Utilization of the Sex-Linked Gene for Imperfect Albinism (S*ALS).
1. Effect of Early Weight Loss on Chick Metabolism

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ABSTRACT The sex-linked gene for imperfect albinism (S*ALS) has been associated with slow early growth in some trials but not in others. Albino (59) and nonalbino (73) chicks were raised to 3 d of age to study early growth. At 3 d of age, plasma β-hydroxybutyrate (β-HBA) levels were measured and the chicks were euthanatized and dissected to measure liver, gall bladder, and yolk sac weights. Fatty acids of the liver and the yolk sac were also analyzed. On average, albino chicks lost weight between hatch and 3 d of age and nonalbinos gained weight (-2.41 vs 0.74 g/d, P < 0.01). At 3 d of age, livers from albinos contained higher (P < 0.01) levels of docosahexaenoic acid than those of nonalbinos, likely reflecting the dependence on yolk sac nutrients of albinos and on dietary lipids of nonalbinos at this time. Albinos had lower body temperatures (P < 0.01), liver weights (P < 0.01) and gall bladder weights (P < 0.05), and heavier yolk sacs (P < 0.01) than did nonalbins. Plasma levels of β-HBA were higher (P < 0.01) for albinos than for nonalbinos. At similar body weights, chicks of both genotypes had similar body temperatures, gall bladder weights, and plasma β-HBA levels. Linear regressions indicated that in albinos weight loss is associated with larger yolk sacs, smaller livers, larger gall bladders, lower body temperatures, and higher levels of β-HBA. Yolk sac utilization seemed to be correlated with activation of the digestive system. The inability of starving chicks to use the yolk sac nutrients while lipolysis is taking place suggests that yolk sac absorption does not respond to lipolytic hormones. Because under certain conditions a large proportion of albinos (90% in this experiment) show the symptoms of the starve-out syndrome, the S*ALS gene could serve as a model for the study of this syndrome.

(Key words: S*ALS, sex-linked albinism, chicken, lipid metabolism, yolk sac utilization)

INTRODUCTION

Mortality caused by the starve-out syndrome is a problem in the turkey industry (Moran, 1990) and exists to a lesser extent in chickens (Moran, 1988). Chicks suffering from the syndrome do not eat from hatch and they may or may not drink until their death by starvation. It is unknown why these chicks do not start to eat, and the inability to predict which birds will suffer from this condition makes it difficult to obtain sufficient numbers for an in-depth study.

Houpt (1958) described the effects of starvation on chicks deprived of feed from hatch. During the first 24 h, the chicks are distressed, chirp shrilly, and have lower body temperatures and plasma glucose levels. After 24 h of starvation, what Houpt (1958) described as a slight depression appears, plasma glucose levels become normal (150 mg/100 mL or higher), body temperature remains low, and the chicks assume a hunched posture. They become inactive but can be stimulated into activity by prodding. Two to 3 d after the initiation of starvation, the chicks sit down on their hocks and appear sleepy and droopy. Their body temperature drops even lower, and their plasma glucose level drops to between 80 to 100 mg/100 mL. Chicks without feed from hatch die after 5 to 6 d of starvation (Houpt, 1958). During the 4 or 5 h preceding death, plasma glucose levels drop below 80 mg/100 mL and the chicks become comatose, with closed eyes and curled toes. Post-mortem examination of these chicks (Houpt, 1958) revealed emaciation, distended gall bladders, and discolored livers.

The increase in plasma glucose observed after 24 h of starvation (Houpt, 1958) may be attributed to the strong glycolytic, gluconeogenetic, and lipolytic effect of glucagon in response to the low glucose levels (Hazelwood, 1987) that occur during the first 24 h of starvation. To fill its glucose requirements, the chick can either use dietary nutrients or gluconeogenesis. If the chick does not eat, its only alternative once the glycogen reserves are depleted is gluconeogenesis, which cannot occur with adipose or yolk lipids as substrate. The starving chick must first use glucogenic amino acids as substrate, and once these are exhausted, protein catabolism of...
Commercial turkey poults are often beak-trimmed, desnooded, and toe clipped at hatching, and these stressful and traumatic practices discourage the initiation of nutrient intake and accentuate early deaths (Lewis and Hurnik, 1979). These stresses accentuate the depletion of hepatic glycogen, which occurs during hatching (Freeman and Vince, 1974). Chicks suffer similar traumatic treatments, such as debeaking and vaccination, in the hatchery prior to transportation and placement. Delays in transportation and late placement in brooding facilities complicate the problem of glycogen depletion (Fanguy et al., 1977), and according to Donaldson and Christensen (1991) further stress prior to hatching (Freeman and Vince, 1974). Chicks suffering from the starve-out syndrome. The objective of this experiment was to use the S*ALS gene to study the effect of early weight loss (and presumably of early starvation) on chick metabolism and yolk sac utilization.

**MATERIALS AND METHODS**

A cross of males heterozygous for the gene S*ALS to albino females was used to produce approximately 50% albino and 50% nonalbino chicks in two series of matings and a total of three hatches. Eggs were identified individually and weighed before incubation and after 17 d of incubation, when they were transferred to individual compartments in a hatching chamber to allow identification of the egg from which each chick hatched. The chicks of each hatch were transferred with genotypes intermingled to a single metal battery brooder after 21.5 d of incubation (at 1 d of age) and were weighed at 1, 2, and 3 d of age. At 3 d of age, body temperature was measured with an electronic thermometer inserted into the cloaca to a depth of approximately 1 cm. A blood sample of approximately 1.5 mL was taken from each 3-d-old chick by cardiac puncture using a heparinized syringe. The blood was immediately put on ice and was centrifuged (3,000 × g, 4 C, 10 min) within 1 h of sampling. The resulting plasma was stored at −20 C. The chicks were killed by cervical dislocation immediately after blood sampling and then dissected to determine sex and liver, gall bladder, and yolk sac (including the contents) weight. The liver and the yolk sac were lyophilized and stored at −20 C for later lipid analysis. The DM content and the total lipid content (percentage on a DM basis) of these tissues were determined. Fatty acid concentrations were determined by gas chromatography as described by Santos and Silversides (1996).

**Plasma Analysis**

The plasma levels of \( \beta \)-hydroxybutyrate (\( \beta \)-HBA) were measured using a quantitative enzymatic method.\(^2\) The frozen plasma was allowed to thaw prior to analysis.
EFFECT OF EARLY WEIGHT LOSS ON METABOLISM

TABLE 1. Egg weight, BW from hatch to 3 d of age, liver weight, gall bladder weight, liver and gall bladder DM, total fat, yolk sac weight, body temperature, and plasma β-hydroxybutyrate levels at 3 d of age of sex-linked albino and nonalbino chicks

<table>
<thead>
<tr>
<th>Variable</th>
<th>Chick genotype</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Age</td>
<td>Albino</td>
<td>Nonalbino</td>
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<tr>
<td></td>
<td></td>
<td>n</td>
<td>x ± SE</td>
<td>n</td>
<td>x ± SE</td>
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<td></td>
<td></td>
<td>(d)</td>
<td></td>
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<td></td>
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<tr>
<td>Egg weight, g</td>
<td></td>
<td>59</td>
<td>53.7 ± 0.5</td>
<td>73</td>
<td>54.0 ± 0.5</td>
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<tr>
<td>Body weight, g</td>
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<td>1</td>
<td>39.0 ± 0.5</td>
<td>73</td>
<td>38.3 ± 0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>34.5 ± 0.6B</td>
<td>73</td>
<td>37.1 ± 0.5A</td>
</tr>
<tr>
<td>Liver weight, g</td>
<td></td>
<td>3</td>
<td>33.7 ± 0.5B</td>
<td>72</td>
<td>39.4 ± 0.5A</td>
</tr>
<tr>
<td>Liver DM, %</td>
<td></td>
<td>3</td>
<td>1.3 ± 0.0B</td>
<td>72</td>
<td>1.6 ± 0.0A</td>
</tr>
<tr>
<td>Liver total fat, %</td>
<td></td>
<td>3</td>
<td>35.6 ± 1.5</td>
<td>35</td>
<td>36.9 ± 1.9</td>
</tr>
<tr>
<td>Yolk sac weight, g</td>
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<td>3</td>
<td>44.5 ± 1.6</td>
<td>35</td>
<td>41.0 ± 2.0</td>
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<tr>
<td>Yolk sac DM, %</td>
<td></td>
<td>3</td>
<td>53.6 ± 2.0</td>
<td>35</td>
<td>46.4 ± 1.6</td>
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<tr>
<td>Yolk sac total fat, %</td>
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<td>3</td>
<td>44.5 ± 1.6</td>
<td>35</td>
<td>47.6 ± 2.1</td>
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<tr>
<td>Gall bladder weight, mg</td>
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<td>3</td>
<td>73.0 ± 1.0A</td>
<td>67</td>
<td>46.0 ± 1.0B</td>
</tr>
<tr>
<td>Body temperature, C</td>
<td></td>
<td>3</td>
<td>40.6 ± 0.1B</td>
<td>71</td>
<td>41.1 ± 0.0A</td>
</tr>
<tr>
<td>β-hydroxybutyrate, mg/dL</td>
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<td>3</td>
<td>11.3 ± 1.4A</td>
<td>36</td>
<td>3.7 ± 1.3B</td>
</tr>
</tbody>
</table>

A, B Means in a row with no common superscript differ significantly (P < 0.01).

RESULTS

Egg weights prior to incubation and BW at 1 d of age were similar (P > 0.05) for both chick genotypes (Table 1). At 2 and 3 d of age, albino chicks had lower BW than nonalbino chicks. On average, nonalbino chicks lost weight between 1 and 2 d of age, at which time they started to gain weight (Table 1). Albino chicks also lost weight up to 2 d of age, but on average they did not start to gain weight at this time (Table 1). After transfer to the batteries, many of the albino chicks took a characteristic hunched position with closed eyes. Nonalbinos did not react in this way even though the two genotypes were in the same environment and were subjected to the same treatments. At 3 d of age, albino chicks had lower body temperatures, smaller livers, larger yolk sacs, larger gall bladders, and higher plasma β-HBA levels than did nonalbino chicks (Table 1). The average change in weight from 1 to 3 d of age was negative for albinos and positive for nonalbinos (~2.41 vs 0.74 g/d, P < 0.01). Up to 3 d of age, 90% of albinos and 32% of nonalbinos lost weight (Table 2).

The percentage DM and the lipid content (on a DM basis) of the livers and yolk sacs of albinos and nonalbinos were similar at 3 d of age (P > 0.05, Table 1). Table 3 shows the fatty acid composition of the livers and yolk sacs of albinos and nonalbinos at 3 d of age. Hepatic docosahexaenoic acid was higher for albinos than for nonalbinos; other differences were nonsignificant between genotypes.

Three series of labeled spectrophotometer tubes were prepared for the reagent blank, the calibrator, and for the plasma samples. Three milliliters of β-HBA reagent were added to each tube, which was then placed in a water bath at 37 C for 2 min. After warming the samples, 0.05 mL of deionized water, calibrator, controls, or plasma samples was added to the respective tubes and the tubes were mixed by gentle inversion. An initial absorbance reading was taken at 340 nm and 0.05 mL of the enzyme catalyzing the transformation of β-HBA into acetoacetate (with the formation of NADH) were added to each tube. After incubating the tubes at 37 C for 10 to 15 min, a final absorbance reading was taken at 340 nm. The β-HBA level of each plasma sample was then calculated using the concentration of the calibrator and the difference between the initial and the final absorbance readings of the sample, the blank, and the calibrator.

Statistical Analysis

All data were initially analyzed using the General Linear Models (GLM) procedure of SAS® (Littell et al., 1991) using a model with genotype as the main effect. Mating and hatch nested within mating were used as blocking factors, and the general error term was used to test the main effects of the model. Sex was used as a main effect in the initial model but as it was not significant it was not included in the final model.

Correlations between the different parameters, including the percentage change in BW from placement to 3 d of age, were calculated for each genotype separately and for the two genotypes together using the PROC CORR procedure of SAS® (Littell et al., 1991). Linear regressions of each parameter on the change in BW to 3 d of age were also calculated for each genotype separately. A covariate analysis (Littell et al., 1991) using mating and hatch within mating as blocking factors and the percentage gain to 3 d of age as the independent covariate was used to test heterogeneity of the slopes of the regressions on the percentage change in BW to 3 d of age.
Table 4 shows correlations between the parameters studied. For albinos and nonalbinos analyzed separately the correlation between liver and gall bladder weights was negative, indicating that large livers were associated with small gall bladders. For nonalbinos, the change in BW to 3 d of age was negatively correlated with yolk sac weight and $\beta$-HBA levels. For albinos, the BW gain to 3 d of age was positively correlated with body temperature, liver weight, and negatively with gall bladder weight, yolk sac weight, and $\beta$-HBA levels. For albinos, the BW gain to 3 d of age was positively correlated with body temperature, liver weight, and gall bladder weight.

The regressions of liver weight, gall bladder weight, yolk sac weight, body temperature, and plasma $\beta$-HBA level on the percentage change in BW to 3 d of age for the two genotypes are shown in Figures 1 and 2. For albinos, the regressions of gall bladder weight, liver weight, and body temperature on the percentage change of body weight to 3 d of age were significant, indicating that smaller livers, larger gall bladders, and lower body temperatures are associated with negative rates of gain between 1 and 3 d of age. Albino chicks that lost little weight had liver weights, gall bladder weights, and body temperatures that were similar to those of nonalbino chicks having similar rates of gain to 3 d of age. The regressions of yolk sac weight and plasma $\beta$-HBA on weight gain to 3 d of age were significant, indicating that the greater the weight of the yolk sac and the greater the $\beta$-HBA levels, the greater the weight of the yolk sac and the greater the level of $\beta$-HBA. To 3 d of age, similar rates of gain or loss of BW resulted in similar yolk sac weights and $\beta$-HBA levels for the two genotypes.

Within the albinos that lost between 10 and 30% of their initial BW, there appeared to be two distinct groups. Despite the weight loss, some of the chicks used their yolk sac contents and had low $\beta$-HBA levels, whereas most chicks that suffered a loss of weight to 3 d of age had high $\beta$-HBA levels and large yolk sacs. The covariate analysis using the percentage change in BW to 3 d of age as an independent variable showed that the slopes of the regressions of yolk sac weight ($P \leq 0.07$) were negatively correlated with body temperature and negatively with liver weight and $\beta$-HBA levels for the two genotypes.
and liver weight ($P \leq 0.01$) on the percentage change in BW to 3 d of age were different from the slopes of the same regressions for nonalbinos. There was no difference between genotypes in the slopes of the regressions on the percentage change in BW to 3 d of age for body temperature ($P < 0.16$), gall bladder weight ($P < 0.63$), and $\beta$-HBA levels ($P < 0.40$).

**DISCUSSION**

The negative effect of the $S^{\text{ALS}}$ gene on growth to 3 d of age is similar to that reported by Silversides et al. (1992) and Santos and Silversides (1996). The hunched position that neonatal albinos often assume under routine stresses such as catching for BW measurement or transfer to the brooding environment is remarkably similar to that which was described by Houpt (1958) for starving chicks, and may reflect a depletion of liver glycogen and a dependence on gluconeogenesis for energy. The absence of this response to stress in nonalbino siblings suggests that albinos and nonalbinos react differently to stress.

Santos and Silversides (1996) found that albino chicks had smaller livers than nonalbinos at hatch. This finding may reflect reduced hepatic glycogen and reduced hepatic accumulation of cholesteryl esters produced from hydrolysis of chylomicron remnants due to the slower transfer of yolk lipids during the last 3 d of incubation. Glycogen reserves are minimal after hatch because glycogen is used as a preferred energy source during the hypoxia associated with pipping and hatching (Freeman and Vince, 1974). Fear, immobilization, and other stimuli perceived as imposing a threat to survival or well-being increase corticosterone secretion, and acute stressors such as restraint cause the release of norepinephrine and epinephrine (Harvey et al., 1987), which may further deplete liver glycogen and reduce nutrient intake and early growth. Low glycogen levels prior to hatch could put albinos in a precarious position when faced with additional stressors.

The decrease in BW of albino chicks observed in this experiment and the smaller liver size of albinos at 3 d of age may result from such an exaggerated stress response. Some albino chicks may have been in a situation of starvation for the first 3 d resulting in BW loss, which was associated with large gall bladders, small livers, low body temperature, high plasma $\beta$-HBA levels, and surprisingly, large yolk sacs. At comparable weight gain or weight loss, all these parameters were similar for both albinos and nonalbinos.

The covariate analysis confirmed this observation. The slopes of the linear regressions of liver weight and yolk sac weight of albinos were probably different from those of nonalbinos because the effect of weight loss on these parameters is greater with extreme weight loss, which was more common for albinos than for nonalbinos. The slopes of the linear regressions of plasma $\beta$-HBA levels, gall bladder weights, and body temperatures were similar for both genotypes, suggesting that these parameters vary even with small BW changes. All the parameters measured were similar for both genotypes at similar BW changes, suggesting that the effect of the $S^{\text{ALS}}$ gene is to decrease BW to 3 d of age resulting in physiological changes, the extent of which depends on the degree of BW loss.

The high levels of arachidonic and docosahexaenoic acid in the livers of albinos may reflect hepatic synthesis of these FA late in incubation and soon after hatch. This synthesis has been reported for arachidonic acid (Noble and Shand, 1985; Noble, 1987), but Noble and Moore
FIGURE 1. Regressions of body temperature, liver weight, and gall bladder weight of sex-linked albino (△) and nonalbino (○) chicks on the percentage change in body weight from 1 to 3 d of age.

(1965, 1967a,b) suggested that the hepatic accumulation of docosahexaenoic acid late in incubation resulted from preferential absorption of yolk phosphatidylethanolamine species by the yolk sac membrane. Preferential yolk absorption is not likely in this case because the yolk sacs of both albinos and nonalbinos contained little docosahexaenoic acid. Nonalbino chicks gained weight to 3 d of age and the observation that their livers contained lower levels of docosahexaenoic acid than those of albinos suggests that the hepatic docosahexaenoic acid...
The level of β-HBA reflects that of ketone bodies, which are high for chicks that are not eating and using fatty acids as an energy source. However, albino chicks that lost weight had high β-HBA plasma levels and large yolk sacs. High ketone levels and large yolk sacs suggest that these chicks successfully tap adipose reserves but are unable to use yolk sac nutrients. This finding leads to the conclusion that hormones controlling the transfer of lipids from adipose tissue to the liver do not control the rate of yolk sac utilization. Independent of genotype, chicks that eat and gain weight successfully use their yolk sac nutrients, suggesting that yolk sac absorption responds to digestive system stimulation. Without this stimulation, there is only a basal level of yolk absorption. This conclusion agrees with the findings of Bierer and Eleazer (1965, for chicks) and Moran and Reinhart (1980, for poultis), who found that absorption of yolk nutrients is complementary to that of feed nutrients during the 1st wk of life. Murakami et al. (1992) found that posthatch starvation caused a decrease in carcass lipid content but did not modify the disappearance rate of yolk reserves. It is possible that these starving chicks...
could access their yolk sac reserves only because they were hungry and their digestive systems were stimulated by this hunger. Some albino chicks in this experiment did not eat when offered feed, suggesting that their digestive systems were not sufficiently stimulated to allow yolk sac absorption.

Glucagon inhibits secretions of the digestive system (Duke, 1987), thus diminishing the heat of digestion. The decreases of digestion, anabolic activity, and basal metabolic rate that accompany feed deprivation likely explain the lower body temperatures of albinos. Bile production by the liver is continuous and bile will accumulate in the gall bladder in the absence of gastric digesta in the duodenum. Feed deprivation lowers the bile release rate and leads to distension of the gall bladder (Moran, 1982), which explains the larger gall bladder (Moran, 1982), which explains the larger gall bladders of albinos. In pigs, liver size and weight are associated to the gene on S*ALS (albinos lie au sexe) chez la poule associees au gene s (Sturkie, 1967), thus diminishing the heat of digestion. The slower differential reaction to stress, lead to the appearance of the symptoms of voluntary feed restriction, including lower heat of digestion, a drop in the metabolic rate and anabolic activity (reflected in lower body temperatures), inhibition of digestive activity (suggested by larger gall bladders), low fatty acid and glycogen levels in the livers (reflected in smaller livers), and increased lipolysis (detected as higher plasma ketone levels). The slower absorption of the yolk sac nutrients may result from low activity of the digestive system.

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REFERENCES


