ross		71 ^a	128 ^a	103 ^a

^{a,b} Different superscripts in same column indicate significant ($P < 0.05$) differences in values

RS wave complex amplitude

In this Trial no significant differences were seen in the RS wave complex amplitude between AS-R and AS-S birds except on Day 30, when the in the ASA-S it was higher (Table 3-9). The Ross group showed similar values as the AS-R birds. In Trial 2 the AS-S birds showed higher values at Days 7 and 21 (but was not examined at a later age).

Table 3-9. RS wave amplitude (μ V) in broilers, by group and age

Group	Age (d)			
	1	14	30	34
Resistant	72 ^a	104 ^a	145 ^b	158 ^{ab}
Susceptible	79 ^a	103 ^a	235 ^a	197 ^a
Ross		106 ^a	178 ^b	139 ^b

^{a,b} Different superscripts in same column indicate significant ($P < 0.05$) differences in values

P wave amplitude

On the three first measurements (Days 1, 14 and 30) the PWA was higher in the AS-S birds than in the AS-R birds (Table 3-10), although on Day 14 the difference was not significant. On Day 34 there were not enough measurements to interpret differences. The PWA of the Ross birds was significantly lower than the AS-S birds on Days 14 and 30. No significant differences were seen between the Ross broilers and the AS-R birds on Days 14 and 30. As seen both in last years trials, and in Trial 2, the PWA can give a reliable prediction as to AS susceptibility.

Table 3-10. P wave amplitude (μ V) in broilers, by group and age

Group	Age (d)			
	1	14	30	34
Resistant	20 ^b	23 ^{ab}	19 ^b	
Susceptible	28 ^a	28 ^a	37 ^a	32 ^a
Ross		20 ^b	23 ^b	24 ^a

^{a,b} Different superscripts in same column indicate significant ($P < 0.05$) differences in values

P wave duration

In this Trial, the PWD was significantly higher in the AS-S birds in comparison to the AS-R birds on Days 1 and 30 (Table 3-11). On Day 34 there were not enough parameters to represent the resistant group. On Day 14 no differences were observed between the groups. On Day 30 the PWD in the Ross group was significantly higher than the AS-R birds and significantly lower than the AS-S birds. On Day 34 the Ross group PWD was significantly lower than the AS-S birds. In Trial 2 the AS-S birds had generally higher PWD up to 21 d of age.

Table 3-11. P wave duration (msec) in broilers, by group and age

Group	Age (d)			
	1	14	30	34
Resistant	13 ^b	14 ^a	13 ^c	
Susceptible	16 ^a	14 ^a	18 ^a	19 ^a
Ross		13 ^a	16 ^b	13 ^b

^{a,b,c} Different superscripts in same column indicate significant ($P < 0.05$) differences in values

Electrolytes in serum

In this trial electrolytes were measured in the serum of broilers on Days 14 and 28 (Ross broilers were measured only on Day 28). No significant difference was observed in the sodium and chloride levels between the groups (Tables 3-12 & 3-13). In contrast, the potassium was significantly higher in the AS-S birds than in the AS-R birds in both the measurements. On Day 28 the potassium levels in the Ross group were higher than in the two other groups. In Trial 2, no statistical difference in Potassium was observed on D 27 between sensitive and resistant groups.

Table 3-12. Electrolytes (meq/L) in broilers' blood on Day 14, by group

Group	Electrolyte		
	K ⁺	Na ⁺	Cl ⁻
Resistant	6.3 ^b	142 ^a	104 ^a
Susceptible	7.1 ^a	141 ^a	103 ^a

^{a,b} Different superscripts in same column indicate significant (P<0.05) differences in values

Table 3- 13. Electrolytes (meq/L) in broilers' blood on day 28, by group

Group	Electrolyte		
	K ⁺	Na ⁺	Cl ⁻
Resistant	6.3 ^c	144 ^a	102 ^a
Susceptible	9.3 ^a	143 ^a	104 ^a
Ross	8.1 ^b	144 ^a	103 ^a

^{a,b,c} Different superscripts in same column indicate very significant (P<0.01) differences in values

Trial 4

Oxygen saturation of the blood SaO₂

SaO₂ values showed (Table 4-1) that the designation of individual birds in high, medium or low groups was valid, as these values were consistent over all the days measured, Days 30, 31, 36 and 37.

Table 4-1. Oxygen saturation of arterial blood (SaO₂) in broilers, by SaO₂ group and age

Group	Age (d)				
	30	31	34	36	37
HSG	96.2 ^a	93.2 ^a	89.4 ^a	89.2 ^a	90.8 ^a
MSG	94.6 ^{ab}	91.1 ^{ab}	88.3 ^a	89.4 ^a	87.8 ^a
LSG	91.6 ^b	89.6 ^b	86.1 ^a	82.1 ^b	80.8 ^b

^{a,b} Different superscripts in same column indicate very significant (P<0.01) differences in values

Hematocrit

No significant differences were seen between the HSG, MSG and LSG in all the measurements (Table 4-2). On Day 37 there were not enough data to represent the low group.

Table 4-2. Hematocrit (%) in broilers, by SaO₂ group and age

Group	Age (d)			
	27	30	33	37
HSG	30.8 ^a	29.9 ^a	34.3 ^a	33.0 ^a
MSG	30.9 ^a	29.5 ^a	34.4 ^a	33.1 ^a
LSG	30.2 ^a	30.4 ^a	34.3 ^a	

^{a,b} Different superscripts in same column indicate significant (P<0.05) differences in values

Heart rate

On Days 30, 31 and 37 no significant differences were observed between the three groups (Table 4-3). On Day 34 the HSG had a significantly higher heart rate than the LSG whereas the MSG did not differ from the two other groups. On Days 35 and 38 the MSG had a significantly higher heart rate from the LSG but not from the HSG group, and no difference was seen between the HSG and LSG). The overall trend was for LSG to have lower values than the HSG.

Table 4-3. Heart rate (beats/min) in broilers, by SaO₂ group and age

Group	Age (d)					
	30	31	34	35	37	38
HSG	391 ^a	375 ^a	378 ^a	365 ^{ab}	365 ^a	352 ^{ab}
MSG	387 ^a	379 ^a	367 ^{ab}	382 ^a	373 ^a	375 ^a
LSG	385 ^a	376 ^a	359 ^b	353 ^b	362 ^a	342 ^b

^{a,b} Different superscripts in same column indicate significant (P<0.05) differences in values

Respiration rate

No significant differences were observed between the three groups at any measurement. On Days 37 and 42 there were not enough data to represent the LSG (Table 4-4).

Table 4-4. Respiration rates (respirations/min) in broilers, by group and age

Group	Age (d)					
	27	30	33	35	37	42
HSG	54.7	64.0	63.5	66.0	53.4	50.6
MSG	55.2	63.8	66.8	64.4	47.0	59.0
LSG	54.0	64.5	62.6	61.5		

RV/TV ratio

RV/TV values were associated with the oxygen saturation grouping (Table 4-5). The mean RV/TV of the LSG was significantly lower than that of the HSG and the control broilers.

Table 4-5. RV/TV ratio of broilers by group

Group	RV/TV ratio
HSG	0.221 ^{bc}
MSG	0.241 ^{ab}
LSG	0.275 ^a
Control	0.199 ^c

^{a,b,c} Different superscripts indicate significant
($P < 0.05$) differences in values

Rectal temperature

In this trial the rectal temperature was measured on Days 27, 30, 33, 35 and 37. No differences were observed between the groups except on Day 33 where the rectal temperature of the HSG was significantly higher than the LSG (Table 4-6). On Day 37 there were not enough parameters to represent the LSG.

Table 4-6. Rectal temperature (°C) in broilers, by group and by age

Group	Age (d)				
	27	30	33	35	37
HSG	41.3 ^a	41.2 ^a	41.5 ^a	41.0 ^a	41.5 ^a
MSG	41.4 ^a	41.2 ^a	41.3 ^{ab}	40.7 ^a	41.3 ^a
LSG	41.2 ^a	41.1 ^a	41.2 ^b	40.9 ^a	

^{a,b} Different superscripts in same column indicate significant ($P < 0.05$)
differences in values

Parameters measured in broilers' lungs on day of sacrifice

In this trial, some lung parameters were measured at the day of sacrifice. All the following parameters are presented in Table 4-7.

Lung weight

The lungs of the control group were significantly heavier than the lungs of the three other groups.

Lung volume

No significant differences in the lung volume were observed among the four groups.

Weight after drying (WAD)

The dried lungs of the control group were significantly heavier than the lungs of the three other groups.

Body weight (BW) of the broiler at the day of sacrifice

The body weight of the control group was heavier than the body weight of the three other groups. No significant differences were observed within those three groups.

Relative lung weight

This parameter is calculated by the weight of the lungs (LW) divided by the body weight of the bird multiplied by 100 which gives the relative weight of the lungs. No significant differences were observed among the four groups of broilers.

Relative lung volume

This parameter is calculated by the volume of the lungs (LV) divided by the body weight of the bird multiplied by 100 which gives the relative volume of the lungs. No significant differences were observed among the four groups of broilers.

Percentage of water in the lung (H₂O)

This parameter is calculated by the formula: $\{(WBD - WAD) \times 100\} / WBD$. The control group had a significant lower percentage of water in the lung than the LSG and the MSG. No significant difference was observed between the HSG, MSG and LSG.

Table 4-7. Parameters in broilers' lungs on day of sacrifice, by group

Group	LW (g)	LV (ml)	WAD (g)	RLW (%)	RLV (%)	H ₂ O in lungs (%)
HSG	8.2 ^b	9.3 ^a	0.92 ^b	0.43 ^a	0.49 ^a	79.2 ^{ab}
MSG	8.0 ^b	10.5 ^a	0.77 ^b	0.45 ^a	0.56 ^a	80.9 ^a
LSG	8.2 ^b	9.4 ^a	0.88 ^b	0.43 ^a	0.50 ^a	79.7 ^a
Control	9.7 ^a	10.9 ^a	1.08 ^a	0.44 ^a	0.49 ^a	77.9 ^b

^{a,b,c} Different superscripts in same column indicate significant (P<0.05)

differences in values

Blood gases, pH and other parameters measured in the left and right ventricular blood

In Trial 4, ventricular blood gases and other parameters were measured immediately before euthanasia. All the following parameters are presented in Table 4-8.

pH of the blood

The pH value of the blood may be the single most valuable factor in the evaluation of the acid-base status. The pH value is an indicator of the balance between the buffer (blood), renal (kidney) and respiratory (lung) systems and one of the most tightly controlled parameters in the body.

The blood of the control group from the left ventricle had a significantly higher pH than the HSG and the MSG groups (Table 4-8). No significant difference was observed between HSG, MSG and LSG. In blood from the right ventricle no significant differences in pH were observed between the four groups.

PCO₂ in blood

This parameter in the arterial blood is used to assess how well the body eliminates carbon dioxide in relation to the metabolic rate of CO₂ production.

In the left ventricular blood the HSG had significantly lower PCO₂ than the LSG. The control group had significantly lower PCO₂ than the three other groups (Table 4-8). No significant

difference was noticed between the MSG and the HSG or LSG. In the right ventricular blood the control and HSG groups had significant lower PCO₂ than the MSG and the LSG.

PO₂ in blood

This parameter in arterial blood is a most important parameter for the evaluation of arterial oxygenation status. Values below the normal PO₂ (arterial hypoxemia) are usually caused by pulmonary, circulatory, or respiratory abnormalities (such as bronchial obstruction, vascular problems, decrease in cardiac output, increased oxygen demand, anatomical heart defect or low inspired O₂ content).

In the left ventricular blood the control group had significantly higher PO₂ than the three other groups (Table 4-8). No significant differences were noticed between the three other groups. In the right ventricular blood the LSG had significant lower PO₂ than the HSG and the control groups. No significant differences were seen between the MSG and the other groups.

Table 4-8. Blood gases and pH in the left and right ventricles on day of sacrifice, by group

Group	Parameter					
	Left ventricle			Right ventricle		
	pH	PCO ₂ (mmHg)	PO ₂ (mmHg)	pH	PCO ₂ (mmHg)	PO ₂ (mmHg)
HSG	7.22 ^b	63.2 ^b	54.1 ^b	7.22 ^a	61.2 ^b	36.3 ^a
MSG	7.22 ^b	69.2 ^{ab}	46.5 ^b	7.19 ^a	77.0 ^a	29.0 ^{ab}
LSG	7.23 ^{ab}	73.1 ^a	52.9 ^b	7.20 ^a	78.0 ^a	28.6 ^b
Control	7.27 ^a	52.4 ^c	71.5 ^a	7.21 ^a	59.4 ^b	36.2 ^a

^{a,b,c} Different superscripts in same column indicate significant (P<0.05) differences in values

The base excess (BE)

This parameter results from the a calculation to determine the titratable base of the blood, which in principle is measured by titration of the blood with a strong acid or base to a pH of 7.4 with PCO₂ = 40 mmHg at 37°C.

$$BE = (1 - 0.014 \times tHb) \times \{(1.43 \times tHb + 7.7) \times (pH - 7.4) - 24.8 + HCO_3\}$$

In blood from the left ventricle the LSG had significantly higher BE than the three other groups between which no significant difference was observed (Table 4-9). In blood from the

right ventricle, the control group had a significantly lower BE than the three other groups while the LSG had significantly higher BE than the HSG.

The total concentration of CO₂ (tCO₂)

This parameter is the sum of dissolved CO₂ and bicarbonate.

$$tCO_2 = HCO_3^- + (0.0307 \times PCO_2)$$

In blood from the left ventricle the LSG had significantly higher tCO₂ than the HSG and the control group (Table 4-9). The control group was also significantly higher than the MSG and the HSG groups. In blood from the right ventricle, the control group had a significantly lower tCO₂ than the LSG and the MSG while the LSG had significantly higher tCO₂ than the HSG group. No significant differences were noticed between the HSG and the MSG, or the LSG and the MSG.

The bicarbonate concentration in plasma (HCO₃⁻)

This parameter results from the calculation below:.

$$HCO_3^- = 0.0307 \times PCO_2 \times 10^{(pH - 6.105)}$$

In the left and the right ventricles, the control group had a significantly lower HCO₃⁻ than the LSG and the MSG while the LSG had also significantly higher HCO₃⁻ than the HSG (Table 4-9). No significant differences were noticed between the HSG and the MSG, or the LSG and the MSG.

The buffer base (BB)

This parameter is the concentration of buffering anions that is available in whole blood to buffer strong acids and consists mainly of protein anions and bicarbonate. Hemoglobin is the most significant protein anion. The calculation of the BB is displayed below:

$$BB = BE + 41.7 + 0.42 \times tHb$$

In the blood from the left ventricle, the LSG had a significantly higher BB than the HSGh and control groups (Table 4-9). No significant differences were noticed between the other groups. In the blood from the right ventricle, the LSG and the MSG groups had a significantly higher BB than the HSG and the control groups.

The base excess at actual oxygen saturation (BEact)

The calculation of this parameter is displayed below:

$$BEact = (1 - 0.0143 \times tHb) \times \{(1.63 \times tHb + 9.5) \times (pH - 7.4) - 24.26 + HCO_3^-\} - 0.2 \times tHb \times (1 - SO_2/100)$$

In the blood from the left ventricle, the LSG had a significantly higher BBact than the three other groups (Table 4-9). No significant differences were noticed between these three groups. In the blood from the right ventricle, the LSG and the MSG had significantly higher BBact than the HSG and the control group.

The base excess of extracellular fluid (BEecf)

This parameter is a quantity that reflects only the non-respiratory components of acid-base balance (tHb = 5 gr/dl). The calculation of the BEecf is displayed below:

$$\text{BEecf} = 16.2 (\text{pH} - 7.4) - 24.8 + \text{HCO}_3^-$$

In the blood from the left ventricle, the LSG had significantly higher BEecf than the HSG and the control group (Table 4-9). No significant differences were noticed between the other groups. In the blood from the right ventricle, the LSG and the MSG had significantly higher BEecf than the HSG and the control group.

Table 4-9. Parameters in broilers' blood from the left and right ventricles on day of sacrifice, by group

Ventricle	Group	Parameter					
		BE (mmol/L)	tCO ₂ (mmol/L)	HCO ₃ (mmol/L)	BB (mmol/L)	BEact (%)	BE ecf (mmol/L)
Left	HSG	-2.8 ^b	26.0 ^h	24.4 ^{bc}	42.5 ^b	-3.3 ^b	-2.7 ^b
	MSG	-1.1 ^b	27.8 ^{ab}	26.3 ^{ab}	44.0 ^{ab}	-2.2 ^b	-0.7 ^{ab}
	LSG	1.3 ^a	29.9 ^a	28.0 ^a	46.7 ^a	0.85 ^a	1.7 ^a
	Control	-2.5 ^b	23.2 ^c	22.5 ^c	42.0 ^b	-3.6 ^b	-3.6 ^b
Right	HSG	-2.6 ^{bc}	26.4 ^{bc}	24.3 ^{bc}	42.9 ^b	-3.6 ^b	-2.8 ^b
	MSG	-0.5 ^{ab}	29.4 ^{ab}	27.4 ^{ab}	46.5 ^a	-0.3 ^a	1.7 ^a
	LSG	1.2 ^a	31.5 ^a	29.2 ^a	46.9 ^a	-0.2 ^a	2.1 ^a
	Control	-3.8 ^c	24.2 ^c	22.2 ^c	41.3 ^b	-4.6 ^b	-3.3 ^b

^{a,b,c} Different superscripts in same column indicate significant (P<0.05) differences in values

The standard bicarbonate of the blood (stHCO₃⁻)

This parameter is defined as the plasma bicarbonate concentration in blood that has been equilibrated at 37°C with a gas mixture having a PCO₂ = 40 mmHg. The calculation of the stHCO₃⁻ is displayed below:

$\text{stHCO}_3^- = 10^{(\text{pHst} - 6.022)}$, when pHst is defined by:

$$\text{pHst} = (0.8262 - 0.01296 \times \text{tHb} + 0.006942 \times \text{BE}) \times \text{Ig} \times (0.025 \times \text{pCO}_2) + \text{pH}$$

In the blood from the left ventricle, the LSG had a significantly higher stHCO₃⁻ than the three other groups (Table 4-10). No significant differences were noticed between these three groups. In the blood from the right ventricle, the LSG had significantly higher stHCO₃⁻ than the HSG. The control group had a significantly lower stHCO₃⁻ than the LSG and the MSG. No significant differences were noticed between the HSG and the MSG or the LSG and the MSG.

The concentration (activity) of hydrogen ions in plasma (cH⁺)

This parameter is calculated by the formula below:

$$\text{CH}^+ = 10^{(9 - \text{pH})}$$

In the blood from the left and the right ventricles no significant differences were noticed between the four groups (Table 4-10).

Total hemoglobin concentration (tHb)

In the blood from the left and right ventricles, the LSG had a significantly higher tHb than the HSG and the control group. No significant differences were noticed between the HSG, the MSG and the control group (Table 4-10).

Total oxygen concentration (TOC)

No significant differences were observed among the groups in bloods from left and right sides of the heart (Table 4-10).

The oxygen saturation of the blood (SaO₂)

In the left ventricle, the control group had a significantly higher SaO₂ than the LSG and the MSG groups (Table 4-10). No significant differences were noticed between the HSG, the LSG and the MSG. In the right ventricle no significant differences were observed between the four groups.

Hematocrit values (HCT)

In the left and right ventricles, the LSG had a significantly higher HCT than the HSG and control group (Table 4-10). No significant differences were noticed between the HSG, MSG and control groups.

The alveolar to arterial oxygen tension gradient (AaO₂)

This parameter is the difference between the alveolar oxygen tension and the oxygen tension of arterial blood. In the blood from the left ventricle, the LSG had a significantly lower AaO₂ than the HSG (Table 4-10).

Table 4-10. Parameters in broilers' blood from the left and right ventricles on day of sacrifice, by group

Ventricle	Group	Parameter						
		stHCO ₃ (mmol/L)	cH ⁺ (mmol/L)	tHb (g/dl)	TOC (g/dl)	SO ₂ (%)	HCT (%)	AaDO ₂ (mmHg)
Left	HSG	21.8 ^b	51.8 ^a	8.6 ^b	10.78 ^a	70.2 ^{ab}	28.4 ^b	50.5 ^a
	MSG	23.2 ^b	52.6 ^a	9.5 ^{ab}	10.59 ^a	66.1 ^b	31.6 ^{ab}	46.0 ^{ab}
	LSG	25.1 ^a	52.0 ^a	9.7 ^a	10.20 ^a	66.9 ^b	32.2 ^a	40.0 ^b
	Control	21.8 ^b	48.3 ^a	8.7 ^b	11.27 ^a	82.9 ^a	29.1 ^b	46.7 ^{ab}
Right	HSG	21.9 ^{bc}	53.5 ^a	8.1 ^{ab}	9.59 ^a	38.2 ^a	26.6 ^b	63.1 ^a
	MSG	23.5 ^{ab}	56.8 ^a	9.0 ^{ab}	9.95 ^a	32.4 ^a	27.7 ^{ab}	59.2 ^a
	LSG	24.8 ^a	57.3 ^a	9.3 ^a	9.72 ^a	28.7 ^a	31.8 ^a	44.0 ^b
	Control	21.1 ^c	50.1 ^a	8.1 ^b	10.40 ^a	40.5 ^a	26.9 ^b	63.6 ^a

^{a,b,c} Different superscripts in same column indicate significant (P<0.05) differences in values

Electrolytes in serum

In this trial serum electrolytes were measured on Days 29 and 36. No significant difference was observed (Table 4-11) in the sodium and chloride levels between the groups in the two measurements. However, the potassium levels were significantly higher in the LSG and the MSG than in the HSG on Day 36. On Day 29 no significant differences were seen between the three groups.

Table 4-11. Electrolytes quantity (meq/L) in broilers' blood, by group and age

Group	Age (d)					
	29			36		
	K ⁺	Na ⁺	Cl ⁻	K ⁺	Na ⁺	Cl ⁻
HSG	7.1 ^a	143.1 ^a	103.4 ^a	7.0 ^b	144.2 ^a	102.8 ^a
MSG	7.5 ^a	142.9 ^a	103.5 ^a	8.1 ^a	144.0 ^a	102.7 ^a
LSG	7.1 ^a	143.8 ^a	103.3 ^a	7.9 ^a	145.2 ^a	102.8 ^a

^{a,b} Different superscripts in same column indicate significant (P<0.05) differences in values

Biochemical parameters in broilers' blood

In this trial biochemical parameters were measured on Days 27 and 34 in the HSG and the LSG (Tables 4-12, 4-13). On Day 34, there were not enough results in some cases to make a meaningful statistical analysis and these data were not depicted.

Albumin (ALB)

On Day 27 the albumin was significantly higher in the low group. No significant differences were observed on Day 34.

Alkaline phosphatase (ALP)

On Day 27 no significant difference was noticed between the two groups. On Day 34 there were not enough data to make a meaningful statistical analysis.

Amylase (Amyl)

On Day 27 no significant differences were noticed between the two groups. On Day 34 there were not enough data to make a meaningful statistical analysis.

Aspartate aminotransferase (AST)

On Day 27 the AST was significantly higher in the LSG. No significant differences were observed on Day 34.

Cholesterol (Chol)

On Day 27 no significant differences were noticed between the two groups. On Day 34 there were not enough data to make a meaningful statistical analysis.

Creatine phosphokinase (CPK)

No significant differences were observed on Days 27 and 34 between the two groups.

Calcium, total (Ca TOT)

No significant differences were observed on Days 27 and 34 between the two groups.

Table 4-12. Biochemical parameters in broilers' blood by day and by group

Age	Group	Parameter						
		ALB (g/dl)	ALP (u/l)	Amyl (u/l)	AST (u/l)	Chol (mg/dl)	CPK (u/l)	Ca TOT (mg/dl)
27	HSG	1.6 ^b	25108 ^a	555 ^a	126 ^b	124 ^a	381 ^a	4.6 ^a
	LSG	1.8 ^a	21978 ^a	540 ^a	145 ^a	132 ^a	1117 ^a	4.7 ^a
34	HSG	1.8 ^a			169 ^a		2330 ^a	5.1 ^a
	LSG	2.0 ^a			162 ^a		2630 ^a	5.7 ^a

^{a,b} Different superscripts in same column indicate significant (P<0.05) differences in values

Magnesium (Mg)

No significant differences were observed on Days 27 and 34 between the two groups.

Protein

On both Days 27 and 34 the protein was significantly higher in the LSG.

Phosphorus (Phos)

No significant differences were observed on Days 27 and 34 between the two groups.

Total bilirubin (T. Bil)

No significant differences were observed on Days 27 and 34 between the two groups.

Triglycerides (TRIG)

On Day 27 no significant differences were noticed between the two groups. On Day 34 there were not enough data to make a meaningful statistical analysis.

Uric acid (uric A)

On Day 27 the uric acid was significantly higher in the low group. No significant difference was observed on Day 34.

Lactic dehydrogenase (LDH)

On Day 27 no significant differences were noticed between the two groups. On Day 34 there were not enough data to make a meaningful statistical analysis.

Table 4-13. Biochemical parameters in broilers' blood by day and by group

Age	Group	Parameter						
		Mg (mg/dl)	Protein (g/dl)	Phos (mg/dl)	T. Bil (mg/dl)	Trig (mg/dl)	Uric A (mg/dl)	LDH (U/l)
27	HSG	1.5 ^a	3.1 ^b	4.4 ^a	0.29 ^a	99 ^a	5.7 ^b	2412 ^a
	LSG	1.7 ^a	3.7 ^a	4.2 ^a	0.32 ^a	114 ^a	7.7 ^a	2470 ^a
34	HSG	1.6 ^a	3.5 ^b	4.9 ^a	0.32 ^a		5.5 ^a	
	LSG	1.7 ^a	3.9 ^a	4.4 ^a	0.26 ^a		4.4 ^a	

^{a,b} Different superscripts in same column indicate significant ($P < 0.05$) differences in values

Body weight

In this trial the body weight was measured on Days 24, 27, 30, 33, 35 and 37 in the HSG, the MSG and the LSG. No significant differences were noticed between the groups during all the measurements (Table 4-14).

Table 4-14. Body weight (g) in broilers, by group and age

Group	Age (d)					
	24	27	30	33	35	37
HSG	868 ^a	1044 ^a	1285 ^a	1629 ^a	1742 ^a	1919 ^a
MSG	845 ^a	1023 ^a	1285 ^a	1616 ^a	1794 ^a	2002 ^a
LSG	869 ^a	1069 ^a	1322 ^a	1623 ^a	1759 ^a	1940 ^a

^{a,b} Different superscripts in same column indicate significant ($P < 0.05$) differences in values

Trial 5

Another change associated with the phenotypic selection for BW has been the reported decreasing levels of tri-iodothyronine (T_3) in growing broilers, with possible detrimental effects on metabolism and cardiac function, and hence on AS. In Year 2, serial determinations of blood levels of T_3 and of the precursor of T_3 , T_4 (thyroxine) were to be determined, as there may be differences in conversion rates by genotype. Blood was taken on 3 occasions from all birds in this trial (Trial 2 of the second year). Retrospective studies of the HCT, SaO_2 levels and HR with dates of ASM could allow us to monitor, in some birds, possible progression of changes in the blood that might be correlated with the subsequent ASM.

T_3 and T_4 in serum

In this trial these hormones were measured in the serum of birds (later classified as AS and no-AS groups) on Days 1, 25 and 39. No significant difference was observed (Table 5-1) in the T_4 levels on Day 1 whereas on Days 25 and 39 the T_4 level was significantly lower in the AS group. No significant differences have been observed in T_3 levels between the two groups during any of the measurements.

Table 5-1. T_3 and T_4 levels (ng/ml) in broilers' serum by AS group and age

Group	Parameter					
	T_4 levels on Day			T_3 levels on Day		
	1	25	39	1	25	39
AS	3.0 ^a	3.6 ^b	1.9 ^b	2.7 ^a	2.9 ^a	1.8 ^a
No-AS	3.3 ^a	4.3 ^a	4.0 ^a	2.8 ^a	2.8 ^a	2.2 ^a

^{a,b} Different superscripts in same column indicate significant ($P < 0.05$) differences in values

T_3 & T_4 in serum by environmental temperature

The statistical differences in T_3 and T_4 were also analyzed by the environmental temperature within the birds were kept (warm and cold). No significant difference was observed in T_3 and T_4 levels on Day 1 whereas on Days 25 and 39 the T_4 level was significantly lower in the cold group. Oppositely, on Days 25 and 39, the T_3 level was significantly higher in the cold group.

Table 5-2. T₃ and T₄ levels (ng/ml) in broilers' serum by environmental temperature and age

Group	Parameter					
	T ₄ levels on Day			T ₃ levels on Day		
	1	25	39	1	25	39
Cold	3.1 ^a	3.8 ^b	2.7 ^b	2.8 ^a	2.9 ^a	2.4 ^a
Warm	3.5 ^a	5.2 ^a	5.5 ^a	2.6 ^a	2.3 ^b	1.4 ^b

^{a,b} Different superscripts in same column indicate very significant ($P < 0.01$) differences in values

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Project Title:

An Integrated Approach to Evaluating Inherited Predictors of Resistance to Pulmonary Hypertension Syndrome (Ascites) in Fast Growing Broiler Chickens

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II. ABSTRACT

Clinically healthy broilers have greater oscillations in growth velocity and acceleration than PHS-susceptible broilers, and artificial neural networks are capable of accurately distinguishing differences in daily growth dynamics between normal broilers and those susceptible to PHS. The relationship between daily patterns of growth dynamics and susceptibility of broiler chickens to PHS has been confirmed. Additional minimally invasive diagnostic indices (heart rates, hematocrit, ECG wave amplitudes and durations, oximetry values for the percentage saturation of hemoglobin with oxygen, plasma amino acid profiles, plasma troponin T) did not universally differentiate PHS-resistant from PHS-susceptible sub-populations of broilers, however the oximetry, hematocrit, and ECG Lead II S-wave amplitude were the most consistent minimally invasive predictors of PHS susceptibility. Analyses of plasma constituents suggest cardiac taurine and troponin T are released into the plasma as a protective mechanism in response to the induction of pulmonary hypertension and hypoxemia; however, plasma taurine and troponin T are not predictive of the apparently healthy broilers that subsequently succumb to ascites. Pulse oximetry systems validated in these studies currently are being used by two major broiler breeding companies to eliminate PHS-susceptible individuals from their genetic stocks. The unilateral pulmonary artery occlusion technique was used in collaboration with a major broiler breeding company to demonstrate conclusively that dominant genes for PHS susceptibility exist, and to create a commercial broiler line that is highly resistant to PHS induced by fast growth and cool temperatures. Cardio-pulmonary characteristics typical of preascitic broilers when compared with the PHS-resistant line include increases in pulmonary arterial pressure, pulmonary vascular resistance, cardiac output, hematocrit, and the partial pressure of CO₂ in arterial blood, venous congestion (increased venous volume) reflected by venous hypertension (increased venous pressure), and lower values for the percentage saturation of hemoglobin with oxygen, heart rate, systemic arterial pressure, and total peripheral resistance. The responses of preascitic broilers to breathing 100% oxygen provided definitive proof that hypoxemia arises as a consequence of a diffusion limitation triggered by blood flowing too rapidly past the gas exchange surfaces of the lungs. Measurements of pulmonary arterial wedge pressures provided definitive proof that pulmonary hypertension is initiated as a consequence of excessive pulmonary arteriole resistance. Systemic hypotension stimulates renal retention of solutes and water that ultimately comprise the ascitic fluid. Highly variable pulmonary hemodynamic responses to vasoactive mediators among individual broilers may be related to innate or acquired variability, and may be relevant to individual differences among broilers in their susceptibility to pulmonary hypertension syndrome. Genetic analyses demonstrate that many traits related to PHS are significantly heritable and hence can potentially be altered by selection, however only the percentage saturation of hemoglobin with oxygen and possibly heart rate are suggested as early predictors for development of AS when birds are exposed to cool temperatures. Therefore selection for high blood oxygenation and high pulse rate in young birds from a commercial broiler stock may genetically reduce the propensity to develop AS.

III. OBJECTIVES OF THE ORIGINAL RESEARCH PROPOSAL

Original Objectives: The objectives of the original research proposal were to: (1) evaluate the growth dynamics of individuals within breeding stocks as a potentially highly informative and directly measured heritable index of individual susceptibility or resistance to PHS, and to select genetic lines exhibiting smooth (SM) or aphasic (AP) growth dynamics; (2) examine the progeny of SM and AP selected lines and compile data on diagnostic indices known to be predictive for PHS, during exposure to three experimental protocols known to trigger PHS; (3) conduct detailed physiological evaluations of broilers having SM and AP growth dynamics to determine if specific differences in cardio-pulmonary function can be detected; (4) compile data on growth dynamics and other diagnostic indices in existing lines selected for susceptibility or resistance to PHS; and, (5) integrate growth dynamics and other diagnostic data within appropriate statistical procedures to provide geneticists with highly predictive indices that accurately characterize resistance or susceptibility to PHS.

Modifications to the Objectives: Originally, the central focus of Objectives 1 to 3 was to correlate patterns of daily growth dynamics with the susceptibility or resistance of broilers to pulmonary hypertension syndrome (PHS, ascites). In order to fully accomplish these objectives, it was essential to acquire accurate body weights at least once daily for each broiler being evaluated. The Peckcode Weigh Platform/Individual Bird I.D. System offered technology that initially appeared suitable for automatically recording body weights many times per bird per day. However, as indicated in the 1st Annual Report, detailed evaluations demonstrated the weights recorded by the Peckcode system were not sufficiently accurate or reproducible. The lack of reproducibility was due to a fundamentally inaccurate load cell that generated values having an instantaneous variance of more than half the anticipated daily body weight gain when a single live broiler was repeatedly placed on the weigh platform. The Peckcode system also did not automatically compensate for accumulated litter, and the Pecklog software was cumbersome and inefficient. Subsequently, the US and Israeli teams embarked on a series of highly labor intensive trials involving manually weighing broilers to accomplish the first part of Objective 1. These trials convinced both teams that manually weighing hundreds of broilers on a daily basis could not be seriously considered as a strategy for selecting genetic lines based on growth dynamics. Because the anticipated labor-saving technology was not available, the decision was made to forgo addressing the second part of Objective 1. Instead, as outlined in the 1st and 2nd Annual Reports, Objectives 2 to 5 were refocused to compare growth dynamics and other key diagnostic indices in broilers known to be clinically healthy or developing PHS (preascitic and ascitic), and in broiler lines selected for susceptibility or resistance to PHS based on criteria other than growth dynamics (unilateral pulmonary artery occlusion, hypobaric hypoxia).

The involvement of geneticists from two of the major international broiler breeding companies (Cobb-Vantress and Hubbard ISA) led to the realization that obtaining multiple diagnostic indices for incorporation into a statistical model is too expensive and labor intensive for commercial genetic selection programs. Accordingly, Objective 5 was refocused toward finding a single, easily obtained and reliable

diagnostic index of PHS susceptibility or resistance for use in commercial broiler selection programs. The US team collaborated with Dr. M. H. Maxwell (Roslin Institute, Scotland, UK) to evaluate his Troponin T marker as a promising additional minimally invasive diagnostic index related to ascites. Dr. Maxwell previously reported a strong genetic correlation between the presence of this marker and ascites susceptibility. Further work on a potential plasma marker was initiated by the US team, assessing key plasma amino acid and metabolite profiles in broilers with cold- and pulmonary artery clamp-induced ascites. Based on the success of surgical unilateral pulmonary artery occlusion as a technique for selecting PHS-resistant genetic lines, the US team evaluated several injectible chemicals for their potential as pulmonary vasoconstrictors in broilers. The chemical vasoconstrictor studies were combined with experiments conducted to satisfy Objective 3, to determine if specific differences in cardio-pulmonary function can be detected between clinically healthy and preascitic broilers. Specific differences in cardio-pulmonary function associated with PHS-susceptibility were identified. The unilateral pulmonary artery occlusion technique was used in collaboration with a commercial geneticist (Dr. Howard French) and a major broiler breeding company to demonstrate conclusively that dominant genes for PHS susceptibility exist, and to create a commercial broiler line that is highly resistant to PHS induced by fast growth and cool temperatures.

I V. RELEVANT DATA, METHODOLOGY, RESULTS AND DISCUSSION

(Note: Appendix A contains all text and figures for published papers, and manuscripts that have been submitted or are in preparation).

1. EVALUATION OF BROILER GROWTH VELOCITY AND ACCELERATION IN RELATION TO PULMONARY HYPERTENSION SYNDROME (PHS). *Current Status:* Published, see Appendix A (Roush, W. B., and Wideman, R. F. Evaluation of growth velocity and acceleration in relation to pulmonary hypertension syndrome (PHS). Poultry Sci. 79:180-191, 2000).

1.A. Summary: An evaluation was made of the relationship between individual daily growth patterns and susceptibility of broiler chickens to pulmonary hypertension syndrome (PHS). In the first experiment, 46 male broilers were weighed for each of 50 d, during which time 13 developed PHS. Three temporal phases (0-15, 16-35, and 36-50 d) of broiler growth velocity and acceleration were examined. Correlation Dimensions and Lyapunov exponents provided evidence of chaos in growth velocity and acceleration, but the absence of detectable differences between broilers in the normal and PHS categories led us to reject the hypotheses that growth is more chaotic in normal broilers than in broilers susceptible to PHS. Growth velocity and acceleration values for mean and SD were statistically evaluated as response variables for each growth phase. Mean values for velocity during the third phase were different between broilers in the normal and PHS categories (velocity: 68.8 vs. 48.9 g/d, $p = 0.03$, respectively) and (acceleration: 0.3 vs. - 1.4 g/d², $P = 0.07$, respectively). The third phase SD (reflecting oscillation for velocity and acceleration), was greater for normal as compared to PHS birds (velocity: 26.1 vs. 21.3 g/d, $P = .13$,

respectively and acceleration: 39.7 vs. 28.2 g/d², $P = 0.03$, respectively). The hypothesis was accepted that normal birds have greater oscillations in growth velocity and acceleration than birds susceptible to PHS. A General Regression Neural Network (GRNN) with genetic adaptive calibration was trained to predict PHS based on the individual growth phases and their combinations. Data representing the first, first two, and all three phases of growth were determined to have potential for computerized diagnostic weighing. Using the GRNN, birds in all three data sets were successfully classified (100%) with or without PHS. A third hypothesis therefore was accepted that artificial neural networks could be used to distinguish the difference between normal broilers and those susceptible to PHS. In the second experiment, only one bird was diagnosed with PHS. Velocity and Acceleration neural networks from Phase 1 and Phases 1-2 in the first experiment were applied to the experiment 2 growth velocity and acceleration data. The Phase 1 neural networks were the most promising in that they correctly identified 71.6 and 72.4% of the birds as normal for velocity and acceleration data, respectively. In general, data in the second experiment exceeded the neural network range of training for both velocity and acceleration. This reflected the increased oscillation during the second phase of growth.

1.B. Objectives Addressed: This project is relevant to the first part of Objective 1, as well as Objectives 2 and 5. The Peckcode System was demonstrated to be inadequate for our purposes. The manufacturers of this system ultimately were unable to incorporate engineering changes according to our requirements (personal communication, Mr. Alwyn Havard, NEDAP Agri, BV). Our problematic experiences involving the Peckcode system played an important role in the decision by at least one broiler breeder company (Cobb-Vantress) to forgo purchasing of these systems. As proposed by Roush *et al.* (1994), the relationship between individual daily growth patterns and susceptibility of broiler chickens to ascites has been confirmed. Individual broilers exhibit intervals of growth stasis followed by episodes of growth saltation. Clinically healthy fast-growing broilers tend to exhibit more frequent and higher amplitude transitions between saltation and stasis than do broilers developing PHS during the Day 35-49 interval. Clinically healthy birds have greater oscillations in growth velocity and acceleration than birds that are susceptible to ascites. A Genetic Adaptive General Regression Neural Network (GRNN) with genetic adaptive calibration was trained to predict ascites susceptibility based on the individual growth phases and their combinations. Data representing the first, first two, and all three phases of growth were determined to have potential for computerized diagnostic weighing. Using the GRNN, birds in all three data sets were successfully classified (100%) with or without PHS. Therefore, artificial neural networks can be used to distinguish the difference between normal broilers and those susceptible to PHS. These observations confirm the fundamental premise of the grant proposal. Additional growth rhythms of longer sinusoidal durations also were observed in the "4-day average standard deviation" calculations for both experiments. The meaning of these apparently synchronized pulses of flock variability remain to be explained from a biological perspective.

2. EVALUATION OF MINIMALLY INVASIVE INDICES FOR PREDICTING ASCITES SUSCEPTIBILITY IN THREE SUCCESSIVE HATCHES OF BROILERS EXPOSED TO COOL TEMPERATURES. *Current Status:* Published, see Appendix A (Wideman, R. F., Wing, T., Kirby, Y. K., Forman, M. F., Marson, N., Tackett, C. D., and Ruiz-Feria, C. A. Evaluation of minimally invasive indices for predicting ascites susceptibility in three successive hatches of broilers exposed to cool temperatures. *Poultry Sci.* 77:1565-1573, 1998).

2.A. Summary: Broilers from three consecutive hatches were exposed to cool temperatures to amplify the incidence of pulmonary hypertension syndrome (PHS, ascites). The largest apparently healthy individuals on Day 42 were evaluated using minimally invasive diagnostic indices (percentage saturation of hemoglobin with oxygen, hematocrit [HCT], heart rate, electrocardiogram [ECG] Lead II, body weight), then they were subjected to the ongoing pressures of fast growth and cool temperatures to determine which of these indices are predictive of the subsequent onset of PHS. Approximately 20% of the males and females evaluated on Day 42 subsequently developed PHS by Day 51. When data for all hatches were pooled and broilers that subsequently developed ascites were compared with those that did not (nonascitic), body weights, heart rates, and percentage saturation of hemoglobin with oxygen were lower on Day 42 for ascitic than for nonascitic males, and HCT was higher in ascitic males and females than in nonascitic males and females, respectively. Comparisons of the ECG Lead II wave amplitudes for all hatches pooled indicated the RS-wave amplitude was larger in ascitic than in nonascitic males, and the ECG Lead II S-wave amplitude was more negative in ascitic males and females than in nonascitic males and females. Necropsies conducted on Day 51 revealed higher right:total ventricular weight ratios in ascitic than in nonascitic broilers, whereas normalizing the left ventricle plus septum weight for differences in body weight generated similar values for ascitic and nonascitic males and females, respectively. These results support a primary role for pulmonary hypertension but not cardiomyopathy in the pathogenesis of ascites triggered by cool temperatures. Values obtained for minimally invasive diagnostic indices on Day 42 also establish predictive thresholds that can be used to evaluate the PHS susceptibility of large and apparently healthy male and female broilers.

2.B. Objectives Addressed: This project is relevant to Objectives 1 and 5. The study was designed to evaluate minimally invasive diagnostic indices using a protocol that is applicable to commercial genetic selection programs. The study was conducted in cooperation with Cobb-Vantress, Inc. Of the males and females identified as the largest apparently healthy individuals on Day 42, an average of 20% across all three hatches subsequently developed ascites by Day 51. None of the minimally invasive diagnostic indices consistently differentiated the apparently healthy males and females that subsequently developed ascites, suggesting the predictive value of these indices may vary when applied to individual birds of different genders. To address this dilemma within a commercial selection program, multiple diagnostic indices (body weight, hematocrit, and oximetry coupled with the ECG Lead II S-wave amplitude) can be evaluated, and the data for each bird can be compared with a series of threshold values known to be predictive for the genotype, gender and environmental conditions. For example, under the conditions of the study, an aggressive threshold for culling on Day 42 might be set near the mean values for nonascitic

birds (36% for HCT, 75% for oximetry, -0.066 mV for ECG Lead II S-wave amplitude), or a more relaxed threshold might be set near the mean values for birds that subsequently developed ascites (40% for HCT, 63% for oximetry, -0.150 mV for ECG Lead II S-wave amplitude). The data from this study were provided to Dr. Roush for incorporation into a neural net model. Geneticists from two of the major international broiler breeding companies (Cobb-Vantress and Hubbard ISA) indicated that obtaining multiple diagnostic indices for incorporation into a statistical model would be considered too expensive and time consuming for a commercial genetic selection program. Both of these broiler breeding companies currently are using the pulse oximetry system we validated in this study (Vet/Ox™ 4403 pulse oximeter and universal “C” sensor) to help identify and then eliminate PHS-susceptible individuals from their genetic stocks.

3. BROILER BREEDER SURVIVORS OF CHRONIC UNILATERAL PULMONARY ARTERY OCCLUSION PRODUCE PROGENY RESISTANT TO PULMONARY HYPERTENSION SYNDROME INDUCED BY COOL TEMPERATURES, AND ASCITES RESISTANCE OF PROGENY FROM BROILER BREEDERS SELECTED FOR TWO GENERATIONS USING CHRONIC UNILATERAL PULMONARY ARTERY OCCLUSION. *Current Status:* Published, see Appendix A (Wideman, R. F., and French, H. Broiler breeder survivors of chronic unilateral pulmonary artery occlusion produce progeny resistant to pulmonary hypertension syndrome induced by cool temperatures. *Poultry Sci.* 78:404-411, 1999; and, Wideman, R. F., and French, H. Ascites resistance of progeny from broiler breeders selected for two generations using chronic unilateral pulmonary artery occlusion. *Poultry Sci.* 79:396-401, 2000).

3.A. Summary: Chronic occlusion of one pulmonary artery triggers a high incidence of pulmonary hypertension syndrome (PHS, ascites) in broilers. In the present study, the left pulmonary artery was chronically occluded in 295 male and 255 female chicks pedigreed from 18 sire families, leading to PHS in 74% of the males and 45% of the females. Survivors were reared to breeding age and served as the first generation (GEN1) parents for the resulting PHS-resistant chicks (Resistant), whereas control chicks were produced from the base population for this line (BASE). In two experiments, male and female Resistant and BASE chicks were reared separately by sex but mixed by group within environmental chambers, where they were exposed to cool (14 C) temperatures. In both experiments, the incidence of PHS was at least 50% lower in the Resistant males and females when compared with the BASE males and females, respectively. When compared within a gender, the BASE and Resistant broilers surviving to the end of both experiments did not differ in final body weight or body weight gain, nor did their right:total ventricular weight (RV:TV) ratios differ. These results demonstrate that broiler breeders capable of thriving after having their entire cardiac output forced to flow through one lung, subsequently produced male and female progeny with substantially improved resistance to the onset of PHS induced by fast growth and exposure to cool environmental temperatures. Fast growth and cool temperatures are primary triggers for PHS under most conditions of commercial broiler growout. In both experiments, final necropsies revealed higher RV:TV ratios in ascitic than in nonascitic broilers, whereas normalizing the left ventricle plus septum weight for differences in body weight generated similar values for ascitic and nonascitic males or females, respectively. These results support a primary role

for pulmonary hypertension but not cardiomyopathy in the pathogenesis of ascites triggered by cool temperatures in both the BASE and Resistant populations.

To create the parent stock for the second generation (GEN2) ascites resistant line, progeny from the GEN1 line were subjected to unilateral pulmonary artery occlusion, and survivors were reared to breeding age. In two experiments, male and female chicks from three lines (exp. 1: BASE, GEN1, GEN2) or two lines (exp. 2: BASE, GEN2) were reared separately by sex but mixed by line within environmental chambers. They were grown as rapidly as possible and exposed to cool (14 C) temperatures from 17 to 49 d of age. The incidences of ascites in the first experiment were 31% (48/157), 15% (8/52), and 4% (3/69) for BASE, GEN1, and GEN2 males, and 10% (13/128), 11% (5/46), and 3% (1/36) for BASE, GEN1, and GEN2 females, respectively. The incidences of ascites in the second experiment were 44% (71/163) and 6% (7/110) for BASE and GEN2 males, and 12% (19/155) and 0% (0/92) for BASE and GEN2 females. Within a sex and line, Day 1 and intermediate (Day 17 or 21) body weights (BW) were not predictive of susceptibility to ascites, and the final BW was consistently lower for ascitic than for nonascitic broilers. Within a sex, the final BW for nonascitic broilers did not differ across lines in experiment 1. However, in experiment 2 the final BW was lighter for nonascitic GEN2 males ($2,915 \pm 43$ g) and females ($2,382 \pm 17$ g) than for nonascitic BASE males ($3,088 \pm 42$ g) and females ($2,493 \pm 22$), respectively. Trends toward differences in the final BW for the BASE and GEN2 lines presumably were due to the relatively relaxed selection for BW gain in the GEN2 population, as compared with intensive selection for this trait in the BASE population. Right:total ventricular weight (RV:TV) ratios were higher for ascitic than nonascitic broilers in both experiments. Elevated RV:TV ratios in ascitic broilers were attributed to elevated relative right ventricular weights (RV/BW), whereas relative left ventricle plus septum weights did not differ between ascitic and nonascitic broilers. Elevated RV:TV ratios and relative right ventricular weights confirm the primary role for pulmonary hypertension in the pathogenesis of ascites. The combined results from both experiments demonstrate ongoing improvement in the ascites resistance of progeny from broiler breeders that, for two consecutive generations, have survived the rigorous selection pressure imposed by unilateral pulmonary artery occlusion. The rapid pace of improvement attainable using this method of selection confirms the central contribution of an inadequate pulmonary vascular capacity (anatomical or metabolic) to the ascites susceptibility of broilers grown as rapidly as possible during exposure to cool temperatures. As a caveat, trends toward slower growth also may have contributed to the improved ascites resistance of the GEN2 line.

3.B. Objectives Addressed: These projects are relevant to Objectives 1, 2 and 4. In lieu of selecting genetic lines exhibiting smooth or aphasic growth dynamics as originally proposed in Objective 1, unilateral pulmonary artery occlusion was used to select for broad-based resistance to ascites by rigorously eliminating PHS-susceptible individuals. Working in close cooperation with Hubbard ISA, the resulting PHS-resistant lines and Base population provided the biological. (genetic) basis for further evaluations of growth dynamics and minimally invasive diagnostic indices. Broiler breeders that thrived in spite of having one pulmonary artery occluded, subsequently produced first generation (GEN1) male and

female progeny exhibiting a > 50% reduction in the incidence ascites. Furthermore, growth performance was not reduced in attaining this improvement in ascites resistance, in spite of the fact that the breeder parent line for the chicks of the Base population (controls) had experienced an additional generation of selection for growth that necessarily could not be imposed on the breeder parents for the Resistant chicks. A second generation (GEN2) of this ascites-resistant line again was selected based on progeny that survived unilateral pulmonary artery occlusion, and additional improvements in ascites resistance were achieved with a minimal impact on growth. The dramatic improvement in ascites resistance within two generations of selection served as definitive proof to commercial geneticists that, with appropriately focused and sufficiently rigorous selection pressures, major advances can be accomplished in reducing the frequency of undefined genes that seem to be partly responsible for ascites susceptibility in commercial broilers. In this context, the success of the unilateral pulmonary artery clamp technique confirms that the anatomical or metabolic capacity of the pulmonary vasculature to accommodate an increase in blood flow is crucial to the ability of broilers to resist the onset of pulmonary hypertension leading to ascites. The gene or genes involved in ascites susceptibility appear to be dominant, indicating that ongoing proactive exposure and elimination of susceptible individuals will be necessary to achieve an overall improvement in ascites resistance. By analogy, familial primary pulmonary hypertension in humans appears to involve vertical transmission of a dominant gene having incomplete penetrance and "genetic anticipation", contributing to the unpredictable onset of pulmonary hypertension at progressively earlier ages in successive generations. A major achievement of this research is that one of the dominant international broiler breeder companies, Hubbard ISA, now possesses a pedigree line that is highly resistant to ascites when grown as rapidly as possible during exposure to cool temperatures.

4. ELECTROCARDIOGRAPHIC AND GENETIC EVALUATION OF GIANT JUNGLE FOWL, BROILERS, AND THEIR RECIPROCAL CROSSES FOLLOWING UNILATERAL BRONCHUS OCCLUSION AND ELECTROCARDIOGRAPHIC EVALUATION OF BROILERS FOLLOWING UNILATERAL OCCLUSION OF AN EXTRAPULMONARY PRIMARY BRONCHUS. *Current Status:* Published, see Appendix A (Kirby, Y. K., Anthony, N. B., Hughes, J. D., McNew, R. W., Kirby, J. D., and Wideman, R. F. Electrocardiographic and genetic evaluation of giant jungle fowl, broilers, and their reciprocal crosses following unilateral bronchus occlusion. *Poultry Sci.* 78:125-134, 1999; and, Kirby, Y. K., McNew, R. W., Anthony, N. B., Marson, N. E., Hughes, J. D., Kirby, J. D., and Wideman, R. F. Electrocardiographic evaluation of broilers following unilateral occlusion of an extrapulmonary primary bronchus. *Poultry Sci.* 78:125-134).

4.A. Summary: Electrocardiography is useful as a noninvasive technique for detecting right ventricular hypertrophy in birds developing pulmonary hypertension (PH) and pulmonary hypertension syndrome (PHS, ascites). The objective of this study was to identify every aspect of the Lead II ECG wave form (amplitude or duration) that can be correlated with right ventricular hypertrophy (increased RV:TV ratios) indicative of PH across a broad genetic background. Sham operations were conducted (SHAM), or PH was induced by occluding one extrapulmonary primary bronchus (BRONCHUS CLAMP) in 14 d old

chicks produced from matings of broilers (BxB), Giant Jungle Fowl (JxJ), and their reciprocal crosses (BxJ and JxB). Standard three lead electrocardiograms (ECG) were recorded on Days 28 and 42, and final necropsies were conducted to evaluate the incidence of ascites, confirm sex, and obtain ventricular weights for calculating the right:total ventricular weight ratios (RV:TV). Ascites did not develop in the JxJ, and one each of the BxJ and JxB chicks developed ascites, consequently only data from birds that did not develop ascites were compared. Heart rate was recorded, and the following amplitudes and durations were measured or calculated for three consecutive wave cycles of the Lead II ECG: base of R to the peak of R (R_bR), peak of R to base of S (RS), base of S to peak of RN (SRN), S, peak of RN to base of RN ($RNRN_b$), and base of S to peak of T (ST). Differences between the ECG of SHAM and BRONCHUS CLAMP groups were more prominent in BxB and BxJ than in JxB, and bronchus occlusion did not affect the ECGs, growth, or RV:TV ratios of JxJ. In contrast, gender influences were more prominent in JxJ and JxB than in BxJ and BxB. These observations suggest a paternal pattern of inheritance for Lead II ECG wave forms, with crosses sired by broilers (BxB, BxJ) exhibiting susceptibility to PH and few ECG differences related to gender, whereas crosses sired by Giant Jungle Fowl (JxJ, JxB) exhibited resistance to PH and numerous ECG differences related to gender.

The second study provides a comprehensive evaluation of both the amplitudes and durations of the Lead II ECG in nonascitic and ascitic broilers. At 14 d of age, male and female broiler chicks were sham-operated (SHAM, $n = 27$), or pulmonary hypertension was initiated by occluding one extrapulmonary primary bronchus (BRONCHUS CLAMP, $n = 57$). Lead II ECGs and body weights were recorded on Days 28 (ECG1) and 42 (ECG2), necropsies were conducted on all birds dying after Day 28, and final necropsies were conducted on Day 49. Data collected at necropsy included the presence (ASCITIC) or absence (NONASCITIC) of ascites, gender, and ventricular weights for calculating the right:total ventricular weight ratio (RV:TV), which serves as a reliable index of pulmonary hypertension. In each bird, three consecutive ECG1 and ECG2 wave cycles were quantified for both amplitude and duration of the following wave segments: $R_b - R$, $R - S$, $S - RN$, $RN - RN_b$, and $S - T$. The S wave amplitude was calculated by subtracting $R - S$ from $R_b - R$ and heart rate (HR) was measured from the peak of one T wave to the peak of the next. In the majority comparisons, ASCITIC and BRONCHUS CLAMP broilers had larger S , $RN - RN_b$, and $S - T$ amplitudes, longer $R - S$, $RN - RN_b$, and $S - T$ durations, and a slower HR than NONASCITIC and SHAM broilers, regardless of gender. The differences in ECG wave forms and durations between ASCITIC and NONASCITIC broilers were greater on Day 42 (ECG2) than on Day 28 (ECG1), but when both ECGs were used to develop a regression equation to estimate RV:TV, the R^2 was 0.79. The most important Lead II ECG parameters associated with the development of ascites were an increasingly negative S wave amplitude and greater amplitudes and durations for $RN - RN_b$ and $S - T$ as well as a decrease in the HR.

4.B. Objectives Addressed: These projects are relevant to Objectives 2, 4 and 5. The data clearly establish an increasingly negative ECG Lead II S-wave amplitude as a marker for pulmonary hypertension and right ventricular hypertrophy induced by unilateral bronchus occlusion. The results

further demonstrate a genetic- and growth rate- related component of the susceptibility to pulmonary hypertension. The results exclude the need to assess the duration components of the ECG wave forms, permitting us to reliably focus on components of the negative ECG Lead II S-wave amplitude as the primary wave segment of interest for evaluating the onset of pulmonary hypertension leading to ascites. The ECG changes associated with unilateral bronchus occlusion are identical to those previously shown to be predictive of pulmonary hypertension induced by cool temperatures or after unilateral pulmonary artery occlusion. Consequently, the use of the bronchus clamp model to generate pulmonary hypertension for studies of growth dynamics and other minimally invasive diagnostic indices has been substantiated (Objective 2).

5. CARDIAC OUTPUT IN 4, 5, AND 6 WEEK OLD BROILERS, AND HEMODYNAMIC RESPONSES TO INTRAVENOUS INJECTIONS OF EPINEPHRINE. *Current Status:* Published, see Appendix A (Wideman, R. F. Cardiac Output increases in 4, 5 and 6 week old broilers, and hemodynamic responses to intravenous injections of epinephrine. Poultry Sci. 78:392-402, 1999).

5.A. Summary: Female broilers were evaluated at 4, 5 and 6 wk of age (1.2, 1.8 and 2.3 kg BW, respectively) to assess changes in cardiac output and related hemodynamics associated with BW gain, and to evaluate cardio-pulmonary hemodynamic adjustments occurring secondary to i.v. injections of epinephrine (0.1 mg/kg BW). Cardiac output increased with BW (253, 348 and 434 mL/min at 4, 5 and 6 wk, respectively) due to increases in stroke volume (0.70, 1.03 and 1.33 mL/beat) that more than compensated for reductions in heart rate (362, 337 and 328 bpm). Normalization for BW eliminated the differences in cardiac output and stroke volume. Increases in cardiac output were not associated with age- or BW-related increases in mean systemic arterial pressure (101.5, 108.6 and 108.0 mm Hg) due to corresponding reductions in total peripheral resistance (0.41, 0.32 and 0.26 relative resistance units). Epinephrine initially triggered immediate (within 90 s) 3-fold increases in total peripheral resistance and pulmonary vascular resistance, which in turn increased the systemic arterial pressure and pulmonary arterial pressure in spite of concurrent reductions in cardiac output that were associated with diminished venous return and dependent reductions in stroke volume. Within 150 s after epinephrine injection, the systemic and pulmonary vascular resistances returned to pre-injection control levels. By 300 s post-injection, stroke volume and heart rate increased, causing cardiac output to rise above pre-injection control levels, which in turn elicited variable pulmonary arterial pressure responses apparently reflecting individual variability in the capacity for flow-dependent pulmonary vasodilation. These studies demonstrate that chronic (age- and BW-related) and acute (epinephrine-induced) changes in cardiac output in broilers reflect complex interactions among hemodynamic variables that include stroke volume, heart rate, and systemic and pulmonary vascular resistances.

5.B Objectives Addressed: This project is relevant to Objectives 3 and 5. We quantified age- and body-mass dependent increases in cardiac output, stroke volume, and ventricular weight, providing direct evidence that the right ventricle was forced to propel a rapidly increasing cardiac output through the

pulmonary vasculature. The growth-associated increments in cardiac output are attributable wholly to increases in stroke volume and not heart rate. When considering heart rate as a minimally invasive diagnostic index, care must be exercised to avoid confounding age-related reductions in heart rate with the equally modest reductions in heart rate occurring during the onset of PHS in rapidly growing broilers. Epinephrine profoundly constricts the pulmonary vasculature, however its actions are too transient to be considered for the prolonged pulmonary vasoconstriction needed to eliminate PHS-susceptible broilers.

6. THROMBOXANE MIMICS THE PULMONARY BUT NOT SYSTEMIC VASCULAR RESPONSES TO BOLUS HCl INJECTIONS IN BROILER CHICKENS. *Current Status:* Published, see Appendix A (Wideman, R. F., Maynard, P., and Bottje, W. G. Thromboxane mimics the pulmonary but not systemic vascular responses to bolus HCl injections in broiler chickens. *Poultry Sci.* 78:714-721, 1999).

6.A. Summary: Bolus i.v. injections of 1.2 N HCl elicit a rapid but transient pulmonary vasoconstriction in broiler chickens. In mammals, the pulmonary vasoconstrictive response to bolus acid injection depends on increased synthesis of thromboxane A_2 , however the vascular responsiveness of domestic fowl to thromboxane previously had not been evaluated. In the present study we tested the hypothesis that, if HCl triggers pulmonary vasoconstriction by stimulating thromboxane A_2 synthesis in broilers, then bolus i.v. injections of the potent thromboxane A_2 mimetic U44069 (9,11-dideoxy-9 α ,11 α -epoxy-methanoprostaglandin $F_{2\alpha}$; 1 micromole per mL; 0.5 mL injected volume) should trigger hemodynamic responses similar to those elicited by HCl (1.2 N; 1.5 mL injected volume). Both HCl and the thromboxane mimetic elicited $\geq$ twofold increases in pulmonary vascular resistance, which in turn increased pulmonary arterial pressure by 50% despite concurrent reductions in cardiac output. The reductions in cardiac output were associated with reductions in stroke volume but not heart rate. The thromboxane mimetic also increased the total peripheral resistance, which minimized the reduction in mean systemic arterial pressure associated with the decrease in cardiac output. In contrast, HCl injections did not increase total peripheral resistance; consequently the reduction in cardiac output caused the mean systemic arterial pressure to decrease by 30 mm Hg. Mannitol (2.5%; 1.5 mL) was injected i.v. as a volume control, and had no influence on any of the variables. This study provides the first direct evidence that thromboxane is a potent pulmonary vasoconstrictor in broilers, and provides support for the hypothesis that thromboxane mediates the pulmonary vasoconstrictive response to bolus i.v. injections of HCl. The differential response of the systemic vasculature to the thromboxane mimetic and HCl may indicate the cardiopulmonary responses to HCl injections are not mediated solely via thromboxane production. Alternatively, a direct dilatory effect of elevated hydrogen ion concentrations on the systemic vasculature may have counteracted any tendency for simultaneously evolved endogenous thromboxane to elicit systemic vasoconstriction.

6.B. Objectives Addressed: This project is relevant to Objectives 3 and 4. We now are capable of identifying broilers with growth dynamics that are characteristic of resistance to ascites. It next will be necessary to determine if specific differences in cardio-pulmonary function can be detected (Objective 3),

under the assumption that such information can help us identify key diagnostic indices (Objective 4). Thromboxane is involved in pulmonary vasoconstriction under a wide variety of circumstances impacting broiler chickens, including hypoxia, metabolic acidosis, and respiratory diseases. Thus the levels of thromboxane synthesized in response to appropriate challenges, or the pulmonary vascular responsiveness to thromboxane, may play a central role in the susceptibility of broilers to ascites. For example, bolus i.v. injections of HCl trigger the release of endogenous thromboxane, and large differences in the responsiveness of individual broilers to bolus HCl injections have been documented (Wideman *et al.*, 1998. The infusion rate dependent influence of acute metabolic acidosis on pulmonary vascular resistance in broilers. *Poultry Sci.* 77:309-321). We now have established the protocols for evaluating the cardio-pulmonary responsiveness of susceptible and resistant broilers to HCl and Thromboxane injections. Presumably susceptible broilers will be hyper-responsive and resistant broilers will be hypo-responsive. These data also provide the basis for reducing the incidence of ascites by treating affected broilers with inhibitors of thromboxane synthesis that do not simultaneously inhibit synthesis of the important vasodilator prostacyclin.

7. VENOUS BLOOD PRESSURE IN BROILERS DURING ACUTE INHALATION OF 5% CARBON DIOXIDE OR UNILATERAL PULMONARY ARTERY OCCLUSION. *Current Status:* Published, see Appendix A. (Wideman, R. F., Maynard, P., and Bottje, W. G. Venous blood pressure in broilers during acute inhalation of five percent carbon dioxide or unilateral pulmonary artery occlusion. *Poultry Sci.* 78:1443-1451, 1999).

7.A. Summary: We evaluated the hypothesis that venous congestion (increased venous volume), as reflected by venous hypertension (increased venous pressure), can arise when the right ventricle is unable to elevate the pulmonary artery pressure sufficiently to propel the cardiac output through an anatomically inadequate or inappropriately constricted pulmonary vasculature. Changes in venous pressure were evaluated in clinically healthy broilers during modest increases in pulmonary vascular resistance induced by inhalation of 5% CO₂, and during large increases in pulmonary vascular resistance accomplished by acutely tightening a snare around one pulmonary artery. Inhalation of 5% CO₂ induced a pronounced respiratory acidosis, as reflected by increases the partial pressure of CO₂ and the hydrogen ion concentration in arterial blood. Inhalation of 5% CO₂ also increased pulmonary artery pressure by approximately 3 mm Hg, and increased venous pressure by approximately 1 mm Hg when compared with the pre-inhalation venous pressure. Tightening the pulmonary artery snare increased the pulmonary artery pressure by approximately 10 mm Hg, and this degree of pulmonary hypertension was sustained until the snare was released. When compared with the pre- and post-snare intervals, tightening the pulmonary artery snare induced a sustained increase in venous pressure of ≥ 1 mm Hg. Veins have highly compliant walls that permit an approximate doubling in volume with only small (4 to 6 mm Hg) increases in central venous pressure. Presumably the apparently modest 1 mm Hg increase in venous pressure we measured after CO₂ inhalation or unilateral pulmonary artery occlusion reflects a large increase in venous volume, and thus substantial venous congestion. These observations support the

hypothesis that increases in pulmonary vascular resistance can initiate increases in venous pressure by challenging the capacity of the right ventricle to propel all of the returning venous blood through the lungs. Central venous congestion predisposes broilers to the onset of cirrhosis and ascites by impeding the outflow of hepatic venous blood and increasing the hydrostatic pressure within hepatic sinusoids.

7.B. Objectives Addressed: This project is relevant to Objectives 3 and 4. Broilers susceptible to ascites progress through a phase of right sided congestive heart failure. Blood that cannot be efficiently pumped by the right ventricle through the lungs must accumulate in and distend the systemic veins. The accumulated venous blood also congests the sinusoids within the liver, contributing to pressure- or hypoxemia-induced cirrhosis and plasma leakage through the surface of the liver. Venous congestion can promote the onset of pulmonary edema by slowing lymph drainage from the lungs into the vena cava. It is relatively easy to puncture the wing vein, insert a cannula, and measure central venous pressure as a potential diagnostic index of the predisposition to right-sided congestive heart failure. We also wanted to develop a technique for evaluating the cardio-pulmonary responsiveness to elevated carbon dioxide levels in the air. Administering carbon dioxide via an inhalation mask while evaluating the ensuing hypoxemia, respiratory acidosis, or venous hypertension potentially could provide crucial diagnostic information. Inhalation of 5% CO₂ created a profound respiratory acidosis but only modestly increased the pulmonary vascular resistance. Considerable individual variability in the responsiveness to 5% CO₂ was observed. Large increases in pulmonary vascular resistance were obtained by acutely tightening a snare around one pulmonary artery. In both cases, venous pressure increased only by approximately 1 mm Hg. This low magnitude of venous hypertension was judged inadequate to serve as a reliable diagnostic index. Nevertheless, this study validated our assessment of the pathophysiological progression leading through central venous congestion to terminal ascites. It also demonstrated that the pulmonary vasculature of broilers, like that of mammals, is minimally responsive hypercapnia (elevated CO₂ in the arterial blood) and the consequent respiratory acidosis.

8. PLASMA TAURINE LEVELS IN BROILERS WITH PULMONARY HYPERTENSION SYNDROME INDUCED BY UNILATERAL PULMONARY ARTERY OCCLUSION. *Current Status:* Published, see Appendix A (Ruiz-Feria, C. A., Beers, K. W., Kidd, M. T., and Wideman, R. F. Plasma taurine levels in broilers with pulmonary hypertension syndrome induced by unilateral pulmonary artery occlusion. *Poultry Sci.* 78:1627-1633).

7.A. Summary: Low plasma levels of taurine are associated with losses of cardiac sarcomeric proteins leading to heart failure in mammals. Recently, it was proposed that cardiac taurine depletion serves to defend the heart against injury caused by regional ischemia. The role of taurine has not been well documented in broilers, particularly in relation to pulmonary hypertension syndrome (PHS, ascites). Three independent experiments evaluated plasma taurine in male broilers by utilizing the following treatments: unoperated controls (CONTROL, n=10 in each experiment); sham operated (SHAM, n=11, 12, and 10); or, unilaterally pulmonary artery clamped (PAC, n=18, 29, and 24) that did (PAC-ASCITES) or did not (PAC-NORMAL) develop ascites within 12 d post-surgery. Plasma samples were collected 9

and 11 d post-surgery in Experiment 1 and 2, respectively, and 2 d before and 4, 8, and 12 d after surgery in Experiment 3. Plasma taurine was analyzed by HPLC. Twelve days post-surgery, the birds were euthanatized and ventricles were weighed for calculating the right to total ventricular ratio (RV:TV). Data were analyzed with the PROC GLM of SAS® and means compared by the SNK method (Experiment 1 and 2), or a repeated measures ANOVA using SigmaStat (Experiment 3). As a result of the sustained increase in pulmonary vascular resistance, the RV/TV ratio of PAC birds (>0.35) consistently was higher ($P<0.01$) than that of CTL and SHAM birds (<0.27 and 0.25 , respectively). In Experiments 1 and 2, plasma taurine was higher ($P<0.05$) in PAC-ASCITES (380 and 370 nmol/mL) than in SHAM broilers (183 and 186 nmol/mL), whereas CTL (262 and 278 nmol/mL) and PAC-NORMAL (362 and 300 nmol/mL) broilers tended to have intermediate plasma taurine levels. In Experiment 3, PAC birds had higher ($P<0.05$) plasma taurine at 8 and 12 d post-surgery when compared with presurgery levels, while plasma taurine was unchanged over time in CTL and SHAM birds. These results suggest cardiac taurine may be released into the plasma as a protective mechanism in response to the induction of pulmonary hypertension, hypoxemia, and right-sided heart failure, similar to the mechanism reported for protecting cardiac muscle from ischemia in mammals.

8.B. Objectives Addressed: This project is relevant to Objectives 3 and 4. As indicated in the 1st Annual Report, work on a potential plasma marker was initiated by Mr. Ciro Ruiz-Feria, a graduate student in Dr. Wideman's lab. Key plasma amino acid and metabolite profiles were assessed in broilers with cold- and pulmonary artery clamp-induced ascites. Three independent experiments evaluated plasma taurine in male broilers by utilizing the following treatments: unoperated controls; sham operated; or, unilaterally pulmonary artery clamped. Plasma taurine was higher in ascitic than in sham-operated broilers. These results suggest cardiac taurine may be released into the plasma as a protective mechanism in response to the induction of pulmonary hypertension, hypoxemia, and right-sided heart failure. Plasma taurine has potential as an easily obtained index of cardiac status in broilers.

9. RENAL RESPONSES OF NORMAL AND PREASCITIC BROILERS TO SYSTEMIC HYPOTENSION INDUCED BY UNILATERAL PULMONARY ARTERY OCCLUSION. *Current Status:* Published, see Appendix A (Forman, M. F., and Wideman, R. F. Renal responses of normal and preascitic broilers to systemic hypotension induced by unilateral pulmonary artery occlusion. Poultry Sci. 78:1773-1785, 1999).

9.A. Summary: During the pathophysiological progression of pulmonary hypertension syndrome (PHS, ascites) broilers concurrently develop systemic hypotension (low mean systemic arterial pressure) that may initiate renal retention of water and solute, contributing to fluid accumulation in the abdominal cavity (ascites). In male Single Comb White Leghorns, the glomerular filtration rate is autoregulated over a systemic arterial pressure range of 110 to 60 mm Hg, and corresponding reductions in urine flow are attributed to a phenomenon known as pressure natriuresis. Acute unilateral pulmonary artery occlusion was used in the present study to reduce systemic arterial pressure toward the lower autoregulatory limit for glomerular filtration, and to evaluate kidney function in normal and preascitic broilers. Preascitic broilers

characteristically exhibited lower ($P < 0.05$) values for mean systemic arterial pressure (91 vs 100 mm Hg) and percentage saturation of hemoglobin with oxygen (73% vs 84%), higher hematocrits (HCT; 35 vs 30%), heavier right ventricles (3.44 vs 2.32 g), and higher right:total ventricular weight ratios (RV:TV; 0.32 vs 0.24) than normal broilers. Preascitic broilers exhibited larger ($P \leq 0.05$) dependent reductions in glomerular filtration, urine flow, osmolal clearance, and solute excretion and had a higher free water clearance than normal broilers in response to pulmonary artery occlusion. The differences observed between normal and preascitic broilers demonstrate that systemic hypotension can trigger renal mechanisms contributing to fluid and solute retention during the development of PHS.

9.B. Objectives Addressed: This project is relevant to Objectives 3 and 4. The experiments validated systemic hypotension (a low systemic arterial pressure) as an early indicator of the ascites pathogenesis in preascitic broilers, and demonstrated that systemic hypotension stimulates the kidneys to retain the solutes and water that ultimately comprise the ascitic fluid. Systemic hypotension develops contemporaneously with the onset of pulmonary hypertension, as demonstrated in broilers and Giant Jungle Fowl when large acute increases in pulmonary vascular resistance were induced by tightening a snare around one pulmonary artery, or when a generalized pulmonary vasoconstriction was induced by hypoxic or pharmacological treatments. The acutely challenged right ventricle is too weak to elevate the pulmonary arterial pressure sufficiently to propel all of the returning venous blood through a substantially constricted pulmonary vasculature. Blood that is not pumped through the lungs congests the systemic veins and increases the venous pressure near the right atrium. The acute reduction in flow to the left side of the heart causes dependent reductions in stroke volume, cardiac output, and mean systemic arterial pressure.

Unilaterally tightening a pulmonary arterial snare triggers a diffusion:perfusion mismatch by forcing the unobstructed lung to receive an elevated blood flow despite the overall reduction in cardiac output. The resulting systemic hypoxemia in turn contributes to the onset of systemic hypotension by causing the systemic precapillary arterioles to dilate. The systemic hypotension that coincides with an acute increase in pulmonary vascular resistance primarily reflects the inability of the relatively weak right ventricle to sustain a normal cardiac output. In contrast, the hypertrophied right ventricle of preascitic broilers and broilers chronically exposed to cool temperatures develops a pulmonary arterial pressure that is sufficient to propel an elevated cardiac output through the pulmonary vasculature. The accompanying hypoxemia causes a net reduction in total peripheral resistance, thereby increasing blood flow and O_2 delivery to the tissues. The elevated cardiac output propelled by the hypertrophied right ventricle evidently is inadequate to maintain a normal systemic arterial pressure in the face of the proportionally greater blood outflow from the arterial pressure reservoir to the tissues, leading to a persistent systemic hypotension in preascitic broilers. These observations clarify key steps in the pathogenesis of ascites, and identify systemic hypotension as a reliable index for identifying preascitic individuals.

10. CARDIO-PULMONARY FUNCTION IN BROILERS REARED AT WARM OR COLD TEMPERATURES:

INHALATION OF 100% OXYGEN DOES NOT REDUCE PULMONARY ARTERIAL PRESSURE. *Current Status:*

Published, see Appendix A (Wideman, R. F., and Tackett, C. D. Cardio-pulmonary function in broilers reared at warm or cool temperatures: Effect of acute inhalation of 100% oxygen. Poultry Sci. 79:257-264, 2000).

10.A. Summary: Fast growth and cool temperatures are the primary triggers for pulmonary hypertension syndrome (PHS, ascites) during commercial broiler growout. We evaluated cardio-pulmonary function in broilers reared at warm or cool temperatures. Male broiler chicks obtained from a commercial hatchery were placed on fresh litter in environmental chambers, and were brooded at 33, 29, and 27 C on Days 1 to 5, 6 to 10, and 11 to 23, respectively. Thereafter, 40 of the largest and apparently healthiest individuals were randomly assigned to chambers maintained at 28 C (WARM group, n = 20) or 16 C (COLD group, n = 20). Comparisons of the COLD and WARM groups on Day 29 revealed no differences in body weight (1,172 vs 1,175 g), however the COLD group had a lower percentage saturation of hemoglobin with oxygen (%HbO₂: 77 vs 85%), a lower heart rate (HR: 401 vs 420 beats/min), and a higher hematocrit (HCT: 36.2 vs 31.8%) than the WARM group. Cardio-pulmonary evaluations were conducted when the broilers were 35 to 42 d of age (body weights = 1,731 and 1,783 g for COLD and WARM, respectively). They were prepared for cardio-pulmonary and arterial blood gas measurements as described previously (Wideman et al., 1996, 1998). While breathing room air (21% O₂), the COLD group had a higher cardiac output (380 vs 300 mL/min) and pulmonary arterial pressure (21 vs 17 mmHg) than the WARM group. The COLD and WARM groups did not differ in mean systemic arterial pressure (104 vs 94 mmHg), partial pressure of oxygen (90 vs 99 mmHg), or partial pressure of carbon dioxide (33.8 vs 33.7 mmHg) while breathing room air. Inhalation of 100% O₂ for 30 min caused equivalent increases the arterial partial pressure of oxygen (399 vs 388 mmHg) and the %HbO₂ ($\geq 99\%$) in both groups, without reducing the pulmonary arterial pressure, the cardiac output, or the mean systemic arterial pressure in either group. None of the broilers in the WARM group and two in the COLD group developed PHS by Day 42. These observations demonstrate cardio-pulmonary characteristics in the COLD group typical of those associated with the pathophysiological progression leading to PHS. Furthermore, it was an elevated cardiac output rather than hypoxemic pulmonary vasoconstriction that made the major contribution to the existing increase in pulmonary arterial pressure in the COLD group.

10.B. Objectives Addressed: This project is relevant to Objectives 3 and 4. The experiments validated the role of cool temperatures in increasing the pulmonary arterial pressure and cardiac output. Providing pure oxygen to breathe did not attenuate the pulmonary arterial pressure, which was associated primarily with the elevated cardiac output rather than hypoxemic pulmonary vasoconstriction.

11. CARDIO-PULMONARY FUNCTION IN PREASCITIC (HYPOXEMIC) OR NORMAL BROILERS INHALING

AMBIENT AIR OR 100% OXYGEN. *Current Status:* Published, see Appendix A (Wideman, R. F., Fedde, M.

R., Tackett, C. D., and Weigle, G. E. Cardio-pulmonary function in preascitic (hypoxemic) or normal broilers inhaling ambient air or 100% oxygen. *Poultry Sci.* 79:415-425, 2000).

11.A. Summary: We evaluated the influence of the percentage saturation of hemoglobin with oxygen (HbO_2) on the pulmonary artery pressure in normal and preascitic (hypoxemic) broilers breathing ambient air or 100% O_2 . In experiment 1, unanesthetized preascitic broilers (right:total ventricular weight ratios $[\text{RV:TV}] = 0.32 \pm 0.02$) breathing ambient air had initial values of 67% for HbO_2 and 32 mm Hg for pulmonary artery pressure. The HbO_2 increased to $\geq 96.6\%$ during inhalation of 100 % O_2 , however pulmonary artery pressure was not reduced. In experiment 2, anesthetized normal ($\text{RV:TV} = 0.23$; $\text{HbO}_2 = 88\%$) and preascitic broilers ($\text{RV:TV} = 0.28$; $\text{HbO}_2 = 76\%$) were compared. The groups did not differ in body weight or respiratory rate, but preascitic broilers had lower values for mean arterial pressure, total peripheral resistance, and partial pressure of O_2 in arterial blood, and higher values for pulmonary artery pressure. Inhaling 100% O_2 increased HbO_2 to 99.9% in both groups, however pulmonary artery pressure remained higher in preascitic than in normal broilers, and the pulmonary vascular resistance was not reduced by 100% O_2 inhalation. Cardiac output was higher in preascitic than in normal broilers before and after, but not during, 100 % O_2 inhalation. Mean arterial pressure and total peripheral resistance increased in the preascitic but not in the normal group during 100 % O_2 inhalation. Low coefficients of determination (R^2) were obtained for linear regression comparisons of HbO_2 vs. pulmonary artery pressure in both experiments. Overall, acutely reversing the systemic hypoxemia in preascitic broilers had little direct impact on pulmonary hypertension, providing no evidence of hypoxemic pulmonary vasoconstriction. Instead, acutely reversing the systemic hypoxemia primarily increased the total peripheral resistance and normalized the mean arterial pressure and cardiac output. A sustained reduction in cardiac output theoretically should attenuate pulmonary hypertension, but this was not observed due to the overriding influence of sustained pulmonary vascular resistance.

11.B. Objectives Addressed: This project is relevant to Objectives 3 and 4. We know that oximetry provides an important minimally invasive diagnostic index of ascites susceptibility. We don't know if the cyanotic hypoxemia (low oxygen content of arterial blood) in preascitic broilers is a primary cause of pulmonary hypertension (hypoxemic pulmonary vasoconstriction), or a consequence of pulmonary hypertension (diffusion:perfusion mismatch). The impact of hypoxemia (low oxygen levels in arterial blood) on cardio-pulmonary hemodynamics during the pathogenesis of ascites is complex. Hypoxemia contributes directly to the reduced total peripheral resistance, increased venous return, elevated cardiac output, pulmonary hypertension, and systemic hypotension in preascitic broilers. Chronic hypoxemia weakens the cardiac muscle and may accelerate the onset of congestive heart failure during the terminal stages of ascites. It also is clear that exposure to low atmospheric oxygen (hypoxia) triggers a generalized pulmonary vasoconstriction and the immediate onset of pulmonary hypertension leading to the development of ascites in domestic fowl. However, a direct influence of hypoxemia on the pulmonary vasculature remained unproven. For example, tightening a snare around one extra-pulmonary primary bronchus directs the entire respiratory minute volume toward the unoccluded lung, whereas returning

venous blood continues to perfuse both lungs. Consequently, the blood exiting the unventilated lung remained "venous" in its gas composition, leading to a rapid onset of hypoxemia and a modest increase in pulmonary arterial pressure. Presumably the modest pulmonary hypertension triggered by tightening the bronchial snare reflects the increased right ventricular work needed to overcome the pulmonary vasoconstriction triggered by local air capillary hypoxia or systemic arterial hypoxemia. The degree of hypoxemia induced by occluding one bronchus equals or exceeds that induced by occluding one pulmonary artery, however unilateral pulmonary artery occlusion triggers a greater increase in pulmonary arterial pressure, and consistently initiates a pathophysiological progression terminating with a larger increase in RV:TV ratios a higher incidence (> 70%) of ascites mortality when compared with unilateral bronchus occlusion. Additional evidence indicates that flow-dependent pulmonary vasodilation reduces the onset of pulmonary hypertension in both broilers and Giant Jungle Fowl by minimizing the increment in pulmonary arterial pressure needed to propel an elevated blood flow through the lungs, regardless of the sustained hypoxemia attributed to a persistent diffusion:perfusion mismatch. In our most recent study, permitting preascitic broilers to inhale 100% O₂ dramatically increased the partial pressure of O₂ in arterial blood without significantly reducing pulmonary vascular resistance. Instead, inhaling 100% O₂ increased the total peripheral resistance and mean systemic arterial pressure, and marginally reduces the cardiac output. Evidently hypoxemia in preascitic broilers is not a primary cause of pulmonary vasoconstriction. Instead, hypoxemia is the consequence of a diffusion:perfusion mismatch created by blood flowing too rapidly through the pulmonary vasculature. Currently, the incidence of ascites induced by various experimental models appears primarily to be correlated with the associated increment in RV:TV ratios and pulmonary arterial pressures, rather than the magnitude of the accompanying hypoxemia.

12. REVIEW: CARDIO-PULMONARY HEMODYNAMICS AND ASCITES IN BROILER CHICKENS. *Current*

Status: Review Article Published, see Appendix A (Wideman, R. F., Cardio-pulmonary hemodynamics and ascites in broiler chickens. *Avian and Poultry Biology Reviews* 11:21-43, 2000).

Summary: Ascites occurs worldwide in broiler chickens reared under a variety of conditions. An elevated blood pressure within the pulmonary circulation (pulmonary hypertension) initiates a distinctive pathophysiological progression that includes the sequential development of hypoxemia, right-sided congestive heart failure, central venous congestion, cirrhosis of the liver, and transudation of ascitic fluid into the abdominal cavity. Consequently, the terms *pulmonary hypertension syndrome* (PHS) and *ascites syndrome* are widely accepted as synonyms. The pathogenesis of ascites can be influenced by environment, management, nutrition, and disease, as summarized previously. Fast growth and cool temperatures are the primary triggers for ascites under most conditions of commercial broiler growout. An undefined genetic component of ascites susceptibility also has been implicated. Many of these factors previously were incorporated into a physiological model designed to bring cohesion to our understanding of the pathogenesis of ascites (Wideman and Bottje, 1993). The model was based on the hypothesis that susceptible broilers have an inherent potential to outgrow their cardio-pulmonary capacity, primarily due

to limitations of the pulmonary circulation, including the right ventricle of the heart and blood vessels of the lungs (pulmonary vasculature). In this context, cardio-pulmonary capacity was defined as the ability of the lungs to accept the requisite cardiac output at blood flow rates and pressures that are sufficiently low to avoid triggering systemic hypoxemia, right-sided congestive heart failure, and ascites. The objective of the present review is to reappraise this model and its assumptions, taking into consideration the most recent evidence relevant to the cardio-pulmonary hemodynamics of fast growing broilers. The current evidence demonstrated that substantial progress has been achieved in integrating our understanding of cardio-pulmonary hemodynamics and the biological basis for the ascites syndrome in fast growing broilers. Highly focused research consistently supports the hypothesis proposed previously (Wideman and Bottje, 1993). Based on this hypothesis, we are able to predict the contributions of seemingly independent factors to the overall incidence of ascites. This hypothesis also served as the theoretical basis in the design of successful strategies for selecting broiler lines that are highly resistant to ascites. Ongoing research efforts must be directed toward developing practical techniques for selecting broilers that are highly resistant to pulmonary hypertension, as well as continuing to develop practical treatments for reducing the incidence of ascites in commercial broiler flocks.

13. PLASMA LEVELS OF ARGININE, ORNITHINE, AND UREA, AND GROWTH PERFORMANCE OF BROILERS FED SUPPLEMENTAL L-ARGININE DURING COOL TEMPERATURE EXPOSURE. *Current Status:* Submitted for Publication, see Appendix A (Ruiz-Feria, C. A., Kidd, M. T., and Wideman, R. F. Plasma levels of arginine, ornithine, and urea, and growth performance of broilers fed supplemental L-arginine during cool temperature exposure. *Poultry Sci.*, *submitted*).

13.A. Summary: Two experiments (Exp. 1 and 2) were conducted to evaluate growth performance, ascites mortality, and plasma arginine, urea, and ornithine concentrations in male broilers raised in floor pens (2 x 4 factorial experiment, 6 pens for treatment) and exposed to cool temperatures averaging 16 C after 21 d of age. Broilers were fed low or high CP diets in both experiments. In Exp. 1, Arg treatments consisted of: control (no supplemental Arg); 0.15 or 0.3% supplemental Arg in the diet (low and medium Arg feed, respectively); and 0.3% supplemental Arg in the drinking water (Arg-water). Arginine levels were increased in Exp. 2 and consisted of: control (no supplemental Arg); 0.3 or 0.85% supplemental Arg in the diet (medium and high Arg feed, respectively); and 0.6% supplemental Arg in the drinking water (Arg-water). The water treatment followed a three-day cyclic regimen, with supplemental Arg being provided for 24 h, followed by tap water for 48 h. When the broilers reached 37 d of age and all groups had consumed tap water for the previous 48 h, blood samples were collected from one bird per pen (time 0, 0700 h), then supplemental Arg was provided in the Arg-water group and additional blood samples were collected from the control and Arg-water groups at 3, 6, 12, and 36 h after time 0. Plasma amino acids were analyzed using HPLC. Birds fed the high CP diet were heavier at 49 d than birds fed the low CP diet in Exp. 1, but not in Exp. 2. No differences were found in feed conversion or ascites mortality due to CP or Arg treatment in either experiment. In both experiments, plasma Arg was similar for all groups at

time 0, but increased in the Arg-water group at 3, 6, and 12 h after Arg was provided in the water. Within 12 h after returning to tap water, plasma Arg levels of the Arg-water group did not differ from the control group. Plasma urea and ornithine paralleled plasma Arg concentrations, and the high CP diets resulted in higher plasma levels of urea and ornithine compared with low CP diets. These results indicate that kidney arginase was readily activated by Arg provided in the water, resulting in an immediate increase in plasma urea and ornithine. Plasma Arg was increased significantly, but no effects were observed in ascites mortality.

13.B. Objectives Addressed: This project is relevant to Objectives 3 and 4. As indicated in the 1st Annual Report, work on a potential plasma marker was initiated by Mr. Ciro Ruiz-Feria, a graduate student in Dr. Wideman's lab. Key plasma amino acid and metabolite profiles were assessed in broilers with cold- and pulmonary artery clamp-induced ascites. Under a variety of nutritional and environmental challenges, Plasma arginine, urea and ornithine levels have not been correlated with susceptibility to PHS.

14. Pulmonary Wedge Pressures Confirm Pulmonary Hypertension in Broilers is Initiated by an Excessive Pulmonary Arterial (Precapillary) Resistance. *Current Status:* Submitted for Publication, see Appendix A (Chapman, M. E., and Wideman, R. F. Pulmonary wedge pressures confirm pulmonary hypertension in broilers is initiated by an excessive pulmonary arterial (precapillary) resistance. Poultry Sci., *submitted*).

14.A. Summary: In the present study, unanesthetized male broilers from an ascites resistant line, the base population from which the resistant line was derived, and a separate unselected line were used to determine whether changes in wedge pressure are predictive of differences in the pulmonary arterial pressure of clinically healthy and pre-ascitic broilers. Venous, right atrial, right ventricular, pulmonary arterial and wedge pressures were obtained by inserting a catheter into a wing vein and progressively advancing the catheter into a pulmonary branch artery until the catheter tip became wedged in and occluded the flow through a terminal artery. Mean right ventricular and pulmonary arterial pressures were lower in the resistant line than in the base population, but wedge pressures did not differ between the resistant, base and unselected lines. Right/total ventricular weight ratios (RV:TV) and the percentage saturation of hemoglobin with oxygen in arterial blood ranged in value from 0.18 to 0.44 and 65 to 96%, respectively. Wedge pressure, however, remained similar when preascitic broilers with high RV:TV values and low oximetry values were compared with clinically healthy broilers. In all birds, whether healthy or showing pre-ascitic characteristics, the wedge pressure was slightly higher than the right atrial pressure, but substantially lower than pulmonary arterial pressure. These observations provide definitive proof that pulmonary hypertension is initiated as a consequence of excessive pulmonary arterial or arteriole resistance. Pulmonary venous pressure is estimated by measuring the pulmonary arterial wedge pressure, and high wedge pressures would be evident if pulmonary hypertension was caused by the elevated downstream resistances associated with left sided heart failure.

14.B. Objectives Addressed: This project is relevant to Objectives 3 and 4. The male progeny of PHS-Resistant broiler breeders selected using the unilateral pulmonary artery occlusion technique (Wideman and French, 1999, 2000) were shown to have lower pulmonary arterial pressures than male progeny from the Base population. Elevated pulmonary arterial pressures were not associated with elevated pulmonary wedge pressures, demonstrating that pulmonary hypertension is initiated as a consequence of excessive pulmonary arterial or arteriole resistance rather than elevated downstream resistances associated with left sided heart failure. This study provides definitive proof of our underlying hypothesis that broilers are susceptible to PHS when their cardio-pulmonary capacity is inadequate to accommodate the requisite cardiac output at a sufficiently low pulmonary arterial pressure.

15. INTRAVENOUS ENDOTOXIN TRIGGERS PULMONARY VASOCONSTRICTION AND PULMONARY HYPERTENSION IN BROILER CHICKENS. Current Status: Submitted for Publication, see Appendix A (Wideman, R. F., Erf, G. F., and Chapman, M. E. Intravenous endotoxin triggers pulmonary vasoconstriction and pulmonary hypertension in broiler chickens. *Poultry Sci.*, *submitted*).

15.A. Summary: Bacterial endotoxins stimulate endothelin-mediated thromboxane-dependent increases in pulmonary vascular resistance in mammals, and thromboxane has been shown to cause an immediate but transient pulmonary vasoconstriction in broiler chickens. In the present study, i.v. injections of 1 mg endotoxin into anesthetized male broilers caused a pulmonary vasoconstrictive response that was delayed in onset by 15 min, and that elevated the pulmonary arterial pressure by 10 mm Hg within 25 min post-injection. Thereafter, pulmonary hemodynamic variables gradually (≥ 15 min) returned toward pre-injection levels, and supplemental injections of 4 mg endotoxin during this recovery period failed to reinitiate pulmonary hypertension. In contrast, injecting the thromboxane A_2 mimetic U44069 during the endotoxin recovery period triggered pulmonary vasoconstriction and pulmonary hypertension similar in magnitude to the responses triggered by U44069 before endotoxin had been administered. The time course and magnitude of the pulmonary hemodynamic responses to endotoxin were highly variable among individual broilers, whereas the individual responses to U44069 were more consistent. Unanesthetized broilers resembled anesthetized broilers in the time course, magnitude, and variability of their pulmonary hemodynamic responses to endotoxin. Overall, these observations are consistent with the hypothesis that endotoxin initiates a biochemical cascade culminating in the delayed onset of pulmonary vasoconstriction and pulmonary hypertension within 20 min post-injection. Subsequently, the pulmonary vasculature remains responsive to large bolus injections of exogenous thromboxane mimetic, however depletion of endogenous vasoconstrictive components of the endotoxin-mediated cascade, a compensatory increase in endogenous vasodilators, or the induction of a transient cellular tolerance to endotoxin, prevented 4-fold higher doses of endotoxin from reversing the return toward a normal pulmonary vascular tone. Individual differences among broilers in their susceptibility to pulmonary hypertension syndrome (ascites) may be related to innate or acquired variability in their pulmonary vascular responsiveness to vasoactive mediators.

15.B. Objectives Addressed: This project is relevant to Objectives 3 and 4. Based on the success of surgical unilateral pulmonary artery occlusion as a technique for selecting PHS-resistant genetic lines, the US team evaluated several chemicals for their potential as pulmonary vasoconstrictors in broilers. The chemical vasoconstrictor studies were combined with experiments conducted to satisfy Objective 3, to determine if specific differences in cardio-pulmonary function can be detected between susceptible and resistant broilers. In mammals, various particulates and bacterial endotoxins stimulate endothelin-mediated thromboxane-dependent increases in pulmonary vascular resistance and pulmonary arterial pressure. The pulmonary vasoconstriction and pulmonary hypertension triggered by endotoxin and thromboxane have a functional significance related to the presumed contribution of poor air quality and respiratory disease to the ascites syndrome. Accordingly, it has been proposed that inhaled dust (endotoxins) and pathogens (sepsis) may cause focal damage and pulmonary vasoconstriction by increasing the intrapulmonary production of thromboxane. Bacterial endotoxins are ubiquitous in the environment of broilers, and any factor capable of increasing the pulmonary vascular resistance potentially can accelerate the pathogenesis of PHS. Accordingly, three independent experiments were conducted to evaluate the pulmonary hemodynamic responses of anesthetized and unanesthetized male broilers to endotoxin. Intravenously administered endotoxin triggered pulmonary hypertension without increasing the cardiac output, providing conclusive evidence that the increase in pulmonary arterial pressure was caused by pulmonary vasoconstriction. Specific differences in cardio-pulmonary function were detected among individual broilers (Objective 3). A maximal i.v. injection dose of endotoxin was used; nevertheless a wide range of variability in the time of onset, amplitude, and duration of the pulmonary hemodynamic responses to endotoxin were evident among broilers that had been hatched and reared together within each experiment, and regardless of the presence or absence of general anesthesia or invasive instrumentation across all three experiments. A similar degree of variability previously was observed in the pulmonary hemodynamic responses of individual broilers to bolus acid (HCl) injections (Wideman et al., 1998). In mammals, the pulmonary vasoconstrictive responses to bolus injections of acid and endothelin-1 are associated with the rate-dependent production of thromboxane A₂. In the present study, individual broilers were much more consistent in their responses to the thromboxane mimetic U44069 than to endotoxin. The widely varying responses to endotoxin among individual broilers may be innate, perhaps reflecting a genetic component of susceptibility to pulmonary hypertension (Conary et al., 1994; Delong et al, 1999; Wideman and French, 1999, 2000). Understanding the mechanisms underlying the individually variable pulmonary hypertensive responsiveness to endotoxin will likely contribute to our understanding of the multi-factorial pathogenesis of PHS in broilers. Presumably such information will lead to the identification of key diagnostic indices related to PHS susceptibility (Objective 4).

16. CARDIAC DAMAGE ASSESSMENT USING TROPONIN T IN BROILERS WITH UNILATERAL OCCLUSION OF THE LEFT PULMONARY ARTERY. *Current Status:* Chapter in a Doctoral Thesis to be submitted by J.

Bautista-Ortega, see Appendix A (subsequently to be published as: Bautista-Ortega, J, Robertson, G. W., Maxwell, M. H., Ruiz-Feria, C. A., and Wideman, R. F. Cardiac damage assessment using troponin T in broilers with unilateral occlusion of the left pulmonary artery; *in preparation*)

16.A. Summary: In human medicine cardiac troponin T (TnTc) is used as a biochemical marker for myocardial damage. The TnTc assay has found several applications related to coronary syndromes including: 1) diagnostic confirmation of acute myocardial infarction; 2) a late marker for patients in the late phase of infarction; 3) exclusion of myocardial necrosis in patients with unstable angina pectoris; 4) monitoring of the outcome of thrombolysis therapy; and, 5) non-invasive estimation of the size of the infarction. It has been demonstrated that unilateral pulmonary artery occlusion (UPAO) in fast-growing broilers leads to pulmonary hypertension due to the inability of the pulmonary system of the perfused lung to accommodate a two-fold increase of the blood flow. Consequently, induced pulmonary hypertension initiates a progression of pathological events leading to ascites. One of these pathological changes is the hypertrophy of the right ventricle accompanied by hypoxia and necrosis of the cardiomyocytes. Unilateral pulmonary artery occlusion may prove useful for dissociating the multiple physiological factors suspected of contributing to the overall susceptibility of broilers to ascites (AS), and for studying physiological changes in the broiler chickens once pulmonary hypertension has been induced, such as the release of proteins into the circulation due to heart injury; namely TnTc. This is the first study to use UPAO to induce chronic heart damage and AS in broiler chickens in order to assess the release kinetics of TnTc from injured myocytes. The present study was carried out with the intention of identifying the start of cardiac necrosis and to characterise the release kinetics of TnTc in the circulation of fast-growing broilers subjected to UPAO by weekly measurements of TnTc concentration in plasma from the time of occlusion (18 and 19 day-old) to 42 days of age. In the present study, TnTc was not predictive of those broilers that developed ascites subsequent to the PAC surgery. The TnTc release curve observed in the CONTROL and SHAM groups followed the same pattern as the one observed in other strains of fast-growing broilers. Briefly, at four weeks of age there is an increase in the TnTc released from the cardiomyocytes of broilers reared under conditions similar to commercial poultry houses. The highest TnTc levels observed at this age have been associated with a fast growing period and a high AS mortality. The TnTc levels then return to basal levels at older ages if the broilers did not succumbed to AS. Cardiac TnTc released from injured myocytes in birds from the PAC group was significantly lower than in birds from the other two groups four days after surgery (in the PAC and SHAM birds). The fact that the birds from the PAC group were exposed to an acute (UPAO) rather than a chronic inducer of ascites may partially explain this observation. The injured cardiomyocytes of these birds may have released all the available (systolic) TnTc within hours following surgery.

16.B. Objectives Addressed: This project is relevant to Objective 4. The USA team collaborated with Dr. M. H. Maxwell's lab group (Roslin Institute, Scotland, UK) to evaluate the Troponin T marker as a promising additional minimally invasive diagnostic index related to ascites. Dr. Maxwell has reported a strong genetic correlation between the presence of this marker and the spontaneous onset of ascites. Dr.

Wideman collected plasma and tissue samples from CONTROL and SHAM-operated broilers, and from broilers that did or did not develop PHS following chronic unilateral pulmonary artery occlusion. These plasma samples, as well as fixed heart tissues from the same birds, were shipped to Roslin Institute, where Mr. J. Bautista-Ortega conducted the Troponin T assays as part of his Doctoral research. Plasma Troponin T was not found to be predictive of susceptibility to PHS.

17. MINIMUM DAILY GROWTH VELOCITY DATA COLLECTION FOR PULMONARY HYPERTENSION SYNDROME (PHS) AS DETECTED BY ARTIFICIAL NEURAL NETWORKS. *Current Status:* Manuscript in preparation, see Appendix A (Roush, W. B., Wideman, R. F., A. Cahaner, and Cravener, T. L. Minimal daily growth velocity data collection for pulmonary hypertension syndrome (PHS) as detected by artificial neural networks. *Poultry Sci., in preparation*).

17.A. Summary: Previously, evaluating the first two-weeks of daily growth velocity with an artificial neural network (ANN) provided an effective non-invasive approach for predicting the susceptibility of broilers to pulmonary hypertension syndrome (PHS). This study was conducted to define the minimum number of days of growth data and the type of ANN required for the best prediction of PHS susceptibility. Four experiments were conducted in which broilers were weighed daily at 0800 h. In Exp. 1, Hubbard male broilers were reared to 50 d of age, with 13 developing PHS and 33 remaining normal (N), for a PHS:N ratio of 13:33. In Exp. 2, ANAK broilers were exposed to cool temperatures (16-17 C) from 17 to 42 d of age, resulting in a PHS:N ratio of 16:46 for males and 11:255 for females. In Exp. 3 and 4, Hubbard male and female chicks from a Base population and a PHS-Resistant line were exposed to cool temperatures from 17 to 42 (Exp. 3) or 49 d of age (Exp. 4). The PHS:N ratios were 40:68 and 6:96 for males and females in Exp. 3, and 26:91 and 10:58 for males and females in Exp. 4. Four ANNs, Back Propagation (BP3), Ward Back Propagation (WardBP), Probabilistic (PNN) and General Regression (GRNN), were evaluated for their ability to predict PHS in the shortest number of d based on daily growth rate and growth velocities ($BW_{d+1}-BW_d$). A 100 % prediction of PHS and N birds was considered the criterion of success. Starting with 14 d of data, each ANN was trained on daily growth velocity, and the number of predictive d was reduced or added with each run of the ANN. The best ANN was a GRNN, which correctly diagnosed PHS and N male broilers on 4 and 6 d of growth velocity data for Exp. 1 and 2, respectively. The results were poorer with the BP3, WardBP, and PNN. The diagnostic ability of the neural network was not consistent over all four experiments. In Exp. 2, a minimum of 6 d was required for 100% PHS detection for males, whereas the best value for females was 82% PHS detection at 15 d. In Exp. 3, the best diagnostic value for males was 93% PHS detection and 100% N detection at 15 d. For females, the 100% PHS detection occurred at a minimum of 8 d. In Exp. 4, males had 100% PHS and N detection at a minimum of 11 d. Females had a 100% PHS and N detection at a minimum of 10 d. An attempt to build a single neural network that would detect PHS susceptibility in both Hubbard (Exp. 1) and Anak (Exp. 2) broilers was unsuccessful. The application of a neural network between experiments also was not successful (data not presented). These studies demonstrate that within a breed or line reared

under similar conditions, a GRNN based on the first 14 d of growth velocity can detect, with at least 82% accuracy, broilers susceptible to PHS.

17.B. Objectives Addressed: This project is relevant to Objectives 1, 2, and 5. As an alternative to the Peckcode system, two complete Biomedic BMDS-5001 systems for logging DATE/TIME/ID/BW data electronically were purchased by the US team. This system automatically detects a unique microchip ID when each bird is manually placed on an electronic balance, and records the ID, date, time and body weight. The system then downloads these data directly into the MS-EXCEL spreadsheet. The US team used this system to collect daily body weight data (Days 1-42) from Hubbard male and female broilers of the PHS-Resistant and Base populations (Wideman and French, 1999, 2000). The Israeli team produced a full pedigree population of chicks from ANAK parents of known growth potential, spanning the range of broilers with top body weight (BW) gain of 5 years ago, through birds with top BW gain of today, and also incorporating types that will form the nucleus of broilers of this strain in the coming years. Chicks were identified with plastic tags attached to the skin of the neck and having both numbers and barcodes. The birds were weighed daily up to 21 d of age, and 3 times a week thereafter. The manual weighing was expedited by identification of the barcode tags with a barcode reader/data logger (Symbol PDT 3100, Symbol Technologies Inc., Bohemia NY, USA) connected to a scientific balance (Sartorius BP 6100, Sartorius AG, Goettingen, Germany). These data were analyzed for growth dynamics by Dr. Roush. Four ANN architectures, Back Propagation (BP3), Ward Back Propagation (WardBP), Probabilistic (PNN) and General Regression (GRNN), were evaluated for their ability to predict birds with ascites within a short number of days based on daily growth rate (velocity). Settings for the parameters of each of the neural networks were customized to optimize the diagnosis of ascites. The neural networks were conducted on an IBM compatible PC using NeuroShell 2. A 100% prediction of both normal (N) and PHS-susceptible (PHS) was considered to be the criterion of success.

The best neural network was a GRNN in which males were correctly diagnosed using only 4 d of BW data collection in the 1st US experiment, and 6 d of BW data for the Israeli experiment. The BP3, WardBP, and PNN were less predictive. With these three neural networks it was apparent that a better diagnosis was made of N than PHS. The better diagnosis of N broilers was most likely do to the larger number of patterns in the training set representing normal birds than the number patterns representing birds that subsequently developed PHS. The diagnostic ability of the neural network was not consistent. In the Israeli experiment, a minimum of 6 d was required for 100% detection of males, whereas the best value for the females was a 82% PHS detection at 15 d. In the 2nd US experiment, the best diagnostic value for male birds was a detection rate of 93% for PHS and 100% for N birds at 15 d. For females the 100% PHS detection occurred at 15,14,13,12, 11, 10 and 8 d. In the 3rd US experiment, male birds had 100% N and PHS detection at 15,14 and 11 d. Female birds had a 100% N and PHS detection at 15,14, 12, and 10 d. It is interesting that in all Exp., the neural network was able to identify 100% of the PHS-susceptible males before the birds had been exposed to cool temperatures to induce ascites, and correct diagnoses were made weeks before the clinical onset of PHS in male or female broilers. An attempt to

build a single neural network based on combined data from Hubbard (US Exp. 1) and Anak birds (Israeli EXP) that would define PHS for both sources of birds was not successful. Also an attempt to apply the neural network from one experiment to another experiment was not successful (data not presented). There was quite a difference among the experiments for these values, possibly explaining the reason that the GRNN neural networks were not consistent between experiments. A drawback for GRNN neural networks is that the GRNN will not predict values beyond the input values on which it was trained. It is apparent that although the growth rates were different between healthy birds and birds that have developed ascites (Roush and Wideman, 2000), the ANN method used in these experiments was less general and less sensitive to divergent selection than the diagnosis procedures involving multiple physiological values as reported by Roush et al., (1996, 1997). Nevertheless, these studies suggest that within the Hubbard and ANAK strains of bird, under similar selection pressures, the first two weeks of growth velocity analyzed by GRNN could be used to predict, at a minimum of 82%, chicks having the propensity to develop PHS.

18. Oxygenation of Arterial Blood in Normal and Cold-Exposed Broilers with Reference to the Ascites Syndrome. *Current Status:* Manuscript in preparation, see Appendix A (M. Bellaichel, A. Rosner¹, A. Cahaner², S Druyan² and A. Shlosberg¹ ¹Kimron Veterinary Institute, PO Box IZ Bet Dagan 50250, Israel ² Hebrew University of Jerusalem, Faculty of agriculture, PO Box 12, Rehovot 76100, Israel).

18.A. Summary: The ascites syndrome (AS) is manifested in affected broilers by an hypoxemia that often appears to be a critical factor leading to the death of the bird. The hypoxemia is rapidly followed by a compensatory rise in hematocrit and thence hemoglobin. T studies were made to study the development of hypoxemia in broilers. One study monitored the oxygen saturation of arterial blood (SaO₂ values) of 150 broilers at day hatch and on Days 1, 5, 10, 16, 25, 35 and slaughter at Day 44, in birds kept under normal (NT) and under ascites-inducing cold (CT) temperatures. High levels (means about 95%) were found at all days up to and including Day 16 in both no-AS and birds. Hypoxemia (<85%) was recorded only 3 times up to and including Day 25 (o bird on Day 16 and 25, and another bird on Day 25). However, by Day 35, 25 % of t NT birds and 47 % of the CT birds showed such hypoxemia, and by Day 42, 17 % 42 % of NT and CT birds were hypoxemic. AS was recorded in 4 % and 42 % of and CT broilers respectively. Both hematocrit (HCT) and hemoglobin (Hb) levels rose in CT birds, but this was manifested only in AS CT birds. There were no significant changes in total oxygen content (TOC) of the blood between no-AS NT and AS groups, whereas no-AS CT had elevated TOC. These results showed that despite lowering of SaO₂, compensatory elevations in HCT and Hb effectively restored TOC

18.B. Objectives Addressed: This project is relevant to Objectives 2, 4, and 5. Previous studies examined only SaO₂, mainly in broilers aged 21 days and older. Multiple SaO₂ and TOC determinations from day of hatch were conducted in one trial in this study, which is apparently the first time that such a

multi-age study has been made. This study was compared to a different trial performed on an identical full pedigree population of chicks, both under AS-inducing cold temperatures. A full pedigree population of chicks was produced from parents from a commercial dam-line of ANAK Breeders Ltd (Netanya, Israel). Each of 30 males was mated with 12 females to produce half-sib sire families representing the stock's genetic variation for growth rate and other traits. In Trial 1 only males ($n = 150$), and in Trial 2 both sexes ($n = 210$) were used. Standard broiler feeding was used. In Trial 1 chicks were brooded in a tiered brooder battery and in Trial 2 the chicks were floor-brooded on wood shavings (11 birds / M²). Both hatches underwent cold-exposure by ambient temperature reduction, starting with 35°C on Day 1, 33°C on Day 4, 31°C on Day 7, and 29°C on Day 10. On Day 11, the temperature was reduced by 1°C every day, down to 20°C on Day 19, when the birds were transferred to individual cages in two CT-controlled chambers. Temperature was maintained at 20°C until Day 21, when it was further reduced to 16°C until slaughter at 6 wk of age. SaO₂ was measured using a portable veterinary oximeter, the 8600V (NONIN Medical Inc., Plymouth, MN, USA) with a model 2000T sensor. In Trial 2 measurements were made in 150 birds randomly selected on day of hatch (Day 0) and usually in the same birds on Days 1, 5, 10, 16, 25, 35 and 42. Hematocrit (HCT) and hemoglobin (Hb) levels were measured periodically. AS was recorded in 36 % of the overall population in Trial 1 and in 33 % in Trial 2. AS mortality (ASM) was seen at an earlier age in Trial 1. Of the AS-associated parameters measured, SaO₂ values showed the first response surprisingly as early as 7 d of age in Trial 1, whereby the mean value of AS birds was significantly lower than apparently healthy (no-AS) birds. This difference was also seen at all the other measurements, on Days 23, 28, 37 and 42. The SaO₂ values in the no-AS birds showed a very mild reduction over the total growth period, whereas the AS birds showed close to abnormal levels as early as Day 23. In Trial 2, AS was recorded in 41 % of the males and 30 % of the females; in the birds in the normal rearing temperatures (NT), 1 of the 27 males and 3 of the 28 females developed AS.

In both trials, hypoxemia and a high incidence of AS were induced by cold-exposure. The earlier mortality in Trial 1 may have been initiated by an outbreak of *Mycoplasma* infection that caused some mortality in chicks in the first week after hatch. Sub-clinical infection may have predisposed other birds to AS by deleteriously affecting cardiac or pulmonary function. In Trial 1 AS birds also showed an early (7 d) lowering of SaO₂ levels (TOC was not determined in this experiment), but this was not seen in the much more comprehensive study in Trial 2.

19. ELECTROCARDIOGRAPHIC FINDINGS IN NORMAL AND COLD-EXPOSED BROILERS WITH REFERENCE TO THE ASCITES SYNDROME. *Current Status:* Manuscript in preparation, see Appendix A (A. Rosner, M Bellaiche¹, A. Cahaner, S. Druryan², and A. Shlosberg³ 'Kimron Veterinary Institute, PO Box 12, Bet Dagan 50250, Israel ²Hebrew University of Jerusalem, Faculty of agriculture, PO Box 12, Rehovot 76100, Israel).

19.A. Summary: Electrocardiography (ECG) was performed in two experiments to study the development of the ascites syndrome (AS) in broilers; in Trial 1 all birds were cold exposed (CT), and in

Trial 2 some birds were also grown at normal temperatures (NT). Pulse rate (PR) was lower in AS birds from Day 15 (Trial 1) and from Day 36 (Trial 2). CT AS birds had lower PR than no-AS birds, whether CT or NT. Lead II S wave amplitudes (SWA) were higher in AS birds, in Trial 1 from 26 d of age and in Trial 2 from 36 d. There was also increased SWA, from Day 36 in Trial 2. As with PR, rises in SWA were manifested mainly in AS CT birds, less so in no-AS CT birds and least in no-AS NT birds. Elevated SWA is indicative of right ventricular hypertrophy and may be predictive of AS. Elevated P wave amplitudes (possibly indicative of right atrial hypertrophy) were seen from Day 6 and on most d of measurement in AS birds in Trial 1. Elevated P wave durations (possibly indicative of left atrial hypertrophy) were seen on Day 35 in AS birds. These studies show that broilers have diverse reactions to cold, and that ECG changes may indicate both ventricular and atrial abnormalities in AS birds.

19.B. Objectives Addressed: This project is relevant to Objectives 2, 4, and 5. Electrocardiography (ECG) is a virtually non-invasive, easily performed monitor of electrical activity, and thence a measurement of function, of the heart, a vital part of cardiovascular function. ECG has been used for some years by leading AS research groups to monitor and predict susceptibility to AS (Odom *et al.*, 1991; Owen *et al.*, 1995). Of most importance has been the finding that an increased S wave amplitude in standard limb lead II indicates increased susceptibility to AS (Wideman *et al.*, 1998). Also, a slower heart rate (bradycardia) has been found in birds developing AS (Olkowski and Classen, 1998; Shlosberg *et al.*, 1998). Two trials to mainly test non-invasive parameters for their potential to predict AS susceptibility in seemingly healthy birds were conducted in broilers. A full pedigree population of chicks was produced from parents from a commercial dam-line of ANAK Breeders Ltd (Netanya, Israel). Each of 30 males was mated with 12 females to produce half-sib sire families representing the stock's genetic variation for growth rate and other traits. In Trial 1 only males ($n = 150$), and in Trial 2 both sexes ($n = 210$) were used. Standard broiler feeding was used. The electrocardiograph (ECG) of the birds was measured using a Biopac MPIOO data acquisition system, three ECG100A amplifiers and Acqknowledge software (Biopac Systems, Goleta, CA 93117, USA) and stored on a laptop computer. Unanaesthetized birds were placed in dorsal recumbency with legs and wings restrained with rubber bands fixed to one of 3 wooden boards of varying sizes, depending on body weight. ECG leads ending in crocodile clips were connected to 23 gauge needles inserted subcutaneously in the skin in the left and right ventral parts of the wing close to the body, and at the medial proximal part of the left leg where it joins the body.

The S wave amplitude (SWA) in broilers is negative in lead II. SWA was generally elevated in AS birds. In Trial 1 this was seen on Day 26 and was probably indicative of developing AS rather than being predictive. The P wave in broilers is positive. In lead II the P wave represents the depolarization of the atria, causing contraction and ejection of their complement of blood into the ventricles. AS caused consistent increases in P wave amplitude, indicating right atrial hypertrophy; P wave duration changes were less marked and can only suggest possible left atrial changes. These studies show that broilers have diverse reactions to cold, and that ECG changes may indicate both ventricular and atrial abnormalities in AS birds.

20. GENETICS OF BLOOD OXYGENATION, PULSE, & ELECTROCARDIOGRAPHY WAVEFORMS, AND THEIR ASSOCIATION WITH ASCITES IN BROILERS. *Current Status:* Manuscript in preparation, see Appendix A (A. Cahaner¹, S. Drayan¹, M. Bellaiche², A. Rosner¹, A. Shlosberg¹, ¹Hebrew University of Jerusalem, Fac. of Agriculture, POB 12, Rehovot 76100, Israel ²Kimron Veterinary Institute, POB 12, 50250 Bet Dagan, Israel).

20.A. Summary: There is evidence for genetic variation in broilers' propensity for development of ascites syndrome (AS). If specific parameters to predict AS resistance are sought in order to assess their worth as selection criteria in breeding, it is necessary to estimate the heritability of these parameters, and their genetic correlation to consequent AS development under ascites-inducing conditions (AIC). The first trial consisted of 150 male chicks from 30 sire-families; a second trial consisted of 210 male and female chicks from the same families. In both trials, chicks were exposed to AIC by rearing in individual cages under low temperatures ($\pm 6^\circ\text{C}$) from Day 20 to Day 44. Birds that died were necropsied and recorded as AS. Body weight (BW), Electrocardiography (ECG), blood oximetry, hematocrit (HCT), and hemoglobin (Hb) were determined several times in each trial from Day 1 to Day 44, when all surviving birds were killed and autopsied. Some were diagnosed as sick with AS, and the rest (65%) were found to be healthy. Heritability estimates of all traits in almost all ages were significant, ranging mostly between 0.4 to 0.8. Percentage of AS per family (%AS), combined from the two trials, ranged from zero to 92%; it was significantly correlated ($r = 0.5$) between the first and second trial, indicating the existence of genetic variation. The heritability (0.4 & 0.7) of the RV/TV ratio, a trait highly correlated (0.53 & 0.73) with %AS, also indicated the existence of genetic variation. Significant negative genetic correlation were found between %AS and blood oxygen and pulse in most ages ($r = -0.43$ to 0.56). The results suggest that although all traits measured are significantly heritable, only blood oxygen and pulse are suggested as early predictors for development of AS in birds under AIC.

20.B. Objectives Addressed: This project is relevant to Objectives 2, 4, and 5. It is apparent that the continuous selection of broilers for a more rapid growth has been increasing their propensity to develop AS, probably due to their higher basal metabolic rate. This conclusion is supported by results from several comparisons between chicken stocks differing substantially in their growth rate. Combining the findings from the comparisons between commercial broilers and distant stocks (Jungle Fowl, Athens-Canadian population, and Label stocks), and among broiler parental lines, one may reach a unified conclusion, namely that higher propensity to AS is always associated with higher growth rate. To facilitate successful commercial selection against AS-sensitivity, one needs to identify traits that are (1) genetically associated with AS development; (2) noninvasive and expressed in broilers under normal conditions, allowing all of them to fully express their genetic potential in other traits, (3) easy to measure thus allowing to apply this selection in large populations with minimal increase in costs. Two trials to mainly test non-invasive parameters for their potential to predict AS susceptibility in seemingly healthy birds were conducted. A full pedigree population was produced by parents from a commercial dam-line of

ANAK Breeders Ltd (Netanya., Israel). Each of 30 males was mated with 12 females to produce half-sib sire families. In Trial 1 only males ($n = 150$), and in Trial 2 both sexes ($n = 210$) were used for genetic evaluations. BW was determined 3 times a week in both trials; other measurements included electrocardiography (ECG), hemoglobin (Hb), the oxygen saturation of Hb in the arterial blood (SaO₂) and HCT. They were determined several times in each trial, from day of hatch to Day 44, when all surviving birds were killed and necropsied to visually determine whether ascites fluid, hydropericardium or right ventricular hypertrophy were present; such birds were regarded as AS positive, and the rest (65% of total chicks) were considered to be healthy. The experimental population was designed to allow estimation of two genetic parameters: heritability of the measured traits, and genetic correlation between pairs of these traits, and between each of them and percentage of AS birds per family. Therefore the population consisted of sire families of half-sibs, where each chick in the family was the progeny of the same sire, but of a different dam. In this genetic design, the total phenotypic variance (V_p) is divided to two variance components: "between sire-families" ("sire var") and "within sire-family" ("residual var").

Significant estimates of heritability were found for all traits. The estimates were mostly similar across ages, but not always between trials (Table 1). The actual number of birds (n) measured was reduced with age due to mortality. There was, however, some random variation in n , due to incomplete sampling of birds, or faulty measurements. In general, however, most estimates of heritability ranged between 0.5 to 0.7, with some lower (0.3 to 0.5) and higher (0.7 to 1.00) estimates. Heritability of BW up to 5 wk of age ranged from 0.4 to 0.7. These estimates are in agreement with numerous reports, hence indicating the genetic validity of the populations and the biometrical analysis used in this study, and the reliability of the data. A lower heritability (0.14 and 0.23 in Trials 1 and 2, respectively) was found for BW at 6 wk of age, apparently reflecting the cumulative effects of the AIC via reduced growth in birds with AS, and the elimination of families with high %AS. Direct estimation of heritability of %AS is not straightforward because it is a discrete "yes-or-no" variable. However, there was a highly significant correlation ($r = 0.5$; $P < 0.001$) between family %AS in trials 1 and 2, indicating that a significant portion of the variation in %AS, ranging among families from 0 to 92%, is genetically controlled in our population, as in the broiler lines studied by Lubritz *et al.* (1995). The quantitative trait most closely related to %AS was RV/TV ratio, determined for each bird on day of death, or on Day 44. Significant heritability was found for RV/TV in Trials 1 and 2 (0.72 and 0.42), also in agreement with Lubritz *et al.* (1995). HCT exhibited the most consistent heritability estimates across ages and trials. Hb was highly heritable in Trial 1, but less so and quite inconsistent in Trial 2, possibly due to some differences in measurement protocols. In both trials, blood SaO₂ was highly heritable and consistent over ages. Heart rate measured from the ECG readings was termed Pulse. High and similar estimates of heritability (ranging mostly between 0.6 to 0.9) were found for Pulse in both trials. Highly significant estimates of heritability were found for the amplitude of the S wave, especially in Trial 1 where most estimates were close to 1 (Table 1). In Trial 2, heritability estimates of S wave were around 0.6, being very consistent across ages. The R wave amplitude was less heritable, with most estimates ranging between 0.2 to 0.5 in both trials (Table 1). The rate of AS in

of birds (4 to 8 per family in each trial). This correlation indicates the existence of genetic variation among families in propensity to develop AS under AIC. Proven existence of genetic variation for AS, allows estimation of genetic correlation with supposedly related traits. Consistently significant estimates of correlation with %AS are presented (Table 2); it was negatively associated with SaO₂; correlation values ranged from -0.44 in the 1st week to -0.55 in the last week. %AS was negatively correlated (around -0.5) also with Pulse, but only after 3 weeks of age, hence genetically controlled lower pulse may reflect secondary steps of developing AS. Significant genetic correlation between l/oAS and BW was found only in Trial 1 (negative in all ages, data not shown). Lower incidence of AS was found in families that exhibited higher BW at all ages in Trial 1, but in Trial 2, there was no correlation between BW and %AS. It is possible that under the unique conditions of Trial 1 (brooding in batteries; mycoplasma infection starting from hatch), average growth rate of families with higher propensity to AS was reduced already during embryonic development and brooding, long before the birds manifested AS. Similar to previous studies, %AS was highly correlated with the RV/TV ratio in both trials (Table 2). As l/oAS, also RV/TV was negatively correlated with SaO₂ at 1 or 2 weeks, with Pulse at 3 or 5 weeks (data not shown). These results confirm that the RV/TV ratio, widely used as a phenotypic indicator of developing AS, can be used reliably also to study genetic parameters of the propensity to AS. None of the ECG waveforms was genetically correlated with %AS or RV/TV ratio. R wave at 3 to 4 weeks of age was negatively correlated with BW at hatch, but the meaning or usefulness of this correlation is unclear. R wave was negatively correlated with S wave, but only at 6 weeks of age. At that age, S wave was negatively correlated with BW. These latter correlation could hardly be relevant to AS resistance, because most susceptible birds had died before then.

The results suggest that although all traits measured are significantly heritable and hence can potentially be altered by selection, only SaO₂, and possibly Pulse, are suggested as early predictors for development of AS when birds are exposed to AIC. Therefore selection for high blood SaO₂ and high pulse rate in young birds from a commercial broiler stock may genetically reduce the propensity to develop AS.

21. BROILER LINES SELECTED FOR SUSCEPTIBILITY OR RESISTANCE TO ASCITES INDUCED BY HYPOBARIC HYPOXIA PRODUCE PROGENY WITH A CORRESPONDING SUSCEPTIBILITY OR RESISTANCE TO ASCITES INDUCED BY COOL TEMPERATURES. *Current Status:* Manuscript in preparation, see Appendix A (Xiaofang Liu and Nicholas B. Anthony, Department of Poultry Science, University of Arkansas, Fayetteville AR 72701).

21.A. Summary: Hypoxia (low atmospheric oxygen) increases pulmonary vascular resistance by provoking an endogenous reactive pulmonary vasoconstriction designed to match the regional distributions of blood flow and ventilation. Generalized pulmonary vasoconstriction in broilers exposed to hypoxia causes an immediate elevation in the pulmonary arterial pressure and, when sustained chronically, leads to terminal PHS/ascites. Birds used in this study originated from a random bred

population derived from a pedigree male line currently maintained by Cobb-Vantress. This population exhibits PHS incidences of 8.4% and 68.9% when reared under local (390 m) and high altitude (2900 m) conditions, respectively (Hughes et al., 1996). In addition to the random bred population, two lines were divergently selected from the same stock for six generations, using hypobaric hypoxia to create lines which now exhibit susceptibility or resistance to PHS when exposed to a simulated altitude of 2900 m. Family selection was utilized with selection for both lines based on the performance of progeny reared under hypobaric conditions. At each generation, tested progeny were generated from 24 sires and 48 dams per line. Replacement progeny were maintained from families selected for degree of resistance or susceptibility of sibs. Progeny from LPHS and HPHS lines were generated through pedigree hatches for use in the evaluation under PHS-inducing protocols, which include fast growth and cool temperature exposure, and unilateral obstruction of an extra-pulmonary primary bronchus. Fast growth and cool temperatures are the primary triggers for ascites under conditions of broiler production at normobaric altitudes. Unilateral bronchus occlusion prevents air flow to one lung, thereby acutely initiating hypoxemia associated with increases in pulmonary vascular resistance and pulmonary arterial pressure, and chronically inducing PHS in broilers.

21.B. Objectives Addressed: This project is related to Objectives 1, 2, and 4. Approximately 200 chicks (males and females combined) from each line (Random Bred, Resistant, Susceptible) were hatched, wing banded, and distributed mixed by sire family among six environmental chambers. All chicks were brooded normally, were exposed to a 23 hour Light :1 hour Dark photoperiod, and received a 23% protein broiler ration *ad libitum*. Obvious culls were eliminated. On Day 17 the temperature in four chambers was reduced to 15 C (Cool Temperature challenge) until the termination of the experiment on Day 49. The temperature remained normal (24 C) in the remaining chambers, however on Day 17 these birds were subjected to surgical unilateral occlusion of an extra-pulmonary primary bronchus (Bronchus Clamp). The Cool Temperature challenge induced 38.8% ascites in the Susceptible line, 27.1% ascites in the Random Bred line, and 16.2% ascites in the Resistant line ($P \leq 0.01$). Differences in ascites susceptibility were not associated with differences in growth performance; the Random Bred line grew fastest, followed by the Resistant line and then the (slowest growing) Susceptible line. The Bronchus Clamp induced 86% total mortality (surgical + post-surgical + ascites) in the Susceptible line, and 80% total mortality in both the Random Bred and Resistant Lines ($P > 0.1$). However, ascites mortality was higher in the Resistant line (48%) than in the Susceptible (37%) or random Bred (34%) lines. Overall, these observations confirm a strong genetic component of ascites susceptibility, and demonstrate key commonalities between the genetic resistances to ascites induced by hypobaric hypoxia and cool temperatures. These studies also confirm that selection for ascites resistance need not predispose toward a loss of growth performance.

V. DESCRIPTION OF THE COOPERATION

Multiple elements of cooperation occurred. All of the Peckode (BARD 97.1, 97.2) data files as well as the MS-EXCEL program for analyzing daily body weight and various permutations of body weight (growth velocity and acceleration, exponential growth, 3-day running standard deviations, etc.) were provided to the Israeli investigators. Dr. Shlosberg visited Dr. Wideman's lab and learned to perform the bronchus clamp surgical procedure, and to use the electronic physiograph for recording ECG's. Oximeters were compared during the same visit. Dr. Cahaner visited Dr. Roush's lab to evaluate the Neural Net software, and to discuss future research plans with Dr. Roush and Dr. Wideman. Dr. Cahaner shared Israeli growth velocity data with Dr. Roush, and these data were evaluated using the neural net program. Wideman, Cahaner and Shlosberg are collaborating in the evaluation of a large ECG data set, which includes the amplitudes and durations for up to 80 consecutive ECG Lead II wave cycles for each of 25 broilers. Dr. Wideman has recommended to Hubbard ISA that Dr. Cahaner should participate in evaluating the pedigree information for the Base and Resistant populations; which will help further characterize the genetic relationships related to ascites resistance. Dr. Wideman provided Dr. Shlosberg with examples of ECG's recorded using different electronic physiograph setups (alligator clip vs. needle electrodes; awake vs. anesthetized; Hi-Pass and/or Lo-Pass filters on vs. off; standard ECG limb leads vs. non-standard positioning). Dr. Roush currently is evaluating Israeli growth velocity data using the neural net approach.

V I. EVALUATION OF THE RESEARCH ACHIEVEMENT

As proposed by Roush *et al.* (1994), the relationship between individual daily growth patterns and susceptibility of broiler chickens to PHS was confirmed. Individual broilers exhibit intervals of growth stasis followed by episodes of growth saltation. Clinically healthy fast-growing broilers tend to exhibit more frequent and higher amplitude transitions between growth saltation and stasis than do broilers developing PHS during the Day 35-49 interval. Clinically healthy broilers have greater oscillations in growth velocity and acceleration than birds that are susceptible to PHS. A Genetic Adaptive General Regression Neural Network (GRNN) with genetic adaptive calibration was trained to predict ascites susceptibility based on the individual growth phases and their combinations. Data representing the first, first two, and all three phases of growth were determined to have potential for computerized diagnostic weighing. Using the GRNN, birds in all three data sets were successfully classified (100%) with or without PHS. Therefore, artificial neural networks can be used to distinguish the difference between normal broilers and those susceptible to PHS, based on daily growth dynamics. These observations confirm the fundamental premise of the original grant proposal.

Studies designed to evaluate minimally invasive diagnostic indices using protocols applicable to commercial genetic selection programs indicated that none of the indices (heart rates, hematocrit, ECG wave amplitudes or durations, oximetry values for percentage saturation of hemoglobin with oxygen, plasma amino acid profiles, troponin T) universally differentiated PHS-resistant males and females from

those that subsequently developed ascites. The predictive value of these indices apparently varied when applied to individual broilers of different genders, breeds, or when reared under different management regimens. When data from apparently healthy broilers that subsequently developed ascites were compared with those that did not, body weights, heart rates, and percentage saturation of hemoglobin with oxygen were lower for susceptible than for resistant males, and HCT was higher in susceptible males and females than in resistant males and females, respectively. Comparisons of the ECG Lead II wave amplitudes indicated the RS-wave amplitude was larger in susceptible than in resistant males, and the ECG Lead II S-wave amplitude was more negative in susceptible males and females than in resistant males and females. These ECG characteristics were consistent for different breeds of domestic fowl, as well as for broilers in which pulmonary hypertension was induced by different experimental models (cool temperatures, bronchus clamp, pulmonary artery clamp). For example, the ECG changes associated with unilateral bronchus occlusion were identical to those previously shown to be predictive of pulmonary hypertension induced by cool temperatures or after unilateral pulmonary artery occlusion. Consequently, the use of the bronchus clamp model to generate pulmonary hypertension for studies of growth dynamics and other minimally invasive diagnostic indices was substantiated. The modest reductions in heart rate associated with the onset of PHS in rapidly growing broilers were confounded by gradual age-related reductions in heart rate, thus heart rate must be considered an unreliable diagnostic index for PHS-susceptibility. Analyses of plasma constituents suggest cardiac taurine and troponin T are released into the plasma as a protective mechanism in response to the induction of pulmonary hypertension and hypoxemia; however, plasma taurine and troponin T apparently are not predictive of the apparently clinically healthy broilers that subsequently succumb to ascites when challenged with unilateral pulmonary artery occlusion. Overall, hematocrit, oximetry, and the ECG Lead II S-wave amplitude were the best minimally invasive predictors of ascites susceptibility. The pulse oximetry systems validated in these studies currently are being used by two major broiler breeding companies to eliminate PHS-susceptible individuals from their genetic stocks.

In lieu of selecting genetic lines exhibiting smooth or aphasic growth dynamics as originally proposed in Objective 1, unilateral pulmonary artery occlusion was used to select for broad-based resistance to ascites by rigorously eliminating PHS-susceptible individuals. Working in close cooperation with Hubbard ISA, the resulting PHS-resistant lines and Base population provided the biological (genetic) basis for further evaluations of growth dynamics and minimally invasive diagnostic indices. Within two generations, broiler breeders that thrived in spite of having one pulmonary artery occluded, subsequently produced male and female progeny exhibiting a > 80% reduction in the incidence PHS induced by fast growth and cool temperatures. Furthermore, growth performance was minimally reduced in attaining this improvement in PHS resistance, in spite of the fact that the breeder parent line for the chicks of the Base population had experienced two additional generations of selection for growth that necessarily could not be imposed on the breeder parents for the Resistant line. The dramatic improvement in PHS resistance within two generations of selection served as definitive proof to commercial geneticists that, with

appropriately focused and sufficiently rigorous selection pressures, major advances can be accomplished in reducing the frequency of undefined genes that seem to be partly responsible for PHS susceptibility in commercial broilers. In this context, the success of the unilateral pulmonary artery clamp technique confirms that the anatomical or metabolic capacity of the pulmonary vasculature to accommodate an increase in blood flow is crucial to the ability of broilers to resist the onset of pulmonary hypertension leading to PHS. The gene or genes involved in ascites susceptibility appear to be dominant, indicating that ongoing proactive exposure and elimination of susceptible individuals will be necessary to achieve an overall improvement in ascites resistance. A major achievement of this research is that an international broiler breeder company, Hubbard ISA, now possesses a pedigree line that is highly resistant to ascites when grown as rapidly as possible during exposure to cool temperatures. This line currently is being multiplied for the commercial broiler market.

Cardio-pulmonary function was evaluated based on the hypothesis that broilers are susceptible to PHS whenever their pulmonary vascular capacity is inadequate to accept and oxygenate, at a sufficiently low pulmonary arterial pressure, the cardiac output required to support tissue metabolism. The pulmonary vascular capacity is defined broadly to encompass anatomical constraints related to the compliance and volume of the blood vessels, as well as metabolic limitations related to the tone (state of contracture) maintained by the resistance vessels. Genetic selection experiments using the unilateral pulmonary artery occlusion technique confirmed that susceptibility to PHS depends substantially on an inherent inability of the pulmonary vasculature to accommodate the requisite cardiac output. Consequently, any factor contributing to an increase in cardiac output or an overall reduction in the pulmonary vascular capacity theoretically can accelerate the pathogenesis of PHS if the right ventricle of the heart must increase the pulmonary arterial pressure to propel blood flow through the lungs. Fast growth and cool temperatures are the primary triggers for PHS during commercial broiler growout. Growth dependent increases in cardiac output, stroke volume, and ventricular weight were quantified, providing direct evidence that the right ventricle of rapidly growing broilers must propel a rapidly increasing cardiac output through the pulmonary vasculature. The growth-associated increments in cardiac output are attributable wholly to increases in stroke volume and not heart rate. Venous congestion (increased venous volume), as reflected by venous hypertension (increased venous pressure), was shown to develop when the right ventricle was unable to elevate the pulmonary artery pressure sufficiently to propel the cardiac output through an anatomically inadequate or inappropriately constricted pulmonary vasculature. Male broilers chronically exposed to cool temperatures had a lower percentage saturation of hemoglobin with oxygen, a lower heart rate, a higher hematocrit, a higher cardiac output, a higher pulmonary arterial pressure, and a marginally lower systemic arterial pressure than broilers reared under warm temperature conditions. It was the elevated cardiac output rather than hypoxemic pulmonary vasoconstriction that made the major contribution to the existing increase in pulmonary arterial pressure in broilers acclimated to cool temperatures. Comparisons of clinically healthy and preascitic broilers revealed no differences in body weight or respiratory rate, but preascitic broilers had lower values for mean arterial pressure, total

peripheral resistance, and partial pressure of O₂ in arterial blood, and higher values for pulmonary artery pressure, cardiac output, and partial pressure of CO₂ in arterial blood. Inhaling 100% O₂ increased HbO₂ to 99.9% in both groups, however pulmonary artery pressure remained higher in preascitic than in normal broilers, and the pulmonary vascular resistance was not reduced by 100% O₂ inhalation. Acutely reversing the systemic hypoxemia in preascitic broilers had little direct impact on pulmonary hypertension, providing no evidence of hypoxemic pulmonary vasoconstriction. Instead, acutely reversing the systemic hypoxemia primarily increased the total peripheral resistance and normalized the mean arterial pressure, total peripheral resistance, and cardiac output. Systemic hypotension was shown to be an early indicator of the pathogenesis in preascitic broilers, and detailed experiments demonstrated that systemic hypotension stimulates the kidneys to retain the solutes and water that ultimately comprise the ascitic fluid. Venous, right atrial, right ventricular, pulmonary arterial and wedge pressures were obtained by inserting a catheter into a wing vein and progressively advancing the catheter into a pulmonary branch artery until the catheter tip became wedged in and occluded the flow through a terminal artery. Mean right ventricular and pulmonary arterial pressures were lower in the PHS-resistant line than in the base (PHS-susceptible) population, but wedge pressures did not differ between the resistant, base and unselected lines. These observations provide definitive proof that pulmonary hypertension is initiated as a consequence of excessive pulmonary arterial or arteriole resistance.

Based on the success of surgical unilateral pulmonary artery occlusion as a technique for selecting PHS-resistant genetic lines, we evaluated the responsiveness of broilers to several chemical mediators of pulmonary vasoconstriction, including epinephrine, metabolic acid, thromboxane, endotoxin, and carbon dioxide gas. Presumably susceptible broilers will be hyper-responsive and resistant broilers will be hypo-responsive to pulmonary vasoconstrictors. Various particulates and bacterial endotoxins stimulate endothelin-mediated thromboxane-dependent increases in pulmonary vascular resistance and pulmonary arterial pressure in mammals. Thromboxane is involved in pulmonary vasoconstriction under a wide variety of circumstances impacting broiler chickens, including hypoxia, metabolic acidosis, and respiratory diseases. Similar interactions between locally induced pulmonary vasoconstrictors may be involved in the pathogenesis of ascites triggered in broilers by intratracheal inoculation with *E. coli* and infectious bronchitis virus. Our experimental observations in broilers are consistent with the hypothesis that endotoxin initiates a biochemical cascade culminating in the delayed onset of thromboxane-mediated pulmonary vasoconstriction and pulmonary hypertension within 20 min post-injection. Epinephrine and thromboxane triggered pulmonary vasoconstriction of consistent amplitude and duration among individual broilers, whereas less consistent individual pulmonary hemodynamic responses were observed following endotoxin injections, induced metabolic acidosis, and artificially elevating the partial pressure of CO₂ in the arterial blood. Individual differences among broilers in their susceptibility to pulmonary hypertension syndrome (ascites) may be related to innate or acquired variability in their pulmonary vascular responsiveness to vasoactive mediators.

Trials were conducted in Israel to test non-invasive parameters for their potential to predict AS susceptibility in seemingly healthy birds. In both trials, chicks were exposed to cool temperatures. BW was determined 3 times a week in both trials; other measurements included electrocardiography (ECG), hemoglobin (Hb), the oxygen saturation of Hb in the arterial blood (SaO₂) and HCT. The experimental population was design to allow estimation of two genetic parameters: heritability of the measured traits, and genetic correlation between pairs of these traits, and between each of them and percentage of AS birds per family. In each trial, family means were calculated for each measurement. Significant estimates of heritability were found for all traits. The estimates were mostly similar across ages, but not always between trials. The results suggest that although all traits measured are significantly heritable and hence can potentially be altered by selection, only SaO₂, and possibly Pulse, are suggested as early predictors for development of AS when birds are exposed to cool temperatures. Therefore selection for high blood SaO₂ and high pulse rate in young birds from a commercial broiler stock may genetically reduce the propensity to develop AS.

VII. CONCLUSIONS

The major objectives have been accomplished. The unilateral pulmonary artery occlusion technique was used in collaboration with a major broiler breeding company to demonstrate conclusively that dominant genes for PHS susceptibility exist, and to create a commercial broiler line that is highly resistant to PHS induced by fast growth and cool temperatures. Clinically healthy broilers were demonstrated to have greater oscillations in growth velocity and acceleration than PHS-susceptible broilers, and artificial neural networks were capable of accurately distinguishing differences in daily growth dynamics between normal broilers and those susceptible to PHS. The relationship between daily patterns of growth dynamics and susceptibility of broiler chickens to PHS was confirmed.. Minimally invasive diagnostic indices were evaluated (heart rates, hematocrit, ECG wave amplitudes and durations, oximetry values for the percentage saturation of hemoglobin with oxygen, plasma amino acid profiles, plasma troponin T). Genetic analyses demonstrate that the percentage saturation of hemoglobin with oxygen is a highly heritable and accurate early predictor of ascites susceptibility. Pulse oximetry systems validated in these studies currently are being used by two major broiler breeding companies to eliminate PHS-susceptible individuals from their genetic stocks. Cardio-pulmonary characteristics typical of preasitic broilers when compared with the PHS-resistant line were described, further solidifying our confidence in advising commercial breeders regarding appropriate selection procedures.

VIII. LIST OF PUBLICATIONS

Refereed Journal Publications (chronological order)

- Wideman, R. F., Wing, T., Kirby, Y. K., Forman, M. F., Marson, N., Tackett, C. D., and Ruiz-Feria, C. A. Evaluation of minimally invasive indices for predicting ascites susceptibility in three successive hatches of broilers exposed to cool temperatures. *Poultry Sci.* 77:1565-1573, 1998.
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- Chapman, M. E., and Wideman, R. F. Pulmonary wedge pressures confirm pulmonary hypertension in broilers is initiated by excessive pulmonary arterial (precapillary) resistance. *Poultry Sci.* (*submitted*).
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I X. PATENTS

Provisional Patent Approved: (Title and ID number confidential; General Topic: Technology for rapid selection of ascites-resistant poultry).