EFFECT OF NEONATAL HEAT STRESS ON GROWTH, MORTALITY AND BLOOD CHARACTERISTICS OF JUVENILE BROILERS EXPOSED TO HIGH AMBIENT TEMPERATURE

by

ANIBAL AUGUSTO ARJONA

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APPROVED:

D. M. Denbow, Chairman

W. D. Weaver, Jr.

H. P. Van Krey

J. H. Wolford

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(ABSTRACT)

Previous studies indicated that exposure of broiler cockerels to acute heat for 24 hr at five days of age increased their survivability when exposed to high ambient temperature prior to market. Since they were conducted at low relative humidity, the aims of the present study were to determine if higher relative humidity affected the response and to investigate the physiological basis of the response.

The early, neonatal, heat stress consisted of increasing ambient temperature to between 35.0 to 37.8 C for 24 hr at five days of age in half of the pens (early heat stress) while the remaining pens were kept at 29.4 C (early control). At 6 wk of age a second, late, juvenile, heat challenge was administered. Ambient temperature in half of the early heat stressed pens and early control pens was gradually increased to between 35.0 to 37.8 C for 8 hr on two consecutive days. Significantly lower mortality during the second heat challenge was observed in the early heat stressed birds. This reduction in mortality ranged from 75 to 90% of that seen in the early control birds. Additionally, there were no deleterious effects on body weight, body weight gain or feed efficiency caused by exposure of birds to early heat stress.

Feed restriction or administration of a commercially available electrolyte package to the water had no effect on the ability of the birds to withstand high ambient temperature. Water consumption, core and surface body temperature were increased upon exposure to late heat; however, there were no significant differences between the early heat stressed and early control groups.

There was a significant reduction in plasma T_3 concentration in the late heat stressed birds. No significant differences in plasma glucose were observed among the heat treatment groups. A significant increase in total plasma protein occurred during the first sampling period during late heat stress, with values returning to control levels during the second sampling period. No significant differences between the early heat stressed and early control groups were observed in plasma T_4 and total plasma protein during late heat. Heterophil to lymphocyte ratio was lower in the early heat stressed group than in the early control group during the second day of late heat exposure

These results indicate that thermotolerance can be induced by exposing broiler chicks to 35.0 to 37.8 C for 24 hr at 5 days of age with no adverse effects on performance. Although the mechanism by which Early, neonatal, heat exposure induces thermotolerance is unknown, it is clear that it does not resemble acclimation.

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INTRODUCTION

The development of homeothermy has provided mammalian and avian species the advantage of determining their own activities, up to a point, rather than have them determined by the environment. The control of body temperature within a narrow range assures that biochemical reactions take place in an efficient manner.

Normal body temperature represents the point at which rate of heat dissipation and production are balanced. Numerous theories have been proposed to explain why body temperature of homeotherms range between 36 and 43 C. Calder (1986) proposed that a higher body temperature would facilitate the transfer of body heat to the environment by creating a temperature gradient. Dunnitz and Benner (1986), on the other hand, suggested that the temperature found in homeotherms represents the point at which viscosity and changes in the hydrophobic properties of water are at a maximum and minimum level, respectively. McArthur and Clark (1987) proposed that body temperature in homeotherms assures the maintenance of heat balance in the animal, as proposed by Calder (1986), and also water conservation.

It is well known that birds have a higher body temperature than mammals of a similar body weight. This is related to their higher metabolic rate and usually lower rate of heat loss (McNab, 1966). While Moreng and Shaffner (1951) showed that an increase in the tolerance of the embryo to temperature fluctuations takes place at about the seventh day of incubation, at the time of hatching, the degree of heat production or conservation is not sufficient to maintain body temperature at a constant level in response to changes in ambient temperature. A gradual increase in body temperature occurs within the first week of life which has been correlated to an increase in metabolic rate (Card, 1921; Lamoreaux and Hutt, 1939). Freeman (1965) proposed that replacement of the yolk sac with active metabolizing tissue was partially responsible for the increase in body temperature.

Thermal stress occurs whenever heat dissipation mechanisms are unable to balance heat production, thus leading to an increase in body temperature. High temperature is known to affect cellular membranes, enzyme activity and rate of biochemical reactions (Brandts,

1967). Hence, thermoregulation is of the utmost importance for the efficient performance and survival of an animal.

Acclimatization, or the physiological change occurring within the lifetime of an organism which reduces the strain caused by stressful changes in the natural environment, has been intensively studied (Calder and King, 1974). Hutchinson and Sykes (1953) reported that tolerance to high environmental temperature was induced by exposing birds to 24 daily 4 hr exposures at a temperature of 37.2 C. The increased resistance in the acclimated birds was reflected as lower body temperatures, higher panting rates, decreased evaporative water loss, and depressed O_2 consumption. May *et al.* (1987) exposed broilers to either a constant (21 C) or an acclimating cycle of 24-35-24 C for four days prior to exposure to acute heat. Although body temperature increased in both groups upon exposure to high environmental temperature, body temperature of acclimated birds plateaud, whereas it continued to increase in unacclimated birds. A similar response has been observed in Leghorns (Sykes and Fataftah, 1986a). Bohren *et al.* (1982) reported that tolerance to acute heat stress at 56 days of age in lines selected for fast and slow growth at 32.2 and 21.1 C was improved if they were previously exposed to mild heat stress.

Heat tolerance is known to be affected by food intake. Sykes and Fataftah (1986b) observed a failure of laying hens to acclimate to high ambient temperatures if the hens were previously transferred from 30 C to 5 C but not from 5 C to 30 C. This failure to acclimate was due to the increased food consumption of the birds transferred to the lower temperature. If the later birds were feed restricted, acclimation to high ambient temperature was not hindered. Increased energy intake has also been shown to reduce tolerance to high environmental temperature (Sykes and Salih, 1986).

Exposure to a mild stressor can increase tolerance to an acute stressor (Satterlee *et al.*, 1983). Bowen and Washburn (1984a) reported that handling broilers for 4 days from 21 to 24 days of age, immediately prior to exposure to high temperature, increased heat stress survival time. This effect was transient since by three days after the cessation of handling, survival time between handled and nonhandled groups was the same. Furthermore,

increased heat tolerance was not observed with White Leghorns, a response probably due to the excitability of these birds.

The etiology of heat prostration has been studied in detail. Cardiovascular failure is the primary cause of death due to high ambient temperature (Whittow *et al.*, 1964; Smith and Oliver, 1971). This is supported by the fact that reserpine, a compound known for its sparing effects on the cardiovascular system increased resistance to heat stress (Edens and Siegel, 1974). Edens and Siegel (1975) proposed that a factor which predisposes chickens to cardiovascular failure during periods of high temperature was acute adrenocortical insufficiency. It was found that the increase in corticosterone concentration, which occurred upon exposure to heat, was followed by a sharp decline just prior to death due to a lower rate of corticosterone synthesis by the adrenal gland. The involvement of corticosterone in heat prostration was further strengthened when it was reported that corticosteroid levels did not decrease when agents known to improve heat tolerance were administered (Edens and Siegel, 1976). Moreover, Edens and Siegel (1975) reported that strains selected for low ACTH responsiveness had greater survival time than those selected for high ACTH responsiveness when exposed to high ambient temperature.

A group of compounds that may also have contributed to the cardiovascular failure observed during heat prostration are the prostaglandins. Prostaglandins, in particular $PGF_{2\alpha}$, have pressor activities under both hot and neutral environments. A reduction in the concentration of these compounds can cause a decrease in the venous return resulting in lowered blood pressure. Oliver and Birrenkott (1982) reported that the PGF plasma concentration was markedly reduced at the time of death due to heat stress. The administration of Banamine®, a prostaglandin synthesis inhibitor, in the drinking water of broilers subjected to acute heat provided some protection as evidenced by lower mortality and longer survival times. Adding 0.15 and 0.3% aspirin to the diet has also been shown to increase survival time of heat stress broilers (Glick, 1963). Broilers fed diets containing 0.6% aspirin had increased mortality and decreased body weights, an effect probably due to aspirin toxicity.

Cardiovascular adjustments in response to high environmental temperature take place before thermal panting begins (Darre and Harrison, 1987). Such adjustments included a decrease in blood pressure (Whittow *et al.*, 1964; Harrison and Biellier, 1969) and peripheral resistance (Whittow *et al.*, 1964; Darre and Harrison, 1987) and an increased in heart rate (Linsley and Burger, 1964; Whittow *et al.*, 1964). and cardiac output (Darre and Harrison, 1987). These adjustments decreased the rate of heat production and increase heat loss. Darre and Harrison (1979) reported that an atropine-induced increase in heart rate lead to a 13% increase in energy expenditure, as evidenced by increased oxygen consumption. Hence, a reduction in cardiac activity helped reduce the heat load in the bird during periods of high ambient temperature.

Kohne and Jones (1975) reported that turkeys exposed to high environmental temperature had a decrease in the plasma levels of sodium and total calcium and an increase in potasium. Similar responses were observed in heat stressed chickens (Edens, 1978). In addition, there was an increase in the plasma level of catecholamines (El-Halawani *et al.*, 1973; Edens and Siegel, 1976), which through their glycogenolytic effects, account for the initial increase in plasma glucose concentration (Edens, 1978). Hemoglobin and hematocrit values were also lowered by high ambient temperature (Subaschandran and Balloun, 1967; Parker and Boone, 1971). This change could be accounted for by increased water consumption (Fox, 1951; Hillerman and Wilson, 1955) which resulted in hemodilution (Parker and Boone, 1971). Moreover, plasma protein levels decreased when birds were subjected to constant high ambient temperature (Deaton *et al.*, 1969; Vo *et al.*, 1978).

The effect of the thyroid gland on metabolism is well established. Decreased thyroid size (Huston and Carmon, 1962; Clark and Das, 1974) and activity (Reineke and Turner, 1945; Hahn *et al.*, 1966) in response to high environmental temperature have been reported. A reduction in thyroid activity by radiothyroidectomy or administration of thiouracil increased heat stress survival time (Fox, 1980; Bowen *et al.*, 1984).

As in mammals, triiodothyronine (T_3) is the metabolically active hormone in birds (Klandorf *et al.*, 1978, 1981) and it originates from the peripheral deiodination of thyroxine (T_4)

by the liver and kidneys (Astier and Newcomer, 1978). Reverse T_3 formation has been suggested as the pathway through which T_4 is deactivated under conditions of high ambient temperature (Rudas and Pethes, 1984). Conflicting reports are found in the literature regarding changes on plasma T_3, reverse T_3 and T_4 concentrations in response to heat stress. Some of these differences can be accounted for by the type of heat treatment used (chronic vs acute; May *et al.*, 1986) and feeding status of the birds (restricted vs *ad libitum*; May, 1978).

No diurnal pattern in T_3 and T_4 plasma concentration has been observed in broilers maintained at constant temperature under continuous lighting with feed and water provided *ad libitum* (May, 1978; Smoak and Birrenkott, 1986). May *et al.* (1986) reported that broilers acclimated to either constant or cyclic moderate temperatures for three days and exposed to 41 C on the fourth day had no consistent changes on T_3 and T_4 concentrations. Rudas and Pethes (1984) observed that upon exposure to 35 C, T_4 concentration decreased while T_3 concentration was not affected. Reverse T_3 plasma levels were undetectable at control temperatures but its concentration increased to detectable levels under heat stress conditions. Sinurat *et al.* (1987) reported decreased plasma T_3 and increased plasma T_4 concentrations in broilers exposed to 35 C for 5 hr per day. These birds were able to readjust plasma T_3 and T_4 levels to control values when returned to 21 C. Reverse T_3 concentration was not altered by heat stress.

Selye (1937) described the nonspecific changes which occur in an organism in response to noxious stimuli. These changes were described in a theory he termed the General Adaptation Syndrome consisting of three stages: the stage of alarm, the stage of resistance and the stage of exhaustion. Although increased adrenal activity provides certain advantages to the animal when challenged by a noxious stimulus, this heightened state of activity, as it occurs during the stage of resistance, can have a detrimental effect on the immune response (Thaxton *et al.*, 1968). Corticosteroids are known for their lympholytic activity. Gross *et al.* (1980) reported a regression of lymphoid organs such as the thymus, spleen and bursa of Fabricious with corticosterone administration in the feed. Corticosterone also causes a depletion of lymphocytes from the germinal centers and decreases in the

number of circulating lymphocytes. The latter effect is due mainly to an increased incorporation of corticosterone by these cells (Siegel and Gould, 1982). The number of other white blood cells, on the other hand, is increased by corticosterone. This shift in the ratio of circulating lymphocytes to other white blood cells, (*e.g.* heterophils) makes birds more susceptible to viral diseases (Gross, 1972) and more resistant to bacterial diseases (Gross and Siegel, 1965).

Several studies have been conducted to identify physiological parameters that can be used as reliable indicators of stress. Some of these physiological indicators include: changes in adrenal weight (Wolford and Ringer, 1962; Freeman 1970), adrenal ascorbic acid (Wolford and Ringer, 1962; Freeman, 1970), adrenal cholesterol (Wolford and Ringer, 1962), leucocyte number (Chancellor and Glick, 1960; Wolford and Ringer, 1962; Ben Nathan, 1976), plasma corticosterone (Gould and Siegel, 1985), glucagon (Freeman and Manning, 1976) and growth hormone levels (Sinurat *et al.*, 1987). Disagreement exists as to whether some of these measurements can be used to make an accurate assessment of how stressful a situation is perceived by the bird. Some require sacrificing the animal while others are influenced by the collection procedure (Freeman and Flack, 1980).

Gross and Siegel (1984) reported that the heterophil to lymphocyte ratio appeared to be a reliable and easy method to indicate stress. Changes in this ratio were found to be a better indicator of social stress and corticosterone levels in the feed than were plasma corticosteroid levels. However, heterophil/lymphocyte ratio measures a physiological change in response to a stressor over a relatively long time while increases in plasma corticosterone indicate an immediate response to a stressor.

Evaporative heat loss is one of the main avenues of heat dissipation available to the bird. The higher panting rate observed as ambient temperature increases (Hillerman and Wilson, 1955; Whittow *et al.*, 1964) induces respiratory alkalosis (Calder and Schmidt-Nielsen 1966, 1968; Teeter *et al.*, 1985; Odom *et al.*, 1986). At high ambient temperatures, thermoregulatory activities take precedent over other systems (*e. g.* digestive system, Bottje and Harrison, 1986). This shift in priorities leads to a greater loss of CO_2 from the blood

resulting in an increase in blood pH. When CO_2 loss exceeds a critical level, chemoreceptors exert an inhibitory effect on the panting center in an attempt to return blood pH to normal levels. Decreased panting rates result in an increase in body temperature which eventually results in a collapse of the cardiovascular system leading to death. As long as panting rates are maintained, body temperature can be kept from reaching lethal levels.

Several studies have been conducted in an attempt to improve the acid-base balance during periods of high ambient temperature. Bottje and Harrison (1985a) reported that administration of either carbonated water (CW), 2% sodium bicarbonate, or 3.5% calcium chloride into the crop of cockerels exposed to acute heat stress improved acid-base balance. Although an improvement in blood pH occurred with the administration of these compounds, high levels of sodium bicarbonate and calcium chloride were found to induce a severe alkalosis and acidosis, respectively, thereby suggesting caution in their use as acid-base correctors during periods of high ambient temperature. The improvement of acid-base balance by CW resulted in greater heat stress survival time, feed efficiency and average daily gain (Bottje and Harrison, 1985b).

Other compounds have also been used to reduce the changes in blood pH which occur during thermal polypnea. Teeter and Smith (1986) evaluated the effects of supplemental NH_4Cl and KCl on the acid-base balance of birds exposed to elevated environmental temperature. Supplementation of the drinking water with 0.2% NH_4Cl reduced blood pH to normal values, increased body weight gain by 23% and feed efficiency by 7.7%. Potassium chloride supplementation also increased body weight gain (46%) and feed efficiency but did not affect blood pH.

Although great advances have been made regarding management during hot weather, the deleterious effects of high environmental temperature on the performance of birds is still a common occurrence. Selection for high body weight has resulted in birds with low heat resistance, with body weight being negatively correlated to heat stress survival time (Reece *et al.*, 1972; Wilson *et al.*, 1975; Bowen and Washburn, 1984b). Differences in heat stress survival time have been found among breeds (Hutt, 1938; Lee *et al.*, 1945) and families (Kheireldin and

Shaffner, 1957). Heavy breeds, when compared to White Leghorns, are known for their low resistance to high ambient temperature. The greater tolerance to heat of White Leghorns has been observed in adults (Hutt, 1938; Lee *et al.*, 1945) as well as day old chicks (Wilson and Plaister, 1951). At least part of this difference in thermotolerance may be related to the continuous consumption of water by leghorns during exposure to elevated temperatures (Fox, 1951).

Since birds decrease food intake as temperature increases (Kleiber and Dougherty, 1934; Prince *et al.*, 1961; Parker *et al.*, 1972), and decreased food intake decreases metabolic rate (Berman and Snapir, 1965), fasting during periods of high environmental temperature has been suggested as a mean of decreasing mortality caused by heat stress. McCormick *et al.* (1979) reported that fasting for 24, 48 and 72 hr progressively increased the survival time of broilers exposed to acute heat. This response was attributed to a decrease in the heat load of the birds and to a decrease in blood pH due to the presence of ketone bodies which prevented the drastic alteration of acid-base status which normally occurs during thermal polypnea. Smith and Teeter (1986) obtained similar results with fasting times ranging from 0 to 24 hr prior to the onset of heat stress. Removal of feed after the beginning of heat stress did not affect heat stress survival time. The latter effect may be explained by the fact that heat production is not reduced until 3 to 8 hr after feed removal (Van Kampen, 1977).

The energy and protein ratio of the diet is commonly adjusted during periods of high ambient temperature in an attempt to maintain nutrient intake. The growth rate depression observed during high temperature is due to a decrease in food intake and is not due to a change in the nutrient requirements of the bird. March and Biely (1972) performed a series of experiments in which chicks were reared in two temperature groups kept at 20 C and 31.1 C and fed diets containing graded levels of lysine. As expected, upon exposure to heat stress a reduction in body weight gain and feed consumption was observed. The depressed gain was not due to a change in the nutrient requirement of the chicks because when the lysine content of the diet was plotted against body weight gain, it was found that birds fed diets containing similar levels of lysine had comparable rates of growth.

Excess amino acids in the diet may impair feed intake and growth rate under heat stress conditions. Waldroup *et al.* (1976) performed a series of experiments in which amino acids were fed at the minimum requirement in the diets of broilers subjected to high ambient temperatures. Broilers fed such diets had significantly better growth rates under high environmental temperature than those which received a conventional diet.

The heat increment of the diet is lower when a large percentage of the energy is derived from lipids (Polin and Wolford, 1976). Several studies have been conducted based on this premise. Dale and Fuller (1979) reported that broiler cockerels kept at 31.1 C and 20.0 C and fed diets with a relatively high fat content had significantly greater body weight than those fed a diet containing carbohydrates. Furthermore, heat production was lower in birds fed a high fat diet. Sinurat and Balnave (1985), on the other hand, reported that fat supplementation during periods of high environmental temperature had no effect on the performance of the birds.

Experiments conducted at this university indicated that exposure of broiler cockerels to acute heat for 24 hr at five days of age decreased mortality when exposed to acute heat prior to market. This study was conducted to investigate the physiological basis of this response. Furthermore, it investigated whether present commercial management practices during high ambient temperature (i.e. feed restriction and electrolyte supplementation) have a positive or negative effect on this response.

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Chapter 1

EFFECT OF HEAT STRESS EARLY IN LIFE ON MORTALITY OF BROILERS EXPOSED TO HIGH ENVIRONMENTAL TEMPERATURE JUST PRIOR TO MARKETING

INTRODUCTION

As environmental temperature exceeds 35 C, mortality and morbidity of broilers increases substantially. The poultry industry has relied on evaporative cooling systems together with poultry house insulation to reduce the adverse effects of high ambient temperature. Although these improvements in broiler house environment have decreased losses, high mortality is still experienced during the first heat wave of the summer. For example, in 1986, 1 million chickens and turkeys were lost during the month of July in Georgia because of high temperature (Brown, 1986).

Based on the physiological mechanisms involved during heat stress, other management techniques besides evaporative cooling and insulation have been investigated. Some of these approaches have included reducing the heat increment of the diet by fat supplementation (Fuller and Mora, 1973), addition of ascorbic acid to the diet (Pardue *et al.*, 1985), limiting the amount of amino acids in the diet (Waldroup *et al.*, 1976), the addition of NH_4Cl and $NaHCO_3$ to drinking water (Branton *et al.*, 1986), addition of NH_4Cl, $NaHCO_3$ and $CaCl_2$ to the diet (Teeter *et al.*, 1985), addition of $NaHCO_3$ to the drinking water (Bottje and Harrison, 1985), acclimation of the birds (Hutchinson and Sykes, 1953; Reece *et al.*, 1972; Bohren *et al.*, 1982), fasting (McCormick *et al.*, 1979; Smith and Teeter, 1986), and preconditioning by handling (Bowen and Washburn, 1984).

Preliminary results obtained at this university indicated that exposing broilers to a period of acute heat stress at five days of age increased their tolerance to high environmental temperature later in life (Denbow and Weaver, unpublished). The purpose of this experiment

was to expand on these preliminary results and evaluate the effects of feed restriction during periods of high environmental temperature.

MATERIALS AND METHODS

Broiler cockerels, one day of age, were vaccinated for Marek's disease and randomly assigned to 32 floor pens (1.52 x 3.66 m) such that each pen had 62 birds. The birds were provided a starter (22% protein; 3141 kcal Metabolizable Energy (ME) / kg), grower (19.5% protein; 3196 ME kcal/ kg) and withdrawal (16.5% protein; 3196 ME kcal/ kg) diet from 0 to 21 days, 22 to 42 days, and 43 to 48 days of age, respectively. The starter and grower diets contained 50 mg of salinomycin[1] per kg feed. The birds were kept under continuous lighting with an intensity of 18.3 lux.

A brooding temperature of 29.4 C was maintained for the first week and then decreased 1.7 C /wk to 21.1 C which was then maintained for the duration of the experiment. The temperature in each pen was monitored and recorded by means of a multi-channel potentiometer (Model 111741, Honeywell, Fort Washington, PA) using T-type thermocouples.

Through 42 days of age, the experiment was conducted using a completely randomized design with two treatments. The two treatments included 16 control pens and 16 pens which were exposed to early heat stress in which the temperature was increased to between 35.0 and 37.8 C for 24 hr at five days of age.

At 42 days of age, the experiment was designed as a 4 x 2 factorial in which heat and feed restriction were the main effects, with four replicate pens per treatment combination. The heat treatments were as follows: 1) Heat exposure at five days of age followed by control temperature throughout the experiment (Early Heat-Control) ; 2) Heat exposure at five days of age followed by high environmental temperature at 44 and 45 days of age (Early Heat-Late Heat) ; 3) Control temperature followed by high environmental temperature at 44 and 45 days

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[1] BioCox ®, AgriBio, Gainsville, Ga.

of age (Control-Late Heat) ; 4) Control temperature throughout the experiment (Control-Control). The early heat treatment was as described above. At 44 and 45 days of age, those pens assigned to the late heat treatment were exposed to temperatures ranging from 35.0-37.8 C for 8 hr per day. After the 8-hr heat cycle at 44 days, the temperature was reduced to 26.4 C and after the 8-hr heat cycle at 45 days the temperature was reduced to 21 C. The feed restriction treatments included: 1) removal of feed for 8 hr per day from 43 to 45 days of age, and 2) controls which were fed *ad libitum*. In the feed restricted groups, feed was removed during the period of the day when temperature was elevated. In this study, relative humidity was not controlled but remained below 30%.

The birds and feed were weighed when the birds were 28, 42 and 48 days of age and mortality was recorded daily. Feed intake, body weight, body weight gain and feed efficiency were analyzed using the General Linear Model of the Statistical Analysis System (SAS Inst. Inc., 1985). The model used through 42 days of age was:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where μ is the mean of Y_{ij}, T equals the heat treatment and e_{ij} is the random variation unique to each pen. The data at 48 days of age were analyzed using the model:

$$Y_{ijk} = \mu + T_i + B_j + (TB)_{ij} + e_{(ij)k}$$

Where T and B represent heat treatment and feed restriction effects, respectively. Significant treatment effects were separated using Duncan's multiple range test (Duncan, 1955). Since the mortality data were not normally distributed, these data were subjected to Chi Square analysis (Steel and Torrie, 1980). Significant differences imply $P \leq .05$.

RESULTS

At 28 days of age, there was a significant decrease in body weight, body weight gain and feed consumed due to heat stress at five days of age (Table 1). By 42 days of age, there were no longer any significant differences in body weight, body weight gain (not shown) or feed consumption between control and early heat stressed birds. There was, however, a significant improvement in feed efficiency at 42 days of age due to early heat stress.

At 48 days of age, after the imposition of feed restriction and late heat stress, there were no significant differences in body weight, body weight gain from 42 to 48 days of age, or feed efficiency due to feed restriction (Table 2). Early heat stress, not followed by heat stress later in life, significantly improved body weight gain from 42 to 48 days of age as well as cumulative feed efficiency at 48 days of age.

Feed restriction had no significant effect on mortality, therefore, results are not shown. Late heat stress at 44 and 45 days of age caused a significant increase in mortality of birds not exposed to early heat stress at five days of age (Table 3). In other words, exposing birds to a heat stress at five days of age significantly (12.3% *vs* 0.8%) reduced mortality when birds were exposed to heat stress again at 44 and 45 days of age.

DISCUSSION

The results of the present study show that exposure of broilers to environmental temperatures of 35.0 to 37.8 C for 24 hr at 5 days of age can decrease mortality resulting from a heat stress later in life by as much as 93% (12.3% *vs* 0.8%). While neither the physiological basis of the response nor the length of time the "protective" effect induced by early heat stress is known, it appears that broilers can be protected through market age.

Previous work has demonstrated that the domestic fowl can be protected from the effects of acute heat stress. For example, Hutchinson and Sykes (1953) showed that chickens could be acclimatized to a hot, humid environment by 24 daily 4-hr exposures to elevated

temperatures. The increase in heat tolerance resulting from acclimatization is due to a decrease in body temperature, decreased insensible heat loss, decreased oxygen consumption and increased panting rates (Sykes and Fataftah, 1986a). While increasing the environmental temperature can lead to increased heat tolerance, lowering the environmental temperature can reduce the bird's ability to acclimate to heat stress (Sykes and Fataftah, 1986b; Sykes and Silah, 1986). This latter effect appears attributable to the increased food intake which accompanies a decrease in the environmental temperature since restricting the food intake will restore the ability to acclimate. Broilers can also be acclimated to high temperature following relatively short (i.e. 4 days) exposure to elevated temperatures (May *et al.*, 1987). In the latter study, it was not determined how long such acclimation lasts. Since acclimatization requires relatively long term exposure to elevated temperatures, it is unlikely that the present results are due to such a process.

McCormick *et al.* (1979) reported that either fasting or feeding a carbohydrate-free diet significantly increased the survival time of broiler cockerels exposed to acute heat stress. Fasting for 24, 48 or 72 hr progressively increased the survival time of birds exposed to acute heat stress. That fasting increased survival time is not surprising since this would decrease heat production, a process similar to what occurs during acclimatization. The results of McCormick *et al.* (1979) indicate that altering the metabolic or nutritional state of the animal affects the ability to withstand heat stress. Since birds normally decrease food intake as environmental temperature increases (Prince *et al.*, 1961; Hurwitz *et al.*, 1980), and decreasing food intake decreases heat production (Berman and Snapir, 1965), restricting feed during the hottest periods of the day has been suggested as a means of decreasing mortality during heat stress. However, feed restriction in the present study had no significant effect on mortality. Restricting feed during only the hottest period of the day is probably insufficient time to induce a decrease in heat production. Kuenzel and Kuenzel (1977) reported that 5-week old broilers must be fasted for 40 hr in order to produce a postabsorptive state. In addition, since feed intake does decrease as temperature increases, the birds that were not feed restricted in this study probably greatly reduced or stopped eating during the imposed heat stress.

Prior exposure to a nontemperature stressor can increase the ability of chickens to withstand acute heat stress. Bowen and Washburn (1984) demonstrated that repeated handling of chickens for 4 consecutive days increased the survival time to acute heat stress occurring the following day. However, this effect was transient since, by three days after the cessation of handling, the birds had normal survival time when exposed to acute heat.

That stress early in life can improve selected production parameters is well documented in Leghorns. Exposing lines of Leghorn chickens selected for either high (HA) or low (LA) antibody response to sheep red blood cells to 46 C for 6 hr at 6 days of age resulted in a 6% increase in body weight of the LA line and a 2% increase in the HA line (Gross and Siegel, 1980). Feed efficiency was improved 8% in the LA line at 18 weeks of age. Removal of water from 6 to 10 days of age had effects similar to that of heat stress.

Seyle (1956) originally proposed the concept of heterostasis. Heterostasis refers to "the establishment of a new steady state by treatment with agents which stimulate the physiologic adaptive mechanisms through the development of normally dormant defensive tissue reactions". Heat stress at 5 days of age may be viewed as heterostasis in that such treatment does strengthen the birds own natural adaptive mechanisms.

While it has been suggested that it is unlikely in practical situations that poultry at risk of severe heat stress can be acclimatized in advance (Sykes and Fataftah, 1986a), the present results demonstrate that it is possible to "protect" the birds from the effects of acute heat stress by using a simple management procedure. Since supplemental heat is provided to the birds during the first weeks of life, it is easy to increase the environmental temperature to 35 C for 24 hr at five days of age. Once the birds have been exposed to such early heat stress, they appear to withstand acute heat stress later in life.

SUMMARY

A study was conducted to investigate the effects of heat stress during the first week of life, and feed restriction on subsequent mortality resulting from exposure to high

environmental temperature just prior to marketing of broiler cockerels. Birds were raised under standard husbandry procedures except, at five days of age, half of the broilers were heat stressed by exposure to an environmental temperature ranging from 35.0 to 37.8 C for 24 hr while the remaining birds were held at 29.4 C. At 44 and 45 days of age, half of the control pens (unstressed) and half of the pens which were stressed at five days of age were exposed to temperatures ranging from 35.0 to 37.8 C for 8 hr per day. In a factorial arrangement of treatments, the effect of restricting feed for 8 hr per day on days 43, 44 and 45 was also examined.

Exposing the birds to high environmental temperatures at five days of age resulted in a significant decrease in mortality when exposed to a high environmental temperature later in life. In addition, feed efficiency was improved significantly in early-heat stressed birds whereas body weight and body weight gain were not affected. Feed restriction had no significant effect on mortality, body weight or feed efficiency. It appears, therefore, that exposing broiler cockerels to mild heat stress for 24 hr at five days of age can significantly decrease mortality resulting from high environmental temperature later in life.

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Table 1. The effect of early heat stress on body weight, feed consumption and feed efficiency at 28 and 42 days of age.

Measurement	Treatment[1]		Pooled SE[2]
	Control	Heated	
	28 days of age		
Body weight (kg)	1.187[a]	1.160[b]	0.006
Feed consumed (kg)	1.832[a]	1.763[b]	0.012
Feed efficiency[3]	0.626[a]	0.635[a]	0.004
	42 days of age		
Body weight (kg)	2.195[a]	2.189[a]	0.012
Feed efficiency[3]	0.547[a]	0.558[b]	0.002

[1] The heated birds were exposed to 35.0 to 37.8 C at 5 days of age in comparison to 29.4 C for the control birds.

[2] Standard error of the treatment means.

[3] kg body wt gain/kg feed consumed.

[a,b] Means within row with different superscripts are significantly different ($P \leq .05$).

Table 2. Effect of heat treatment and feed restriction on body weight, body weight gain, feed consumption and feed efficiency[1]

Feed Treatments	Heat treatments[2]				Mean
	Control-control	Control-late	Early-control	Early-late	
			Body weight, kg, 48 days		
Feed Restricted	2.556 ± 0.031	2.625 ± 0.046	2.628 ± 0.021	2.619 ± 0.013	2.607 ± 0.016
Ad libitum	2.626 ± 0.013	2.555 ± 0.020	2.643 ± 0.009	2.619 ± 0.041	2.611 ± 0.014
Mean	2.591 ± 0.021[x]	2.589 ± 0.027[x]	2.636 ± 0.011[x]	2.619 ± 0.019[x]	
			Gain, 42-48 days		
Feed Restricted	0.388 ± 0.018	0.392 ± 0.046	0.439 ± 0.012	0.422 ± 0.015	0.410 ± 0.013
Ad libitum	0.425 ± 0.013	0.379 ± 0.013	0.476 ± 0.008	0.419 ± 0.030	10.424 ± 0.012
Mean	0.406 ± 0.013[y]	0.385 ± 0.022[y]	0.457 ± 0.009[x]	0.420 ± 0.016[xy]	
			Cum. feed efficiency[3], 1-48 days		
Feed Restricted	0.515 ± 0.004	0.500 ± 0.011	0.533 ± 0.003	0.525 ± 0.003	0.518 ± 0.004
Ad libitum	0.510 ± 0.005	0.506 ± 0.004	0.525 ± 0.002	0.518 ± 0.008	0.515 ± 0.003
Mean	0.513 ± 0.003[yz]	0.503 ± 0.006[z]	0.529 ± 0.002[x]	0.522 ± 0.004[xy]	
			Feed consumption, kg, 42-48 days of age		
Feed Restricted	0.992 ± 0.016	1.157 ± 0.097	1.049 ± 0.025	1.022 ± 0.009	1.055 ± 0.028
Ad libitum	1.131 ± 0.056	1.050 ± 0.028	1.134 ± 0.015	1.103 ± 0.026	1.105 ± 0.018
Mean	1.061 ± 0.038[x]	1.040 ± 0.051[x]	1.092 ± 0.021[x]	1.062 ± 0.019[x]	
			Feed efficiency[3], 42-48 days		
Feed Restricted	0.391 ± 0.017	0.346 ± 0.054	0.418 ± 0.006	0.413 ± 0.015	0.392 ± 0.015
Ad libitum	0.378 ± 0.020	0.361 ± 0.016	0.419 ± 0.005	0.380 ± 0.027	0.385 ± 0.010
Mean	0.385 ± 0.012[xy]	0.354 ± 0.026[y]	0.419 ± 0.004[x]	0.397 ± 0.016[xy]	

[1] Values represent mean ± standard error.

[2] At 5 days of age, birds maintained at 29.4 C (Control) or exposed to 35 to 37.7 C for 24 hr (Early) while at 44 and 45 days of age the birds were exposed to either 21 C (Control) or exposed to 35 to 37.8 C for 8 hr per day (Late).

[3] kg body weight gain/kg feed consumed.

[x,y] Means within a row with different superscripts are significantly different ($P \leq .05$).

Table 3. Effects of heat treatment on mortality

Treatment[1]	0 to 42 days		43 to 45 days		0 to 48 days	
	No (dead/total)	%	No (dead/total)	%	No (dead/total)	%
EL	22/496	4.4[a]	4/474	.08[a]	29/496	5.8[a]
EC	16/496	3.2[a]	0/480	.0[a]	16/496	3.2[a]
CL	18/496	3.6[a]	59/478	12.3[b]	78/496	15.5[b]
CC	17/497	3.4[a]	0/480	0[b]	23/497	4.6[b]

[1] First letter.- C, Control, no heat stress; E, Early heat stress (5 days of age, 35.0-37.8 C); Second letter.- L, Late heat stress (44-45 days of age; 35.0-37.8 C); C, control no heat stress.

[a,b] Means with the same superscript are not significantly different ($P \leq .05$).

Chapter 2

EFFECT OF NEONATAL HEAT STRESS AND ELECTROLYTE SUPPLEMENTATION ON WATER CONSUMPTION, MORTALITY, SURFACE AND CORE TEMPERATURE OF JUVENILE BROILERS EXPOSED TO ACUTE HEAT

INTRODUCTION

Significant changes in plasma electrolytes occur during exposure to high ambient temperature. Kohne and Jones (1975) reported that adult turkey hens exposed to 32 C exhibited a significant decrease in plasma sodium, total calcium, magnesium and inorganic phosphorus and an increase in plasma potassium levels. Edens (1978) observed a similar response in broiler cockerels exposed to acute heat. An increase in the rate of potassium excretion was also observed in heat stress hens (Deetz and Ringrose, 1976).

Increases in environmental temperature cause panting (Hillerman and Wilson, 1955; Whittow *et al.*, 1964) which can lead to respiratory alkalosis (Calder and Schmidt-Nielsen, 1966, 1968; Teeter *et al.*, 1985). The addition of compounds to the drinking water or diet in order to correct the plasma pH has been shown to reduced the deleterious effects of high ambient temperature. Bottje and Harrison (1985) reported that heat stressed broilers provided carbonated water had better survival time, feed efficiency and average daily gain than those receiving tap water. Smith and Teeter (1987) reported that potassium supplementation with 1.5% to 2.0% or 0.24% to 0.3% in the feed and water, respectively, improved body weight gain of broilers exposed to chronic heat stress. Furthermore, Branton *et al.* (1986) showed that addition of ammonium chloride or sodium bicarbonate to the drinking water of broilers exposed to high ambient temperature resulted in improved performance. This effect, however, was related to an increase in water consumption rather than to an effect on the acid-base balance.

Feed consumption decreases as ambient temperature increases (Cowan and Mitchie, 1977; Cerniglia *et al.*, 1983). Supplementation of the diet with ascorbic acid (Thornton and

Moreng, 1959; Perek and Kendler, 1962), vitamin A (Heywang, 1952; Ascarelli and Bartov, 1963) and thiamine (Mills *et al.*, 1947) during periods of high ambient temperature has been shown to improve the bird's performance. Based on these facts, electrolyte/vitamin packages have been developed in an attempt to maintain the water balance and electrolyte profile of the birds under conditions of high ambient temperature.

The control of body temperature within a narrow range is important for survival. At or below thermoneutral temperatures, sensible heat loss is the primary mechanism of heat dissipation, whereas at high environmental temperatures evaporative cooling becomes more important (Mount, 1974). Therefore, as humidity increases during periods of elevated environmental temperatures, heat loss in birds is impaired (Hutchinson, 1954). Wilson *et al.* (1969) observed a greater increase in body temperature of Leghorn hens kept at 32 C and 60% relative humidity compared to those kept at 22% relative humidity. Similarly, Reece *et al.* (1972) reported that mortality of broilers exposed to 40.6 C for 6 hr was influenced by relative humidity.

The possibility of increasing thermotolerance of ready to market broilers by exposure to temperatures between 35 to 37.8 C for 24 hr at five days of age has been reported (Chapter 1). In that study, relative humidity remained below 30%, a value lower than generally observed during the summer. The aims of this study were to investigate the effects of neonatal heat stress on mortality, water consumption, core and surface body temperature of juvenile broilers exposed to acute heat. Furthermore, the effects of constant humidity ($50 \pm 5\%$) and addition of electrolytes to the water were also studied.

MATERIALS AND METHODS

Broiler cockerels, one day of age, were vaccinated for Marek's disease and randomly assigned to 32 floor pens (1.52 X 3.66 m) such that each pen had 63 birds. A starting brooding temperature of 29.4 C was decreased 1.7 C/wk to 21.0 C which was maintained for the duration of the experiment, except as otherwise noted. The relative humidity was maintained at

50±5% throughout the experiment. Wet and dry bulb temperatures were monitored and recorded with a multichannel potentiometer (Model 111741, Honeywell, Fort Washington, PA) using T-type thermocouples. Light intensity was maintained at 18.3 lux.

Standard commercial starter (21.5% protein; 3250 kcal Metabolizable Energy (ME)/kg), grower (19.7% protein; 3250 kcal ME/kg) and finisher (18.2% protein; 3250 kcal ME/kg) diets were fed from 0 to 24, 25 to 40 and 41 to 49 days of age, respectively. The starter and grower diets contained nicarbazin (125 mg/kg) and monensin (121 mg/kg), respectively. Feed and water were provided *ad libitum*.

The experiment was designed as a 4 X 2 factorial in which heat treatment and electrolyte supplementation were the main effects with 4 replicate pens per treatment combination. Heat treatment consisted of the following levels: 1) neonatal heat exposure at five days of age followed by control temperature throughout the experiment (EC); 2) neonatal heat exposure at five days of age followed by high environmental temperature at 43 and 44 days of age (EL); 3) control temperature at 5 days of age followed by high environmental temperature at 43 and 44 days of age (CL); 4) control temperature throughout the experiment (CC). Neonatal, early, heat was administered as follows: at five days of age, the temperature in half of the pens was increased to between 35.0 to 37.8 C for 24 hr while the remaining pens were kept at 29.4 C. Juvenile, late, heat consisted of increasing the temperature from 21.0 C to between 35.0 and 37.8 C for 8 hr at 43 and 44 days of age. After the 8 hr heat cycle at 43 days of age, the temperature was reduced to 26.4 C and after the 8 hr heat cycle at 44 days temperature was reduced to 21.0 C.

The electrolyte supplementation treatment consisted of two levels: 1) control (C) which received tap water and 2) tap water which contained a commercially available electrolyte package (ElectroR, I. D. Russell Co., Kansas City, Mo.) 36 hr prior to the imposition of late heat (42 days of age). Water consumption was measured by placing a 20 L reservoir in each of the pens with loss due to evaporation being determined by placing a similar container in each of the rooms. These reservoirs were attached to the water system by plastic hoses. Water consumption was monitored and recorded twice a day from 41 to 45 days of age.

At 43 and 44 days of age, core and surface body temperatures were recorded from two randomly selected birds/pen using thermistor probes (T2605 and T2625 ; Yellow Springs International, Yellow Springs, OH) connected to a digital thermometer (LED, Fisher Scientific, Raleigh, N.C.) Temperatures were recorded 6 hr after the initiation of the daily heat stress.

Birds and feed were weighed at 1, 28, 42 and 49 days of age and mortality was recorded daily. Body weight, feed consumption, body weight gain, water consumption and core and surface body temperatures were analyzed using Analysis of Variance from the Statistical Analysis System (SAS Inst. Inc., 1985). The model used through 42 days of age was:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where μ is the mean of Y_{ij}, T equals the heat treatment and e_{ij} is the random variation unique to each pen. The data at 48 days of age were analyzed using the model:

$$Y_{ijk} = \mu + T_i + B_j + (TB)_{ij} + e_{(ij)k}$$

Where T and B represent heat treatment and water supplementation effects, respectively. Significant treatment effects were separated using Duncan's multiple range test (Duncan, 1955). Mortality data were subjected to Chi Square analysis (Steel and Torrie, 1980). Significant differences imply $P \leq .05$.

RESULTS

At 28 and 42 days of age there were no significant differences in body weight, feed consumption or feed efficiency between the early heat stressed and early control groups (Table 1). There were no significant differences among the heat treatments or electrolyte

supplemented groups regarding body weight, feed consumption or feed efficiency at 49 days of age (Table 2) and for the period between 43 and 49 days of age (Table 3).

Daily water consumption from 41 through 45 days of age is shown in Table 4. A significant increase in water consumption was observed with the imposition of late heat. Electrolyte supplementation had no significant effect on water consumption within heat treatment groups.

The EL group had 78.3% lower mortality during late heat stress than the CL group (4.34 vs 20.04%) (Table 5). Electrolyte supplementation prior to and during late heat had no significant effect on mortality within heat treatment groups (Table 6).

Core and surface body temperature during late heat stress are shown in Table 7. A significant increase in core and surface temperature was observed between the late heat stressed and control groups. No significant differences were found between the EL and CL groups regarding core and surface temperature. Within heat treatment groups, electrolyte supplementation had no effect on body temperature.

DISCUSSION

The results of this study confirm those previously reported which indicated that mortality associated with high environmental temperature prior to market could be reduced by exposing broilers to an acute heat stress for 24 hr at five days of age (Chapter 1). Although an improvement in performance was not observed at 28 and 42 days of age, no deleterious effects were observed with early heat stress. Even though relative humidity was higher in the present study (50 ± 5%) than in the previous one (≤30%) (Chapter 1), early heat stressed birds were still better able to withstand acute heat just prior to market. Electrolyte supplementation during late heat did not seem to alleviate the effects of late heat stress by stimulating water consumption and replenishing electrolytes.

Increased water consumption during high ambient temperature has been shown to result in greater survival time (Vo and Boone, 1977; Branton *et al.*, 1986), a response which can

be attributed to the replenishment of water loss through evaporative cooling and to the use of water as a heat sink which enables the bird to dissipate greater amounts of heat (Fox, 1951). Although water consumption increased during late heat in the early heat stressed and early control groups, the lack of significant differences between the two may indicate that water intake is not a factor in the greater tolerance to heat observed in the former group. There is no explanation as to why water consumption was significantly greater in the control-late group at 42 days of age.

As expected, an increase in core and surface body temperature took place upon exposure to high ambient temperature. Acclimated birds are able to maintain body temperature at a point which is significantly lower than that of non-acclimated birds (Hutchinson and Sykes, 1953; May *et al.*, 1987). The lack of differences in body temperature between the early heat and early control groups and the lower mortality observed in the former may be a reflection of the early heat group to better withstand higher body temperature. The body temperature response in the early heat stressed birds did not resemble that of acclimated birds since it was not significantly lower than that of the early control group. Additionally, the surface to core ratio did not show any differences between the early heated and early control groups. This suggests that the increased thermotolerance observed in the latter group was not related to a greater amount of heat being dissipated to the surface, a response which differs from that of acclimated birds since an increase in sensible heat loss has been shown to occur during acclimation to high environmental temperature (Deshazer *et al.*, 1970).

The results of this study support an earlier report that exposure of broilers to elevated temperatures at 5 days of age for 24 hr induces thermotolerance (Chapter 1). The mechanism of this response remains to be elucidated; however, it does not appear to be the same as acclimation since it does not involve a change in set point for body temperature, a change in the ability of the birds to lose heat or a change in water consumption.

SUMMARY

A study was conducted to investigate the effects of heat stress at 5 days of age on mortality, water consumption, core and surface temperature of broilers exposed to acute heat at 43 and 44 days of age. In a factorial arrangement of treatments the effect of electrolyte supplementation during high ambient temperature was also studied.

Neonatal, early, heat stress consisted of raising the environmental temperature to between 35 to 37.8 C for 24 hr at 5 days of age in half of the pens. At 43 and 44 days of age temperature was gradually increased to between 35 to 37.8 C for 8 hr/day in half of the early and nonearly heat stressed pens (late heat; juvenile). Relative humidity was maintained at 50±5% throughout the experiment. Electrolyte supplementation via the drinking water was provided 36 hr prior to the imposition of late heat.

Early heat stress significantly reduced mortality during the second heat challenge by 78%. Furthermore, a significant increase in water consumption, core and surface body temperature was observed in the late heat stressed pens. No significant differences were found between the early heat and early control groups regarding water consumption, core and surface body temperature when exposed to late heat. Electrolyte supplementation had no effect on water consumption within heat treatment groups.

It appears, therefore, that subjecting broilers to acute heat for 24 hr at five days of age can increase their survivability when exposed to juvenile heat stress without any deleterious effects on performance.

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Table 1. Effect of early heat stress on body weight, feed consumption and feed efficiency at 28 and 42 days of age[1]

Measurement	Treatment[2]		Pooled SE[3]
	Control	Heated	
	28 days of age		
Body weight (kg)	1.071	1.080	0.004
Feed consumed (kg)	1.560	1.576	0.009
Feed Efficiency[4]	0.658	0.657	0.002
	42 days of age		
Body weight (kg)	2.083	2.095	0.008
Feed consumed (kg)	3.545	3.571	0.017
Feed Efficiency[4]	0.575	0.574	0.001

[1] No significant differences.

[2] The heated birds were exposed to 35.0 to 37.8 C at five days in comparison to 29.4 C for the control birds.

[3] Standard error of the treatment means.

[4] kg of body wt gain / kg feed consumed.

Table 2. Effect of heat treatment and electrolyte supplementation on body weight (kg), feed consumption (kg) and feed efficiency from 0 to 49 days of age[1]

Water treatments	Heat Treatments[2]				Mean
	Control-control	Control-late	Early-control	Early-late	
			Body weight		
Supplemented[3]	2.541 ± 0.015	2.572 ± 0.057	2.586 ± 0.020	2.542 ± 0.038	2.560 ± 0.017
Non supplemented	2.569 ± 0.075	2.516 ± 0.012	2.568 ± 0.017	2.542 ± 0.024	2.549 ± 0.019
Mean	2.555 ± 0.036	2.554 ± 0.029	2.577 ± 0.012	2.542 ± 0.021	
			Feed consumption		
Supplemented	4.684 ± 0.028	4.730 ± 0.055	4.780 ± 0.084	4.677 ± 0.065	4.718 ± 0.029
Non supplemented	4.673 ± 0.065	4.684 ± 0.025	4.775 ± 0.038	4.700 ± 0.012	4.708 ± 0.020
Mean	4.679 ± 0.033	4.707 ± 0.029	4.777 ± 0.042	4.689 ± 0.031	
			Feed Efficiency[4]		
Supplemented	0.533 ± 0.003	0.533 ± 0.003	0.532 ± 0.007	0.534 ± 0.005	0.533 ± 0.002
Non supplemented	0.540 ± 0.015	0.528 ± 0.002	0.528 ± 0.001	0.531 ± 0.004	0.532 ± 0.004
Mean	0.537 ± 0.007	0.531 ± 0.004	0.530 ± 0.003	0.533 ± 0.003	

[1] No significant differences.

[2] First letter.-E, Early heat stress at five days of age; C, early control.
Second letter.-L, Late heat stress at 43 and 44 days of age; C, late control.

[3] Supplemented with electrolyte beginning 36 hr prior to late heat stress.

[4] kg body weight gain / kg feed consumed.

Table 3. Effect of heat treatment and electrolyte supplementation on body weight (kg), feed consumption (kg) and feed efficiency from 43 to 49 days of age[1]

Water treatments	Heat treatments[2]				Mean
	Control-control	Control-late	Early-control	Early-late	
			Gain		
Supplemented[3]	0.464 ± 0.011	0.475 ± 0.049	0.503 ± 0.010	0.439 ± 0.024	0.470 ± 0.014
Non supplemented	0.501 ± 0.069	0.423 ± 0.013	0.470 ± 0.021	0.443 ± 0.021	0.459 ± 0.018
Mean	0.483 ± 0.033	0.449 ± 0.025	0.486 ± 0.012	0.441 ± 0.015	
			Feed consumption		
Supplemented	1.156 ± 0.013	1.142 ± 0.016	1.200 ± 0.035	1.123 ± 0.010	1.155 ± 0.012
Non supplemented	1.161 ± 0.028	1.127 ± 0.013	1.173 ± 0.040	1.148 ± 0.013	1.152 ± 0.012
Mean	1.159 ± 0.014	1.134 ± 0.010	1.187 ± 0.025	1.136 ± 0.009	
			Feed Efficiency[4]		
Supplemented	0.401 ± 0.005	0.415 ± 0.040	0.419 ± 0.013	0.391 ± 0.021	0.407 ± 0.011
Non supplemented	0.431 ± 0.058	0.375 ± 0.009	0.399 ± 0.005	0.385 ± 0.015	0.398 ± 0.014
Mean	0.416 ± 0.027	0.395 ± 0.020	0.409 ± 0.007	0.388 ± 0.012	

[1] No significant differences.

[2] First letter.-E, Early heat stress at five days of age; C, early control.
Second letter.-L, Late heat stress at 43 and 44 days of age; C, late control.

[3] Supplemented with electrolyte beginning 36 hr prior to late heat stress.

[4] kg body weight gain / kg feed consumed.

Table 4. Effect of heat treatment and electrolyte supplementation on water consumption[1]

Treatment[2]	Water consumption (ℓ/bird)				
	Day 41	Day 42	Day 43	Day 44	Day 45
ELC	$0.212^{a} \pm 0.003$	$0.256^{b} \pm 0.005$	$0.294^{a} \pm 0.001$	$0.333^{a} \pm 0.008$	$0.312^{b} \pm 0.012$
ELS	$0.219^{a} \pm 0.006$	$0.247^{b} \pm 0.009$	$0.285^{a} \pm 0.012$	$0.306^{a} \pm 0.007$	$0.277^{abc} \pm 0.014$
ECC	$0.220^{a} \pm 0.008$	$0.230^{b} \pm 0.004$	$0.240^{b} \pm 0.006$	$0.259^{b} \pm 0.008$	$0.262^{a} \pm 0.011$
ECS	$0.232^{a} \pm 0.018$	$0.240^{b} \pm 0.012$	$0.247^{b} \pm 0.009$	$0.267^{b} \pm 0.015$	$0.276^{ab} \pm 0.014$
CLC	$0.223^{a} \pm 0.010$	$0.283^{a} \pm 0.019$	$0.298^{a} \pm 0.014$	$0.316^{a} \pm 0.007$	$0.276^{bc} \pm 0.008$
CLS	$0.226^{a} \pm 0.005$	$0.303^{a} \pm 0.030$	$0.303^{a} \pm 0.005$	$0.325^{a} \pm 0.008$	$0.297^{c} \pm 0.003$
CCC	$0.212^{a} \pm 0.005$	$0.237^{b} \pm 0.009$	$0.249^{b} \pm 0.007$	$0.254^{b} \pm 0.007$	$0.253^{a} \pm 0.00$
CCS	$0.216^{a} \pm 0.008$	$0.243^{b} \pm 0.011$	$0.239^{b} \pm 0.004$	$0.258^{b} \pm 0.004$	$0.252^{a} \pm 0.004$

[1] Values represent mean ± standard error.

[2] First letter.- E, Early heat stress at five days of age ; C, early control.
Second letter.- L, Late heat stress at 43 and 44 days of age ; C, late control.
Third letter.- S, Supplemented with Electrolytes 36 hr prior to late heat ; C, control (tap water).

[a,b,c] Means in a column with different superscripts are significantly different ($P \leq 05$).

Table 5. Effect of heat treatment on mortality

Treatment[1]	0-42 days		43-44 days		0-49 days	
	dead/total	% mortality	dead/total	% mortality	dead/total	% mortality
EL	21/505	4.16[a]	21/484	4.34[b]	45/505	8.91[b]
EC	11/504	2.18[a]	3/493	0.61[c]	19/504	3.77[c]
CL	24/503	4.77[a]	96/479	20.04[a]	122/503	24.25[a]
CC	19/504	3.77[a]	3/485	0.62[c]	25/504	4.96[c]

[1] First letter.- E, Early heat stress at five days of age; C, early control.
Second letter.- L, Late heat stress at 43 and 44 days of age; C, late control.

[abc] Means in a column with different superscripts are significantly different ($P \leq .05$).

Table 6. Effect of heat treatment and electrolyte supplementation on mortality[1]

Treatment[2]	0-42 days		43-44 days		0-49 days	
	dead/total	% mortality	dead/total	% mortality	dead/total	% mortality
ELC	6/253	2.37	16/247	6.48	23/253	9.09
ELS	15/252	5.95	5/237	2.11	22/252	8.73
ECC	5/252	1.98	3/247	1.21	9/252	3.57
ECS	6/252	2.38	0/246	0	10/252	3.96
CLC	12/252	4.76	43/240	17.92	56/252	22.22
CLS	12/252	4.76	53/240	22.08	66/252	26.19
CCC	7/251	2.78	1/244	0.41	10/251	3.98
CCS	12/252	4.76	2/240	0.83	15/252	5.95

[1] No significant differences between supplemented and non supplemented groups within a heat treatment.

[2] First letter.- E, Early heat stress at five days of age; C, early control.
Second Letter.- L, Late heat stress at 43 and 44 days of age; C, late control.
Third letter.- S, Water supplemented with electrolyte, 36 hr prior to late heat ; C, control (tap water).

Table 7. Effect of heat treatments on rectal and footpad temperature at 43 and 44 days of age[1]

Treatment[2]	Rectal (C)		Footpad (C)	
	Day 43	Day 44	Day 43	Day 44
EL	43.2[a] ± 0.20	43.1[a] ± 0.16	39.7[a] ± 0.23	40.2[a] ± 0.16
EC	41.6[b] ± 0.04	41.4[b] ± 0.07	36.0[b] ± 0.26	36.2[b] ± 0.15
CL	43.6[a] ± 0.26	43.1[a] ± 0.11	40.3[a] ± 0.19	40.3[a] ± 0.15
CC	41.5[b] ± 0.05	41.3[b] ± 0.05	35.4[b] ± 0.27	36.8[b] ± 0.20

[1] Values represent mean ± standard error.

[2] First letter.- E, Early heat stress at five days of age; C, early control.
Second letter.- L, Late heat stress at 43 and 44 days of age; C, late control.

[ab] Means in a column with different superscripts are significantly different ($P \leq .05$).

Chapter 3

THE EFFECT OF NEONATAL HEAT STRESS ON PLASMA THYROXINE (T_4), TRIIODOTHYRONINE (T_3), HETEROPHIL/LYMPHOCYTE RATIO, GLUCOSE AND TOTAL PLASMA PROTEIN OF JUVENILE BROILERS EXPOSED TO ACUTE HEAT

INTRODUCTION

Exposure to elevated environmental temperatures causes changes in plasma hormone levels. For example, prolonged exposure of chickens to elevated temperatures decreased plasma T_3 while causing either no change (Cogburn and Harrison, 1980; Klandorf *et al.*, 1981) or a decrease in plasma T_4 concentrations (Williamson *et al.*, 1985). In contrast, Bobek *et al.* (1980) reported that exposure of quail to high temperatures decreased plasma T_4 but increased T_3 concentrations. Plasma corticosterone concentration increased in chickens (Edens and Siegel, 1975) and turkeys (El-Halawani *et al.*, 1973) upon exposure to acute heat.

Other blood parameters have also been shown to change in response to heat exposure. There was a significant decrease in plasma sodium, calcium, magnesium and inorganic phosphorus and an increase in potassium levels in turkeys (Kohne and Jones, 1975) and chickens (Edens, 1978) exposed to elevated temperatures. While some authors have reported a significant decrease in total plasma protein during heat stress (Deaton *et al.*, 1969; Vo *et al.*, 1978), others have shown no changes (Squibb et al., 1959) or an increase on total protein plasma concentration (Pardue *et al.*, 1985). These discrepancies may be due to difference in heat treatments and duration, depressed food intake (Prince *et al.*, 1961; Cowan and Mitchie, 1977), increased water consumption (Wilson, 1948) or to the catabolic activities of glucocorticoids.

To date, most approaches for the induction of heat tolerance have included exposure of birds to controlled rises in environmental temperature immediately prior to acute heat stress (Hutchinson and Sykes, 1953; Reece *et al.*, 1972; Sykes and Fataftah, 1986ab; May *et al.*, 1987). Recently it was shown that exposure of broiler cockerels to elevated temperatures at

five days of age improved their survivability when exposed to acute heat prior to market (Chapter 1; Chapter 2). Since the mechanism of the response is unknown, the purpose of this study was to measure certain blood parameters known to be involved in the response of birds to high ambient temperature.

MATERIALS AND METHODS

Broiler cockerels, one day of age, were vaccinated for Marek's disease and randomly assigned to 16 floor pens (1.52 X 3.66 m) such that each pen had 63 birds. A starting brooding temperature of 29.4 was decreased 1.7 C/wk to 21.0 C which was maintained for the duration of the experiment, except as otherwise noted. Relative humidity was maintained at 50±5% throughout the experiment. Wet and dry bulb temperatures were monitored and recorded using a multichannel potentiometer (Model 11174I, Honeywell, Fort Washington, PA) using T-type thermocouples. Light intensity was maintained at 18.3 lux.

Standard commercial starter (21.5% protein; 3250 kcal Metabolizable Energy (ME) /kg), grower (19.7% protein; 3250 kcal ME /kg) and finisher (18.2% protein; 3250 kcal ME /kg) diets were fed from 0 to 24, 25 to 40 and 41 to 49 days of age, respectively. The starter and grower diets contained nicarbazin (125 mg/kg) and monensin (121 mg/kg), respectively. Feed and water were provided *ad libitum*.

Neonatal, early, heat stress consisted of increasing temperature to between 35.0 to 37.8 C for 24 hr at five days of age in half of the pens (early heat stressed) while the remaining pens were kept at 29.4 C (early control). At 43 an 44 days of age temperature in half of the early heat stressed and early control pens was gradually increased to between 35.0 to 37.8 C for 8 hr per day (late heat; juvenile). After the 8 hr heat cycle at 43 days of age temperature was reduced to 26.4 C and after the 8 hr heat cycle at 44 days of age temperature was reduced to 21.0 C.

At 43 and 44 days of age blood samples were collected from 3 randomly selected birds/pen immediately prior to increasing the environmental temperature and after 6 hr of heat exposure. No bird was bled more than once.

Plasma was collected and stored at -70 C until analysis. Blood smears were prepared by placing a drop of blood on each slide and centrifuging with a Larc spinner (Corning Glass Works, Scientific Instruments Division, Metfield, Massachussets). Smears were stained within 10 days of preparation with May-Grunwald-Giemsa stain. A total of 60 cells were read and heterophil to lymphocyte ratios were determined.

Plasma T_3 and T_4 concentrations were analyzed using a double antibody RIA procedure described by McNabb and Hughes (1983). Assay sensitivity was 0.125 ng/ml and 1.25 ng/ml for T_3 and T_4, respectively. Plasma glucose concentration was determined colorimetrically by the glucose oxidase method (Raabo and Terkildsen, 1960). Total plasma protein was assayed using the procedure of Bradford (1976) (BioRad Protein assay kit; BioRad Chemical Division, Richmond, Ca). For all assays interassay coefficient of variation was $\leq$11.0%.

Data were analyzed with the General Linear Model of the Statistical Analysis System (SAS Inst. Inc., 1985) using the following model:

$$Y_{ijk} = \mu + T_i + B_j + (TB)_{ij} + e_{(ij)k}$$

Where T and B represent heat treatment and time of the day, respectively. Heterophil to lymphocyte ratios were transformed using the square root transformation (Steel and Torrie, 1980). Significant treatment effects were separated using Duncan's multiple range test (Duncan, 1955). Significant differences imply $P \leq .05$.

RESULTS

There were no significant differences in plasma T_4 concentration among the different heat treatment groups at 43 or 44 days of age; however, a significant decline in plasma T_3 concentration was observed in the groups exposed to late heat (Table 1). Although ambient temperature was decreased following the 8 hr exposure on day 43, plasma T_3 levels remained significantly depressed the following day. No significant differences were found in plasma T_3 concentration between the early heat stressed and early control groups during late heat.

Plasma glucose concentrations were not significantly different among heat treatment groups at each time period (Table 2). Total plasma protein was significantly higher for the late heat stressed group compared with control late group during the second sampling period at 43 days of age (Table 3). No significant differences were found among the heat treatment groups at any other time. Heterophil to lymphocyte ratio was significantly lower in the early heat stressed than the early control group when exposed to high ambient temperature at 44 days of age (Table 4).

DISCUSSION

The decline in plasma T_3 concentration observed in this study upon exposure to high ambient temperature is in agreement with previous reports (Rudas and Pethes, 1984; Williamson *et al.*, 1985). Plasma T_4 concentration, on the other hand, was not affected by heat exposure. Several reports have indicated that the decreased food intake which occurs at high ambient temperature may be responsible for the decline in T_3 (Harvey and Klandorf, 1983; Klandorf and Harvey, 1985). Plasma T_4 concentration, on the other hand, is increased by fasting. The sensitivity of these hormones to fasting appears to be time dependent since May (1978) showed that plasma T_3 declined after a 4.5 hr fast at 32 C while plasma T_4 was increased after 22 hr.

T_3 is the metabolically active hormone in birds (Klandorf *et al.*, 1978, 1981; Rudas and Pethes, 1984; Piearkaszewska *et al.*, 1987) and originates from the peripheral deiodination of T_4 (Astier and Newcomer, 1978). A depression in T_3 concentration has been shown to produce a decrease in heat production (Williamson *et al.*, 1985). Reduction of thyroid activity by radiothyroidectomy or administration of thiouracil has been shown to result in greater survival times under high ambient temperature (Fox, 1980; Bowen *et al.*, 1984). Rudas and Pethes (1984) observed that upon exposure to high ambient temperature, plasma T_3 and rT_3 was decreased and increased, respectively. They suggested that the formation of rT_3 represented the preferred pathway for T_4 deiodination at high ambient temperature. Although rT_3 was not measured, the possibility that the decline in plasma T_3 concentration could have been due to a shift in T_4 deiodination exists.

The fact that heterophil and lymphocyte numbers were affected by high ambient temperature is not surprising since their number are influenced by stressors (Chancellor and Glick, 1960; Wolford and Ringer, 1963; Ben Nathan *et al.*, 1976). Gross and Siegel (1984) observed that the heterophil to lymphocyte ratio was a more reliable indicator of social stress and corticosterone levels in the feed than was plasma corticosterone concentration. The lower heterophil to lymphocyte ratio observed in the early-late group would seem to suggest that these birds were not as affected by high ambient temperature as the non-early heat stressed birds. Gross and Siegel (1986) reported that previous fasting resulted in a reduction in the severity of changes in this ratio when birds were subjected to a second fasting cycle. This response appeared to be specific since water deprivation resulted in a response on the fasted birds which was similar to that of birds not previously exposed to water deprivation.

Edens and Siegel (1976) reported that an increase in plasma glucose concentration occurred within 2 hr of exposure to 41 C which was followed by a rapid decline. Depletion of glycogen stores during periods of high energy demands results in an increased utilization of other substrates (*e.g.* protein, fat). Hence, the lack of differences among the heat treatment groups may be a reflection of the sampling times used since blood samples were collected 6 hr after the initiation of the daily heat cycle.

Plasma proteins are involved in the maintenance of fluid equilibrium between intercellular spaces and the vascular system (Freeman, 1971). Hence, plasma volume influences the efficiency of the cardiovascular system under heat stress conditions. While some reports have indicated that a decline in total plasma protein occurs during high ambient temperature (Deaton, *et al.*, 1969; Vo *et al.*, 1978), increases (Pardue *et al.*, 1985) as well as no alterations (Squibb *et al.*, 1959) in total plasma protein have also been reported. These differences might be related to heat treatment used (chronic *vs* acute), sampling times and food intake. The fact that a significant increase in total plasma protein occurred during the first day of late heat stress may have been related to a failure of the birds to replenish water loss through evaporative cooling as well as to the catabolic effects of glucocorticoids.

The results of this study appear to indicate that the difference in liveability between neonatally heat stressed and neonatal control groups when exposed to acute heat at 43 and 44 days of age may involved different mechanisms than those observed during acclimation to high environmental temperature.

SUMMARY

The purpose of this study was to measure certain blood parameters known to be involved in the response of birds to high ambient temperature. This was done to established the physiological basis for the greater tolerance to high ambient temperature generated by exposure to heat stress at five days of age (Chapter 1; Chapter 2). Neonatal, early, heat stress consisted of increasing temperature to between 35.0 to 37.8 C for 24 hr at five days of age in half of the pens (early heat stressed) while the remaining pens were kept at 29.4 C (early control). At 43 an 44 days of age temperature in half of the early heat stressed and early control pens was gradually increased to between 35.0 to 37.8 C for 8 hr per day (late heat; juvenile). After the 8 hr heat cycle at 43 days of age temperature was reduced to 26.4 C and after the 8 hr heat cycle at 44 days of age temperature was reduced to 21.0 C. Late heat stress significantly reduced T_3 plasma concentration while T_4 concentration was not affected. No

significant differences on glucose were observed in the late heat stress birds. Total plasma protein was significantly different between the late heat stressed and control late birds during the second sampling period at 43 days of age. No significant differences were found at any other time. Heterophil to lymphocyte ratios were lower in the early heat stressed than in the early control groups exposed to heat at 44 days of age. The results of this study appear to indicate that the lower mortality during periods of high environmental temperature in the neonatally heat stressed birds may involved different mechanisms than those observed during acclimation to high environmental temperature.

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Table 1. Effect of heat treatment on T_3 (ng/ml) and T_4 (ng/ml) at 43 and 44 days of age [1,2]

Measurement	Heat treatments[1]			
	Early-late	Early-control	Control-late	Control-control
		Day 43, morning		
T_3	2.404 ± 0.111[a]	2.119 ± 0.062[a]	2.120 ± 0.225[a]	2.171 ± 0.483[a]
T_4	15.758 ± 1.357[a]	15.831 ± 2.145[a]	13.609 ± 1.543[a]	13.267 ± 1.351[a]
		Day 43, afternoon		
T_3	0.677 ± 0.074[b]	2.573 ± 0.098[a]	0.548 ± 0.059[b]	2.698 ± 0.023[a]
T_4	15.852 ± 1.172[a]	13.789 ± 0.916[a]	9.823 ± 1.698[a]	15.905 ± 0.593[a]
		Day 44, morning		
T_3	0.629 ± 0.053[b]	2.559 ± 0.089[a]	0.712 ± 0.076[b]	2.128 ± 0.055[a]
T_4	12.987 ± 0.901[a]	13.437 ± 1.221[a]	15.220 ± 0.607[a]	14.889 ± 1.616[a]
		Day 44, afternoon		
T_3	0.777 ± 0.014[b]	2.079 ± 0.212[a]	0.806 ± 0.093[b]	2.127 ± 0.146[a]
T_4	14.241 ± 1.130[a]	13.183 ± 2.106[a]	13.263 ± 1.530[a]	14.102 ± 0.146[a]

[1] At 5 days of age, birds maintained at 29.4 C (Control) or exposed to 35 to 37.7 C for 24 hr (Early) while at 44 and 45 days of age the birds were exposed to either 21 C (Control) or exposed to 35 to 37.8 C for 8 hr per day (Late).

[2] Values represent mean ± standard error.

[a,b] Means within a row with different superscripts are significantly different ($P \leq .05$).

Table 2. Effect of heat treatment on plasma glucose concentration (mg/dl) at 43 and 44 days of age[1,2,3]

Treatment[4]	Day 43		Day 44	
	Morning	Afternoon	Morning	Afternoon
EL	244 ± 15.2	237 ± 16.6	222 ± 11.6	245 ± 9.8
EC	232 ± 9.5	265 ± 6.8	219 ± 7.7	227 ± 11.9
CL	236 ± 5.8	234 ± 8.6	229 ± 6.3	250 ± 14.3
CC	248 ± 8.3	257 ± 8.9	234 ± 5.7	238 ± 13.5

1 Values represent mean ± standard error.

2 No significant differences between treatments within a time period.

3 Blood samples collected prior to and 6 hr into the heat cycle at 43 and 44 days of age.

4 First letter.- E, Early heat stress at 5 days of age; C, early control.
Second letter.- L, Late heat stress at 43 and 44 days of age; C, late control.

Table 3. Effect of heat treatment on total plasma protein at 43 and 44 days of age[1,2]

Treatment[3]	Day 43		Day 44	
	Morning	Afternoon	Morning	Afternoon
EL	3.7 ± 0.09[a]	3.5 ± 0.05[b]	3.8 ± 0.07[a]	3.5 ± 0.05[a]
EC	4.1 ± 0.06[a]	3.3 ± 0.06[a]	3.9 ± 0.03[a]	3.5 ± 0.05[a]
CL	4.1 ± 0.10[a]	3.6 ± 0.04[b]	3.9 ± 0.06[a]	3.6 ± 0.07[a]
CC	4.1 ± 0.11[a]	3.3 ± 0.06[a]	3.9 ± 0.07[a]	3.6 ± 0.03[a]

1 Values represent mean ± standard error.

2 Blood samples collected prior to and 6 hr into the heat cycle the heat cycle at 43 and 44 days of age.

3 First letter.- E, Early heat stress at five days of age; C, early control.
Second letter.- L, Late heat stress at 43 and 44 days of age; C, late control.

a,b Means within a column with different superscripts are significantly different ($P \leq .05$).

Table 4. Effect of heat treatment on heterophil and lymphocyte numbers at 43 and 44 days of age [1,2,3]

Measurement	Heat treatments[1]			
	Early-late	Early-control	Control-late	Control-control
			Day 43	
Heterophil	11.40 ± 0.67	12.08 ± 0.90	10.08 ± 1.18	11.50 ± 0.77
Lymphocyte	48.50 ± 0.67	47.90 ± 0.90	49.90 ± 1.18	48.40 ± 0.77
Ratio X 100	23.76 ± 1.73[a]	25.70 ± 2.38[a]	20.22 ± 2.68[a]	24.14 ± 1.99[a]
			Day 44	
Heterophil	15.36 ± 0.62	11.33 ± 0.67	17.90 ± 0.77	9.90 ± 0.48
Lymphocyte	44.64 ± 0.62	48.66 ± 0.67	42.10 ± 0.73	50.10 ± 0.48
Ratio X 100	34.40 ± 1.39[b]	23.40 ± 1.67[c]	42.51 ± 1.83[a]	19.89 ± 1.15[c]

[1] At 5 days of age, birds maintained at 29.4 C (Control) or exposed to 35 to 37.7 C for 24 hr (Early) while at 44 and 45 days of age the birds were exposed to either 21 C (Control) or exposed to 35 to 37.8 C for 8 hr per day (Late).

[2] Values represent mean ± standard error.

[3] Samples collected prior to and 6 hr into the heat cycle at 43 and 44 days of age.

[a,b] Means within a row with different superscripts are significantly different ($P \leq .05$).

GENERAL SYNTHESIS

Although improvements in hot weather management have taken place, the deleterious effects of high ambient temperature is still a significant problem in poultry production. The results of this study indicate that induction of thermotolerance later in life is possible through exposure to acute heat for 24 hr at five days of age. Although the mechanism (s) of this response remains to be elucidated, it appears that thermotolerance induced by neonatal heat stress does not resemble that obtained by exposing birds to control rises in ambient temperature immediately prior to exposure to acute heat, since the former is known to decrease when temperature is reduced (Sykes and Fataftah, 1986). Instead, it appears that the improved tolerance to heat in the neonatally heat stressed birds might be related to a reduction of the bird's responsiveness to high ambient temperature. How this is accomplished is not known; however, work performed with rats indicated that stressful events early in life influences their behavioral and physiological responses when exposed to the same stressor later in life (Levine *et al.*, 1967). Naumenko and Dygalo (1983) reported that administration of hydrocortisone to pregnant rats at 16 and 18 days of gestation resulted in altered responsiveness of their offsprings to intracerebroventricular injections of norepinephrine, carbachol or serotonin. Although drawing comparisons between these studies and the response induced by neonatal heat stress might be risky, the possibility exists that neonatal heat stress might be influencing the "normal" establishment of systems involved in the response of birds to high environmental temperature.

Recently, research has shown the presence of intracellular proteins referred to as heat shock proteins (HSPs). Originally reported as a series of new puffs in the polytene gland chromosome of *Drosophyla melanogaster* which were induced by heat, sodium salicilate or dinitrophenol (Ritossa, 1962), this response has been observed in almost all organisms and tissues studied to date (see reviews by, Lindquist, 1982; Nover *et al.*, 1984; Burdon, 1986). The induction of this response is thought to be generated by stress per se since a wide range of compounds and organisms besides heat are known to elicit production of HSPs. Several reports have linked the presence of these proteins to thermotolerance (Henle *et al.*, 1978; Li

and Werb, 1982; Li *et al.*, 1983). This is supported by the following facts: induction of these proteins, in particular heat shock protein 70, by stressors other than heat generates a state of thermotolerance (Li and Hahn, 1978) and inhibition of HSP synthesis is known to result in sensitivity to heat. Dean and Atkinson (1985) demonstrated the production of these proteins *in vivo* in RBC's of heat stressed quail. The possibility that neonatal heat stress might be priming the birds so that a quicker and stronger production of these proteins occurs in response to high ambient temperature remains to be determined.

In conclusion, neonatal heat stress appears to be a practical management technique to reduce the deleterious effects of high ambient temperature. Since no advance knowledge of the weather is required, and new facilities are not needed for the induction of the protective effect, the addition of this management practice to the poultry industry's arsenal for the battle against high ambient temperature seems viable.

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COLORIMETRIC DETERMINATION OF PLASMA GLUCOSE

PRECAUTIONS:

1. Some of the substances that may interfere with the assay include: ascorbic acid, catechols, cysteine, glutathione, acetylsalicilic acid, L-dopa, mercurial diuretics, tetracycline. All tend to decrease the value to some degree.

2. Contact with rubber by reagents or water used in preparing reagents must be avoided since certain substances present in the rubber may inhibit the reaction.

3. A new blank must be prepared for each series of tests (Blank color will increase with time).

4. Distilled water must be used in the preparation of all reagents.

PREPARATION OF SOLUTIONS:

1. PGO Enzymes:

   - Dissolve 1 capsule in distilled water.

   - Bring up to 100 ml. (Gentle shaking).

   - Store in an amber bottle as follows:

     0-5 C, solution is stable for 1 month.

     -20 C, solution is stable for at least 6 months.

2. Dianisidine Dihydrochloride (Color Reagent):

   - Reconstitute vial with 20 ml distilled water.

   - Stable for 3 months at 0-5 C.

3. PGO enzyme solution and Dianisidine Dihydrochloride solution:

   - To 100 ml PGO enzyme solution, add 1.6 ml of Dianisidine Dihydrochloride (color reagent).

   - Solution is stable for up to a month at 0-5 C, unless turbidity develops.

PROCEDURE:

1. Label tubes as follows: blanks, standards, sample 1, sample 2 ect. Run all blanks, standards and samples in duplicate.

2. Set up a standard curve such that values are within range for avian plasma glucose concentration (Refer to Beljan *et al*, 1971).

3. Pipette into the tubes as follows:

	Blank	Sample
Distilled H_2O (ml)	0.500	0.48
Sample (ml)	-	0.02
PGO Enzyme-color reagent sol. (ml)	5.0	5.0

4. Mix each tube thoroughly

5. Incubate all tubes at room temperature (18-26 C) for 45 min or for 30 ± 5 min at 37 C.

   NOTE: AVOID EXPOSURE TO BRIGHT LIGHTS

6. Read and record absorbance (abs) of blanks, standards, and tests by setting the Spectrophotometer at 450 nm. (visible, single wavelength, 6 seconds draw time)

   NOTE: READINGS SHOULD BE COMPLETED WITHIN 30 MIN.

RESULTS:

Plasma glucose concentrations were read from the standard curve.

Expressed in SI (Systeme International) units:

$$\text{Plasma Glucose (mmol / l)} = \frac{\text{Plasma glucose (mg / 100 ml)}}{18}$$

PREPARATION OF TUBES FOR BLOOD COLLECTION

NOTES:

1. At room temperature, glucose undergoes glycolysis at a rate of 5% per hr. Leukocytosis and bacterial contamination accelerate the loss.

2. Fluoridated blood is stable for several hours at room temperature.

PROCEDURE:

1. Prepare by dissolving 4 g of Sodium Fluoride in 100 ml of distilled water. (Heating will facilitate solution)

2. Cool solution to room temperature.

3. Add 0.61 g (140 USP/mg) Heparin Sodium salt.

4. Store at room temperature.

PREPARATION OF HEPARIN / SODIUM FLUORIDE TUBES:

1. Into a tube add 5 drops of Heparin / Sodium Fluoride solution.

2. Oven dry at 60 C. (In order to reduce any contribution to the total volume of the blood sample collected.- small amounts). Not neccessary if large volumes are going to be harvested.

3. Cool and stopper.

4. Stable at room temperature.

REFERENCES

Beljan, J. R., T. I. Madley, A. B. Hellewell, and L. J. Hein, 1971. Determination of selected avian blood plasma chemistry values using the Technicon Autoanalyzer®. Poultry Sci. 50:229-232.

Raabo E., and TC Terkildsen, 1960. On the enzymatic determination of blood glucose. Scan. J. Clin. Lab. Invest. 12:402.

Sigma Tech bulletin No 510 (1-78).

BIORAD BRADFORD PROTEIN ASSAY
REDUCED SAMPLE VOLUME PROTOCOL

PROCEDURES:

1. Dilute BioRad dye reagent concentrate 1:5 with double distilled water. Filter through a Whatman No. 1 paper. Make enough dye reagent to used 2 ml in each standard and unknown and enough to run a single blank between each set of duplicate standards and unknowns. Reason for running blanks between duplicates: dye tends to build up on the inside of the tubing (if a sipper is being used).

2. Reconstitute the Bovine Serum Albumen protein standard provided by the kit so that a 1.4 mg/ml concentration is achieved.

3. Pippete into the tubes as follows:

Tube #	μ BSA Stock	μl Buffer	Dye Reagent (ml)
1,2	0	40	2
3,4	5	35	2
5,6	10	30	2
7,8	15	25	2
9,10	20	20	2
11,12	30	10	2
13,14	40	0	2
Unknowns		total volume 40 μl	

4. Determine dilution required for sample to be within the standard curve (20 μl of a 1:20 dilution was used with plasma from 7 week old broiler cockerels).

5. Mix.

6. Allow to stand for 5 minutes.

7. Measure OD-595: read each set of duplicates, a single blank, then the next set of duplicates.

8. Obtain sample concentration from the standard curve. Adjust for dilution.

9. Spectrophotometer could be clean by running through a 0.1% Sodium Dodecyl Sulfate solution (SDS) several times. Then, it should be rinse thouroughly with double distilled water.

REFERENCES

Bradford, M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein binding utilizing the principle of protein-dye binding. Anal. Biochem. 72:248-254.

MAY GRUNWALD GIEMSA STAIN (HETEROPHIL/LYMPHOCYTE RATIO)

SOLUTIONS

- May-Grunwald Stain.- Do not dilute.-
- Giemsa Stain.-

  Add 20 cc of Giemsa stain to 7 cc of distilled water. (enough for 15 slides).

  This solution must be used within 1 hour of mixing.

PROCEDURE:

1. Flood slides with May-Grunwald Stain. Let it sit for 7 minutes.

2. Add distilled water to the slides.-(just enough to cover the slides).- Mix the two by blowing air over the slides for 4 minutes.-

3. Pour stain off the slides.-

4. Flood the slides with Giemsa Stain. Let it sit for 18 minutes. Mix the stain by blowing air over the slides.- (not as often).-

5. Pour the stain off and dip the slides on Methanol.- (4 to 5 dips).-

6. Dry in Bibulous paper.- (put slides in the book and close it, DO NOT WIPE THE SMEAR SIDE OF THE SLIDES).-

7. Wipe THE BACK of the slides with kimwipes and distilled water if necessary.-

Appendix B Table 1. Analysis of variance for body weight at 28 days of age (Chapter 1).

Source of variation	df	Sum of squares (10^{-4})	F value	P
Trmt	1	56	8.61	0.0064
Error	30	195		
Total	31	252		

Appendix B Table 1. Analysis of variance for feed consumption at 28 days of age (Chapter 1).

Source of variation	df	Sums of Squares (10^{-4})	F value	P
Trmt	1	386	16.53	0.0003
Error	30	701		
Total	31	1087		

Appendix B Table 1. Analysis of variance for feed efficiency at 28 days of age (Chapter 1).

Source of Variation	df	Sums of Squares (10^{-4})	F value	P
Trmt	1	78	3.31	0.0788
Error	30	71		
Total	31	149		

Appendix B Table 1. Analysis of variance for body weight at 42 days of age (Chapter 1).

Source of variation	df	Sums of Squares (10^{-4})	F value	P
Trmt	1	2	0.14	0.7151
Error	30	635		
Total	31	637		

Appendix B Table 1. Analysis of variance for feed efficiency 42 days of age (Chapter 1).

Source of variation	df	Sums of Squares (10^{-4})	F value	P
Trmt	1	9	20.54	0.0001
Error	30	14		
Total	31	23		

Appendix B Table 2. Analysis of variance for body weight at 48 days of age (Chapter 1).

Source of variation	df	Sums of Squares (10^{-4})	F value	P
Feed Treatment	1	1	0.04	0.8525
Heat treatment	3	120	1.32	0.2910
Feed x Heat	3	202	2.22	0.1121
Error	24	730		
Total	31	1054		

Appendix B Table 2. Analysis of variance for body weight gain for the period between 42 to 48 days of age (Chapter 1).

Source of variation	df	Sums of Squares (10^{-4})	F value	P
Feed Treatment	1	15	0.78	0.3848
Heat treatment	3	221	3.63	0.0273
Feed x heat	3	40	0.66	0.5826
Error	24	488		
Total	31	766		

Appendix B Table 2. Analysis of variance for cumulative feed efficiency at 48 days of age (Chapter 1).

Source variation	df	Sums of Squares (10^{-4})	F value	P
Feed Treatment	1	1	0.73	0.4013
Heat Treatment	3	29	7.49	0.0010
Feed x Heat	3	2	0.58	0.6328
Error	24	31		
Total	31	63		

Appendix B Table 2. Analysis of variance for feed consumption for the period between 42 to 48 days of age (Chapter 1).

Source of variation	df	Sums of Squares (10^{-4})	F value	P
Feed treatment	1	194	2.55	0.1230
Heat treatment	3	108	0.47	0.7031
Feed x Heat	3	691	3.03	0.0491
Error	24	1827		
Total	31	2822		

Appendix B Table 2. Analysis of variance for feed efficiency for the period between 42 to 48 days of age (Chapter 1).

Source of variation	df	Sums of Squares (10^{-4})	F value	P
Feed treatment	1	4	0.19	0.6655
Heat treatment	3	175	2.42	0.0910
Feed x Heat	3	25	0.35	0.7923
Error	24	581		
Total	31	787		

Appendix B Table 3. Table of contrasts for Chi Square Analysis for mortality at 42 days of age (Chapter 1).

Contrast	Value of statistic	df	critical value	conclusion
ELC vs ELFR	0.54	1	9.950	ACCEPT
ECC vs ECFR	0.25	1	9.950	ACCEPT
CLC vs CLFR	2.00	1	9.950	ACCEPT
CCC vs CCFR	4.76	1	9.950	ACCEPT
E vs C	0.12	1	9.950	ACCEPT

Appendix B Table 3. Table of contrasts for Chi Square Analysis for mortality at 43 and 44 days of age (Chapter 1).

Contrast	Value of statistic	df	critical value	conclusion
ELC vs ELFR	1.00	1	10.882	ACCEPT
ECC vs ECFR	0.00	1	10.882	ACCEPT
CLC vs CLFR	0.42	1	10.882	ACCEPT
CCC vs CCFR	0.00	1	10.882	ACCEPT
EL vs CL	48.01	1	10.882	REJECT
EL vs CC	0.00	1	10.882	ACCEPT
EC vs CC	0.00	1	10.882	ACCEPT
EL vs EC	1.00	1	10.882	ACCEPT
E vs CC	1.00	1	10.882	ACCEPT
CL vs rest	48.01	1	10.882	REJECT

Appendix C Table 2. Analysis of variance for body weight at 49 days of age (Chapter 2).

Source of variation	df	Sum of squares	F value	P
Supp	1	0	0.00	0.971
Heat	3	121	0.82	0.496
Supp X Heat	3	4	0.27	0.844
Error	24	118		
Total	31	134		

Appendix C Table 2. Analysis of variance for feed consumption at 49 days of age (Chapter 2).

Source of variation	df	Sum of squares	F value	P
Supp	1	6	0.06	0.8156
Heat	3	483	1.43	0.2576
Supp X Heat	3	53	0.16	0.9227
Error	24	2696		
Total	31	3240		

Appendix C Table 2. Analysis of variance for feed consumption at 49 days of age (Chapter 2).

Source of variation	df	Sum of squares	F value	P
Supp	1	6	0.06	0.8156
Heat	3	483	1.43	0.2576
Supp X Heat	3	53	0.16	0.9227
Error	24	2696		
Total	31	3240		

Appendix C Table 2. Analysis of variance for feed efficiency at 49 days of age (Chapter 2).

Source of variation	df	Sum of squares	F value	P
Supp	1	0	0.03	0.8646
Heat	3	2	0.37	0.7751
Supp X Heat	3	2	0.39	0.7630
Error	24	36		
Total	31	40		

Appendix C Table 3. Analysis of variance for body weight gain from 43 to 49 days of age (Chapter 2).

Source of variation	df	Sum of squares	F value	P
Supp	1	0	0.00	0.9823
Heat	3	2	1.59	0.2189
Supp X Heat	3	7	0.66	0.5847
Error	24	792		
Total	31	1015		

Appendix C Table 4. Analysis of variance for water consumption from 41 to 45 days of age (Chapter 2).

Source of variation	df	Sum of squares	F value	P
Heat	3	522	36.95	0.0001
Supp	1	1	0.28	0.5949
Time	4	903	47.87	0.0001
Heat X Time	12	240	4.25	0.0001
Heat X Supp	3	42	2.97	0.0345
Heat X Supp X Time	16	33	0.43	0.9706
Error	120	566		
Total	159	2308		

Appendix C Table 6. Analysis of variance for core and surface body temperature at 43 and 44 days of age (Chapter 2).

Source of variation	df	Sum of squares	F value	P
Heat	3	107.2	117.7	0.0001
Supp	1	0.525	1.73	0.1909
Time	1	1.711	5.64	0.0192
Heat X Time	3	0.948	1.04	0.3772
Heat X Supp	3	1.705	1.87	0.1381
Error	116	35.2		
Total	127	147.3		

Appendix C Table 7. Table of contrasts for Chi Square Analysis for mortality at 42 days of age (Chapter 2).

Contrast	Value of statistic	df	critical value	conclusion
ELC vs ELS	3.86	1	8.052	ACCEPT
ECC vs ECS	0.10	1	8.052	ACCEPT
CLC vs CLS	0.00	1	8.052	ACCEPT
CCC vs CCS	1.32	1	8.052	ACCEPT
E vs C	1.62	1	8.052	ACCEPT
EL vs CL	0.20	1	8.052	ACCEPT
EL vs CC	0.10	1	8.052	ACCEPT
El vs EC	3.12	1	8.052	ACCEPT
EC vs CC	2.12	1	8.052	ACCEPT
CL vs EC	4.82	1	8.052	ACCEPT
CL vs CC	0.58	1	8.052	ACCEPT

Appendix C Table 7. Table of contrasts for Chi Square Analysis for mortality at 43 and 44 days of age (Chapter 2).

Contrast	Value of statistic	df	critical value	conclusion
ELC vs ELS	5.76	1	7.477	ACCEPT
ECC vs ECS	3.00	1	7.477	ACCEPT
CLC vs CLS	1.04	1	7.477	ACCEPT
CCC vs CCS	0.32	1	7.477	ACCEPT
EL vs CL	48.06	1	7.477	REJECT
EL vs CC	13.50	1	7.477	REJECT
EL vs EC	13.50	1	7.477	REJECT
EC vs CC	0.00	1	7.477	ACCEPT

Appendix D Table 1. Analysis of variance for plasma T_4 concentration at 43 and 44 days of age (Chapter 3).

Source of variation	df	Sum of squares	F value	P
Trmt	3	19.29	0.85	0.474
Period	3	3.88	0.17	0.916
Trmt X Period	9	88.39	1.29	0.262
Error	54	410.10		
Total	69	521.68		

Appendix D Table 1. Analysis of variance for plasma T_3 concentration at 43 and 44 days of age (Chapter 3).

Source of variation	df	Sums of Squares	F value	P
Trmt	3	28.68	92.07	0.0001
Period	3	8.94	28.69	0.0001
Trmt X Period	9	15.05	16.11	0.0001
Error	65	6.75		
Total	80	59.41		

Appendix D Table 2. Analysis of variance for plasma glucose concentration at 43 and 44 days of age (Chapter 3).

Source of Variation	df	Sums of Squares	F value	P
Trmt	3	5.6	0.54	0.655
Period	3	6.1	0.60	0.618
Trmt X Period	9	35.6	1.15	0.332
Error	122	419.9		
Total	137	467.3		

Appendix D Table 3. Analysis of variance for total plasma protein at 43 and 44 days of age (Chapter 3).

Source of variation	df	Sums of Squares	F value	P
Trmt	3	0.234	1.52	0.211
Period	3	7.278	47.13	0.001
Trmt X Period	9	0.416	0.90	0.527
Error	166	8.546		
Total	181	16.476		

Appendix D Table 4. Analysis of variance for heterophil to lymphocyte ratio at 43 and 44 days of age (Chapter 3).

Source of variation	df	Sums of Squares (10^{-4})	F value	P
Trmt	3	941.48	7.40	0.02
Period	1	408.96	9.64	0.002
Trmt X Period	3	249.57	1.96	0.126
Error	80	3393.21		
Total	87	4993.22		

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