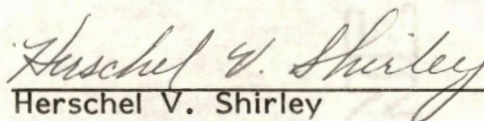
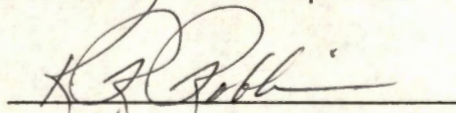
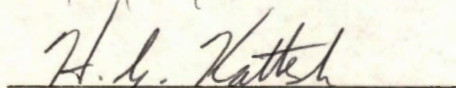


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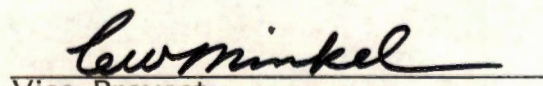
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**A STUDY OF THE EFFECTS OF ENVIRONMENTAL LIGHT
AND TEMPERATURE ON SELECTED BLOOD
AND URINARY FACTORS IN BROILERS**

**A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville**

Waleed Odeh Twal

March 1988

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Thesis

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ABSTRACT

Two experiments were conducted to investigate the effect of light, temperature, and age on some blood parameters and ammonia excretion by male broiler birds. Light treatments were 16L:8D and 24L:0D while temperature treatments were 22°C and 35°C within each light treatment. Samples were taken at 2, 4, and 6 weeks of age.

Results show that light and temperature had no significant effect on blood concentrations of sodium, potassium, chloride, CO_2 , anion gap, or ammonia, kidney asparaginase activity, plasma corticosterone levels, or total ammonia excreted in the feces. However it was found that blood ammonia levels at 2 weeks of age were significantly higher when compared to levels obtained at 4 and 6 weeks of age. It was concluded that this was due to the effect of the high protein diet fed to birds to 3 weeks of age.

Kidney asparaginase activity was also significantly higher at 2 weeks of age which could reflect the effect of the high protein diet and the rapid turnover and restructuring of proteins in the body while birds are growing at a very fast rate.

Plasma corticosterone levels tended to be higher at 2 weeks of age when compared to levels at 4 and 6 weeks. Birds on 24L:0D light treatment had higher corticosterone levels than birds on 16L:0D light treatment but not significantly so, which might indicate the tendency for continuous light to over-stimulate the adrenal gland directly or indirectly and initiate changes in blood chemistry and the normal physiological status of the body.

Atmospheric ammonia was higher in 16L:8D light treated birds at all ages compared to the 24L:0D treated birds. This difference is thought to be due to prevailing conditions in the litter which tended to be more wet in pens with birds under continuous light thus suppressing uric acid and other nitrogenous compounds breakdown to ammonia by anaerobic bacteria.

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CHAPTER I

INTRODUCTION

It is the aim of broiler producers for their birds to reach desired market weights at as young age as possible. Today's broiler strains have been selected for very fast growth rate and high feed efficiency. When coupled with the feeding of well balanced diets and correct management practices, they reach market weight of 2.0 kg at the age of 7 weeks or less. However, as broilers have been selected and managed for faster growth, certain problems have become more prevalent.

Continuous light is one of these practices which have been observed to cause problems. It is a common practice among growers to provide continuous light in broiler houses from 1 day of age. Hogg (1967), Voitle (1969) and Osei (1981) described a condition of eye enlargement and vision impairment resulting from continuous light and suggested that the stress from the absence of diurnal light patterns was responsible. Dejoie (1986) showed that a light treatment of 24L:OD resulted in a very rapid onset of rigor mortis of slaughtered birds when compared to birds kept under a 16L:8D light treatment. He concluded that continuous light apparently interferes with the glycolytic pathway by which muscle energy is generated, thus leading to lower ATP levels in muscles. The rate of rigor mortis onset is directly related to the ATP level in muscles.

In unpublished studies, Shirley (1987) has observed that levels of atmospheric ammonia in pens of birds reared under 24L:OD

treatment were lower than those of pens receiving a 16L:8D treatment. Adekunmisi and Robbins (1987a) found a significantly higher concentration of plasma ammonia in birds housed under 16L:8D light treatment when compared to birds housed under 24L:0D. These results and observations suggest that continuous light results in important alterations of the body's physiological processes which are involved in blood and urine acid-base balance.

The objective of this study is to investigate the effects of light and temperature on ammonia production and blood chemistry and to examine possible mechanisms by which these effects are mediated.

CHAPTER II

LITERATURE REVIEW

Ammonia Synthesis

The end products of nitrogen metabolism in avian species are uric acid, ammonia, urea and other unidentified materials. According to Kroghdahl and Dalsgard (1981), of the total ammonia in excreta, 90% comes from urinary origin and the rest from feces. Ammonia nitrogen constitutes 7% of total urinary nitrogen, 88% from uric acid, 3% from urea and 2% from other sources.

As early as 1921 Nash and Benedict showed that ammonia is formed in kidney cells. Pitts (1964) stated that 35-40% of urinary ammonia nitrogen in rats comes from the amide group of glutamine and the remainder comes from the amino group of glutamine and other amino acids. Makarewicz and Zydowo (1962) showed that adenosine monophosphate can be a substrate for ammonia liberation in avian kidney homogenates and in addition to glutaminase, the enzymes adenylic aminohydase and adenosine aminohydase were also present. Coon and Balling (1984) showed that asparagine and not glutamine is important in regulating acidosis in chickens by acting as an ammonia donor. Thus measuring asparaginase activity in the kidney will indicate ammonia production levels.

The free base ammonia produced in kidney cells is readily soluble in lipids and therefore easily moves out of the cell across the cell membrane to the tubular fluid. The ammonium ions produced in the

tubules upon reaction with hydrogen ions are very poorly soluble in lipids thus cannot diffuse back out of the tubular fluid into the cells and are excreted in the urine. Hydrogen ions are produced by the action of carbonic anhydrase on carbonic acid in renal tubular cells. This process also involves the formation of one bicarbonate ion and reabsorption of one sodium ion for each ammonium ion excreted (Sykes, 1971).

Factors Affecting Ammonia Production

Protein level. According to Tasaki and Okumura (1964) urinary ammonia and uric acid excretion increases linearly with an increase in nitrogen intake. O'Dell et al. (1960) reported higher levels of ammonia excretion in birds fed purified diets compared to birds fed commercial diets and concluded that these differences were due to the altered acid-base balance produced by the experimental diet. Ward et al. (1974) reported an increase in rates of urine flow, uric acid and ammonia excretion of roosters in response to an increase in dietary protein. Urine uric acid concentrations increased while ammonia levels remained constant. They also showed that water restriction increases ammonia excretion and decreases uric acid excretion.

Tasaki and Okumara (1964) noticed that birds fed protein free diets excreted more ammonia than that estimated from the regression equation calculated with data of varying levels of protein and assumed that part of that ammonia was excreted in response to the altered body acid-base balance.

Electrolyte levels. Electrolytes play a major role in maintenance of pH in blood and cells. Hence any changes in their delicate balance in the body results in a change in pH which is then reflected in the amount of ammonia produced to get blood pH within normal limits. Tannen (1978) reported that hyperkalemia was the cause of reduced urinary ammonia excretion in humans. Sebastian et al. (1977) showed an inverse relationship between the level of serum potassium and rate of ammonia excretion in human patients suffering from hypoaldosteronism. Halperin et al. (1985) proposed that the cell (mitochondrial) hydrogen ion concentration is reciprocally related to extracellular fluid K^+ concentration presumably reflecting K^+ exit and H^+ entry during hypokalemia and the reverse during hyperkalemia. Burnell et al. (1974) found a decrease in hydrogen ion secretion and urinary ammonia in dogs fed potassium free diets.

In addition to potassium, phosphorus was also reported to affect ammonia production. Yu et al. (1976) reported stimulation of ammonia production and excretion in rabbits after infusion of sodium phosphate. Emmet et al. (1977) reported decreased ammonia production in rats fed low phosphate diets.

Renal disorders. Loss of nephron mass in the kidney due to chronic renal failure or nephrolithiasis affects ammonia production in humans. Elkington (1957) stated that as nephron population decreases, the rate of ammonia production of the remaining nephrons increase, however the capability for this adaptation is limited and total ammonia excretion diminishes. Metcalf-Gibson et al. (1965) demonstrated that

ammonia excretion was normal in patients with kidney stones exhibiting normal creatinine clearance rates, but was reduced in patients suffering from reduced clearance ability. Walser (1980) showed that ammonia production by the kidney was diminished in patients with chronic renal failure but liver metabolism was not affected. Halperin et al. (1985) suggested that lower ammonia production in patients with decreased glomerular filtration rate (GFR) is due to processes leading to preservation of ATP balance within the proximal tubule cells. Since ammonia production produces ATP which is then utilized by cells to reabsorb sodium by the sodium pump from glomerular filtrates, reduced GFR decreases the amounts of Na^+ ions being transported across cell membranes and ATP needed for this process. Vinay et al. (1976) showed that free fatty acids inhibit ammoniogenesis in acidotic dogs probably through substrate availability and competition to produce ATP within the cell and not through modification of glutamine transport across the mitochondrial membrane.

According to Hills (1973), urinary ammonia concentration increased nearly exponentially as urine pH was progressively reduced and was always accelerated when urinary flow rate was increased due to altered NH_3 pressure gradient between renal tissue and urine.

Acid-base balance. According to Tasaki and Okumara (1964) and O'Dell et al. (1960), ammonia production in the kidney is closely correlated to the state of acid-base balance of the body. Any condition that might affect this balance will be reflected in the amount of ammonia produced, given that the production mechanism in the kidney is not

impaired due to other reasons. Included among these conditions expected to alter ammonia production would be respiratory acidosis and alkalosis. Respiratory alkalosis may result from hyperventilation in response to high environmental temperature. Dubrovsky et al. (1981) stated that humans suffering from Addison's disease which is characterized by hypoglycocorticoidism have metabolic acidosis while patients with hyperglucocorticoidism have metabolic alkalosis. Wolbach (1955) noticed a sharp increase in ammonia production after HCL infusion in birds. Okumara and Tasaki (1968) observed the same effect on birds fed 10-15 mmoles of HCl. They also added 9 mmoles of sodium bicarbonate daily to chickens on protein free diet, and noticed a decrease in urinary ammonia production.

Corticoid levels. It is through the work of Sartorius et al. (1952, 1953), that research on the effect of adrenal hormones on ammonia production and titratable acid (TA) excretion was initiated. Phillips and Jones (1957) identified corticosterone as the major glucocorticoid secreted from the adrenal gland of capons. They found that cortisol is found in small amounts during incubation and hatching but could not be detected after 17 days of age. Welbourne and Francoeur (1977) reported an increase in ammonia production in rats after administering D-aldosterone when coupled with metabolic alkalosis. Hultner et al. (1977) working with adrenalectomized dogs reported a sharp decrease in acid excretion, largely due to reduction in urinary ammonia, when they discontinued administering exogenous mineralocorticoid but maintained the animals on glucocorticoid.

Hughey et al. (1980) found that adrenalectomized rats have decreased circulating levels of the ammoniagenic precursor glutamine and reduced renal glutamine extraction. Glucocorticoid replacement increased plasma glutamine concentrations and restored excretion to normal levels (Sapir et al., 1977). Dubrovsky et al. (1981) reported diminishing acidification and excretion of TA and ammonia in rats suffering from adrenal insufficiency. According to Wilcox et al. (1982) the defect in TA excretion in adrenalectomized rats appears to be related to glucocorticoids rather than mineralocorticoids. Titratable acid, which is made of phosphate and organic anions, and ammonia excretion increase with physiologic replacement doses of glucocorticoids but not of aldosterone. Aldosterone was observed to increase the secretion of H^+ ions thus promoting urinary acidification.

Photoperiod Effects

No report could be found in the literature that describes photoperiod effects on acid-base balance and ammonia production in birds. However other disorders have been observed to occur as a result of certain light treatments. Savory (1976) reported lowered weight gains in chickens reared under continuous light when compared to birds given 12 hrs of light daily. Wilson et al. (1984) and Buckland et al. (1976) reported higher incidence of leg abnormalities with broilers reared under continuous light. Dejoie (1986) studied the effect of light on rigor mortis and meat toughness and concluded that continuous light resulted in a very rapid onset of rigor mortis but had no effect on meat toughness. Polonis (1982) showed that serum cholesterol levels were

significantly higher at 5 weeks of age and significantly lower at 8 weeks in birds reared under 16 hrs of artificial intermittent light when compared to birds receiving 14 hrs of natural day light.

Avian glaucoma results from exposing birds to continuous light or continuous darkness and is thought to be due to the absence of diurnal light rhythms (Voitle, 1969; Osei, 1981). Eye enlargement and elevated intraocular pressure develop in birds subjected to 24 hr light but not in birds under 16L:8D light treatment.

Of the well known effect of photoperiod on birds is its relationship to thyroid activity. It has been shown that the thyroid gland is important in such things as growth, migration and reproduction. Necomer (1974) and Krebietke (1975) showed the occurrence of a diurnal thyroid activity rhythm in birds exposed to natural day length. May (1978) showed that exposing birds to continuous light abolishes the diurnal cycle of T_3 and T_4 .

Light treatments have also been reported to affect corticosterone levels in birds. Buckland et al. (1976) reported up to four fold increase in plasma corticoid levels in birds subjected to continuous light compared to birds under different intermittent light regimes. Osei (1981) showed higher corticosterone levels in birds subjected to continuous light as compared with 16L:8D reared birds. Freeman and Manning (1978) reported that birds subjected to constant illumination did not have the circadian rhythm in the glucagon mediated lipidaemic response. Freeman et al. (1981) also reported an increased sensitivity to corticotrophin in birds subjected to continuous light which

they attributed to adrenal hypertrophy which could be a result of a higher concentration of glucagon. These results indicate a stress condition being imposed on birds subjected to continuous light interfering with their normal physiological rhythms.

Temperature Effects

The area of temperature effect on physiology of birds has been studied by many researchers. It is well known that high temperature induces panting in birds in order to lower their body temperature (Elhadi and Sykes, 1972). The result of this process is the loss of CO_2 and consequently the development of respiratory alkalosis (Koelkebeck and Odom, 1985).

High temperature also affects blood electrolyte levels in birds. Huston (1978) reported lower mean blood potassium concentration of birds acclimated to 30°C compared to birds kept under 8°C . Kohne and Jones (1975) reported that a temperature increase caused a significant decrease in blood sodium, calcium, and magnesium and a significant increase in potassium level. Teeter et al. (1985) fed birds subjected to 32°C , 0.5% sodium bicarbonate (NaHCO_3) or 1% ammonium chloride and noticed that blood pH returned to normal levels of nonpanting non-stressed birds.

Temperature has an effect on thyroid activity. Rudas and Pethes (1984) reported reduced thyroxine (T_4) levels in birds after exposure to temperature of 35°C for 1 hr but noticed no effects on triiodothyronine (T_3) levels. They concluded that the conversion of T_4 to T_3

played a major role in the early phase of temperature acclimation. May et al. (1986) studied the effects of moderate constant or high cyclic temperatures on thyroid hormone concentration in broilers and concluded that the mechanism of short term acclimation involved endocrine or physiological responses other than changes in circulating thyroid hormone levels. Cogburn and Harrison (1980) have also observed suppression of thyroid activity at elevated temperatures. Bobek et al. (1980) reported a 3-fold increase in T_3 levels for the first 2 hrs of cold exposure (8°C) decreasing to a steady concentration about twice the initial value before exposure. Thyroxine levels decreased initially and increased after 12 hrs, which is due to the initial rapid increase in T_4 to T_3 conversion.

High ambient temperatures also affect adrenal function in birds. Edens (1978) reported development of acute adrenal cortical insufficiency (AACI) in birds subjected to a temperature of 43°C for 2 hrs. Acute adrenal cortical insufficiency is characterized by a sharp increase in plasma corticoid levels followed by a rapid decline, and it was not related to loss of their precursor cholesterol. The author concluded that the development of this condition in heat stressed birds may predispose them to an irreversible heat prostration and death due to cardiovascular failure.

CHAPTER III

MATERIALS AND METHODS

Two experiments were conducted to evaluate the effect of light and temperature on ammonia production and excretion, blood electrolytes, and plasma corticosterone levels in male broilers. The two experiments were identical and were run at the same time with 2 weeks difference between the ages of the two groups of birds. Treatments in experiment 1 were assigned to 4 pens on one side of the house while treatments in experiment 2 occupied 4 pens on the other side of the house. Samples were collected at the end of 2, 4, and 6 weeks of age.

Rearing Phase

One hundred and twenty male chicks of a commercial broiler (Peterson) strain in each experiment were brooded in 4 light proof rooms measuring approximately 4 ft x 5 ft. Two rooms were subjected to continuous light (24L:0D) and the other two to alternating light and dark (16L:8D) using one 25 watt bulb per room. Light treatments were imposed at 1 day of age. One room in each light treatment was equipped with an air conditioner to keep temperature at 21-23°C. The other two rooms were maintained at 32-35°C using resistor coil heaters. Feed was offered ad libitum. A ration containing 23% protein and 2835 kcal/kg was fed to 3 weeks of age followed by a ration containing 21% protein and 2884 kcal/kg until 8 weeks of age.

Sample Collection

Blood. Blood was collected by heart puncture from 5 birds in each treatment using lithium heparinate as an anticoagulant. Samples were kept on ice until they were centrifuged at 2000 rpm for 15 min at 5°C to separate the plasma. Blood ammonia was determined immediately and the rest of the samples were stored at -20°C until corticosterone and electrolyte determinations were performed.

Excreta. A group of birds were placed in specially built wire cages measuring 33 x 28 x 35.5 cm equipped with water and feed troughs. Number of birds placed in cages at 2, 4, and 6 weeks of age were 5, 3, and 2 respectively. Birds were allowed to adjust for 24 hrs before the start of collection period. A plastic pan containing 2.0 liters of 6N HCl was placed under the cage allowing all excreta voided in 24 hrs to drop in acid solution. These solutions were sampled for ammonia determination.

Kidney. Kidney samples, weighing approximately 3 g each, were taken from 5 birds in each treatment following cervical dislocation, accurately weighed, and kept frozen at -80°C until enzyme assay was run.

Parameters Measured

Kidney asparaginase activity. Asparaginase activity was determined using the procedure of Coon and Balling (1984) with the following modifications. A 3 g sample of kidney was homogenized in 12 ml of

ice cold TRIS buffer (pH 7.6). The homogenate was centrifuged at 2200 rpm at 5°C for 10 min using a Beckman centrifuge (model TJ6). Protein was determined by a kit procedure (No. P5656 Sigma Diagnostics, St. Louis, Mo.) using 25 μ l of the above supernatant. Ammonia was determined using an ammonia color reagent (catalogue no. 14-2, Sigma Diagnostics, St. Louis, Mo.) and optical density read at 480 nm using a Bausch and Lomb (spectronic 2000) spectrophotometer. Ammonia content was calculated using a standard curve (Figure 1) and asparaginase activity expressed as μ moles NH_3 produced/mg protein/hr.

Blood ammonia. Plasma ammonia was determined by kit procedure (catalogue no. 170-B, Sigma Diagnostics, St. Louis, Mo.) not more than 3 hrs after blood was collected and centrifuged.

Blood electrolytes. Electrolytes were determined using an automatic electrolyte analyzer (Beckman system E4A). Electrolytes analyzed were Na, K, Cl, CO_2 and aniongap. Aniongap is defined as the difference between the sum of Na^+ and K^+ ion blood concentrations and the sum of Cl^- and HCO_3^- ion concentrations all expressed as meq/L.

Plasma corticosterone. Hormone levels were determined based on a technique developed by Foster and Dunn (1974) with modifications. The procedure is given in the Appendix. Rabbit antiserum (Cambridge Medical Diagnostics, Billerica, MA) was prepared against corticosterone-3-bovine serum albumin. Crossreactivity according to Cambridge Medical Diagnostics was 4% for deoxycortisol, 1% for 5 β -pregnanedione, 0.6% progesterone and 0.5% with cortisol. Intraassay coefficient of variation

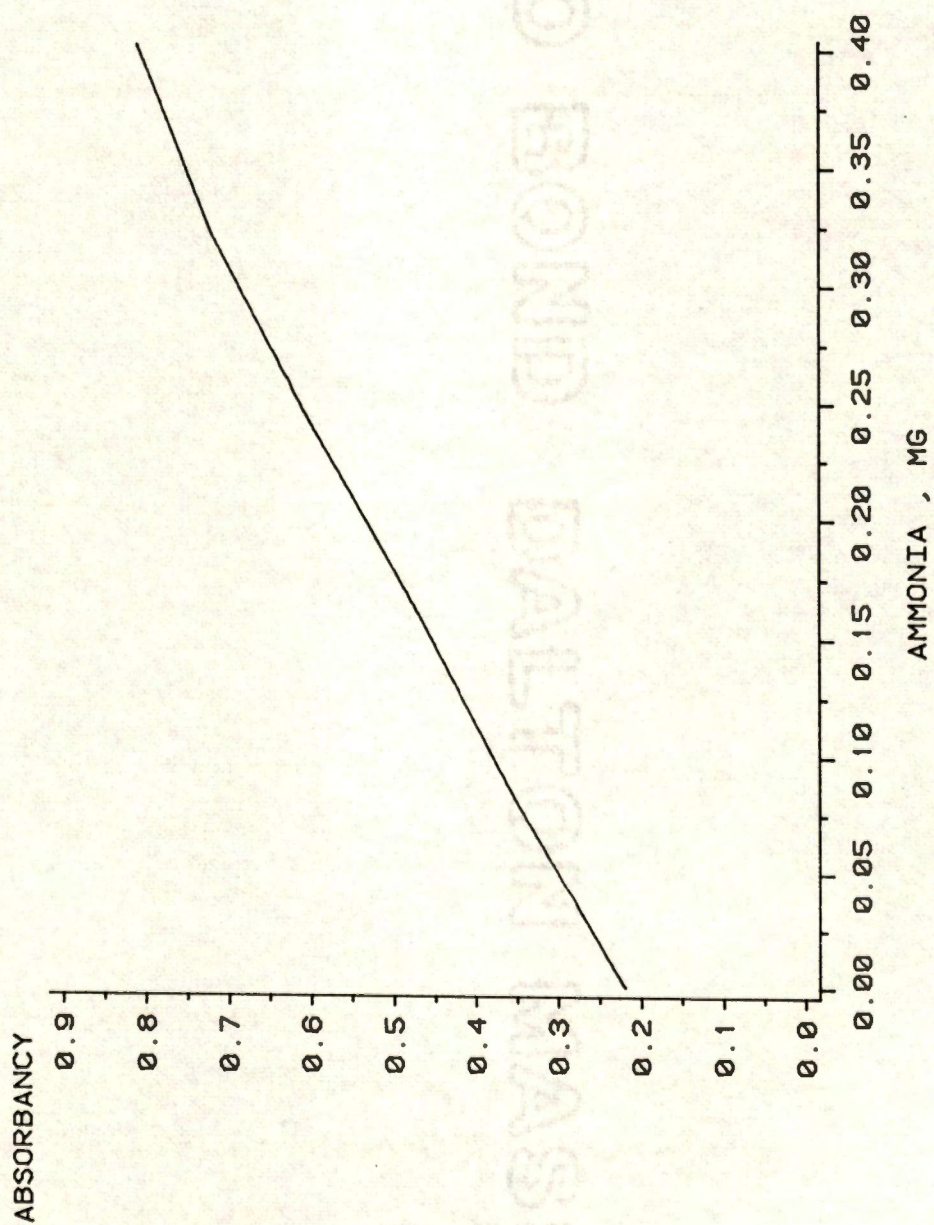


Figure 1. Absorbance of Different Levels of Ammonium Ion in Solution at 480 nm.

was 13.2% while interassay coefficient of variation was 32.5%. Validation of the assay was performed by adding different amounts of corticosterone to 0.5 ml of charcoal stripped plasma. Amounts of added corticosterone were equivalent to 0.5, 1, 2.6, 5, 10 ng/ml of charcoal stripped plasma. The amounts of corticosterone recovered were 0.53, 1.07, 1.9, 2.0, 4.0 ng/ml. Good recoveries were obtained up to 2.6 ng/ml of added corticosterone, and since most samples analyzed were between 0.2 and 3.0 ng/ml with only few values higher than 3.0 ng/ml the assay was considered appropriate for such levels of hormone.

Excreta ammonia. Excreta samples which were collected in pans containing 2 liters of 6N HCl were filtered and the clear solution was assayed for ammonia using an ammonia color reagent (catalogue no. 14-2 Sigma Diagnostic, St. Louis, Mol.) using the following procedure.

1. One milliliter of excreta solution was mixed with 5 ml of distilled water. Two milliliters of 0.3N $\text{Ba}(\text{OH})_2$ and 2 ml of 0.3N ZnSO_4 were added, mixed, and then centrifuged. Twentieth milliliter of the above supernatant was mixed with 5.3 ml of water, and 0.5 ml of ammonia color reagent was then added, mixed, and let stand for at least 10 min for the color to develop. Absorbancy was read at 400 nm using a spectrophotometer. Ammonium ion concentration* was determined using a standard curve.

* Values obtained were multiplied by 2.5 to get final concentration per ml of original sample.

2. Standard curve was prepared according to this procedure.

A solution containing 20 mg NH_4^+ /ml was prepared by dissolving 7.33 g of $(\text{NH}_4)_2\text{SO}_4$ in 100 ml of distilled water. Different volumes of this solution were prepared to contain different amounts of NH_4^+ as shown in Table I. Absorbancy of these standard solutions was read after addition of 0.5 ml ammonia color reagent at a wavelength of 400 nm. Concentration of NH_4^+ vs. absorbancy were plotted as shown in Figure 2.

Atmospheric ammonia. One hundred milliliter of air sample was drawn using special syringe and ammonia analyzer tubes (Gastec Corporation, Yokohawa, Japan). Exhaust fans and air conditioners were turned off 10 minutes before measurements were taken. The sample was drawn over a period of 1 min. Tubes gave reading between 0 and 30 ppm.

Statistical Analysis

Analysis of variance was performed to determine the significance of the main effects and their interaction according to a split plot design using the model

Effect =	Light	Temperature	Light x Temperature	Replication
Light x Temperature x Replication*	Age	Age x Light	Age x Temperature	
Age x Temperature x Light	Age x Light x Temperature	Age x Temperature x Replication**		

* Error A used to test Light Temperature and Light x Temperature.

** Error B used to test Age, Age x Light, Age x Temperature, and Age x Temperature x Light.

Table I. Preparation of Ammonia Standard Curve Tubes.

Tube	Standard Solution ml	Water Added ml	Absorbance at 400 nm	Equivalent to Solution NH_4^+ in mg/ml* ⁴
1	0.0	5.5	0.221	0
2	0.2	5.3	0.353	0.08
3	0.4	5.1	0.468	0.16
4	0.6	4.9	0.599	0.24
5	0.8	4.7	0.719	0.32
6	1.0	4.5	0.8011	0.40

* For 0.5 ml diluted excreta sample.

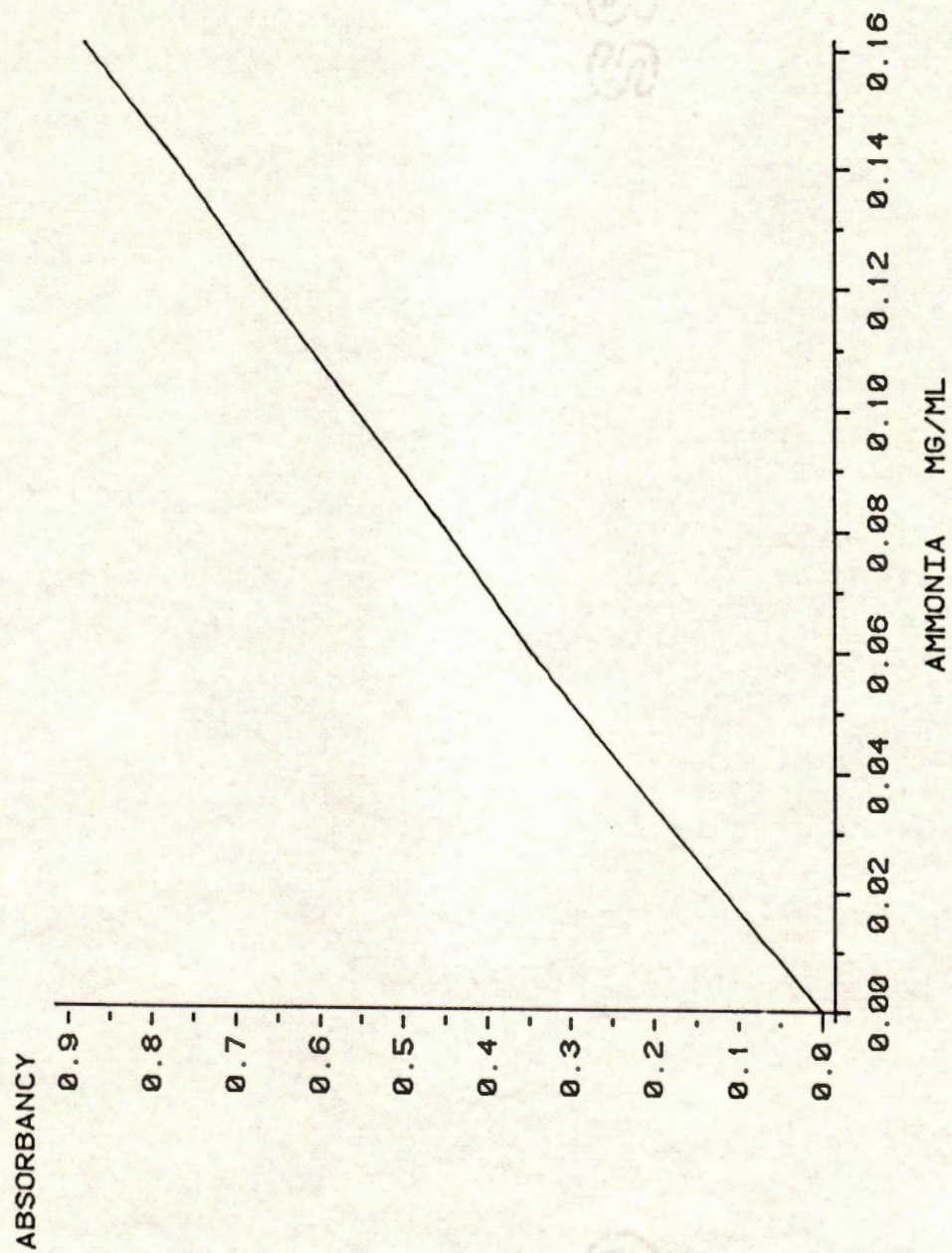


Figure 2. Absorbance of Different Amounts of Ammonia in Solution at 400 nm.

Differences between means were determined using Student-Newman-Keuls method of mean separation.

CHAPTER IV

RESULTS AND DISCUSSION

Plasma Electrolytes

Blood electrolyte levels are presented in Tables II, III, IV, V and VI. Values for experiment 1 at 6 weeks of age are not reported because of an error in analysis.

Sodium. The average levels of plasma sodium are presented in Table II. Light and temperature had no significant ($p > .05$) effect on Na levels.

Potassium. The average levels of plasma potassium are presented in Table III. Light and Temperature had no significant ($p > .05$) effect on K levels.

Chloride. Average chloride levels are presented in Table IV. Light and temperature had no significant ($p > 0.05$) effect on chloride plasma levels.

CO₂. Average values of CO₂ are shown in Table V. These values show no significance ($p > .05$) due to the effects of light and temperature.

Anion-gap. Mean anion-gap values are shown in Table VI. Light and temperature had no significant ($p > .05$) effect on these values. In experiment 1 means were higher at 4 weeks of age when compared to

Table II. Effect of Light, Temperature, Age on Plasma Na⁺ Levels* in Broiler Males.¹

Light	Room Temperature °C	Age					
		2 Weeks		4 Weeks		6 Weeks	
		Experiment 1	Experiment 2	Experiment 1	Experiment 2	Experiment 1	Experiment 2
16L:8D	35	133.2 ^a _A	140.0 ^a _A	145.6 ^a _A	142.2 ^a _A	145.0 ^a _A	145.0 ^a _A
	22	137.6 ^a _A	138.2 ^a _A	146.8 ^a _A	140.8 ^a _A	144.8 ^a _A	144.8 ^a _A
24L:0D	35	126.6 ^a _A	141.8 ^a _A	147.6 ^a _A	142.0 ^a _A	146.8 ^a _A	146.8 ^a _A
	22	133.5 ^a _A	142.3 ^a _A	147.4 ^a _A	142.4 ^a _A	146.4 ^a _A	146.4 ^a _A

*In meq/L.

¹5 observations/mean.a,b Means with same superscript in columns are not significantly different ($p > 0.05$).A Means with same subscript in rows are not significantly different ($p > 0.05$).

Table III. Effect of Light, Temperature, and Age on Plasma K⁺ Levels* in Broiler Males.¹

Light	Room Temperature °C	Age					
		2 Weeks		4 Weeks		6 Weeks	
		Experiment		Experiment		Experiment	
		1	2	1	2	1	2
16L:8D	35	6.04 ^a _B	6.78 ^a _B	7.78 ^a _A	5.72 ^a _B	5.70 ^a _B	
	22	6.58 ^b _A	6.24 ^a _A	8.26 ^a _B	6.04 ^a _A	5.64 ^a _A	
24L:OD	35	5.75 ^a _B	6.70 ^a _A	7.12 ^a _A	5.88 ^a _B	5.66 ^a _B	
	22	6.23 ^b _{BC}	6.70 ^a _B	8.18 ^a _A	5.88 ^a _{BC}	5.62 ^a _{BC}	

*In meq/L.

¹5 observations/mean.

a,b Means with same superscript in columns are not significantly different (p > 0.05).

A,B,C Means with same subscript in rows are not significantly different (p > 0.05).

Table IV. Effect of Light, Temperature, and Age on Plasma Cl^- Levels* in Broiler Males.¹

Light	Room Temperature °C	Age					
		2 Weeks		4 Weeks		6 Weeks	
		Experiment 1	Experiment 2	Experiment 1	Experiment 2	Experiment 1	Experiment 2
16L:8D	35	106.4 ^a _A	111.0 ^a _A	113.8 ^a _A	114.4 ^a _A	115.8 ^a _A	115.8 ^a _A
	22	109.2 ^a _B	108.0 ^a _B	116.2 ^a _A	111.6 ^a _{AB}	116.0 ^a _A	116.0 ^a _A
24L:OD	35	105.8 ^a _B	110.6 ^a _{AB}	114.8 ^a _{AB}	111.2 ^a _{AB}	117.6 ^a _A	117.6 ^a _A
	22	106.0 ^a _A	110.0 ^a _A	115.2 ^a _A	111.4 ^a _A	116.8 ^a _A	116.8 ^a _A

*In meq/L.

¹5 observations/mean.

a,b Means with same superscript in columns are not significantly different ($p > .05$).

A,B Means with same subscript in rows are not significantly different ($p > .05$).

Table V. Effect of Light, Temperature, and Age on Plasma CO₂ Levels* in Broiler Males.¹

Light	Room Temperature °C	Age					
		2 Weeks		4 Weeks		6 Weeks	
		Experiment		Experiment		Experiment	
		1	2	1	2	1	2
16L:8D	35	18.8 ^a _A	17.6 ^a _A	17.0 ^a _A	19.1 ^a _A	20.0 ^a _A	
	22	20.2 ^a _A	20.5 ^a _A	16.3 ^a _A	19.8 ^a _A	19.2 ^a _A	
24L:0D	35	18.4 ^a _{ABC}	21.4 ^a _{ABC}	19.0 ^a _{ABC}	23.0 ^a _{AC}	21.8 ^b _C	
	22	19.5 ^a _A	23.4 ^a _A	18.6 ^a _A	24.1 ^a _A	21.6 ^b _A	

*In meq/L.

¹5 observations/mean.a,b Means with same superscript in columns are not significantly different ($p > .05$).A,B,C Means with same subscript in rows are not significantly different ($p > .05$).

Table VI. Effect of Light, Temperature, and Age on Plasma Anion Gap Levels* in Broiler Males.¹

Light	Room Temperature °C	Age					
		2 Weeks		4 Weeks		6 Weeks	
		Experiment		Experiment		Experiment	
		1	2	1	2	1	2
16L:8D	35	6.34 ^a _B	11.60 ^a _{AB}	13.52 ^a _A	8.82 ^a _{AB}	9.22 ^a _{AB}	
	22	8.02 ^a _{AB}	9.66 ^a _{AB}	14.26 ^a _A	9.50 ^a _{AB}	9.66 ^a _{AB}	
24L:OD	35	7.36 ^a _B	10.00 ^a _B	13.82 ^a _A	8.22 ^a _B	7.52 ^a _B	
	22	7.98 ^a _B	8.90 ^a _B	14.42 ^a _A	7.08 ^a _B	8.18 ^a _B	

*In meq/L.

¹₅ observations/mean.a,b Means with same superscript in columns are not significantly different ($p > .05$).A,B Means with same subscript in rows are not significantly different ($p > .05$).

values at 2 or 6 weeks of age but not significantly so. This increase was not observed in experiment 2.

Total Ammonia in Excreta

Mean values for total ammonia voided in excreta are shown in Table VII. Light and temperature had no significant ($p > .05$) effect on these values. However age of birds had a highly significant ($p < .001$) effect on mean ammonia values. Ammonia in excreta was highest at 2 weeks of age in both experiments, decreasing by 30-40% by 4 weeks of age, and a further nonsignificant decrease at 6 weeks of age in experiment 1 and a slight increase in experiment 2 (Figure 3). Values reported are higher than what Long (1982) has cited from the literature. These differences could be due to the fact that all reported values in literature are for mature birds that had been operated on to collect urine and feces separately and no reports could be found for growing young birds.

Halperin et al. (1985) stated that ammonia excretion is necessary to neutralize the nonvolatile acids produced from the metabolism of certain amino acids. Methionine and cysteine metabolism generate H_2SO_4 while arginine and lysine generate HCl. During the first 3-4 weeks of the bird's life the protein turnover is very high and body mass is increasing rapidly which tend to generate high amounts of nonvolatile acids that must be neutralized and excreted in the urine. This would probably explain the increased levels of ammonia excreted by birds during the first 2 weeks of age and the drop in excretion after the rate of growth and protein turnover decrease sometime between 2 and

Table VII. Effect of Light, Temperature, and Age on Total Ammonia* in Excreta of Broiler Males.¹

Light	Room Temperature °C	Age					
		2 Weeks**		4 Weeks†		6 Weeks††	
		Experiment		Experiment		Experiment	
		1	2	1	2	1	2
16L:8D	35	56.45 ^a _A	46.40 ^a _A	30.75 ^a _B	23.55 ^a _B	22.10 ^a _B	24.57 ^a _B
	22	40.45 ^a _A	41.25 ^a _A	31.63 ^a _B	22.85 ^a _D	18.45 ^a _E	26.90 ^a _C
24L:0D	35	41.05 ^a _A	47.40 ^a _A	35.15 ^a _A	26.35 ^a _A	24.30 ^a _A	27.10 ^a _A
	22	39.45 ^a _A	38.55 ^a _A	38.55 ^a _A	27.10 ^a _A	21.90 ^a _A	32.55 ^a _A

*In mg/100 g body weight/24 hr.

¹2 observations/mean.

**Number of birds/collection is 5.

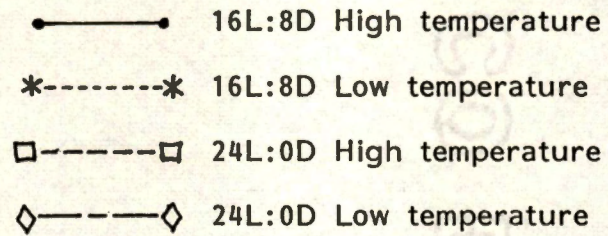
†Number of birds/collection is 3.

††Number of birds/collection is 2.

a Means with same superscript within column are not significantly different ($p > .05$).

A,B,C,D,E Means with same subscript within row are not significantly different ($p > .05$).

Figure 3. Total Ammonia in the Excreta of Broiler Males at 2, 4, and 6 Weeks of Age in Experiments 1 and 2.



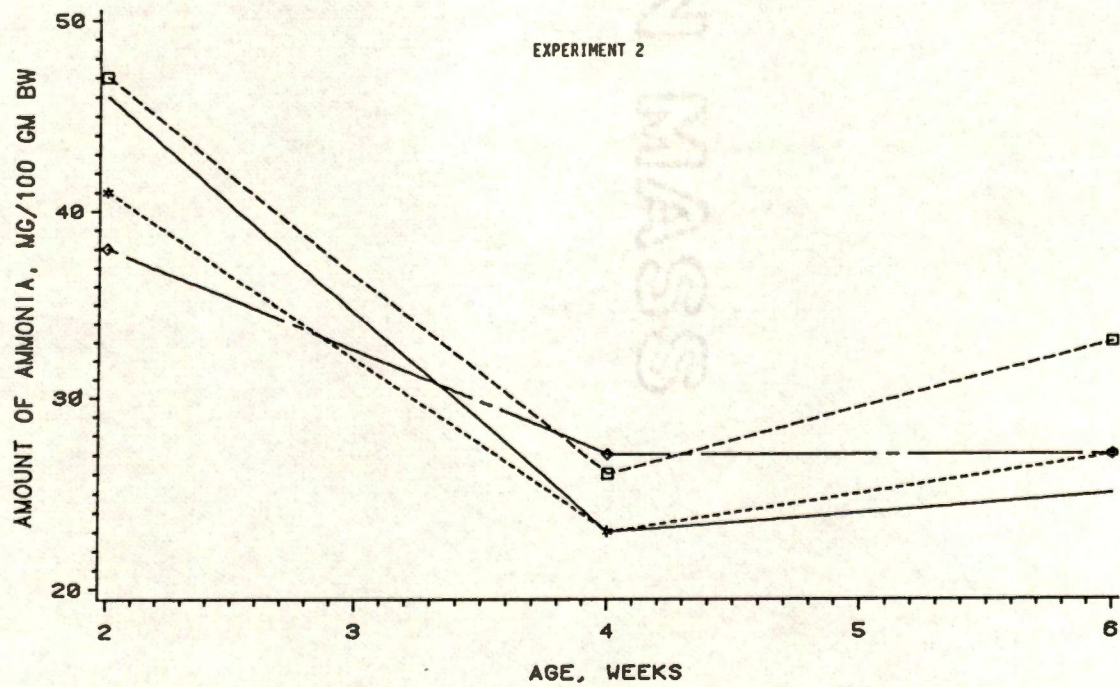
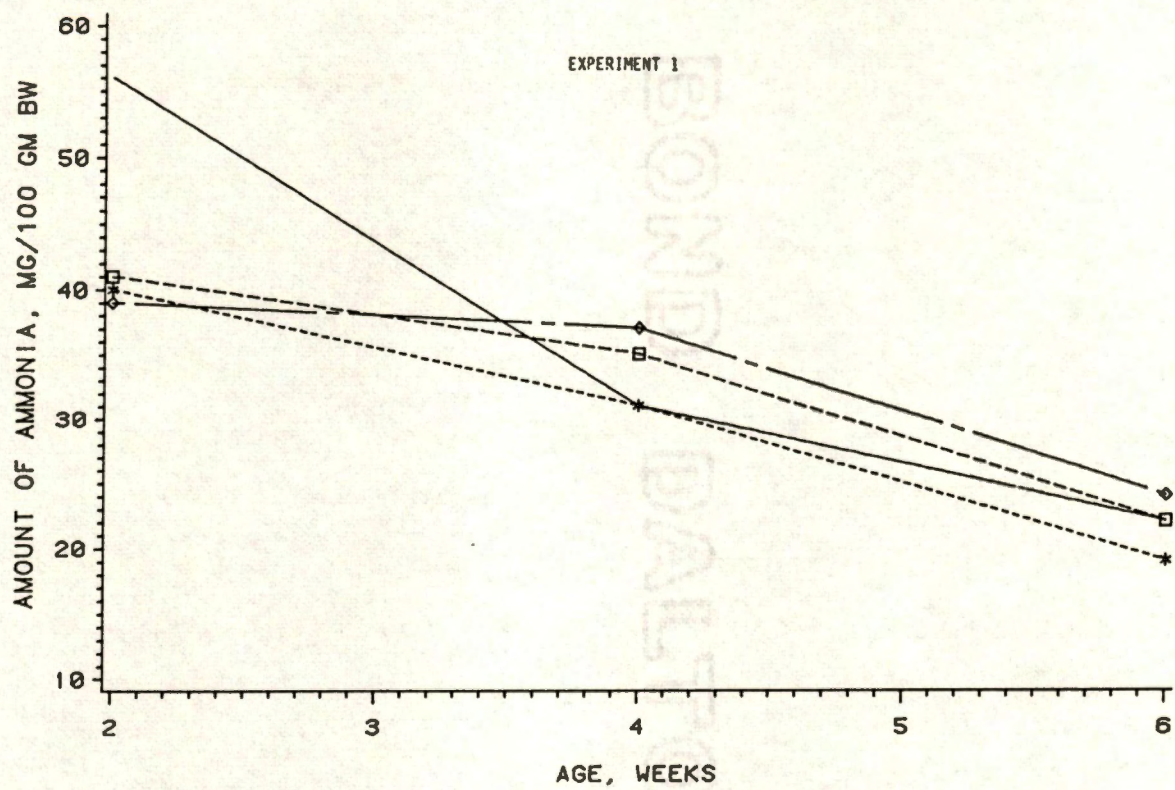


Figure 3 (continued)

4 weeks of age. Change in rations at 3 weeks of age might have also contributed to this drop in excretion levels.

Plasma Ammonia

The average values of plasma ammonia are presented in Table VIII. These values show that light and temperature had no significant ($p > .05$) effects on plasma ammonia. Results obtained are different from what Adekunmisi and Robbins (1987a) have reported in which they found higher levels of blood ammonia in the 16L:8D treatment birds. However values at 2 weeks of age were significantly ($p > .05$) different from values obtained at 4 or 6 weeks of age. This could be attributed to higher levels of protein in the diet of starting chicks and higher rate of body protein turnover.

Kidney Asparaginase Activity

Mean kidney asparaginase activity values are presented in Table IX. Results show no significant ($p > .05$) effect of light or temperature on these values. However asparaginase activity which is a measure of NH_3 produced, tended to be significantly ($p < .05$) lower at 4 weeks of age in experiment 1 which might be due to change of feed. Results obtained in both experiments are lower than values reported by Adekunmisi and Robbins (1987b).

Plasma Corticosterone

Plasma corticosterone levels are presented in Table X. Values obtained show no significant ($p > .05$) effect of light or temperature.

Table VIII. Effect of Light, Temperature, and Age on Plasma Ammonia Levels* in Broiler Males.¹

Light	Room Temperature °C	Age					
		2 Weeks		4 Weeks		6 Weeks	
		Experiment 1	Experiment 2	Experiment 1	Experiment 2	Experiment 1	Experiment 2
16L:8D	35	11.00 ^a _A	9.72 ^a _{AB}	6.36 ^a _{DC}	4.38 ^a _D	7.96 ^a _{BC}	3.87 ^a _D
	22	11.97 ^a _A	9.12 ^a _B	7.48 ^a _{CB}	4.77 ^a _C	7.80 ^a _{CB}	8.18 ^b _{CB}
24L:OD	35	11.35 ^a _B	14.16 ^b _A	7.08 ^a _C	3.54 ^a _D	7.34 ^a _C	7.25 ^b _C
	22	11.97 ^a _A	13.62 ^b _A	6.84 ^a _B	4.14 ^a _C	7.76 ^a _B	7.78 ^b _B

*In µg/ml.

¹ 5 observations/mean.

a,b Means within a column with different superscripts are significantly different ($p > .05$).

A,B,C,D Means within a row with different subscripts are significantly different ($p > .05$).

Table IX. Effect₁ of Light, Temperature, and Age on Kidney Asparaginase Activity* in Broiler Males.

Light	Room Temperature °C	Age					
		2 Weeks		4 Weeks		6 Weeks	
		Experiment		Experiment		Experiment	
		1	2	1	2	1	2
16L:8D	35	0.556 ^a _{AB}	0.624 ^a _{AB}	0.431 ^a _B	0.674 ^a _{AB}	0.856 ^a _A	0.801 ^a _{AB}
	22	0.626 ^a _A	0.650 ^a _A	0.348 ^b _B	0.670 ^a _A	0.831 ^a _A	0.629 ^a _A
24L:OD	35	0.641 ^a _A	0.773 ^a _A	0.308 ^b _B	0.652 ^a _A	0.758 ^a _A	0.609 ^a _A
	22	0.708 ^a _A	0.607 ^a _A	0.285 ^b _B	0.542 ^a _A	0.855 ^a _A	0.677 ^a _A

*In mg NH₄ produced/mg protein/hr.¹5 observations/mean.a,b Means with same superscript within column are not significantly different ($p > .05$).A,B Means with same subscript within row are not significantly different ($p > .05$).

Table X. Effect₁ of Light, Temperature, and Age on Plasma Corticosterone Levels* in Broiler Males.

Light	Room Temperature °C	Age					
		2 Weeks		4 Weeks		6 Weeks	
		Experiment		Experiment		Experiment	
		1	2	1	2	1	2
16L:8D	35	1.78 ^a _A	2.38 ^a _A	1.58 ^a _A	0.90 ^a _A	1.18 ^a _A	0.92 ^a _A
	22	1.58 ^a _A	1.78 ^a _A	1.46 ^a _A	0.79 ^a _A	0.80 ^a _A	0.49 ^a _A
24L:OD	35	3.78 ^b _A	1.90 ^a _B	1.84 ^a _B	0.88 ^a _B	1.10 ^a _B	0.63 ^a _B
	22	2.61 ^b _A	2.73 ^a _A	0.94 ^a _B	1.44 ^a _{AB}	0.96 ^a _B	0.35 ^a _B

*In ng/ml.

¹₅ observations/mean.

a,b Means with the same superscript within column are not significantly different ($p > .05$).

A,B Means with the same subscript within row are not significantly different ($p > .05$).

Birds at 2 weeks of age had significantly ($p < .05$) higher corticosterone levels compared to birds at 4 or 6 weeks of age. Birds on 24L:OD light treatment at 2 weeks of age had higher levels of corticosterone compared to birds on 16L:8D light treatment but not significantly so. This might indicate stimulation of the adrenal gland and oversecretion of corticosterone which leads to an increase in blood pH. Higher blood pH reduces the need for ammonia production since hydrogen ion concentrations are lower. Higher corticosterone levels act also on proximal kidney tubules stimulating absorption of water and sodium ions from kidney filtrate leading to an increase in blood volume and development of hypertension. After the initial period of oversecretion the body tends to regulate adrenal secretion to reduce plasma corticosterone levels. This might explain the lower levels obtained at 4 and 6 weeks of age.

Atmospheric Ammonia

Measurements of ammonia concentration in the atmosphere of the pens are presented in Table XI. These values show that pens with birds on 24L:OD light treatment have lower atmospheric ammonia compared to pens with 16L:8D treated birds. According to Elliot and Collins (1982) ammonia in the atmosphere is the result of aerobic bacterial breakdown of uric acid in the litter. Factors affecting this breakdown include litter temperature, moisture and pH. It has been observed that the condition of litter in the 24L:OD treatment pens is different from 16L:8D treatment in that it contains more moisture. This

Table XI. Effect of Light, Temperature, and Age on Atmospheric Ammonia Levels* in Broiler Pens.**

Light	Room Temperature °C	Age					
		2 Weeks		4 Weeks		6 Weeks	
		Experiment		Experiment		Experiment	
		1	2	1	2	1	2
16L:8D	35	20.0	6.0	35.0	8.0	14.0	20.0
	22	12.0	1.0	45.0	12.0	29.0	24.0
24L:0D	35	12.0	11.0	7.5	6.0	6.0	6.0
	22	2.0	0.0	16.0	7.0	22.0	18.0

*In ppm.

**Number of birds and total body mass is different between sampling times.

might suppress bacterial breakdown since anaerobic conditions prevail in the litter.

Amount of ammonia increased over time in all treatments although number of birds subsequently decreased. This could be due to the accumulation of droppings in the litter as birds get older.

CHAPTER V

SUMMARY AND CONCLUSION

Two similar experiments were conducted to determine the effect of light and temperature on blood electrolytes, plasma corticosterone, plasma ammonia, ammonia produced in kidney, and total ammonia in excreta. Results show no significant effect of light or temperature on any of these parameters.

Mean values for blood ammonia, asparaginase activity, plasma corticosterone and total excreted ammonia were significantly ($p < .05$) affected by age. Levels of blood ammonia, kidney asparaginase activity and total excreted ammonia were higher at 2 weeks of age and dropped at 4 and 6 weeks of age. This is thought to be due to the high protein diet being fed to the chickens to 3 weeks of age and to rapid growth rate. The high turnover of protein in the body produces non-volatile acids that are neutralized in the kidney and excreted as ammonium compounds.

Plasma corticosterone was also significantly ($p < .05$) higher at 2 weeks of age when compared to values at 4 and 6 weeks of age. Values at 2 weeks of age tended to be higher for birds on 24L:OD light treatment even though it was not significantly so. This might indicate a trend in which continuous light might overstimulate the adrenal gland directly or indirectly to produce higher plasma hormone levels. Higher corticosterone levels increase blood pH, and kidney tubules tend to absorb more Na^+ and water leading to blood volume expansion. The

possible increase in blood volume might affect the levels of blood electrolytes and hormones.

Atmospheric ammonia was lower in pens with birds on the 24L:0D light treatment. This is thought to be due to poor litter conditions prevailing in the pens which suppress bacterial breakdown of nitrogenous compounds to ammonia.

Further studies on blood volume expansion and its effect on plasma levels of electrolytes and levels of corticosterone and aldosterone might help explain more clearly the etiology of this syndrome.

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APPENDIX

PROCEDURE FOR DETERMINING CORTICOSTERONE LEVELS
IN CHICKEN PLASMA

1. Preparation of Phosphate Buffer Saline Buffer (PBS) (0.1M, 7.3 pH).

- A. Dissolve 27.8 g of monobasic sodium phosphate in 1000 ml of water (0.2 M).
- B. Dissolve 28.4 g of anhydrous dibasic sodium phosphate in 1000 ml of water (0.2 m).
- C. Working solution. Add 70 ml of solution A to 430 ml of solution B. Then dissolve 9 g of sodium chloride and 1 g sodium azide. Adjust pH to 7.3-7.4 with 6N HCl. Add water to give a final volume of 1000 ml.
- D. Working solution with gel (PBS-Gel). Add 500 mg of Knox Gelatin to 500 ml of PBS-no gel. Heat to solubilize.

2. Preparation of Dextran Coated Charcoal (DCC).

Dissolve 0.0625 g of dextran in 100 ml of PBS-without gel, then add 0.625 g of activated charcoal powder (MCB Manufacturing Chemist, Cincinnati, OH).

3. Preparation of Standard Curve.

- A. Solution A: Dissolve 5 mg of corticosterone (Sigma Chemical Co., St. Louis, MO) in 5 ml of distilled ethanol (1 mg/ml).
- B. Solution B: Dilute 100 μ l of solution A in 9.9 ml of distilled ethanol (10 μ g/ml).

- C. Solution C: Add 100 μ l of solution B in 9.9 ml of distilled ethanol (100 ng/ml).
- D. Solution D (working solution): Dilute 1 ml of solution C with 9 ml of distilled ethanol (10 ng/ml).
- E. Standard curve tubes.

1. Prepare the following tubes for each 50 tube assay.

<u>Tube</u>	<u>Amount added</u>
TCT	Total Count Tube
0	Zero tube
NSB	Nonspecific binding tube
0.01 ng/ml	1.0 μ l of solution D
0.025 ng/ml	2.5 μ l of solution D
0.05 ng/ml	5.0 μ l of solution D
0.10 ng/ml	10.0 μ l of solution D
0.25 ng/ml	25.0 μ l of solution D
0.50 ng/ml	50.0 μ l of solution D
1.00 ng/ml	100 μ l of solution D

2. Dry down all tubes containing solution D in a vacuum oven at 50°C.
3. Add 500 μ l of PBS-gel buffer to all tubes including TCT, 0, and NSB.
4. Vortex all tubes.

4. Plasma preparation.

- A. Add 500 μ l of plasma to 2 ml of PBS with gel (1:10 dilution) and vortex.
- B. Take 500 μ l of the above solution to assay.

5. Preparation of antibody.

- A. Use rabbit anticorticoosterone (Cambridge Medical Diagnostics Inc., Billerica, MA) kept under -80°C until the day the assay is run.
- B. Add 500 μl of 1:16 dilution antibody to 8.8 ml of PBS-with gel to give a final volume of 9.3 ml (1:297.6 dilution).
This amount is enough for 90-92 tubes.

6. Preparation of ^3H -labeled corticosterone.

- A. ^3H -corticosterone (New England Nuclear, Boston, MA) stock solution is dried down under nitrogen.
- B. Add 300 μl of 95:5 Chloroform:Ethanol solution and vortex.
- C. Presoak 5 g of sephadex LH-20 (Pharmacia Fine Chemical, Piscataway, NJ) in chloroform:ethanol solution (95:5) for 24 hr.
- D. Pack a column (0.4 mm in diameter) to a height of 6 cm with the presoaked sephadex and allow excess (chloroform, ethanol) solution to drain.
- E. Add the 300 μl of corticosterone to the column and allow to enter column.
- F. Add 1 ml of chloroform and ethanol solution and collect effluent.
- G. Repeat step F to collect ten 1 ml tubes.
- H. Take three 10 μl aliquot samples from each tube and count it on a beta counter after addition of 4 ml of Scintiverse II scintillation fluid (Fisher Scientific Co., Fairlawn, NJ).

- I. Combine tubes with highest counts.
- J. Vortex and take 3 10 μ l samples and add 4 ml Scintiverse II. Count on a beta counter. Average the three values to get the average count of the solution.
- K. Dry down under nitrogen and add an amount of 95% ethanol to get a average count 35,000-45,000 cpm.
- L. Prepare the working solution by drying an amount of the stock solution in (K) determined using this formula:
Number of tubes $\times$ 5000/average cpm in (K).
- M. After drying add an amount of PBS-gel which would be sufficient for the number of tubes in the assay.

7. Assay Procedure.

- A. Heat all standard curve and sample tubes in a water bath at 70°C for 1 hr to denature proteins.
- B. Cool tubes to room temperature and add 100 μ l of corticosterone antibody except to NSB tube to which 100 μ l of PBS-gel is added.
- C. Add 100 μ l of ^3H labeled corticosterone to all tubes.
- D. Vortex, and incubate at 4°C for 15-16 hr.
- E. Keeping all tubes in an ice bath, add 200 μ l of DCC solution to all tubes except to TCT tube to which 200 μ l PBS-no gel is added.
- F. Vortex, and let stand for 20 min from the start to DCC addition.
- G. Centrifuge all tubes at 2500 rpm for 15 min at 4°C.

- H. Pour supernatant into scintillation vials and add 4 ml of Scintiverse II.
- I. Count vials on a beta counter.
- J. Plot standard curve concentration and counts.
- H. Calculate sample concentration from the standard curve.

VITA

Waleed Odeh Twal was born in Amman, Jordan, on May 27, 1956. He attended Terra Sancta secondary school where he received his high school diploma in 1973. He joined the College of Agriculture at the University of Jordan and graduated with a degree in animal science in 1977. In the same year he started working as a farm manager responsible for one of the biggest poultry establishments in Jordan. In September 1986, he was accepted to continue his higher studies at the University of Tennessee in Knoxville at the Department of Animal Science. He graduated with a Master of Science degree from there in March 1988.