Characterizing physiological and energetic responses of young chicks to stress alleviating measures for long-journey air transport

by

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This is to certify that the Master's thesis of

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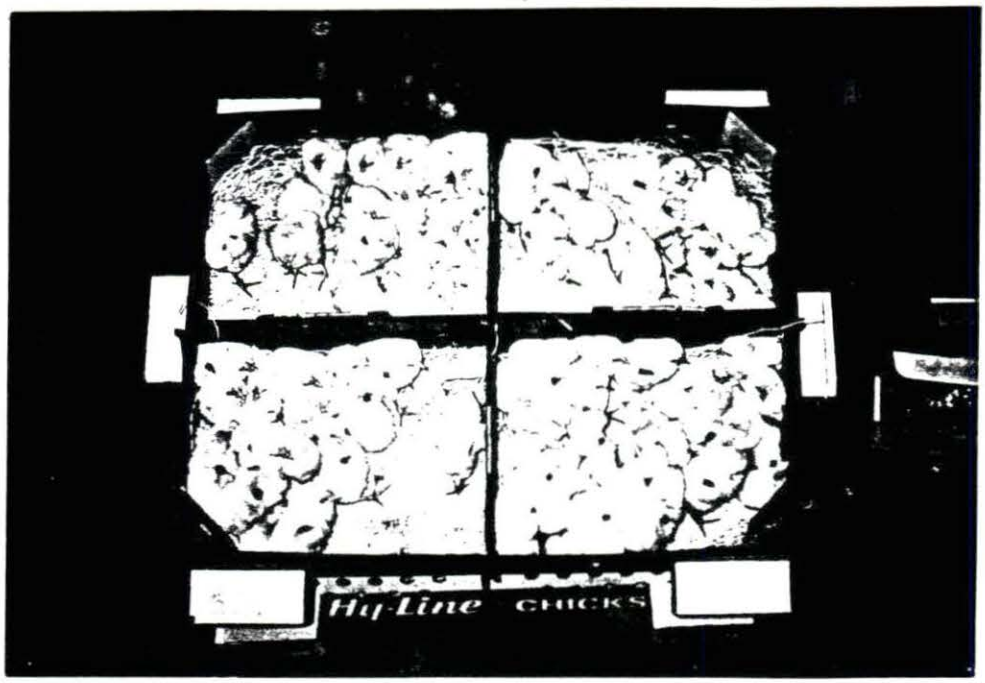
CHAPTER 1. INTRODUCTION

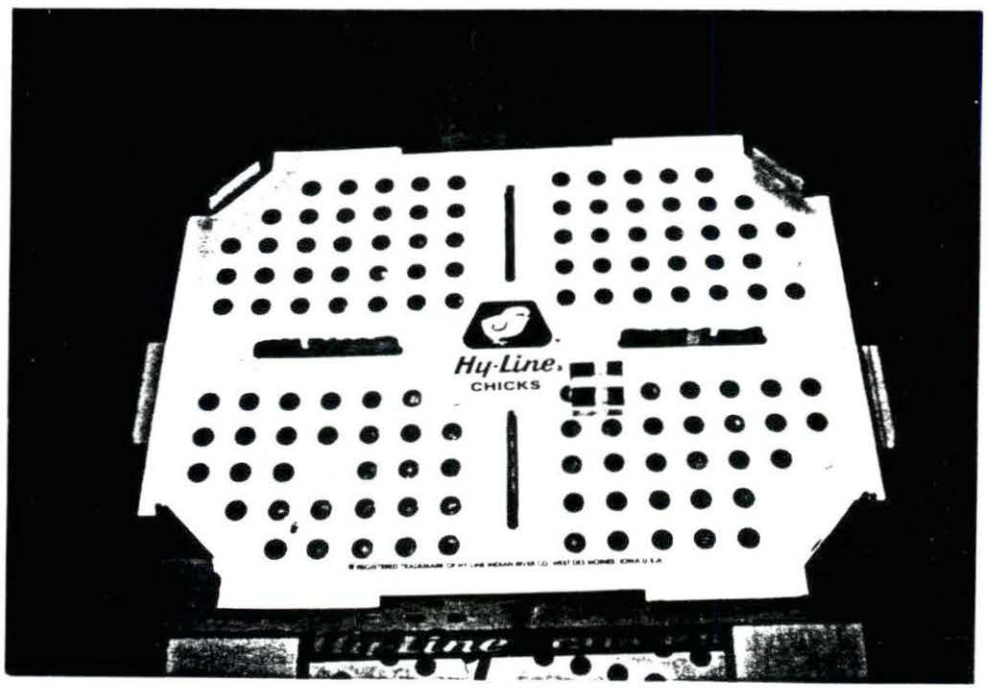
Background

An increasing number of one-day-old breeding chicks are being exported from the U.S.A. to other countries. In this process, many companies place chick containers in the cargo area of commercial airplanes. Upon arriving in the country, the chicks are then delivered to the farm by other transportation means such as bus or truck. Many companies use cardboard shipping boxes such as the one shown in Figure 1. During transportation, the chicks may experience extreme temperatures, relative humidity, and sudden changes in the thermal conditions. Previous experience and studies have shown that chicks undergoing a short-journey transportation (i.e., to countries in Europe or South America) arrived in good condition; however, chicks undergoing a long-journey transportation (i.e., to Asia) often arrived with severe problems such as weight loss or mortality (Xin and Rieger, 1995). Xin and Rieger (1995) found that the shipments to China last 41 to 72 hours although the actual flight time is less than 20 hours and that chick mortality increases with journey duration.

In summer, the chicks in transit are susceptible to heat stress which may be aggravated by: heat production of the chicks, inadequate ventilation inside the container, thermal conditions of the storage area, and the bird's natural tendency to congregate. This is especially the case on hot and humid days. Furthermore, the inaccessibility of water and water evaporation from the chicks' bodies over an extended period can cause dehydration of the chicks. In winter, chicks are likely to suffer from cold stress. The unusually low relative humidity (< 20 %) in the aircraft presumably accelerates the dehydration of chicks (Xin and



(A)



(B)

Figure 1. A) An inside view of the chick container showing four compartments.
B) An outside view of the chick container.

Rieger, 1995). It is generally the policy of the breeding company to credit the customers for, or to replace, mortalities encountered during the first seven days on the farm.

Previous studies indicated that uncomfortable thermal conditions were the major environmental problem during ground transportation of market size poultry (Kettlewell, 1989; Webster et al., 1993). Henken et al. (1987) showed drastic weight loss in chickens during long time exposure to high temperature. Poor health conditions before transportation, physical injury, fasting, handling of chickens, and emotional stress have also been reported as adverse factors on chicks being transported (Nicol and Scott, 1990; Broom, 1990). These stress factors can cause a significant number of dead-on-arrival chicks or severe weight loss (Bayliss and Hinton, 1990) .

Extreme temperature and relative humidity, and sudden changes in the thermal factors are considered detrimental to chicks. The study of Xin and Rieger (1995) revealed that the ambient temperature during transportation ranges from 20 ^{o}C to 38 ^{o}C, the container temperature ranges from 30 ^{o}C to 45 ^{o}C, and the relative humidity (RH) ranges from 12 % to 62 %. This study also showed high fluctuations of both temperature and RH and a positive relationship between chick mortality and transport duration.

To address this important industry issue, research is needed to investigate the mechanisms of the chick stress and thus mortality and to explore practical stress-alleviation means.

Objectives

The objectives of this project were:

1) To test the hypothesis of dehydration being the main cause to high chick mortality associated with long-journey transportation;
2) To explore and evaluate means to alleviate chick stress and early mortality for long-journey transportation, particularly the suitability of a water substitute (Aqua Jel®) for use during transportation; and
3) To evaluate physiological and energetic responses of the chicks to various transportation conditions, specifically body water content, packed cell volume (PCV), and metabolic heat and moisture production.

CHAPTER 2. LITERATURE REVIEW

Heat Production

The body temperature is determined by the balance between the quantity of heat produced in the body and the amount of heat lost from the body to the environment.

Heat Production - Heat Loss = Heat Storage

Heat loss represents the loss of body heat by evaporation, radiation, convection, and conduction. Negative heat loss implies heat gain. Heat storage forms a buffer which enables an animal to balance its thermal equilibrium state for short-time periods in an uncomfortable condition. Compared to large animals, small animals have less heat storage capacity per unit surface area. Due to the small heat storage, the small animal can not withstand rapid changes in environmental temperature as long a time as large animals (Kettlewell, 1989). The heat, a result of metabolic processes in the body, flows from the core to the surface of the body and then to the environment by means of radiation, convection, conduction, and evaporation of water. Specifically, the sum of radiative, convective, and conductive heat losses is referred to as sensible heat loss and the evaporative heat loss is referred to as insensible or latent heat loss. Sensible heat is lost primarily from the outer surfaces of animal, whereas latent heat is lost from the respiratory tracts via expired air (Albright, 1990). The sum of sensible heat and latent heat is also referred to as total heat production (THP).

Convection is the heat transfer by a stream of molecules from a warm part to a cooler part. The blood steam is the convectional heat transport within body, and it has great flexibility as a means of heat transport. During cold weather, peripheral blood flow is

decreased (vasoconstriction) to reduce heat losses. During hot weather, peripheral blood flow is increased (vasodilation) to enhance heat dissipation. Conduction is the heat transfer due to molecular collisions within the material. Heat transport by conduction occurs across the tissues. Radiation is the heat transfer in the form of infrared heat rays, a type of electromagnetic wave. Radiative heat flow depends on the temperature and the nature of the surface of the radiating body. The animal receives radiant heat from objects which are warmer than itself, and conversely gives radiant heat to objects which are cooler than itself. Evaporation heat losses occur through the skin and lungs (unlike mammals, birds do not have sweat glands). Water from the body is continuously and insensibly evaporated and it causes continual heat loss (Guyton, 1991; Kettlewell, 1989; Seagrave, 1971). Evaporation decreases with increasing air humidity, and it will cease when the relative humidity reaches 100 %.

Heat production is commonly determined by either direct or indirect calorimetry. The direct calorimetric method measures the total quantity of heat liberated from the body in a given time, since in a state of thermal equilibrium metabolic heat production equals heat loss (Scott, 1983). The direct calorimeter is an insulated metal container surrounded by water. The animal's heat production is measured by the change in temperature of the water in the walls of the calorimeter (Ganong, 1993). Heat production can also be computed by the indirect calorimetric method which measures gaseous exchange because more than 95 per cent of the energy expended in the body is derived from reactions of oxygen with the different foods (Guyton, 1991). The indirect calorimeter is most commonly used and more flexible in housing the animals (Sturkie, 1986; Nienaber and Maddy, 1985). The following

are algorithms to calculate metabolic rate of animals (Brouwer, 1965) and two modified algorithms to verify the values of metabolic rate for chicks. The algorithm of metabolic heat production (Brouwer, 1965) has the form of

$$H = 3.866 * O_2 + 1.2 * CO_2 - 0.518 * CH_4 - 1.431 * N \quad \text{[Eq 1.]}$$

where H = heat production (kcal)

O_2 = oxygen consumption (liters)

CO_2 = carbon dioxide production (liters)

CH_4 = methane production (liters)

N = urinary nitrogen (g)

Chicks produce negligible amount of methane (CH_4), thus it was omitted from the equation. Urinary nitrogen excretion (N) may be expressed as 0.032 ($\pm$ 0.01 SD) gram per liter oxygen consumption (McLean, 1972). Hence,

$$H = 3.866 * O_2 + 1.2 * CO_2 - 1.431 * 0.032 * O_2 \quad \text{[Eq 2.]}$$

Romijn and Lokhorst (1961[a]) used the following equation to calculate the heat production of birds from gaseous exchange

$$H = 3.871 * O_2 + 1.194 * CO_2 \quad \text{[Eq 3.]}$$

The O_2 consumption and CO_2 production can be expressed as

$$O_2 = v_i x_i - v_o x_o$$

$$CO_2 = v_o y_o - v_i y_i$$

where v_i = the volume of inlet air

v_o = the volume of outlet air

x_i = O_2 concentration in inlet air

x_o = O_2 concentration in outlet air

y_i = CO_2 concentration in inlet air

y_o = CO_2 concentration in outlet air

The oxygen gas analyzer detects O_2 concentration in dry air. The dry air volume is the volume of moist air subtracted by the volume of water vapor in the air.

$$V_{dry\ air} = V_{moist\ air} - V_{water}$$

$$V_{water} = (P_{water} / P_{moist\ air}) * V_{moist\ air}$$

where $V_{dry\ air}$ = the volume of dry air

$V_{moist\ air}$ = the volume of moist air

V_{water} = the volume of water in moist air

P_{water} = the partial pressure of water vapor

$P_{moist\ air}$ = the pressure of moist air

The partial pressure of water vapor can be calculated by one of the following equations:

1. Weiss (1977) equation:

$$P_{water} = 0.61078 \exp \frac{17.2693882t}{t + 237.3} \qquad \text{[Eq 4.]}$$

where t= the dew point temperature of air (^oC)

P_{water} = the partial pressure of water vapor (kPa)

or $$P_{water} = 4.582 \exp \frac{17.27t}{t + 237.2} \qquad \text{[Eq 5.]}$$

where t= the dew point temperature (^{o}C)

P_{water} = the partial pressure of water (mmHg)

2. Albright equation (1990):

$$P_{water} = \phi P_{ws} \quad \text{[Eq 6.]}$$

$$P_{ws} = \exp(-5.8002206E+03/T + 1.3914993 - (48.640239E-03)(T) + (41.764768E-06)(T)^2 - (14.452093E-09)(T)^3 + 6.5459672 \ln(T)) \quad \text{[Eq 7.]}$$

where P_{water} = the actual partial pressure of water (Pa)

P_{ws} = the water vapor saturation partial pressure (Pa)

T = temperature (Kelvin)

ϕ = relative humidity

Latent heat production can be calculated by

$$H_{latent} = M \times h \times \Delta W \text{ (w)} \quad \text{[Eq 8.]}$$

Where M = air mass flow (g/s)

$$M \text{ (g/s)} = \frac{V(l/\min) * 28.9(g/mole)}{22.4(l/mol) * 60(\min/\sec)} \quad \text{[Eq 9.]}$$

h = the heat of vaporization of water (540cal/g)

ΔW (kg/kg) = $(W_o - W_i)$

$$W = 0.62198 * \frac{P_{water}}{(P - P_{water})} \quad \text{[Eq 10.]}$$

where V = volumetric air flow (l/s)

W = humidity ratio (kg/kg)

W_o = humidity ratio of outlet air (kg/kg)

W_i = humidity ratio of inlet air (kg/kg)

P_{water} = actual water vapor pressure (kPa)

P = barometric pressure (kPa).

Sensible heat production was calculated as the difference between the total heat production (THP) and latent heat production (LHP), i.e., SHP = THP - LHP.

Stress

The definition of stress is "force or pressure caused by difficulties in life" (Longman dictionary, 1986). Stress is very subjective and different for different species and different characteristics of the same species (Kettlewell, 1989). Fraser et al., (1975) proposed that "an animal is said to be in a state of stress if it is required to make abnormal or extreme adjustments to its physiology or behavior to cope with adverse aspects of its environment and management". Stress is one of the main reasons for birds' death during transportation (Bayliss and Hinton, 1990; Webster et al., 1993), and physiological responses of poultry to various stresses have been reported by many researchers (Freeman, 1976; Hill, 1983; Kettlewell, 1989; Nicol and Scott, 1990). During transportation, birds may be subjected to both thermal and nutritional stressors. High or low ambient temperature and high or low relative humidity cause thermal stress to the chick. Long-time fasting and deprivation of water also add stress. Among these stresses, the thermal stress is acute to chicken during transportation. Thermal stress can be caused by both ambient temperature and relative

humidity (Egbunike, 1979). A bird maintains its homeothermy by balancing heat production and heat loss. Birds have a body temperature of 41.2 - 42.2 °C (Whittow, 1965). However, the body temperatures of birds greatly depend on size, breed, environmental conditions, and sex. The optimal ambient temperature is approximately 24 °C (75 °F), and the ideal temperature range is from 15 °C to 30 °C for the adult hen (Austic and Nesheim, 1990). At ambient temperatures above 27 °C, the hen has an elevated rectal temperature (Meltzer et al., 1982), and long exposure of a hen to an ambient temperature of 38 °C can be fetal. The upper rectal temperature limit of the hen is 45 °C (Dukes, 1977; Kettlewell and Moran, 1992). Thermal stress can develop sooner for hens than for cockerels at temperatures above 32 °C. Thermal stress can begin more quickly in an environment which has high moisture content (Romijn and Lokhorst, 1961[b]) due to the limit of evaporative heat loss. If birds can not maintain their body temperature under the weather conditions, they are in a state of thermal stress.

Birds can regulate sensible heat loss by adjusting their behavior, such as spreading their wings, selecting a comfortable environment, and huddling, and by physiological responses, such as vasodilation or vasoconstriction (Nicol and Scott, 1990; Schein and Hafez, 1969; Webster et al., 1993). However, if a chicken is in the limited area with others (e.g., in a cage or a container), it has very little ability to carry out the thermoregulatory postures; consequently, its thermal comfort zone is narrowed. At high air temperatures, the chicken reduces sensible heat loss but elevates the evaporative heat less (Farrell and Swain, 1977). When the environmental temperature reaches the body temperature, the sensible heat loss

becomes zero, and heat loss mainly relies on evaporation, and a chicken will experience hyperthermia (Kettlewell, 1989; Van Kampen, 1981; Webster et al., 1993). In addition, if high humidity is associated with high temperature, the evaporative heat loss is limited, and chickens will experience more heat stress.

Excessive heat loss during transportation can occur due to either low environmental temperature or excessive air movement, or both. This forces the chicken to elevate heat production by shivering, or it causes hypothermia. Chicken feathers have great insulatory properties. A well-feathered chicken would be thermally comfortable within a travelling container at 6.5 to 22 ^{o}C in still air, at 15 to 26 ^{o}C when air movement is 0.5 m/s, and at 24 to 32 ^{o}C when air movement is 3.3 m/s (Webster et al., 1993). However, the insulatory properties of feathers are greatly reduced when they are wet (Nicol and Scott, 1990). The insulatory properties of the down of small chicks are unknown.

Fasting is one of the stress factors that results in weight loss of animals. After six hours of fasting, the intestinal content of a chicken becomes minimal (Veerkamp, 1978), and liver glycogen is negligible (Warriss et al., 1988). However, birds are fasted for longer periods during most long-journey transportation. Fasting may reduce heat stress due to reduced metabolic activity (Mitchell and MacLeod, 1988). Fasting of market birds is recommended in hot weather to prevent birds arriving at the processing plant with full intestines (Benoff, 1986). But, after 4 - 6 hours of fasting, weight loss occurs at a rate of 0.2 - 0.5 percent of initial body weight per hour (Veerkamp, 1978). As the blood glucose level declines due to fasting, gluconeogenesis (the synthesis of glucose from protein or lipid

precursors) is accelerated, using glycerol, amino acids, and latic acid. As fasting continues, peripheral tissues further restrict the use of the glucose, and ketone bodies become the primary energy source. As a result, fasting animals gradually become weak and lethargic because peripheral systems are weakened by protein catabolism and changed pH levels (Martini, 1992). Fasting also elicits physiological responses of stress in chicks (Freeman et al., 1980). Kelley (1980) concluded that common stressors alter the immune system of animals, change their susceptibility to infectious disease, and influence the etiology of diseases.

Body Fluid

Body water is very important to the basic physiological function of the body and accounts for almost two-thirds of its total weight. Changing body water content can have fatal consequences because all physiological systems will be affected. The body is composed of trillion of cells, and body fluids fill the interior of these cells and the gaps between them. If body fluids freeze or become hot, cells and tissues will stop working or be destroyed. However, due to the hydrogen bonding structure and high heat capacity of water, the body temperature is stabilized. Body water prevents rapid changes in body temperature and quickly distributes heat from one region to another in the body.

Body water also transports dissolved gases. It brings oxygen from the lungs to the tissues and cells. It distributes nutrients, and it transports metabolic wastes from peripheral

tissues to sites of excretion, such as the kidneys. It regulates the pH and electrolyte composition throughout the body.

The body water is divided into extracellular and intracellular compartments. Fluid inside the cells is called intracellular fluid (ICF), and nearly two-thirds of the total body water content is intracellular fluid. Extracellular fluid (ECF) is any fluid outside the cells. Extracellular fluid is composed of interstitial fluid, plasma of the blood, lymph, cerebrospinal fluid (CSF), synovial fluid, serous fluids, aqueous humor, perilymph, and endolymph (Guyton, 1991; Martini, 1992).

Because young birds have relatively leaner body tissue than adults, young birds have more total body fluid than adults (Sturkie, 1986). The total amount of body water in a one-week old chick (55.1g body weight) is approximately 0.04 liters, averaging 72.4 percent of the chick's total body weight. The body fluid percentage of birds varies with age, and it progressively decreases from young to old age. The total body water of 32 week-old chicken (1.76 kg body weight) is approximately 57.3 percent of its total body weight (Sturkie, 1986) and becomes 52.9 percent of its total body weight at 55 weeks of age (2.054 kg) (Weiss, 1958). The distribution of extracellular and intracellular fluid compartments varies with age (Ganong, 1993). Intracellular fluid progressively increases from the sexually immature condition to sexually maturity, and extracellular fluid significantly decreases from young to old age. Also, the ratio of ECF volume to ICF volume is larger in the immature chickens than in the mature birds(Sturkie, 1986).

Abnormality of body fluid can be detected by hematocrit. The hematocrit is the percentage of red blood cells in whole blood. It is easily measured by centrifugation of blood in a hematocrit tube , which results in blood cells and plasma being separated. Normal erythrocyte volumes of chickens are 29 % for sexually immature female and male chickens, 45 % for sexually mature males and 29 % for sexually mature females (Sturkie, 1986). Specifically, the hematocrit for a 1 week-old female chick is 27.5 ± 1.5 % (Medway and Kare, 1959). In severe anemia, the hematocrit may fall to around 10, barely sufficient to sustain life. In polycythemia (excessive production of red blood cells), the hematocrit rises to 65, the upper limit of the hematocrit level; excessive hematocrit causes the blood to become viscous, and as a result the peripheral vascular tree is plugged.

Edema is the presence of excess fluid in the tissues of the body. Depression of metabolic systems of tissues or lack of adequate nutrition to the cells can cause serious edema. In severe starvation, local blood flow is depressed and delivery of oxygen and other nutrients is too low to maintain normal tissue metabolism, which depresses the cell membrane ionic pumps. As a result, osmotic pressure of the blood declines and fluids begin moving from the blood into peripheral tissues throughout the body. Edema also occurs in inflamed tissue areas. Inflammation makes cell membranes increase their permeability, allowing sodium and other ions to diffuse to the interior of cells, and pump fails to remove these ions. This causes diffusion of water into the cells. Edema is also caused by abnormal leakage of fluid from the blood capillaries, failure of the lymphatic system, and by renal retention of salt and water.

Anemia is a deficiency of red blood cells. It can be caused either by slow production or rapid loss of red blood cells; the oxygen-carrying capacity of the blood is reduced, causing a lack of the delivery of oxygen to peripheral tissues. Anemia causes premature muscle fatigue, a lack of energy, weakness, and lethargy. After hemorrhage, the body replaces the plasma within 1 to 3 days, but there is still a low concentration of red blood cells. Blood viscosity may fall to as low as 1.5 times that of water (the normal value is about 3 times that of water) (Guyton, 1991). The resistance to blood flow is decreased in the peripheral vessels and blood return to the heart is increased. Moreover, tissue vessels' dilation due to hypoxia causes further increased blood return to the heart and increases the cardiac output. The increased work load on the heart is one of the major effects of anemia.

Dehydration

Dehydration is a major problem in hot weather. Dehydration is the loss of body water from all fluid compartments in the body. The main causes of dehydration are:

1) excessive water loss by excretion,

2) inadequate intake of fluid and electrolytes, or

3) failure of the kidneys to reabsorb water and electrolytes.

Due to the lean body and high percentage of extracellular fluid, dehydration develops more rapidly and severely in young chicks. It was reported that dehydration for a few days (3 or more days) caused increased hematocrit, decreased blood volume, decreased blood pressure, increased plasma sodium, and increased heart rates (Koike et al., 1983).

Dehydration causes a decrease in ECF volume and can reduce the total blood volume. This leads to hypovolemic shock which has results very similar to that of hemorragic shock (Ganong, 1993; Guyton, 1991).

CHAPTER 3. MATERIALS AND METHODS

Experimental Chicks

One-day-old TK male chicks (average body weight of 37 to 38 grams/chick), as shown in Figure 1-A, were obtained from a local breeding company (Hy-Line International, Dallas Center, IA). Chicks were delivered within four to ten hours after hatching to the Agricultural Engineering Livestock Environment and Animal Physiology (LEAP) laboratory at Iowa State University. The chicks were randomly divided into eight groups in four environmentally controlled indirect calorimeter chambers. The same air temperature and relative humidity were applied to all four chambers.

Indirect Calorimeter Chambers and Data Acquisition

Figure 2 shows the schematic of the indirect calorimeter chamber system. Four environmentally-controlled indirect calorimeter chambers (1.52 W x 1.83 L m) were used for the experiments. Each chamber was partitioned with a wire-mesh divider into two sections. Two lines of nipple waterers (6 drinkers per line) were placed in each chamber, each near the middle of the section. The commercial excelsior bedding used in shipment was placed on the floor. Two controllable heater/fan units were installed in each chamber to maintain the inside temperature of $27 \pm 0.5\ ^{o}C$ (or $29 \pm 0.5\ ^{o}C$). A temperature-humidity sensor was placed near chick's level in the middle of chamber. Thermoelectric air-mass flow meters were used to measure air flow rate through each chamber. Fluorescent lighting at 26 lux (2.5 foot candles) illumination near the chicks' level was controlled by a programmable electronic timer. For

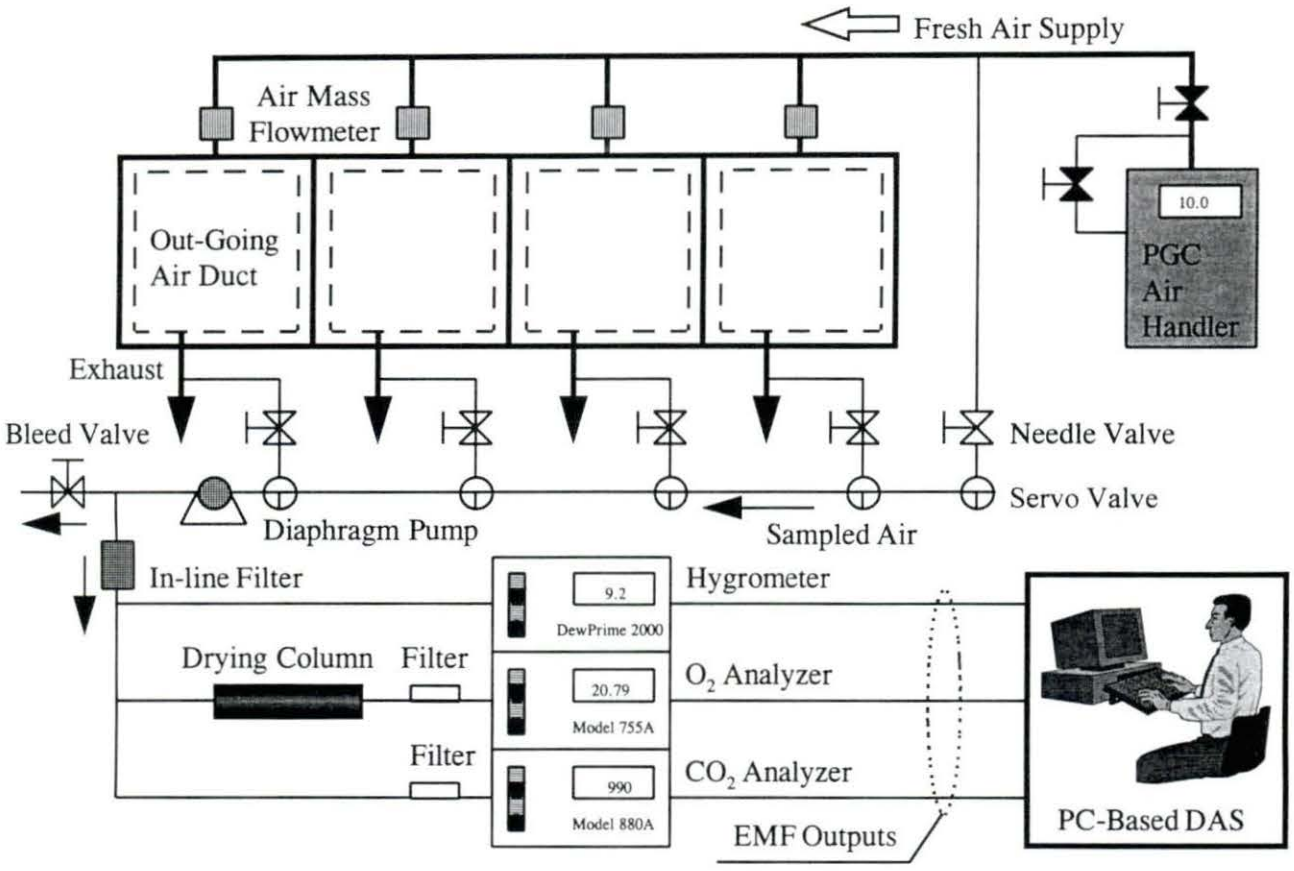


Figure 2. Schematic of the ISU indirect animal calorimetry system (after Xin, 1996).

each chamber, the exhaust air was sampled using copper tubing (0.64 cm diameter) for analyzing gas composition. The air sample lines were kept free of condensation with an electrical heating cable. An air pump delivered sample air to each analyzer. The air flow rate to each gas analyzer was 300 cc/min for the dew point analyzer, 250 cc/min for the oxygen gas analyzer, and 500 cc/min for the carbon dioxide gas analyzer. The data-acquisition system controlled switching of the air sampling at four-minute intervals. Each air sample (one fresh air and four exhaust air samples) was analyzed after a three-minute flushing period. During the fourth minute, air temperature, relative humidity, air flow rate, dew point temperature, CO_2 and O_2 concentrations, and barometric pressure were sampled every 2 seconds and stored as one-minute average with the automatic data acquisition system. The chambers were cleaned after each trial with disinfectant (1 stroke Environ®) to prevent bacterial influence.

Instrumentation

Datalogger and Controller

A fully programmable datalogger and controller (model CR 10, Campbell Scientific, Inc., Logan, UT) was used for measuring the output signals of the sensors and analyzers. The CR10 converts the measured analog inputs to digital values, and stores these values over time for later retrieval. The data logger can take 12 single-ended inputs or 6 differential inputs.

Multiplexer

Due to the limited number of inputs of the CR10, a multiplexer (AM416, Campbell Scientific, Inc.) was used to extend the input capacity to 32 differential measurements or 64 single-ended measurements. The CR10 scanned the multiplexer, transported the data from the multiplexer to the CR10, converted the analog signals to the digital signals, and stored these data in the memory system for retrieval at a later time.

Controller

The two heaters in each chamber were controlled by the SDM-CD16 (Campbell Scientific, Inc.) in conjunction with the CR10 and peripheral relays to maintain the desired chamber air temperature. The controller was also responsible for switching gas sample collection every four minutes. Under the datalogger (CR10) control, the SDM-CD16 activated or deactivated DC powered external relays. These relays in turn actuated heating elements or solenoid valves of the gas sampling system.

Temperature and Relative Humidity Probe (HMP35C, Campbell Scientific, Inc.)

In conjunction with the CR10, this probe measured air temperature and relative humidity (RH) near the chick level. The temperature sensor was a single-ended thermistor. The capacitive RH sensor produces an output of 0 to 100 millivolts corresponding to the relative humidity of 0 to 100 %.

Oxygen Analyzer

Oxygen concentration in the sampled gas was analyzed by a paramagnetic oxygen analyzer (Model 755A, Rosemount Analytical Inc., La Habra, CA). The basic theory of this instrument is that oxygen is a strong paramagnetic gas and an oxygen molecule becomes a temporary magnet when it is located in a magnetic field. The test body in the analyzer has a displacement torque due to the "magnetic buoyancy" effect of the temporary magnetic characteristic of oxygen. The analyzer measures the displacement torque which is proportional to oxygen concentration. Zero calibration was performed with pure nitrogen gas (99.99 %) at a flow rate of 250 cc/min through the analyzer. Span calibration was performed with a gas mixture of 20.9789 % O_2 with N_2 balance.

Carbon Dioxide Analyzer

Carbon dioxide concentration was measured with a non-dispersive infrared analyzer (Model 880A, Rosemount Analytical Inc.). The basic operation of this device is as follows. Two equal infrared beams are directed to each detector; one is through the sample cell and the other is through the reference cell. The existence of gas in the sample cell causes the change of infrared energy and the detector measures the difference of infrared energy between the sample cell and the reference cell. Pure nitrogen gas and 1977 ppm CO_2 with N_2 gas balance was used for zero and span calibration gas, respectively.

Experimental Regimes

Experiment I: The effect of water and feed on body mass and mortality of chicks was investigated. Each group was composed of 150 chicks. The chicks were exposed for 60 hours to one of the three nutritional regimes: two water-only (W) groups, four water-and-feed (WF) groups, and two neither-feed-nor-water (N) groups. The two W groups and two of the four WF groups were compared and the two N groups and the other two WF groups were compared. After 60 hours of nutritional treatment, water and feed were provided to all chicks in W groups and continued for the WF groups. To prevent pasting of chicks in the N groups, water was introduced for 4 hours, followed by feed. Continuous lighting was provided to all groups for the first 60 hours, and then 12 hours of light and 12 hours of darkness (12L:12D) was used. The entire experiment lasted 7 days.

Experiment II: Due to the liquid nature of water, a water supplier such as a nipple has leakage problems and is not adequate during the transportation of chicks. This leakage problem could be eliminated by jelly type water-substitutes such as Aqua Jel ® (Trans-Container Co., Columbus, OH). Aqua Jel ® contains more than 93 % water (by weight) with the remainder being hydrocolloid, phosphoric acid, and potassium sorbate. It had been used as a water supplement for the transport of rodents (De Marco, 1995). However, it had never been tested on poultry. This experiment tested the suitability of Aqua Jel ® for chicks. To test the suitability of Aqua Jel ® as a water substitute, Aqua Jel ® and water groups were compared. This experiment consisted of four Aqua Jel ®-and-feed(JF) groups and four water-and-feed (WF) groups. The Aqua Jel ® was provided to the chicks in 4.54 kg (10, 0.454 kg

plastic packs of Aqua Jel ®) per section (150 chicks) for the first 64 hours. Then Aqua Jel ® was replaced with water. For the WF groups, one nipple water line was placed in each section. Chicks in all sections were fed *ad libitum* from the first day. Continuous lighting was used for all groups for the first 64 hours and then switched to 12L:12D.

Experiment III: From the previous experiments, the 7-day mortality rate of chicks was controlled at approximately 1 % by providing water (or water substitute) and feed with continuous lighting. Even though providing water substitute and feed is practical, providing continuous lighting is unfeasible during transportation. For this reason, studying the physiological responses of chicks to different lighting regimes was necessary. In this experiment, the physiological responses of chicks were compared between continuous light and intermittent light of 1 hour light and 5 hours dark (1L:5D). For the first 60 hours, continuous light was given to one group while another group received the 1L:5D treatment. Thereafter, 12 hours light and 12 hours darkness (12L:12D) conditions were provided to all chambers. Feed and water were introduced to the chicks in both groups from the first day.

Experiment IV: This experiment examined the effects of supplying feed directly on a commercial honey comb bedding, compared to feed supply using troughs. Supply of feed on the honey comb bedding would be more practical than using a feed tray. Lighting conditions for the first 72 hours were 1L:5D and 12 L:12 D thereafter. Aqua Jel ® was replaced with water after 72 hours.

Experiment V: This experiment studied the effects of Aqua Jel ® only for chicks under intermittent lighting conditions of 1L:5D. Specifically, one experimental group was provided

with only Aqua Jel ® (OJ) and the other group was provided with Aqua Jel ®-and-feed (JF) for the first 72 hours. The feed was directly spread on the honey comb bedding. After the initial 72 hour intermittent lighting period, water and feed were provided to both groups with 12 hours light and 12 hours dark. For OJ group, Aqua Jel ® was replaced with feed and water following the 72 hours treatment. This experiment lasted 9 days.

Experiment VI: The suitability of Aqua Jel ® for chicks during air transportation was consolidated through this experiment. The physiological responses of chicks were compared between an Aqua Jel ®-and-feed (JF) and a neither-Aqua Jel ®-nor-feed (N) groups during air transportation. Sixteen boxes of chicks (1,280 total) were used for this experiment. Half of the chicks in each box were provided with both feed and Aqua Jel ®; the other half had neither. They were sent to Miami in the morning and returned to the ISU LEAP laboratory the next morning (24 hours round-trip flight). The ambient and inside the box temperature during the air-transportation are shown in Figure 3. While inside the calorimeter chambers, each group remained in its respective nutritional treatment till 72 hours of age with a 1L:5D photoperiod. Then, feed and water were provided to both groups with 12L:12D photoperiod. The physiological responses were compared between two groups for 2 weeks.

Experiment VII and Experiment VIII: Traditionally, neither feed nor water is provided to chicks during transportation. The heat production characteristics of the fasted chick is necessary for the proper design and operation of the ventilation system. However, information was meager in the literature. In these experiments, six containers of chicks (528 total) were placed in each chamber and the body weight change, mortality, and heat

production were determined with neither water nor feed for 3 days. Two lighting regimes of continuous dark and continuous light were used during the 72-hour trial period. Then feed and water were introduced to both groups with a photoperiod of 12L:12D.

Experiment IX: In a shipment of day-old chicks to China provided with feed and Aqua Jel ®, it was found that a photoperiod of 11 hours dark with 1 hour light during the shipment (Figure 4) led to a low consumption of feed and Aqua Jel ®. It was uncertain however whether the low consumption was due to the photoperiod or the fact that the chicks were in transit. In order to determine this, four containers of chicks (320 total) were placed in each chamber with supply of Aqua Jel ® and feed for 72 hours. Body weight loss and mortality were compared for two different photoperiods of 1L:11D virsus 0.5L:11.5D. After the 72 hours of treatment, the chicks were placed on the chamber floor and provided with feed, water, and a photoperiod of 12L:12D for four more days.

Body Blood and Water Content Tests

Blood Test

Blood of chicks from each nutritional treatment groups was sampled and stored in EDTA coated Monoject® blood collection tubes (Sherwood, Corp., St. Louis, MO) to measure packed cell volume (PCV). The blood in the test tubes was transferred into heparinized Yankee® micro hematocrit tubes (Clay-Adams, Inc., New York, NY) and the blood in hematocrit tubes was spun for five minutes by Adams micro-Hematocrit centrifuge (Clay-Adams, Inc., New York, NY). Three hematocrit tubes were used for testing the PCV

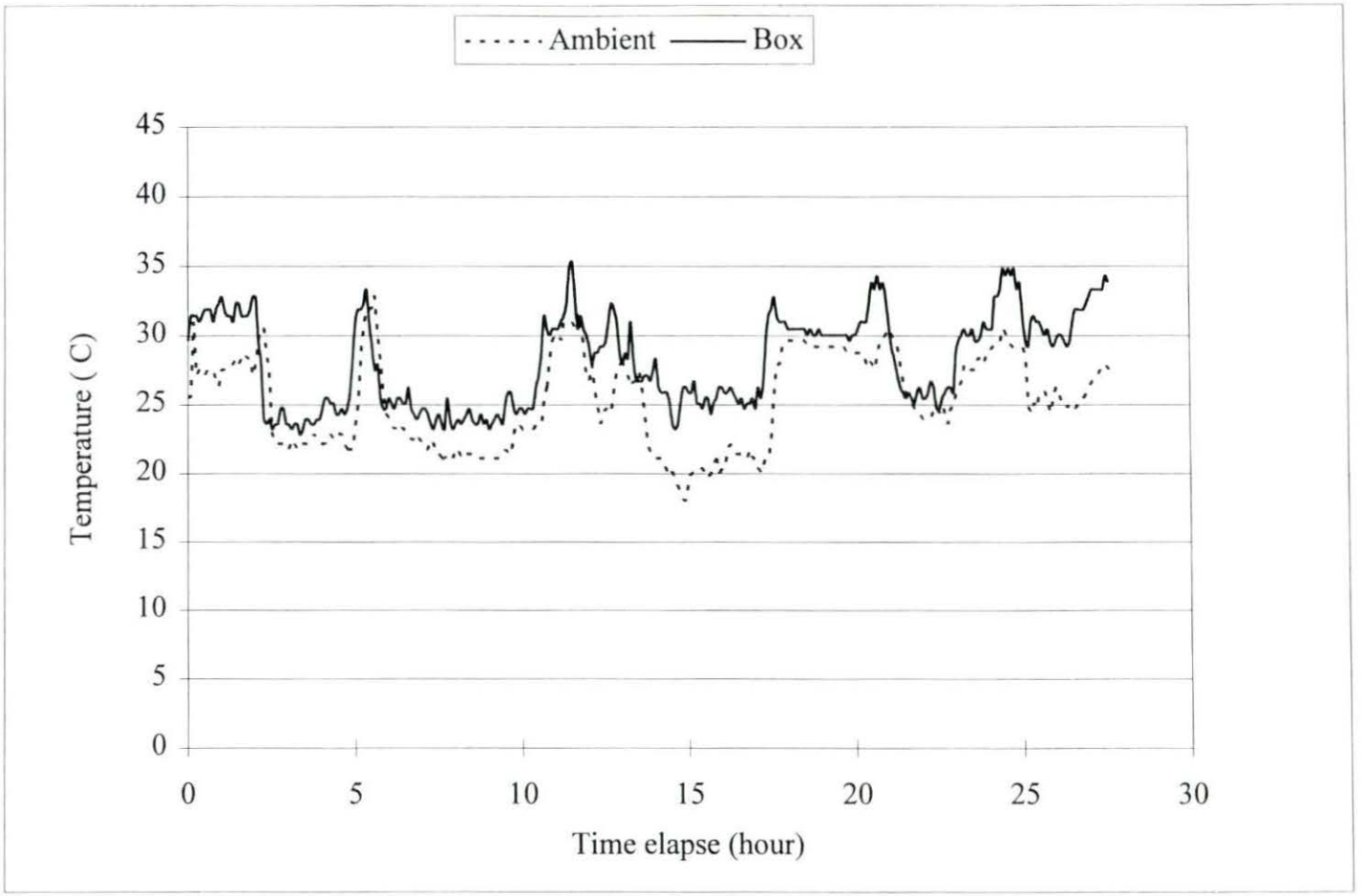


Figure 3. Temperature changes of ambient and inside box during one day air-transportation from Des Moines to Miami and return.

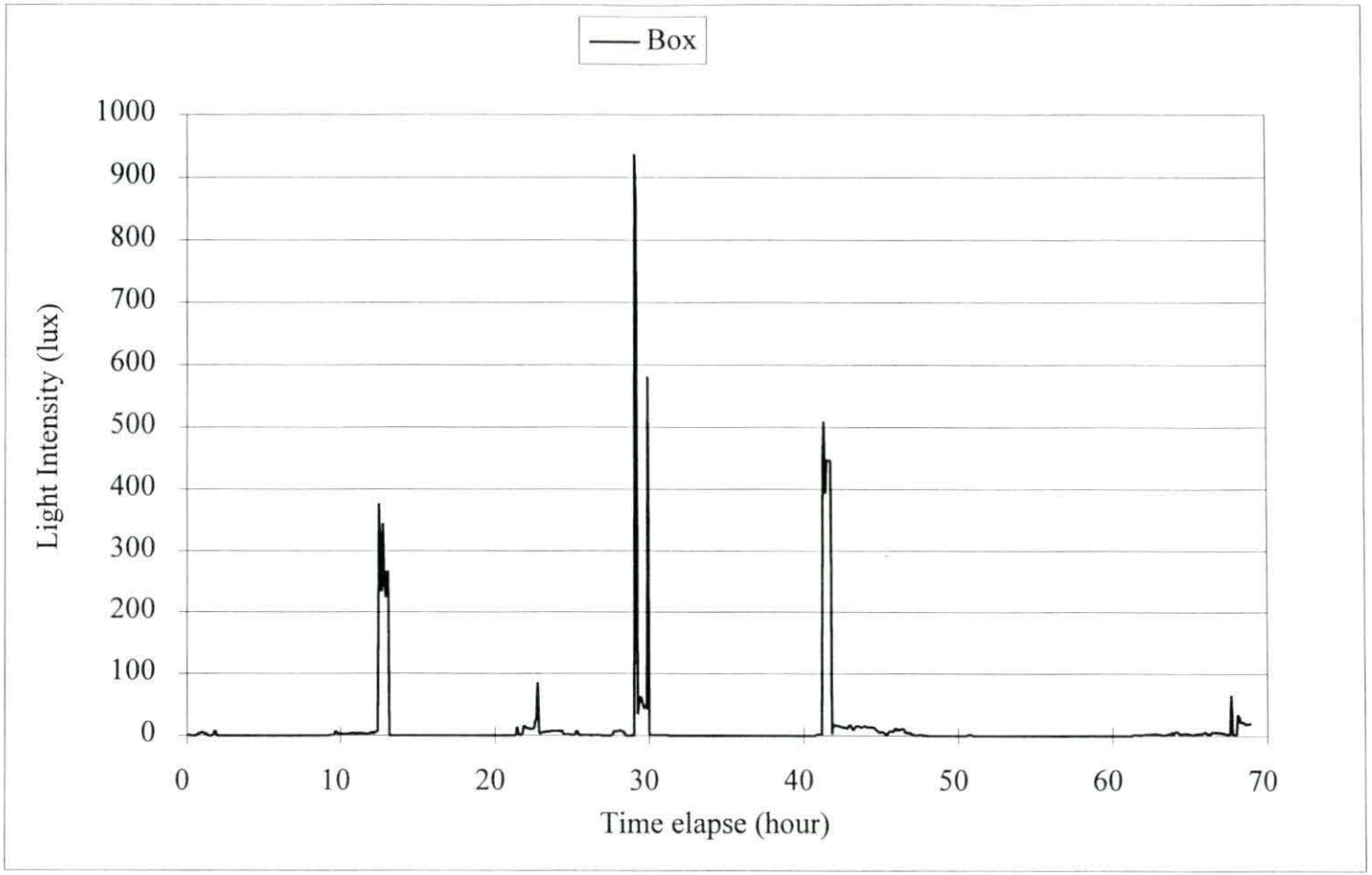


Figure 4. Light intensity change inside chicks' box during a shipment to China provided with feed and Aqua Jel.

of each randomly selected chick through experiments X and XI.

Body Water Content Test

The randomly sampled chicks were sacrificed from each treatment group and the body fluid content estimated. The weight of each chick was measured, the chicks were then sacrificed using CO_2 inhalation (in the laboratory of LAR), and they were placed in a 50 ^{o}C drying oven for a period of one day. After drying, the weight of each chick was measured and the body fluid content was calculated by comparing the wet and dry body weights.

Experiments for Testing PCV and Body Water Content

Experiment X and XI: From the previous experiments, it was revealed that dehydration did not seem to be the reason for the chicks' death, but rather lack of nutrient supply. To further investigate this, two trials were conducted to study the physiological changes, particularly PCV and body water content of chicks subjected to low humidity conditions. For the first experiment, five nutritional treatments were used: water-and-feed (WF), Aqua Jel ®-and-feed (JF), water-only (W), Aqua Jel ®-only (J), and neither feed-nor-water (N). The test lasted three days, during which continuous lighting was provided to all groups (about 40 to 50 lux). The blood samples from seven randomly sampled chicks was collected from each group and analyzed for PCV each day for three days. At the same time, ten chicks were sacrificed and dried to determine the body water and dry matter content. For the second experiment, three nutritional treatments were used: water-and-feed (WF) group,

water-only (W) group, neither-water-nor-feed (N) group. After 72 hours, feed and water were provided to all groups. Blood samples of weak chicks were collected for the PCV test, and dead chicks were dried in the oven. On the fifth day, blood sample of four chicks from each groups was collected for PCV analysis.

Respiratory Tract of Chicks under "Dehydration" Condition

The effects of water absence on the respiratory tract of the chick was studied. Dryness of mucus in the trachea was expected from long-time deprivation of water. Because one of the main functions of mucus is to protect the respiratory tract from viruses, dry mucus makes the trachea more susceptible to virus infection. Five chicks from the treatment of water deprivation for three days were randomly selected. These chicks were sent to the electron microscopy laboratory of Department of Botany of Iowa State University, where they were sacrificed, and trachea tissues were processed. Trachea tissues were observed with 2000 to 3000 times magnification using the transmission electron microscope.

Statistical Analysis

Analysis of variance and Duncans multiple mean comparison were performed on the response variables using SAS program (SAS Institute, 1994).

CHAPTER 4. RESULTS

Chick Mortality and Body Weight Change

Experiment I - Determine the effect of water availability on early chick mortality

Cumulative mortality rate (%), body weight (g/chick), and body weight change (%) for each treatment are shown in Table 1. For the seven day experiment, only one per cent (6 chicks out of 600 total) died in the water-and-feed (WF) treatment group; however, the mortality rate reached to 21.3 % in the neither-feed-nor-water treatment group (N) and 26 % in the water-only group (W). The mortality rate of the WF group slightly changed from 60 hours of age to 144 hours of age (0.83 % to 1.00 %). The mortality rate of both N and W treatment groups, however, greatly increased from 60 hours of age to 144 hours of age (1.00 % to 21.3 % and 2.33% to 26.0 %, respectively). Particularly, one day after the normal nutritional treatment, the daily mortality rate was the highest for both N and W groups. The chicks in the WF treatment constantly gained weight, but the chicks in both N and W treatments lost body weight during the 60 hours of treatment. After 60 hours, they started gaining weight with the availability of feed and water.

Experiment II - Test the suitability of Aqua Jel® for chicks

The cumulative mortality rate (%), body weight (g/chick), and body weight change (%) for each treatment are shown in Table 2. During the 64 hours of treatment, 0.44 % chicks (2 out of 600) died in the water-and-feed (WF) treatment group and 0.67 % chicks (3 out of 600) died in the Aqua Jel ®-and-feed (JF) treatment group. During the subsequent four

Table 1. The mortality rate and body weight of chicks subjected to three nutritional treatments with continuous lighting for the first 60 hours. (Experiment I)

Trt. / Age,hr	Cumulative Mortality (%)			Body Weight (g/chick)			Body Weight Change (%)		
	WF (S.D.)	N (S.D.)	W (S.D.)	WF (S.D.)	N (S.D.)	W (S.D.)	WF (S.D.)	N (S.D.)	W (S.D.)
0	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	38.0 (0.73)	38.7 (1.24)	37.9 (0.19)	0.0 (0.00)	0.0 (0.00)	0.0 (0.00)
24	0.50 (0.64)	0.67 (0.00)	1.33 (0.94)	42.6 (0.23)	34.4 (0.38)	35.9 (0.61)	12.0 (2.25)	-9.9 (1.89)	-5.1 (1.14)
48	0.83 (0.64)	1.00 (0.47)	2.00 (0.94)	46.3 (0.42)	32.5 (0.35)	33.7 (0.06)	21.7 (3.27)	-16.2 (1.78)	-10.9 (0.28)
60	0.83 (0.64)	1.00 (0.47)	2.33 (1.41)	48.4 (0.52)	31.6 (0.25)	33.2 (0.19)	27.4 (1.34)	-18.4 (1.97)	-12.2 (0.08)
72	1.00 (0.67)	4.33 (3.30)	4.67 (2.83)	-	-	-	-	-	-
96	1.00 (0.67)	19.0 (4.24)	23.0 (1.41)	-	-	-	-	-	-
120	1.00 (0.67)	21.3 (7.54)	26.0 (3.77)	-	-	-	-	-	-
144	1.00 (0.67)	21.3 (7.54)	26.0 (3.77)	66.2 (0.70)	50.7 (1.01)	49.6 (1.42)	74.3 (3.43)	31.1 (6.8)	31.0 (3.10)

WF = water and feed; N = neither; W = water only
S.D. = standard deviation
Photoperiod = continuous lighting for the first 60 hours and then 12L:12D
Temperature = 27.1 ± 0.5 °C; Relative humidity = 37 ± 5 %.

days, only four more chicks died in the WF treatment group and none in the JF treatment group. Chicks in the WF group gained more body weight (29.2 %) than the chicks in the JF group (19.7 %) during the first 64 hours of the nutritional treatment. The chicks in JF treatment gained body weight at a larger rate when the Aqua Jel ® was replaced with water after the first 64 hours. However, body weight difference compared to the WF group was not fully recovered during the following four days of growth.

Table 2. The mortality rate and body weight of chicks subjected to two nutritional treatments with continuous lighting for the first 64 hours. (Experiment II)

Trt. / Age,hr	Cumulative Mortality (%)		Body Weight (g/chick)		Body Weight Gain (%)	
	WF (S.D.)	JF (S.D.)	WF (S.D.)	JF (S.D.)	WF (S.D.)	JF (S.D.)
0	0.00 (0.00)	0.00 (0.00)	36.8 (0.63)	36.8 (0.08)	0.0 (0.00)	0.0 (0.00)
24	0.22 (0.38)	0.00 (0.00)	41.0 (0.21)	38.6 (0.43)	11.4 (1.36)	5.1 (0.95)
48	0.22 (0.38)	0.22 (0.38)	45.0 (0.34)	42.1 (0.35)	22.2 (1.41)	14.6 (0.81)
64	0.44 (0.77)	0.67 (0.67)	47.6 (0.36)	44.0 (0.31)	29.2 (1.99)	19.7 (1.02)
72	0.67 (1.15)	0.67 (0.67)	49.2 (0.57)	48.4 (0.82)	33.7 (0.92)	31.6 (2.00)
96	0.67 (1.15)	0.67 (0.67)	53.2 (0.41)	52.4 (0.43)	44.5 (1.64)	42.7 (1.34)
120	1.11 (1.39)	0.67 (0.67)	57.4 (0.32)	56.1 (0.59)	55.8 (1.82)	52.7 (1.91)
144	1.11 (1.39)	0.67 (0.67)	62.2 (0.34)	61.7 (0.96)	68.7 (2.52)	67.8 (2.96)
168	1.33 (1.15)	0.67 (0.67)	68.2 (0.71)	67.3 (1.21)	85.1 (2.76)	83.2 (3.65)

WF = water and feed; JF = Aqua Jel ® and feed
S.D. = standard deviation.
Photoperiod = continuous lighting for the first 64 hours and then 12L:12D
Temperature = 27 ± 1 °C; Relative humidity = 44 ± 7 %.

Experiment III: Determine the physiological responses of chicks to the intermittent lighting condition of 1 hour light and 5-hour dark(1L:5D)

Table 3 shows the cumulative mortality rate (%), body weight (g/chick), and body weight change (%) for each treatment. During the 60 hours of light treatment, the mortality rate was 0.67 % for the continuous light group and 0.17 % for the intermittent light group. Mortality rate was 1.33 % (8 out of 600) for both groups during the seven-day-trial period. At the end of the 60-hour, the body weight difference between continuous lighting and the

Table 3. The mortality rate and body weight change of chicks subjected to two lighting regimes for the first 60 hours, followed by 12L:12D photoperiod. (Experiment III)

Trt. / Age, hr	Cumulative Mortality (%)		Body Weight (g/chick)		Body Weight Gain (%)	
	Light (S.D.)	1L:5D (S.D.)	Light (S.D.)	1L:5D (S.D.)	Light (S.D.)	1L:5D (S.D.)
0	0.00 (0.00)	0.00 (0.00)	37.7 (0.30)	37.9 (0.49)	0.0 (0.00)	0.0 (0.00)
24	0.33 (0.67)	0.00 (0.00)	42.3 (0.25)	39.4 (0.59)	12.3 (0.67)	4.0 (0.58)
48	0.67 (0.94)	0.17 (0.33)	46.0 (0.31)	42.3 (1.07)	22.0 (1.28)	11.4 (1.41)
60	0.67 (0.94)	0.17 (0.33)	47.6 (0.34)	43.4 (0.77)	26.3 (1.31)	14.3 (0.79)
72	1.00 (1.59)	0.50 (0.64)	50.3 (0.22)	50.9 (0.85)	33.4 (0.93)	34.2 (0.85)
96	1.17 (1.91)	1.00 (0.86)	54.2 (0.46)	53.3 (0.81)	43.7 (1.53)	40.4 (0.34)
120	1.33 (1.81)	1.33 (1.22)	58.2 (1.22)	57.7 (1.22)	54.3 (3.26)	52.0 (1.45)
144	1.33 (1.81)	1.33 (1.22)	63.6 (0.70)	62.9 (1.10)	68.6 (2.33)	65.7 (0.82)
168	1.33 (1.81)	1.33 (1.22)	68.7 (0.98)	67.3 (0.99)	82.1 (2.94)	77.4 (1.09)

Light = continuous lighting; 1L:5D = 1 hour light and 5 hours dark
S.D. = standard deviation
Temperature = 27 ± 0.9 °C; Relative humidity = 46 ± 9 %
Nutritional regimes = Aqua Jel® and feed during the first 60 hours treatments; and water and feed thereafter.

intermittent lighting (1L:5D) was 4.2 (g/chick) in favor of the continuous lighting treatment. Specifically, the chicks in the continuous light treatment gained 26.3 % of the initial body weight but the chicks in 1L:5D treatment group gained only 14.3 % of the initial body weight. When the chicks in the 1L:5D treatment received normal light conditions of 12L:12D, their body weight increased faster than those once kept in the continuous light. Thus, there was a compensatory growth for the intermittent light treatment.

Experiment IV: Determine the effects of feed supply methods on chick performance

The cumulative mortality rate (%), body weight (g/chick), and body weight change (%) in each of the treatment groups are shown in Table 4. The mortality rate (%) of chicks in

Table 4. The mortality rate and body weight change of chicks subjected to two feed supply treatments for the first 72 hours. (Experiment IV)

Trt. / Age, hr	Cumulative Mortality (%)		Body Weight (g/chick)		Body Weight Gain (%)	
	HCB (S.D.)	Tray (S.D.)	HCB (S.D.)	Tray (S.D.)	HCB (S.D.)	Tray (S.D.)
0	0.00 (0.00)	0.00 (0.00)	37.3 (0.30)	37.4 (0.49)	0.0 (0.00)	0.0 (0.00)
72	1.00 (0.67)	2.17 (1.14)	39.5 (0.34)	39.1 (0.67)	6.0 (0.82)	4.4 (1.34)
96	2.33 (1.59)	5.17 (1.37)	41.3 (0.48)	40.5 (0.40)	10.6 (0.57)	8.2 (1.28)
120	3.50 (2.13)	6.83 (2.20)	46.0 (0.67)	45.4 (0.70)	23.3 (1.11)	21.5 (1.48)
144	3.50 (2.13)	7.17 (2.33)	53.8 (0.95)	52.6 (0.51)	44.2 (1.42)	40.6 (1.78)
168	3.50 (2.13)	7.17 (2.33)	59.6 (0.75)	58.4 (0.48)	59.8 (0.86)	56.2 (1.35)

HCB = feed supply on honey comb bedding; Tray = feed supply using feed trough
S.D. = standard deviation
Photoperiod = 1L:5D for the first 72 hours and then 12L:12D
Temperature = 27 ± 0.3 ^{o}C; 42.5 ± 6 %.

the honey comb bedding treatment (HCB) was 1 % (6 out of 600) and that of chicks in the feed tray treatment (Tray) was 2.17 % for the first 72 hours. It reached 3.5 % for the HCB group and 7.17 % for the Tray group at the end of 168 hours. Specifically, one day after the normal treatment, the mortality for the Tray group was considerably higher (2.17 % to 5.17 %). The chicks of the HCB group had a slightly higher weight gain (59.8 % of the initial weight) than the chicks in Tray group (56.2 %).

Experiment V: Further determine the suitability of Aqua Jel® under intermittent lighting condition of 1L:5D

Table 5 shows the cumulative mortality rate (%), body weight (g/chick), and body weight change (%) of each treatment. The mortality rate of chicks in the Aqua Jel ®-only (J) treatment was 1 % and that of chicks in the Aqua Jel ®-and-feed (JF) treatment was 0.83 % for the first 72 hours of treatment. However, when the chicks in the J groups received feed and water with normal lighting condition, the mortality rate increased to 14.05 %, and the mortality rate of chicks in JF treatment was 2.17 % for the 9-day trial period. The chicks in J groups lost 17.7 % of their initial body weight, but the chicks in the JF groups lost only 4.1 % of the initial body weight during the first 72 hours of treatment. During the subsequent 4-day growing period under normal nutrition and lighting (12L:12D) conditions, the chicks gained 30.2 % of their initial body weight (37.6 to 48.9 g/chick) for the J groups and 59.7 % (37.5 to 59.8 g/chick) for the JF groups.

Experiment VI: Test the effect of Aqua Jel® on chick during air transportation

Table 6 shows the cumulative mortality rate (%), body weight (g/chick), and body weight change (%) of each treatment. During the one-day air transportation, only one chick in the JF treatment group died. For 72 hours of treatment, two more chicks died in the JF group (0.31 %) and eight chicks (1.25 %) died in the N treatment group. One day after introducing the normal nutritional treatment, the daily mortality rate was the highest in N group. By seven days of age, mortality was 1.25 % in the JF group and 20 % in the N group. In addition, only one more chick in JF groups died during the second week of the trial. The chicks in N treatment group lost 9.2 % of their body weight during the one-day air

Table 5. The mortality rate and body weight change of chicks subjected to two nutritional treatments with intermittent light condition for the first 72 hours. (Experiment V)

Trt. / Age, hr	Cumulative Mortality (%)		Body Weight (g/chick)		Body Weight Gain (%)	
	J (S.D.)	JF (S.D)	J (S.D.)	JF (S.D)	J (S.D.)	JF (S.D)
0	0.00 (0.00)	0.00 (0.00)	37.6 (0.34)	37.5 (0.24)	0.0 (0.00)	0.0 (0.00)
72	1.00 (0.86)	0.83 (0.64)	30.9 (0.31)	35.9 (0.55)	-17.7 (0.79)	-4.1 (1.67)
96	9.70 (2.64)	1.33 (0.94)	36.9 (0.49)	46.3 (0.72)	-1.9 (1.36)	23.6 (2.24)
120	13.38 (2.79)	1.50 (1.26)	41.4 (0.52)	50.5 (0.81)	10.4 (1.77)	34.7 (2.61)
144	13.55 (2.48)	1.83 (1.14)	44.4 (0.73)	55.0 (1.04)	18.2 (2.58)	46.8 (3.14)
168	13.89 (2.17)	2.00 (1.44)	48.9 (1.09)	59.8 (1.36)	30.2 (3.64)	59.7 (4.30)
192	14.05 (2.40)	2.00 (1.44)	53.6 (1.09)	64.1 (1.45)	42.7 (3.89)	71.0 (4.42)
216	14.05 (2.40)	2.17 (1.48)	58.1 (1.66)	69.2 (1.82)	54.8 (5.50)	84.7 (5.44)

J = Aqua Jel ® only: JF = Aqua Jel ® and feed
S.D. = standard deviation
Photoperiod = 1L:5D for the first 72 hours and then 12L:12D
Temperature = 29 ± 0.6 °C; Relative humidity = 41 ± 7 %.

transportation; whereas, the chicks in the JF group gained 1.5 % of their initial body weight. For the next two days of nutritional and light treatments inside the calorimeter chambers, the chicks in the JF treatment group lost 1.8 % of their initial body weight and the chicks in the N group lost 22.5 % of their initial body weight. The chicks in both groups started recovering and regaining their body weight after the normal treatment started.

Table 6. The mortality rate and body weight change of chicks subjected to two nutrition treatments with intermittent light condition for 48 hours after a 24-hour air transportation. (Experiment VI)

Trt. / Age, hr	Cumulative Mortality (%)		Body Weight (g/chick)		Body Weight Gain (%)	
	JF (S.D.)	N (S.D)	JF (S.D.)	N (S.D)	JF (S.D.)	N (S.D)
0	0.00 (0.00)	0.00 (0.00)	38.0 (0.00)	38.0 (0.00)	0.0 (0.00)	0.0 (0.00)
24	0.16 (0.08)	0.00 (0.00)	38.6 (0.53)	34.5 (0.10)	1.5 (1.38)	-9.2(0.27)
72	0.31 (0.36)	1.25 (0.00)	37.3 (0.31)	29.4 (0.36)	-1.8 (0.83)	-22.5 (0.95)
96	0.78 (0.60)	16.72 (5.21)	46.3 (0.59)	37.3 (0.43)	21.9 (1.54)	-1.9 (1.14)
120	0.94 (0.36)	18.75 (6.35)	52.4 (0.74)	41.2 (0.37)	38.0 (1.96)	8.3 (0.97)
144	1.25 (0.51)	19.84 (6.30)	56.8 (0.70)	45.4 (0.57)	49.4 (1.83)	19.5 (1.49)
168	1.25 (0.51)	20.00 (6.12)	61.0 (0.80)	50.4 (0.87)	60.4 (2.11)	32.6 (2.30)
192	1.25 (0.51)	20.00 (6.12)	66.7 (1.05)	55.7 (0.72)	75.6 (2.76)	46.4 (1.91)
216	1.56 (0.81)	20.00 (6.12)	71.4 (0.87)	60.9 (0.81)	88.0 (2.28)	60.2 (2.13)
240	1.56 (0.81)	20.00 (6.12)	76.5 (1.16)	66.9 (0.81)	101.2 (3.05)	75.9 (2.12)
264	1.56 (0.81)	20.00 (6.12)	84.8 (1.40)	76.0 (0.92)	123.2 (3.68)	100.0 (2.41)
312	1.56 (0.81)	20.00 (6.12)	102.1 (1.32)	91.7 (1.54)	168.6 (3.48)	141.3 (4.06)
360	1.56 (0.81)	20.00 (6.12)	113.4 (1.67)	101.9(0.72)	198.4 (4.39)	168.2 (1.91)

JF = Aqua Jel ® and feed; N = neither
S.D. = standard deviation.
Photoperiod = 1L:5D from 24 hour to 72 hour and then 12L:12D
Temperature = 28 ± 1 °C; Relative humidity = 45 ± 7 %.

Specifically, after the subsequent four days of normal nutritional treatment, the chicks in the JF group gained 60.4 % of initial body weight and the chicks in the N group gained 32.6 % of initial body weight. The body weight of the chicks in the N group did not equal that of the chicks in the JF group after 10 days of growth.

Experiment VII and Experiment VIII: Measure the physiological and energetic responses of fasting chicks to different lighting regimes

The cumulative mortality rate (%), body weight (g/chick), and body weight change (%) of each treatment are shown in Table 7. After the first 72 hours of treatment, the mortality rate was similar for both groups: 0.80 % for the Light treatment group and 0.52 % for the Dark treatment group. However, it drastically increased from the fourth day, 10.04 %

Table 7. The mortality rate and body weight of chicks subjected to two light treatments with fasting treatment for the first 72 hours. (Experiments VII and VIII)

Trt. / Age, hr	Cumulative Mortality (%)		Body Weight (g/chick)		Body Weight Gain (%)	
	Light (S.D.)	Dark (S.D.)	Light (S.D.)	Dark (S.D.)	Light (S.D.)	Dark (S.D.)
0	0.00 (0.00)	0.00 (0.00)	37.8 (1.21)	37.8 (1.41)	0.0 (0.00)	0.0 (0.00)
24	0.09 (0.33)	0.09 (0.33)	-	-	-	-
48	0.28 (0.51)	0.28 (0.51)	-	-	-	-
72	0.80 (1.18)	0.52 (0.75)	28.5 (1.13)	30.1 (0.95)	-24.8 (1.45)	-20.6 (0.96)
96	10.04 (2.82)	4.26 (2.01)	25.7 (0.36)	27.2 (0.10)	-30.5 (0.81)	-25.9 (0.71)

Light = continuous lighting; Dark = continuous dark
Temperature = 29 ± 0.3 ^{o}C; Relative humidity = 28 ± 3 %.

for the Light treatment and 4.26 % for the Dark treatment. The chicks in the Light treatment lost 24.8 % of their initial body weight and the chicks in the Dark group lost 20.6 % of their initial body weight during the three days of treatment. Metabolic heat and moisture production of the chicks are presented in the following section on "Metabolic Rate".

Experiment IX: Determine the physiological responses of chicks to two intermittent lighting conditions (1L:11D versus 0.5L:11.5D)

The cumulative mortality rate (%), body weight (g/chick), and body weight change (%) of each treatment are shown in Table 8. The cumulative mortality rate (%) of the chicks in both treatment groups 1L:11D and 0.5L:11.5D for the three-day treatment was 1.41 % for

Table 8. The mortality rate and body weight change of chicks subjected to two intermittent lighting treatments for the first 72 hours. (Experiment IX)

Trt. / Age, hr	Cumulative Mortality (%)		Body Weight (g/chick)		Body Weight Gain (%)	
	1L:11D	0.5L:11.5D	1L:11D	0.5L:11.5D	1L:11D	0.5L:11.5D
0	0.00 (0.00)[1]	0.00 (0.00)	32.1 (0.86)	32.0 (0.49)	0.0 (0.00)	0.0 (0.00)
24	0.00 (0.00)	0.00 (0.00)	-	-	-	-
48	0.47 (0.93)	0.78 (1.76)	-	-	-	-
72	1.41 (1.04)	1.87 (2.41)	28.8 (0.49)	27.1 (0.50)	-10.1 (2.65)	-15.5 (0.89)
96	5.47 (1.55)	5.16 (1.55)	-	-	-	-
120	6.87 (0.88)	7.03 (1.55)	-	-	-	-
144	6.87 (0.88)	7.03 (1.55)	-	-	-	-
168	7.18 (0.44)	7.03 (1.55)	53.1 (0.03)	50.1 (0.36)	65.7 (0.10)	56.3 (0.40)

1L:11D = 1 hours light and 11 hour dark; 0.5L:11.5D = 0.5 hours light and 11.5 hour dark
[1] = mean (standard deviation)
Temperature = 29 ± 0.5 °C; Relative humidity = 28 ± 0.5.

1L:11D and 1.87 % for 0.5L:11.5D. The cumulative mortality rate after the subsequent four days of nutritional and light treatment was 7.18 % for 1L:11D and 7.03 % for 0.5L:11.5D. The chicks in the 1L:11D treatment lost less body weight during the three-day treatment and gained more weight during the subsequent four days of normal treatment than the chicks in the 0.5L:11.5D treatment.

Mathematical Relations of the Body Weight Change

Experiments I through IV

For the first 60-hour nutritional treatment:

WF: $Y = -0.0003\ X^2 + 0.1895\ X + 37.61$ $R^2 = 0.9994$

JF: $Y = 0.0011\ X^2 + 0.0577\ X + 36.724$ $R^2 = 0.9986$

W: $Y = 0.0002\ X^2 - 0.0932\ X + 37.909$ $R^2 = 0.9941$

N: $Y = 0.0011\ X^2 - 0.1836\ X + 38.716$ $R^2 = 0.9998$

where Y = body weight (g/chick); X = age of the chick (hour) ($0 \leq X \leq 60$) .

After 60 hours with feed and water:

WF: $Y = 0.0004\ X_1^2 + 0.1046\ X_1 + 40.207$ $R^2 = 0.9968$

JF: $Y = 0.0001\ X_1^2 + 0.1747\ X_1 + 33.972$ $R^2 = 0.9917$

W: $Y = 0.2278\ X_2 + 17.918$ $R^2 = 1^*$

N: $Y = 0.1948\ X_2 + 21.553$ $R^2 = 1^*$

where Y = body weight (g/chick)

X = age (hour) of the chick ($60 \leq X_1 \leq 168$, $60 \leq X_2 \leq 144$)

* = two data point used to generate the linear equation.

Experiment V

For the first 72 hours treatment: ($0 \leq X \leq 72$)

J: $Y = 37.554 - 0.0925\ X$ $R^2 = 1^*$

JF: $Y = 37.467 - 0.0213\ X$ $R^2 = 1^*$

After 72 hours with normal treatment: ($72 \leq X \leq 216$)

J: $Y = 12.086 + 0.2915\ X - 0.0004\ X^2$ $R^2 = 0.987$

JF: $Y = 8.8412 + 0.4495\ X - 0.0008\ X^2$ $R^2 = 0.9836$

Experiment VI

For first 72 hours treatment: ($0 \leq X \leq 72$)

JF: $Y = 38 + 0.0391\ X - 0.0007\ X^2$ $R^2 = 1$

N: $Y = 38 - 0.1593\ X + 0.0006\ X^2$ $R^2 = 1$

After 72 hours with normal treatment: ($72 \leq X \leq 360$)

JF: $Y = 27.14 + 0.1658\ X + 0.0002\ X^2$ $R^2 = 0.9936$

N: $Y = 18.981 + 0.1483\ X + 0.0002\ X^2$ $R^2 = 0.9953$

Experiment VII

For the first 100 hours treatment: ($0 \leq X \leq 100$)

Light: $Y = 38.346 - 0.1133\ X$ $R^2 = 0.9904$

Dark: $Y = 38.84 - 0.1065\ X$ $R^2 = 0.9978$

Experiment 8

For the first 100 hours treatment: ($0 \leq X \leq 100$)

Light: $Y = 36.867 - 0.121\ X$ $R^2 = 0.9911$

Dark: $Y = 36.655 - 0.1002\ X$ $R^2 = 0.9987$

where Y = body weight (g/chick)

X = age of the chick (hour)

* = two data point used to generate the linear equation.

Metabolic Rate

Experiment V: As shown in Table 9 and Figures 5 through 8, the nutritional and light conditions affected the metabolic rate and moisture production of chicks. The metabolic rate of the JF group exceeded that of the J group during the nutritional treatment period. However, when feed and water were provided (after 72 hours), the metabolic rate per unit body weight for the J group was similar to that for the JF group (Figure 7). The increased metabolic rate presumably arose from the feed intake following the fasting. The peak metabolic rate of the JF group continued to increase till about 48 hours of age and remained relatively constant thereafter. For both groups, there was a large increase in the metabolic rate when the light came on. With normal treatments (a photoperiod of 12L:12D) after 72 hours, the metabolic rates of chicks in both groups continually increased with age (Figures 6 and 7). Figure 6 also shows that the gap of metabolic rate between the two groups was widened under light and narrowed under darkness. Figure 7 shows the total heat production

Table 9: Metabolic rate, moisture production, and respiratory quotient of chicks from two nutritional treatments: Aqua Jel-and-feed (JF) and Aqua Jel only (J).

		Metabolic Rate*		Moisture Production**		Respiratory Quotient	
		J	JF	J	JF	J	JF
1st day	Mean	7.49	8.07	13.29	13.5	0.65	0.77
(a)	Std. Dev.	1.60	1.87	2.06	1.79	0.05	0.07
	Max.	12.62	12.69	19.22	17.81	0.79	0.91
	Min.	5.60	6.08	10.11	10.35	0.55	0.64
2nd day	Mean	8.56	9.69	14.56	12.9	0.63	0.76
(a)	Std. Dev.	1.90	2.09	2.15	1.70	0.04	0.05
	Max.	15.95	15.82	19.3	16.61	0.76	0.89
	Min.	0.51	0.77	9.74	8.28	0.55	0.66
3rd day	Mean	8.02	10.29	12.11	10.75	0.61	0.75
(a)	Std. Dev.	2.09	2.60	1.71	1.70	0.06	0.06
	Max.	14.38	16.82	16.58	14.87	0.80	0.88
	Min.	5.56	7.37	9.74	8.28	0.52	0.62
4th day	Mean	11.9	11.49	16.83	16.29	0.91	1.02
(b)	Std. Dev.	1.41	1.94	2.50	2.74	0.04	0.06
	Max.	15.02	14.44	22.25	21.22	0.98	1.12
	Min.	9.59	9.03	13.22	12.36	0.81	0.87
5th day	Mean	13.51	13.51	19.33	18	0.87	0.96
(b)	Std. Dev.	2.23	2.43	2.52	2.54	0.04	0.04
	Max.	16.56	15.99	22.82	22.38	0.94	1.06
	Min.	10	9.38	14.34	13.26	0.77	0.86
6th day	Mean	13.66	13.52	19.81	16.91	0.85	0.91
(b)	Std. Dev.	2.51	2.59	3.24	2.77	0.04	0.03
	Max.	17.1	17.01	26.06	22.06	0.94	1.02
	Min.	10.56	10.19	14.6	13.08	0.77	0.84
7th day	Mean	14.22	13.66	20.12	16.42	0.83	0.87
(b)	Std. Dev.	2.38	2.75	2.59	2.49	0.03	0.03
	Max.	17.96	17.88	25.19	20.59	0.89	0.93
	Min.	11.41	10.19	16.09	12.89	0.72	0.81

Intermittent lighting: (a) 1L:5D and (b) 12L:12D

* Unit for metabolic rate: kcal/kg-hour

** Unit for moisture production: (g/kg-hour) x 10 ^ -3

Temperature: 29 (0.6) C

Relative humidity : 41 (7) %.

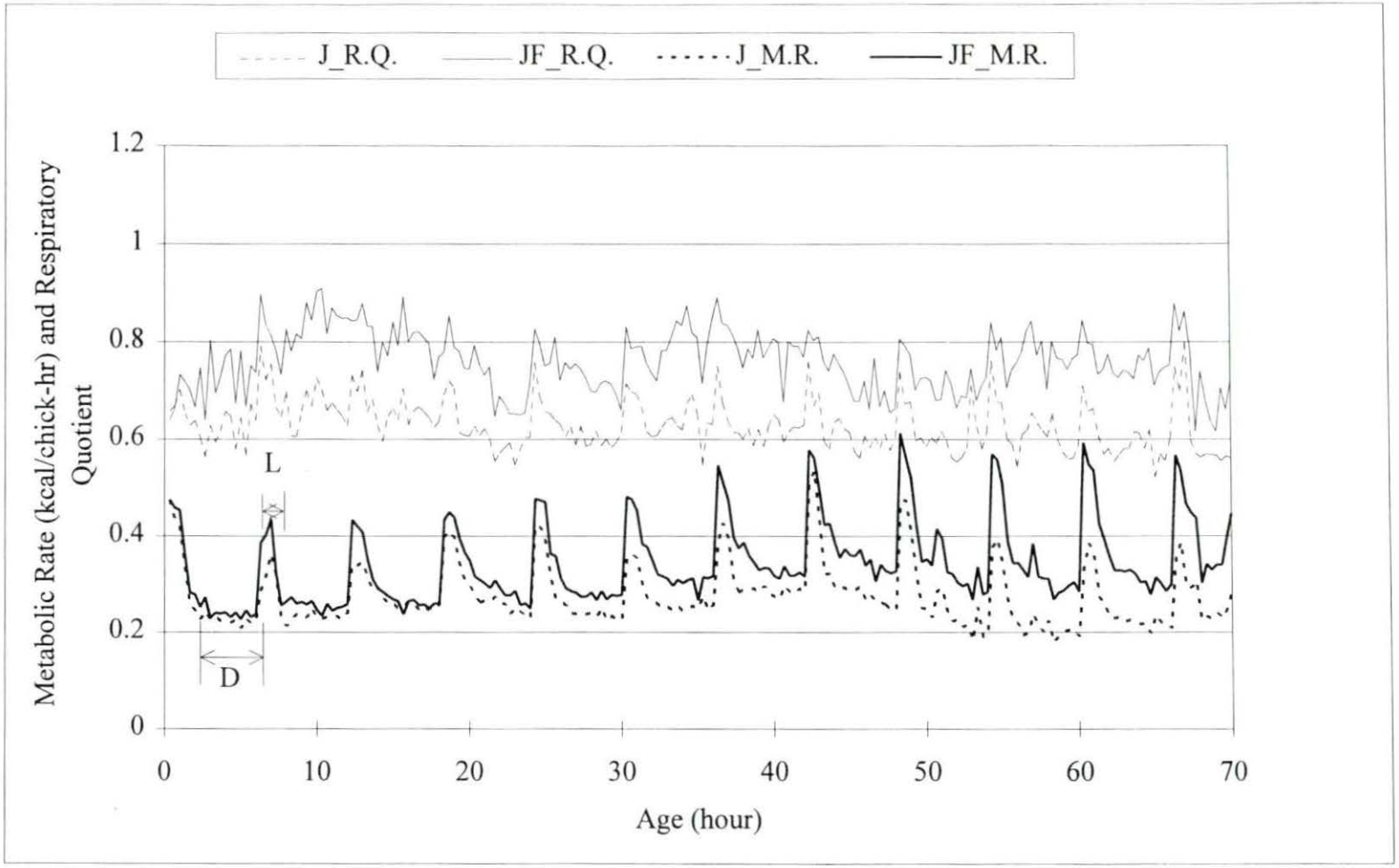


Figure 5: Metabolic rate (M.R.) and respiratory quotient (R.Q.) of chicks from two different nutritional treatments Aqua Jel-and-feed (JF) and Aqua Jel only (J) with a photoperiod of 1L:5D.
"D" stands for dark and "L" stands for light.

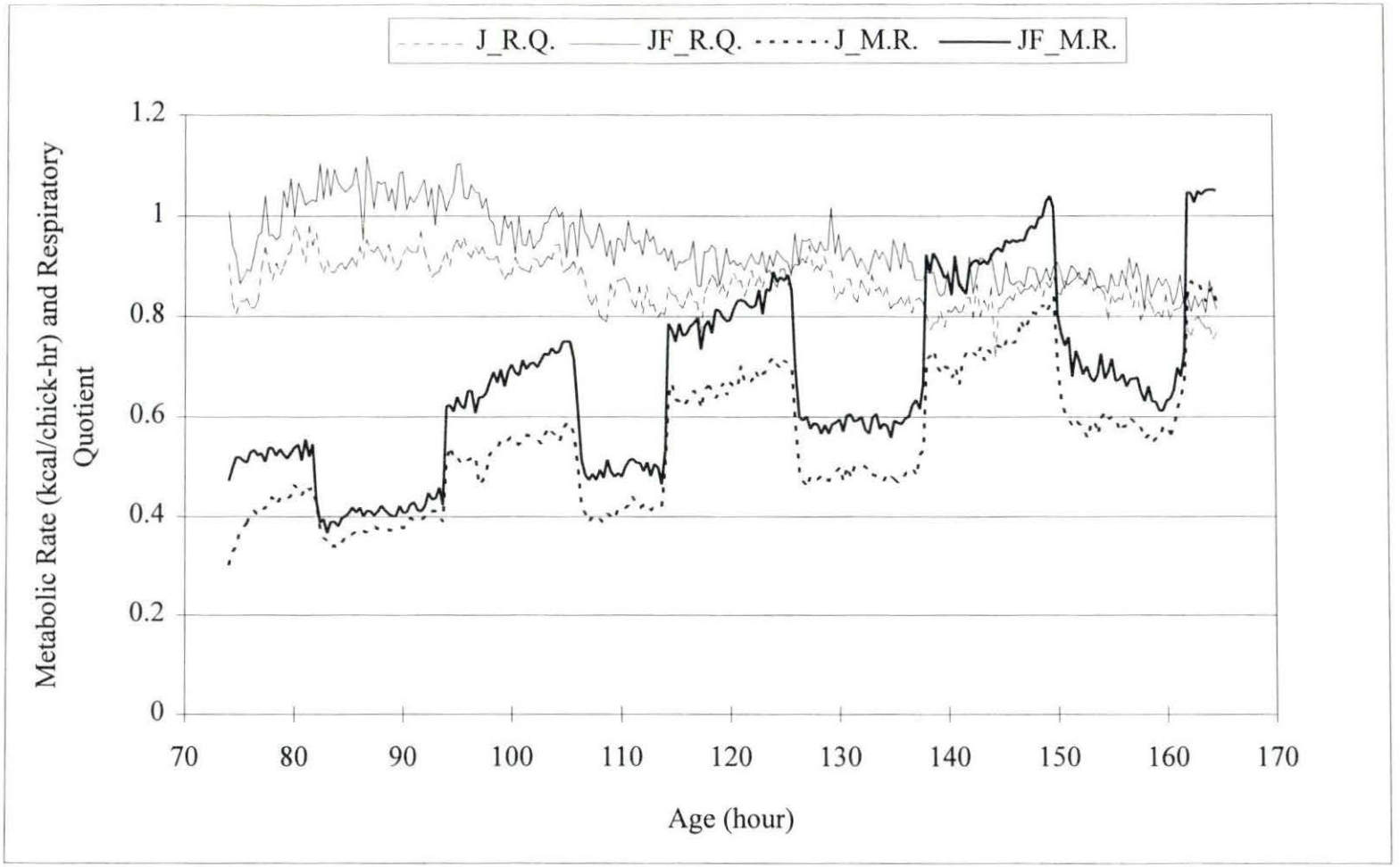


Figure 6: Metabolic rate (M.R.) and respiratory quotient (R.Q.) of chicks from two nutritional treatments of Aqua Jel-and-feed (JF) and Aqua Jel only (J) with a photoperiod of 12L:12D.
(from 72 hour, feed and water were provided to chicks in both groups)

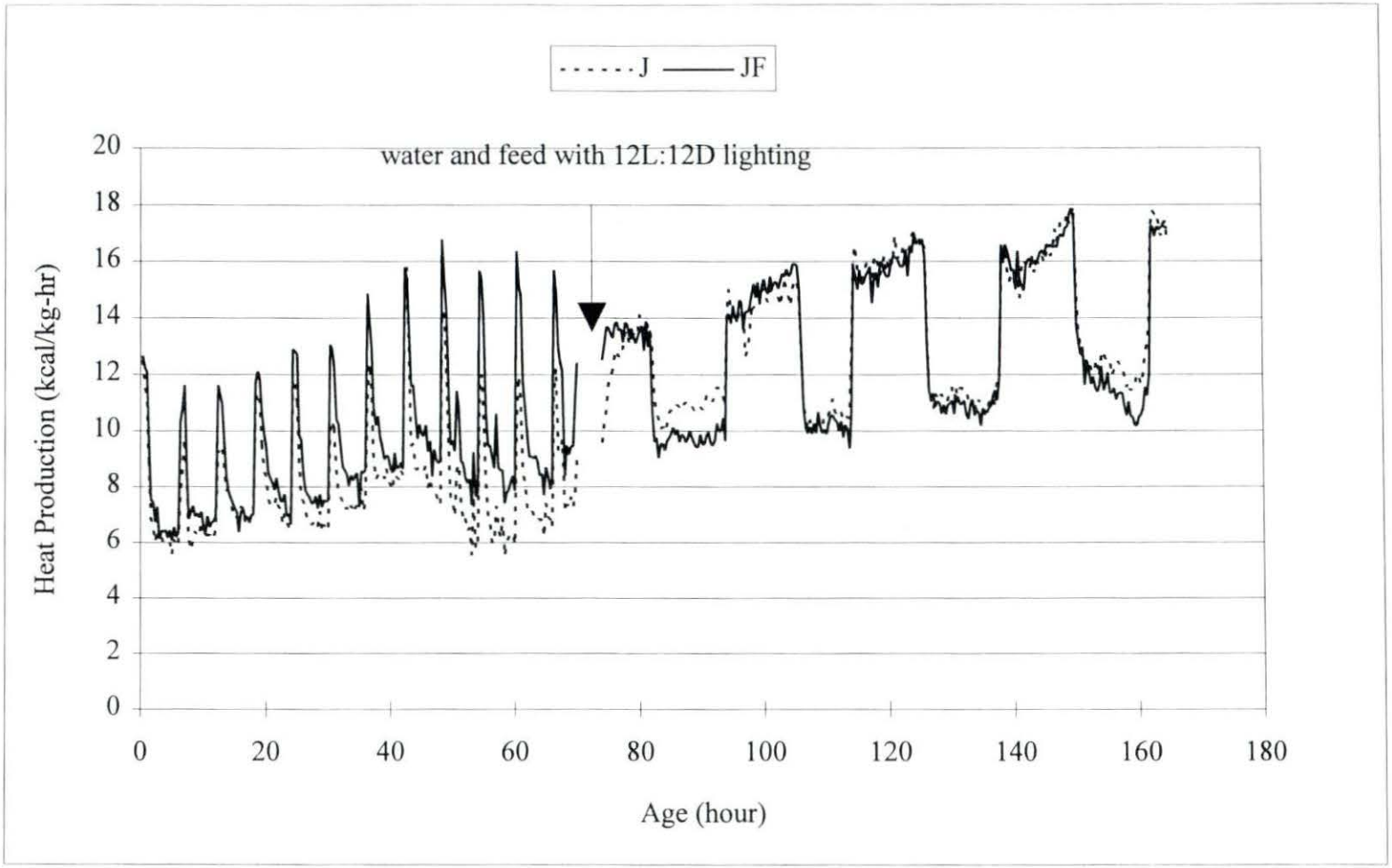


Figure 7: Heat production of chick under two different nutritional regimes of Aqua Jel-and-feed (JF) and Auqa Jel only (J).

* Photoperiods = 1L:5D for the first 72 hours and then 12L:12D for after 72 hour.

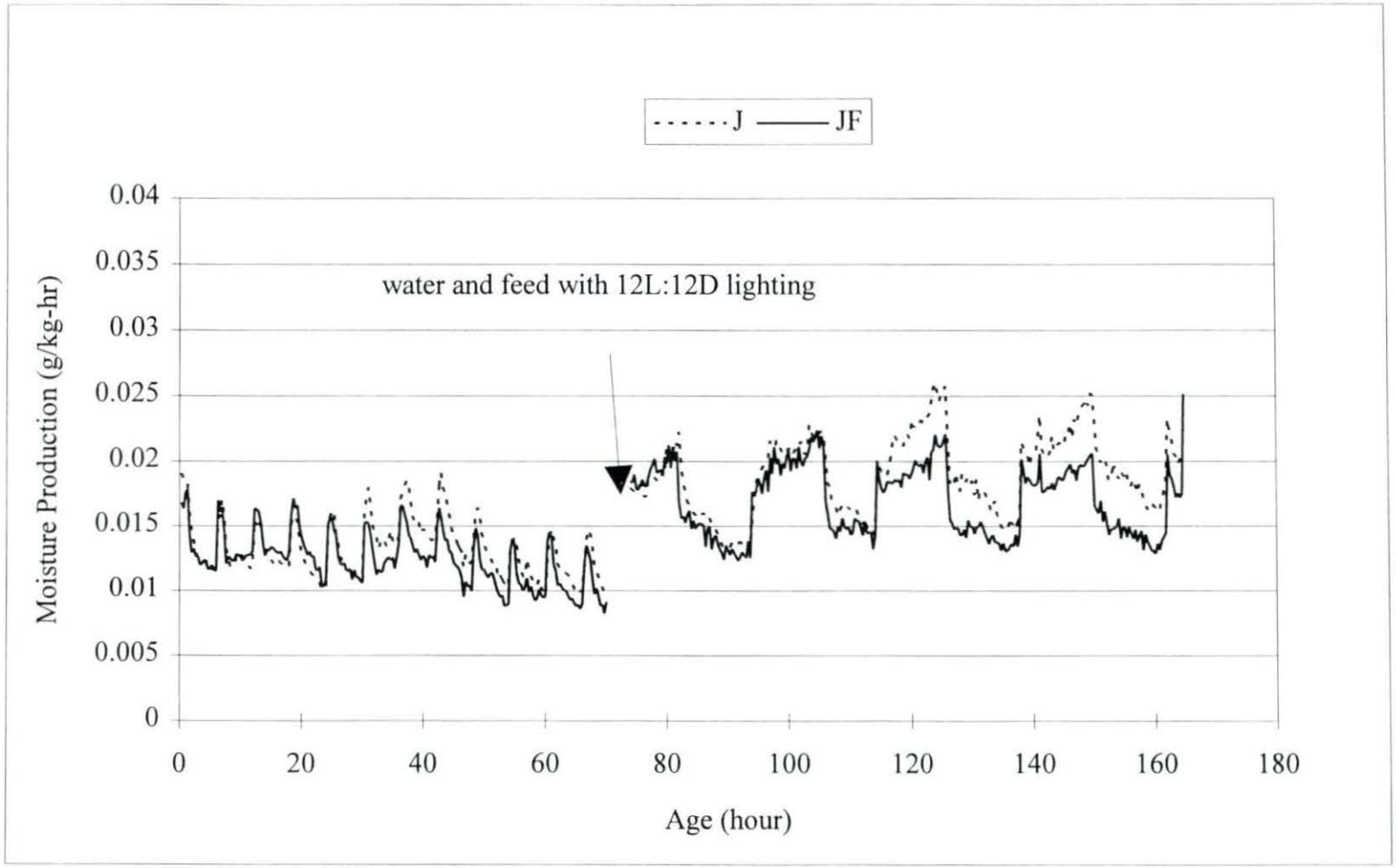


Figure 8: Moisture production of chick under two different nutritional regimes of Aqua Jel-and-feed (JF) and Auqa Jel only (J).

* Photoperiods = 1L:5D for the first 72 hours and then 12L:12D for after 72 hour.

profiles, and Figure 8 shows the moisture production profiles.

Experiment VI: The metabolic rate and moisture production were compared between the neither-Aqua Jel ®-nor-feed (N) treatment and the Aqua Jel ®-and-feed (JF) treatment, and the results are summarized in Table 10. The dynamic heat and moisture production rates are shown in Figures 9 to 12. Both the metabolic rate and moisture production of chicks in the JF group appeared higher than those of chicks in the N treatment for the first 5 days. However, after 5 days, chicks in the N group produced more moisture than chicks in the JF group. Figure 9 shows the result of the metabolic rate and R.Q. of both groups. It shows the metabolic rate and R.Q. of chicks in the JF group appeared greater than those of chicks in N group. The metabolic rate of chicks in both JF and N groups after 72 hours with the same nutritional (water and feed) and light (12L:12D) treatments was shown in Figure 10. Although the same nutritional treatments were provided to both groups, the difference in metabolic rate per kilogram body weight lasted till 6.5 days of age (155 hours) (Figure 11). As already shown in the previous figures, light condition affected the metabolic rate. The heat production quickly increased upon lighting. Under the dark condition, the chicks maintained a certain metabolic rate.

Experiment VII and Experiment VIII: The metabolic rate and moisture production were compared between different lighting conditions (continuous 24-hour light versus 24-

Table 10: Metabolic rate, moisture production, and respiratory quotient of chicks from t nutritional treatments: Aqua Jel-and-feed (JF) and neither (N).

		Metabolic Rate*		Moisture Production**		Respiratory Quotient	
		JF	N	JF	N	JF	N
2nd day	Mean	8.80	6.59	14.76	7.15	0.97	0.75
(a)	Std. Dev.	1.39	1.47	1.80	1.36	0.05	0.06
	Max.	13.46	10.54	21.42	11.93	1.06	0.91
	Min.	7.32	5.09	11.59	5.50	0.86	0.62
3rd day	Mean	9.41	6.63	12.3	6.52	0.92	0.72
(a)	Std. Dev.	1.90	1.36	1.96	1.26	0.05	0.05
	Max.	14.17	10.05	17.01	9.72	1.00	0.90
	Min.	6.73	5.12	7.94	4.80	0.82	0.63
4th day	Mean	11.47		15.61		0.98	0.87
(b)	Std. Dev.	2.28		2.45		0.06	0.09
	Max.	15.36		19.19		1.08	1.03
	Min.	7.28		11.35		0.81	0.66
5th day	Mean	13.43	12.43	16.83	15.92	0.99	0.94
(b)	Std. Dev.	2.37	2.72	2.25	2.55	0.03	0.05
	Max.	18.07	15.88	20.45	19.81	1.06	1.07
	Min.	10.52	9.52	12.86	13.35	0.94	0.82
6th day	Mean	15.45	14.07	17.56	19.34	0.99	0.96
(b)	Std. Dev.	3.06	2.77	3.31	3.11	0.03	0.04
	Max.	20.03	18.63	23.41	25.23	1.07	1.07
	Min.	11.8	10.69	12.78	14.32	0.94	0.88
7th day	Mean	16.31	15.76	17.33	20.06	0.96	0.98
(b)	Std. Dev.	3.44	3.12	3.79	3.85	0.04	0.04
	Max.	20.85	20.75	23.78	26.41	1.02	1.06
	Min.	12.12	11.89	11.69	12.85	0.85	0.85
8th day	Mean	15.48	15.87	16.99	19.26	0.90	0.94
(b)	Std. Dev.	3.10	2.86	3.19	3.23	0.04	0.05
	Max.	20.04	19.29	22.05	24.74	0.96	1.03
	Min.	11.77	12.13	11.7	13.31	0.81	0.81

Intermittent lighting: (a) 1L:5D and (b) 12L:12D

* Unit for metabolic rate: kcal/kg-hour

** Unit for moisture production: (g/kg-hour) x 10 ^ -3

Temperature: 28 (1) C

Relative humidity: 45 (7) %.

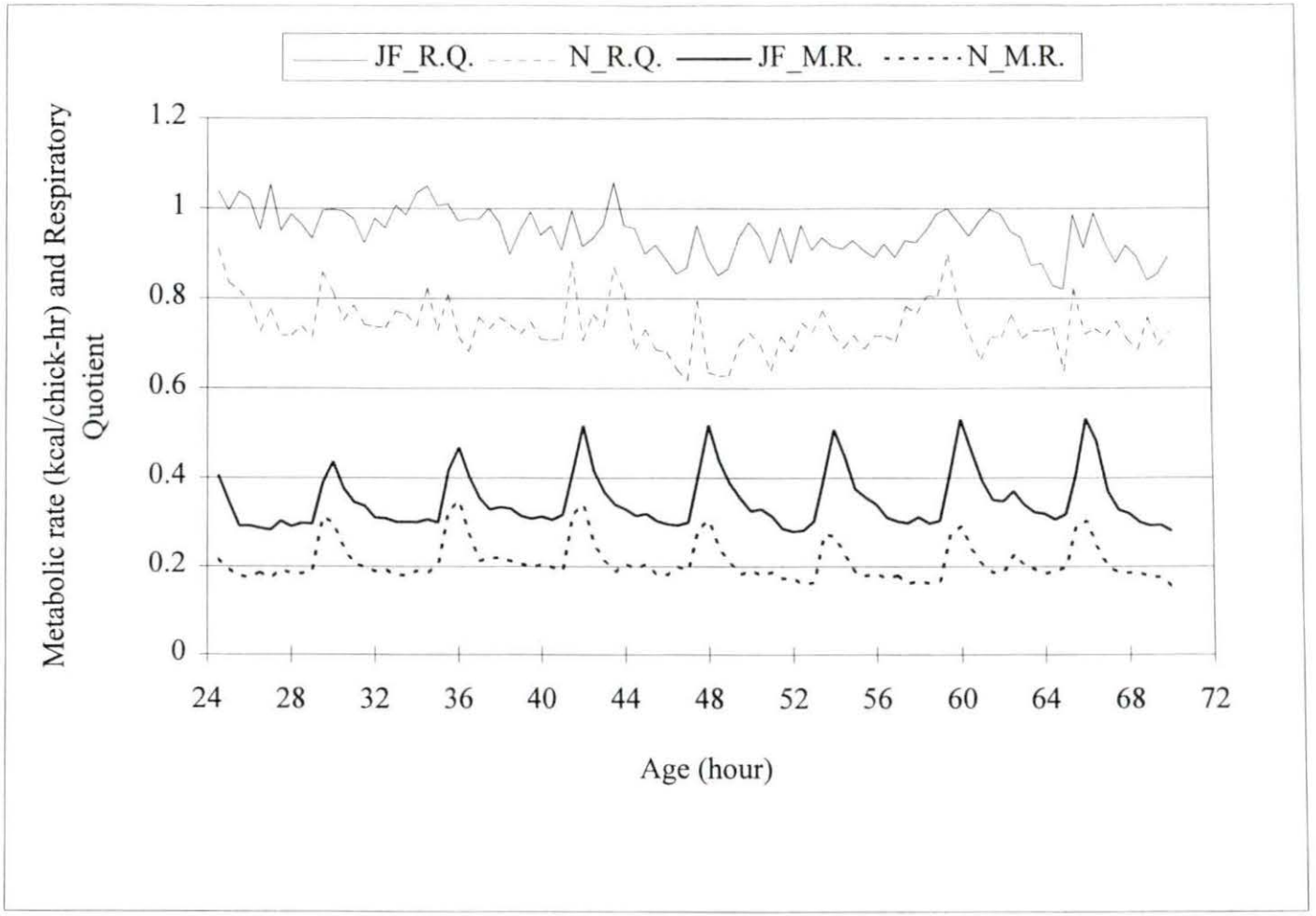


Figure 9: Metabolic rate (M.R.) and respiratory quotient (R.Q.) of chicks from two different nutritional treatments; Aqua Jel-and-feed (JF) and neither (N) with a photoperiod of 1L:5D.

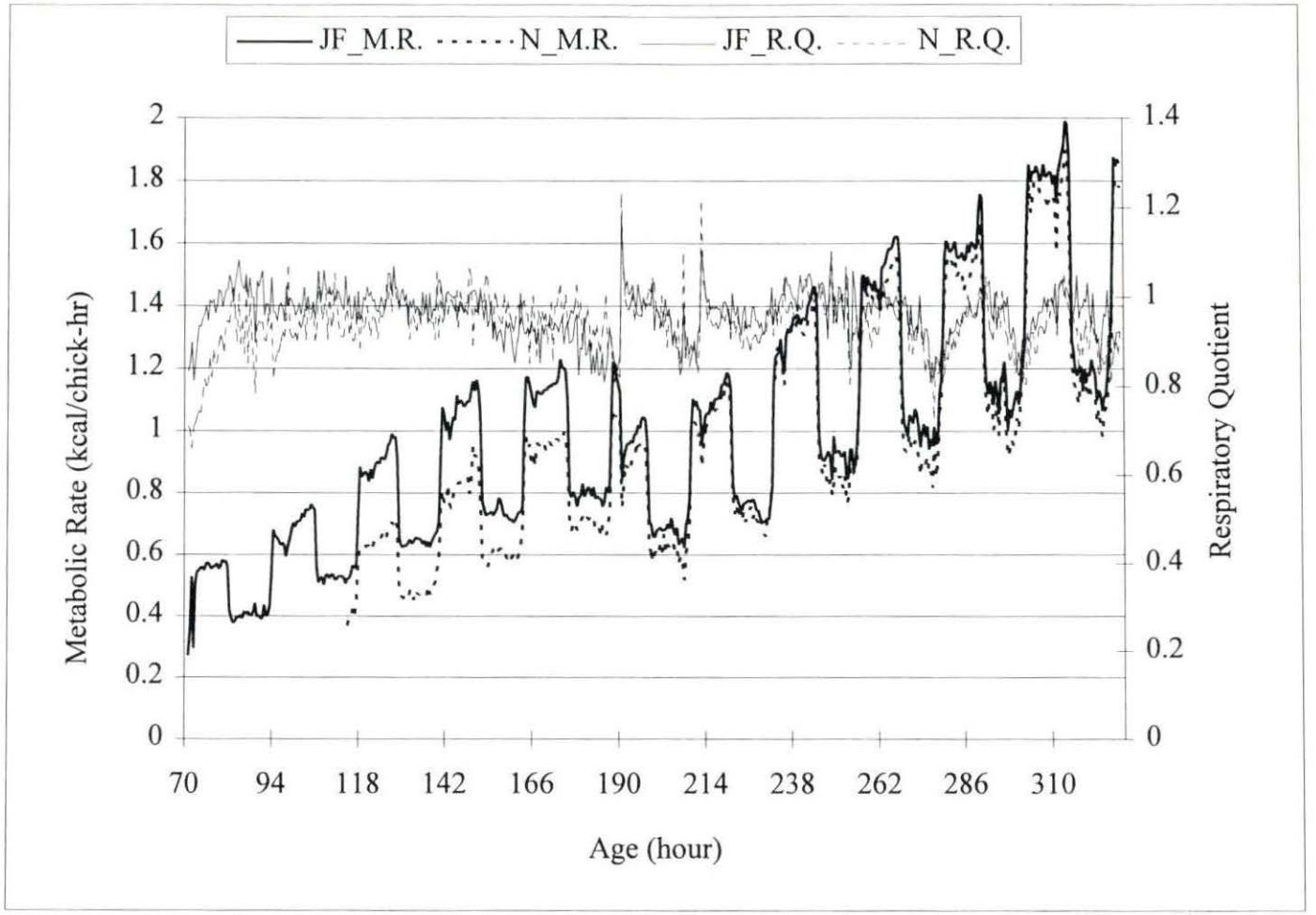


Figure 10: Metabolic rate (M.R.) and respiratory quotient (R.Q.) of chicks from two different nutritional treatments; Aqua Jel-and-feed (JF) and neither (N) with a photoperiod of 12L:12D. (from 72 hour, feed and water were provided to chicks in both groups)

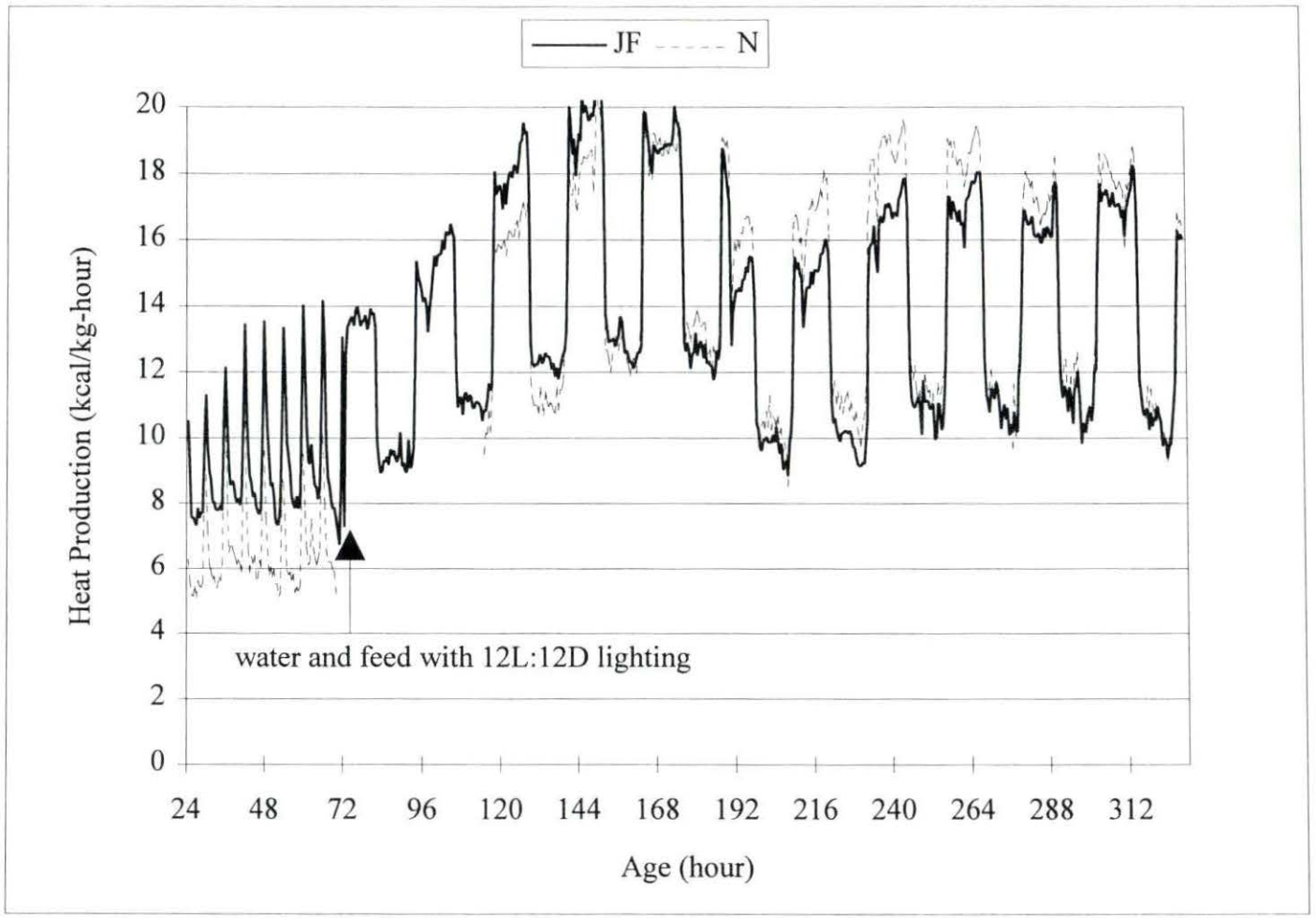


Figure 11: Heat production per unit body mass under two nutritional treatments of Aqua Jel-and-feed (JF) and neither (N).

* photoperiod = 1L:5D for the first 72 hour and then 12L:12D for after 72 hour.

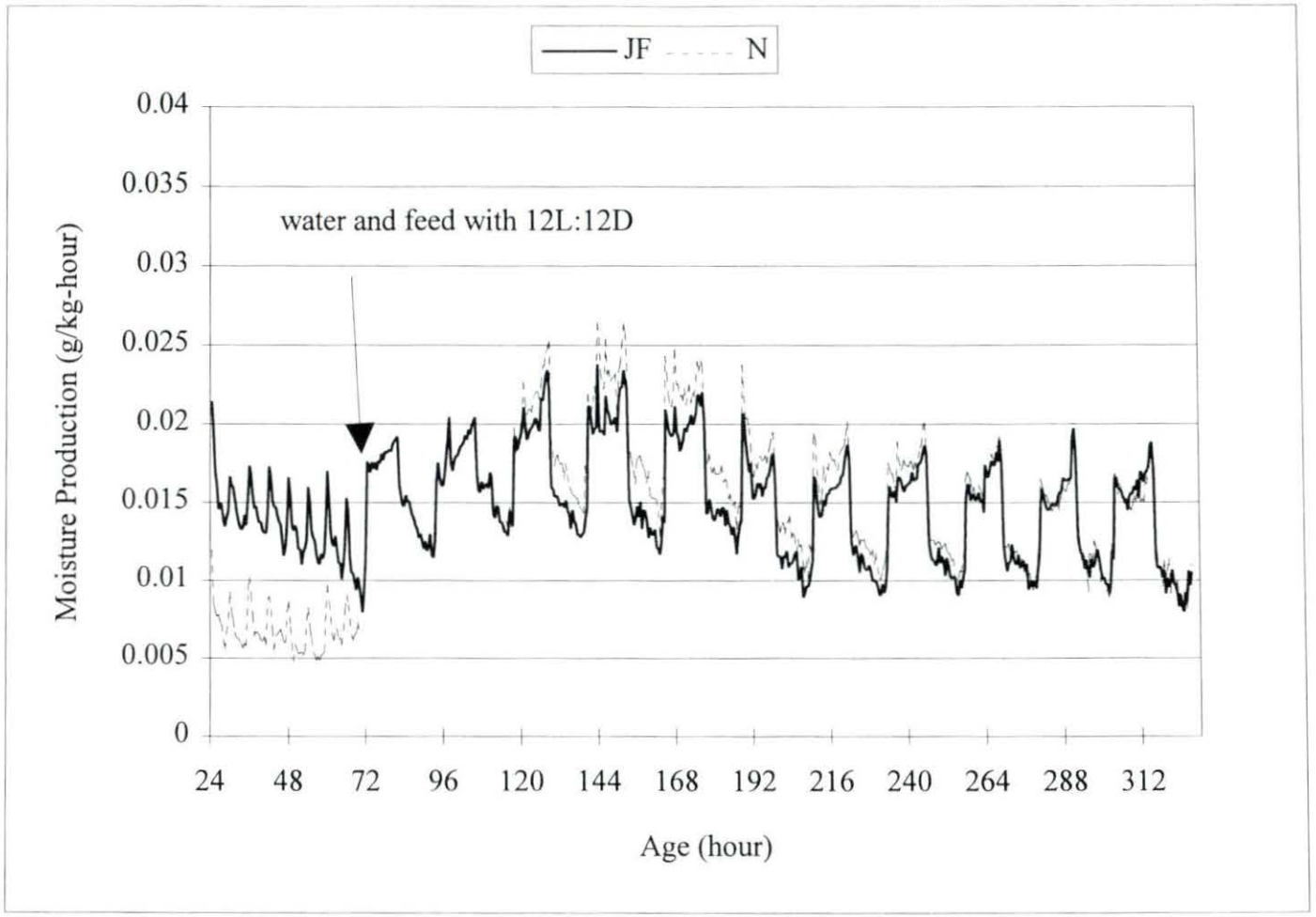


Figure 12: Moisture production per unit body mass under two nutritional treatments of Aqua Jel-and-feed (JF) and neither (N).

* photoperiod = 1L:5D for the first 72 hour and then 12L:12D for after 72 hour.

hour dark) and the results are summarized in Table 11. As shown in the Tables, the metabolic heat production of chicks in the light treatment appeared higher than that of chicks in the dark treatment. The heat production, R.Q., and moisture production of chicks in the fasting groups with different light treatments are shown in Figures 14 and 15.

Table 11: Metabolic rate and moisture production of fasting chicks under the lighting treatments of continuous light (Light) versus continuous dark (Dark).

		Metabolic Rate*		Moisture Production**		Respiratory Quotient	
		Light	Dark	Light	Dark	Light	Dark
1st day	Mean	6.30	5.23	4.92	3.89	0.81	0.83
	Std. Dev.	1.11	0.64	0.84	0.73	0.04	0.07
	Max.	8.32	6.83	7.89	7.65	0.88	0.93
	Min.	4.98	4.26	2.72	2.08	0.71	0.62
2nd day	Mean	6.62	5.70	4.44	3.77	0.79	0.83
	Std. Dev.	0.88	0.54	0.67	0.66	0.03	0.04
	Max.	8.26	6.62	5.35	4.95	0.85	0.88
	Min.	5.43	4.95	3.43	2.86	0.72	0.71
3rd day	Mean	6.64	5.84	4.45	3.64	0.80	0.85
	Std. Dev.	0.73	0.66	0.56	0.69	0.04	0.06
	Max.	7.76	7.03	5.23	4.93	0.90	0.96
	Min.	5.56	4.43	3.33	2.64	0.73	0.71
4th day	Mean	5.77	5.49	5.67	5.40	0.78	0.86
	Std. Dev.	0.51	0.22	0.54	0.57	0.03	0.04
	Max.	6.85	6.10	6.54	6.16	0.84	0.93
	Min.	5.18	5.06	4.93	4.12	0.74	0.77

* Unit for metabolic rate: kcal/kg-hour
** Unit for moisture production: (g/kg-hour) x 10 ^ -3
Temperature: 29 (0.3) C
Relative humidity: 28 (3) %.

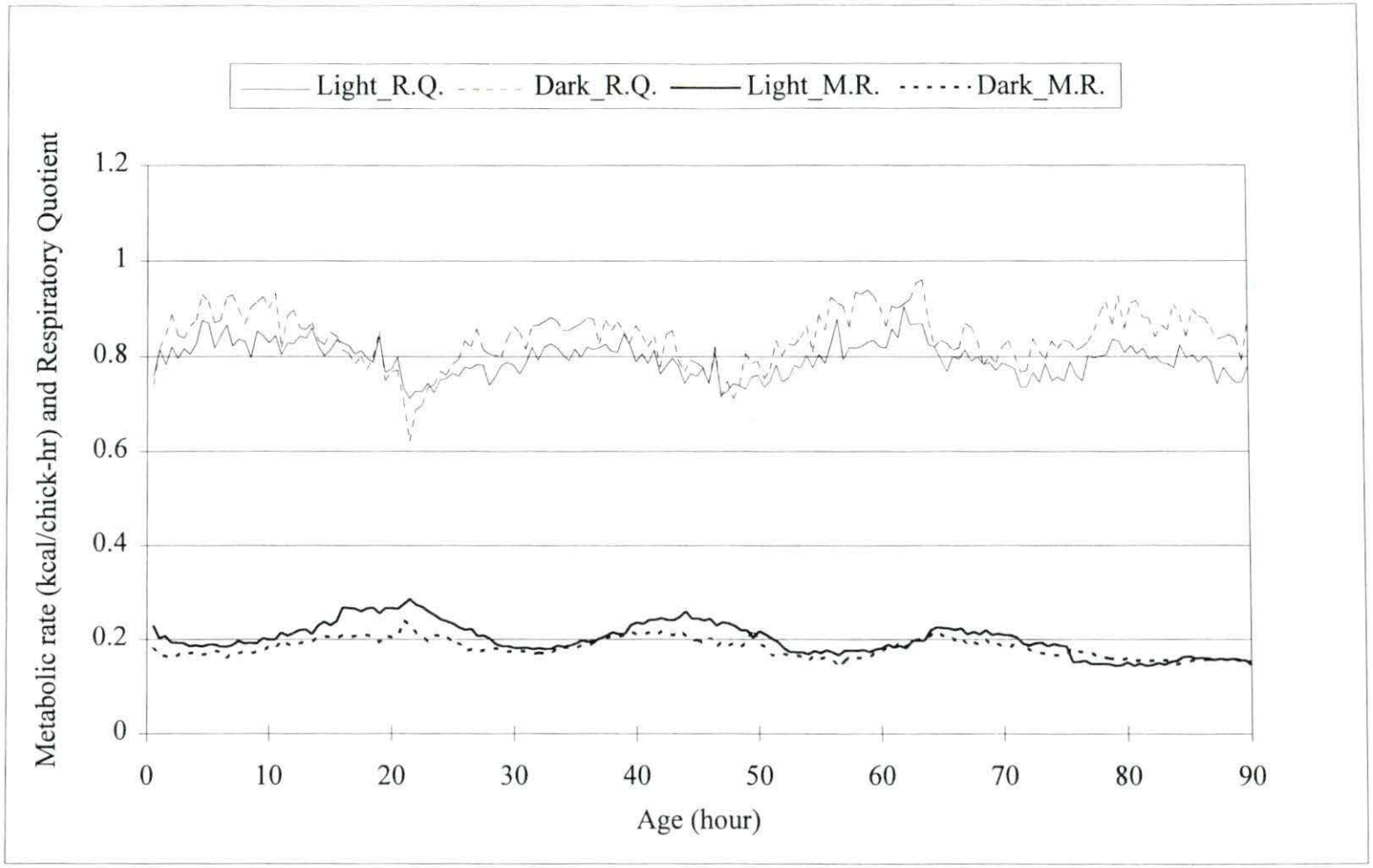


Figure 13: Metabolic rate (M.R.) and respiratory quotient (R.Q.) of fasting chicks under the light treatments of continuous 24 hours light (Light) versus 24 hours dark (Dark).

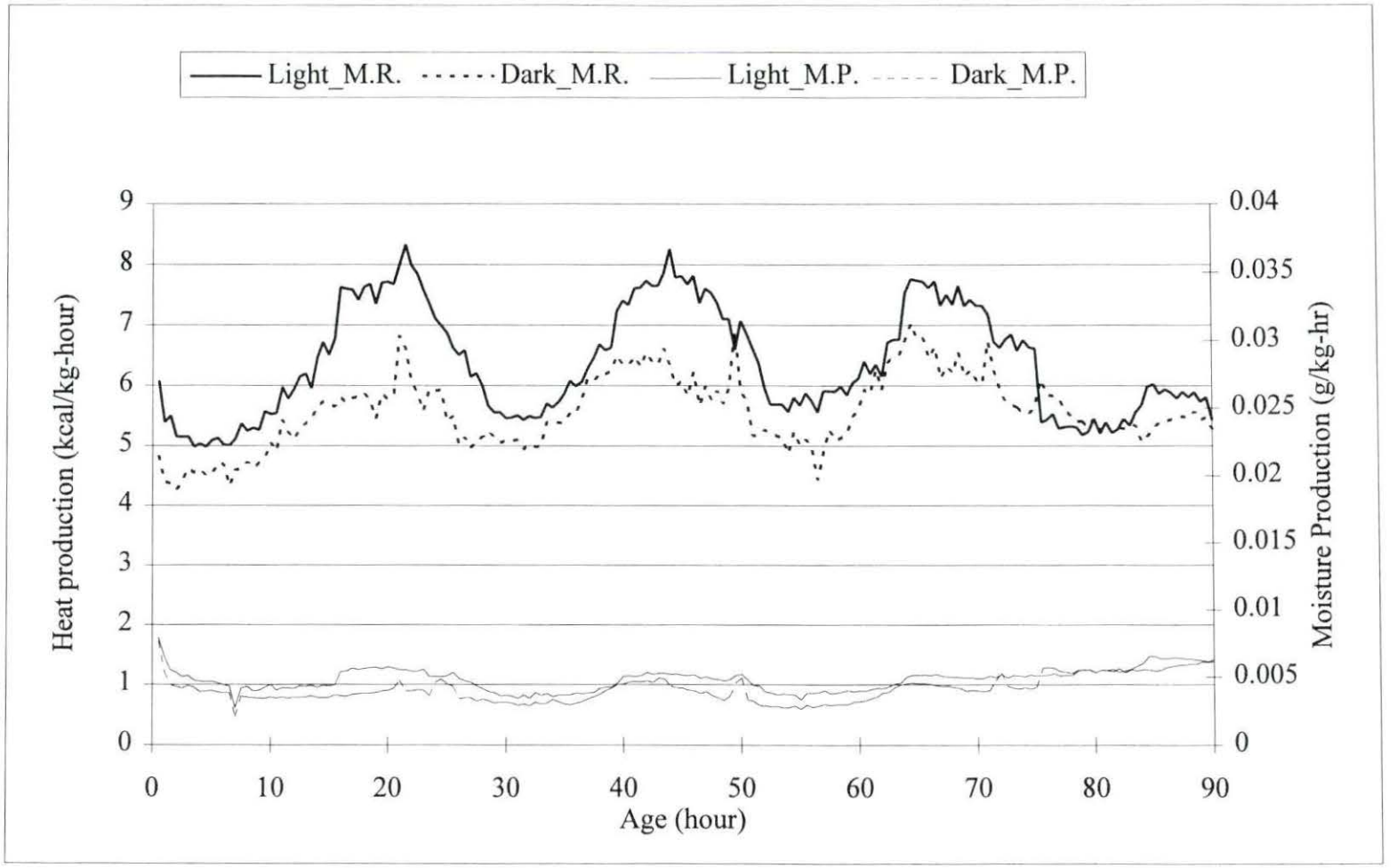


Figure 14: Metabolic rate (M.R.) and moisture production (M.P.) of fasting chicks under the light treatments of continuous 24 hours light (Light) versus 24 hours dark (Dark).

Body Water Content and PCV Test

The body water content of 10 randomly selected chicks (less than half-day old) was 70.9 ± 1.44 % of the total body weight (Table 12). The body water content was not significantly different among the WF, JF, and N groups, and between W and J groups after the three-day nutritional treatments ($p>0.05$). However, the body water content of chicks in the W treatment was significantly higher than that of chicks in WF, JF, or N treatment ($p<0.05$). The body water content increased significantly by 5.42 percent of initial body water content (IBWC) ($p<0.05$) for chicks in the WF treatment and 4.85 % IBWC ($p<0.05$) for chicks in the JF treatment for the 3-day period. Body water content of chicks in W, J, and N treatments increased by 7.84, 6.67, and 4.32 % IBWC ($P<0.05$), respectively. These results show that the chicks in all treatments were not in a dehydration state. Interestingly, even though the chicks in the N treatment had no access to water, their relative body water content did not decrease. The increase in relative body water content of the chicks in the W, J, and N treatments was mainly due to the decrease of dry body weight as shown in Table 13. The body water content of dying chicks from the W treatment showed an average of 76 % body water content.

The packed cell volume (PCV) from 7 randomly selected chicks (less than half-day-old) averaged 25.67 ± 2.37 % (Table 14). The PCV was not significantly different among the WF, JF, J, and N groups after the three-day nutritional treatment ($p>0.05$). However, the PCV was significantly different between WF and W groups ($p<0.05$). PCV of chicks in the WF, JF, J, and N treatments did not show significant change ($p>0.05$) over the 3-day period.

Table 12: Body water content as the percentage of total body weight of chicks subjected to different nutritional treatments.

Age, day	WF (S.D.)	JF (S.D.)	W (S.D.)	J (S.D.)	N (S.D.)
0 †	$70.90(1.44)^{a}_{3}$	$70.90(1.44)^{a}_{3}$	$70.90(1.44)^{a}_{3}$	$70.90(1.44)^{a}_{3}$	$70.90(1.44)^{a}_{2}$
1 *	$70.95(1.27)^{b}_{3}$	$72.19(1.18)^{b}_{2,3}$	$75.43(2.94)^{a}_{2}$	$75.50(1.44)^{a}_{2}$	$75.48(1.95)^{a}_{1}$
2 *	$74.64(1.49)^{b}_{2}$	$73.42(1.53)^{c}_{2}$	$77.33(1.38)^{a}_{1}$	$77.88(1.31)^{a}_{1}$	$75.04(0.86)^{b}_{1}$
3 *	$76.32(1.58)^{b,c}_{1}$	$75.75(2.66)^{c}_{1}$	$78.74(1.30)^{a}_{1}$	$77.57(1.90)^{a,b}_{1}$	$75.22(1.49)^{c}_{1}$

Table 13: Dry body weight of chicks subjected to different nutritional treatments (g/chick).

Age, day	WF (S.D.)	JF (S.D.)	W (S.D.)	J (S.D.)	N (S.D.)
0 †	$12.11\ (1.26)^{a}_{1}$	$12.11\ (1.26)^{a}_{1}$	$12.11\ (1.26)^{a}_{1}$	$12.11\ (1.26)^{a}_{1}$	$12.11\ (1.26)^{a}_{1}$
1 *	$11.24\ (1.00)^{a}_{1}$	$10.08\ (0.69)^{b}_{2}$	$9.62\ (0.75)^{b}_{2}$	$9.47\ (0.64)^{b}_{2}$	$9.48\ (1.09)^{b}_{2}$
2 *	$11.58\ (1.55)^{a}_{1}$	$11.23\ (1.44)^{a}_{1,2}$	$7.76\ (0.87)^{b}_{3}$	$7.99\ (0.47)^{b}_{3}$	$8.32\ (0.71)^{b}_{3}$
3 *	$11.76\ (1.25)^{a}_{1}$	$11.46\ (1.60)^{a}_{1}$	$6.76\ (0.61)^{b}_{4}$	$7.29\ (0.53)^{b}_{3}$	$7.23\ (0.92)^{b}_{4}$

S.D. = standard deviation.

* Values for each treatment are average of 10 chicks for each age group

† Initial body water content or dry body weight (age 0) was based on 10 randomly selected chicks and assumed the same for all nutritional treatments

WF is water and feed, JF is Aqua Jel® and feed, W is water only, J is Aqua Jel® only, and N is neither Aqua Jel® nor feed

Column means with the same subscripts are not significantly different ($p > 0.05$)

Row means with the same superscripts are not significantly different ($p > 0.05$).

Table 14: PCV of chicks subjected to different nutritional treatments for a 3-day period.

Age, day	WF (S.D.)	JF (S.D.)	W (S.D.)	J (S.D.)	N (S.D.)
0 †	25.67 (2.37)$^{a}_{1}$	25.67 (2.37)$^{a}_{1}$	25.67 (2.37)$^{a}_{1}$	25.67 (2.37)$^{a}_{1}$	25.67 (2.37)$^{a}_{1,2}$
1 *	20.26 (2.75)$^{b}_{2}$	24.40 (4.46)$^{a}_{1}$	21.43 (1.90)$^{a,b}_{1,2}$	22.43 (3.48)$^{a,b}_{1}$	22.71 (2.93)$^{a,b}_{2}$
2 *	24.67 (1.41)$^{a}_{1}$	23.10 (1.84)$^{a,b}_{1}$	18.38 (4.44)$^{c}_{2}$	21.10 (3.69)$^{b,c}_{1}$	24.05 (1.77)$^{a,b}_{2}$
3 *	26.88 (1.54)$^{a}_{1}$	25.24 (2.37)$^{a,b}_{1}$	20.29 (6.58)$^{b}_{2}$	22.52 (7.53)$^{a,b}_{1}$	27.14 (3.63)$^{a}_{1}$

S.D. = standard deviation.

* Values for each treatment are average of 7 chicks for each age group

† Initial packed cell volume (age 0) was based on 7 randomly selected chicks and assumed the same for all nutritional treatments

WF is water and feed, JF is Aqua Jel® and feed, W is water only, J is Aqua Jel® only, and N is neither Aqua Jel® nor feed

Column means with the same subscripts are not significantly different ($p > 0.05$)

Row means with the same superscripts are not significantly different ($p > 0.05$).

PCV of chicks in the W treatment significantly decreased by 5.38 percent of initial PCV ($p<0.05$). These results also support that the chicks in all treatments were not in a dehydration state. During the second experiment, the PCV test was conducted on the dying (weak) chicks only. The PCV of chicks in the W treatment averaged 8.83 ± 6.32 % at 3 days of age (4 chicks) and 7.72 ± 3.75 % at 4 days of age (3 chicks). The PCV of chicks in the N treatment averaged 13.93 ± 9.22 % at 4 days of age (5 chicks). The PCV of live chicks at 5 days of age averaged 28 ± 1.15 % for the WF treatment, 28.92 ± 0.69 % for the W treatment, and 27.25 ± 1.71 % for the N treatment. Figures 15 through 21 show the environmental conditions, body weight change, body water content, and PCV change of the two experiments.

Dryness of Respiratory Tract

Figure 22 is a TEM of trachea from a normal chick. It shows the mucus layer over the epithelium. By comparison, Figure 23 is a TEM of trachea from a chick that had been deprived from water for 3 days. From Figure 23, the absence of the mucous layer can be noticed.

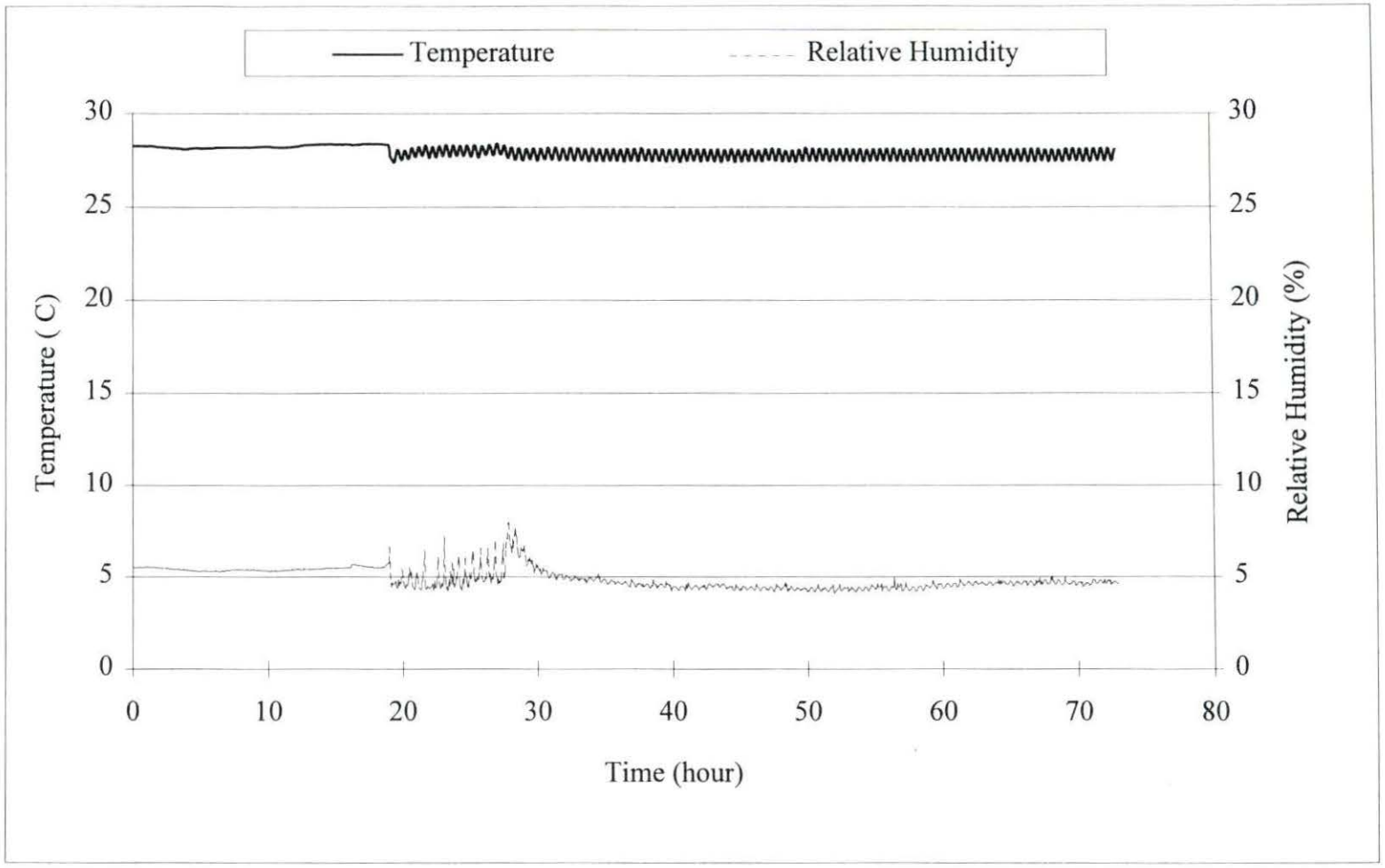


Figure 15: Environmental conditions for *Experiment X* of body water content and PCV test.

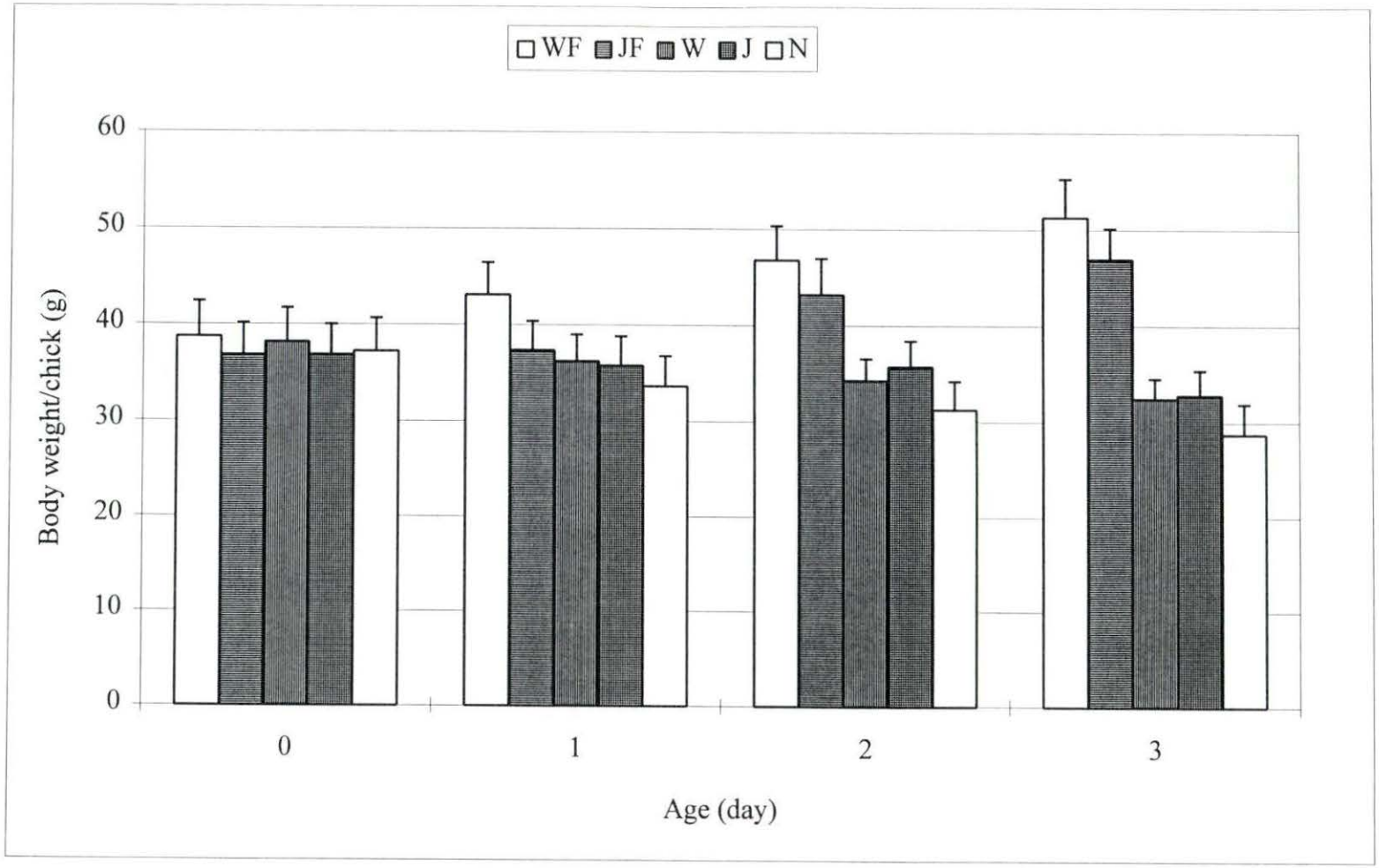


Figure 16: Chick body weight change over time (60 chicks on 0th day, 60 chicks on 1st day, 43 chicks on 2nd day, 26 chicks on 3rd day for each treatment group).
* WF is water and feed, JF is Aqua Jel and feed, W is water only, J is Aqua Jel only, and is neither water nor feed. (The vertical bars stand for standard deviation).

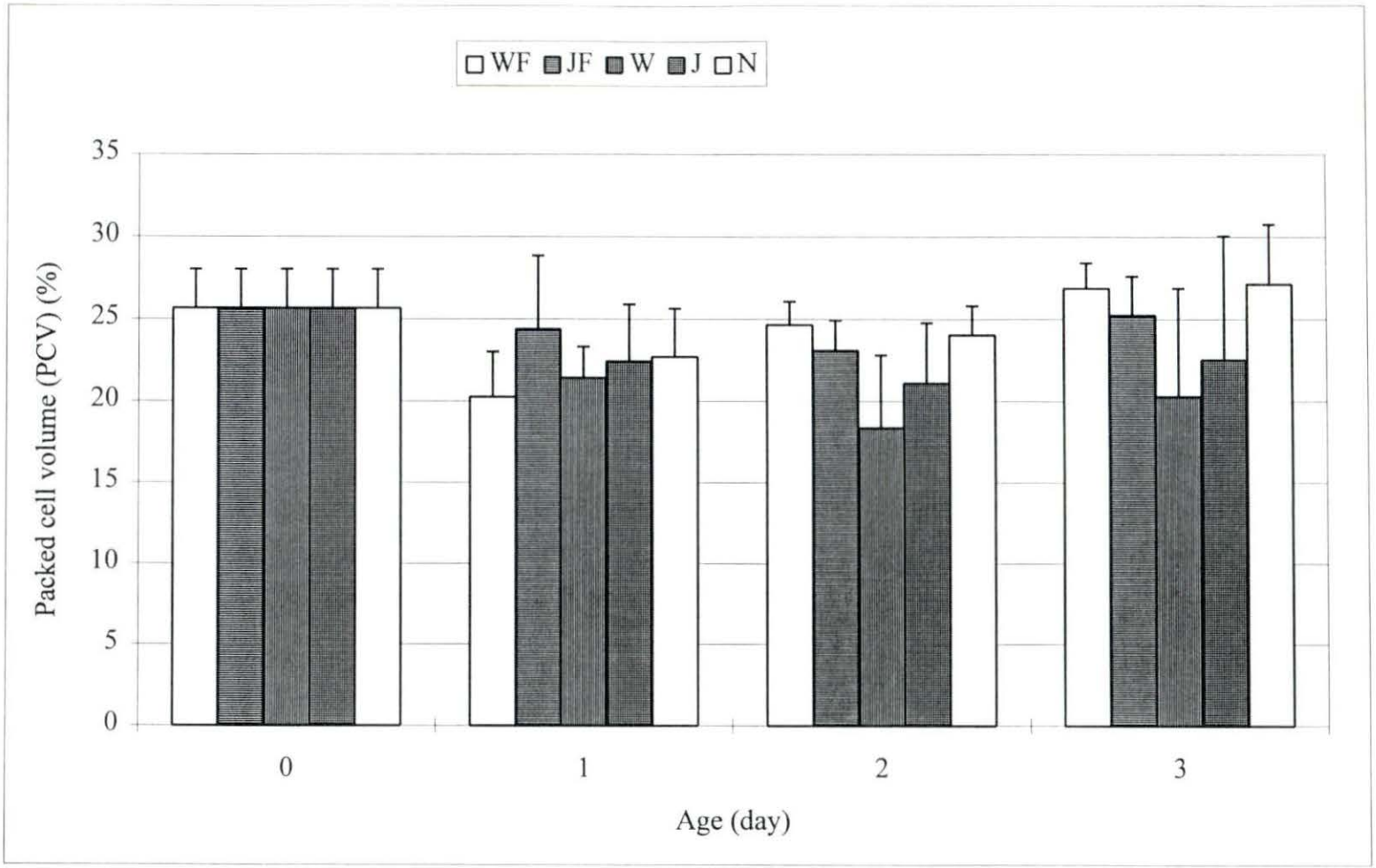


Figure 17: PCV change of chicks for different nutritional treatments (7 chicks from each group per day).
* WF is water and feed, JF is Aqua Jel and feed, W is water only, J is Aqua Jel only, and is neither water nor feed. (The vertical bars stand for standard deviation).

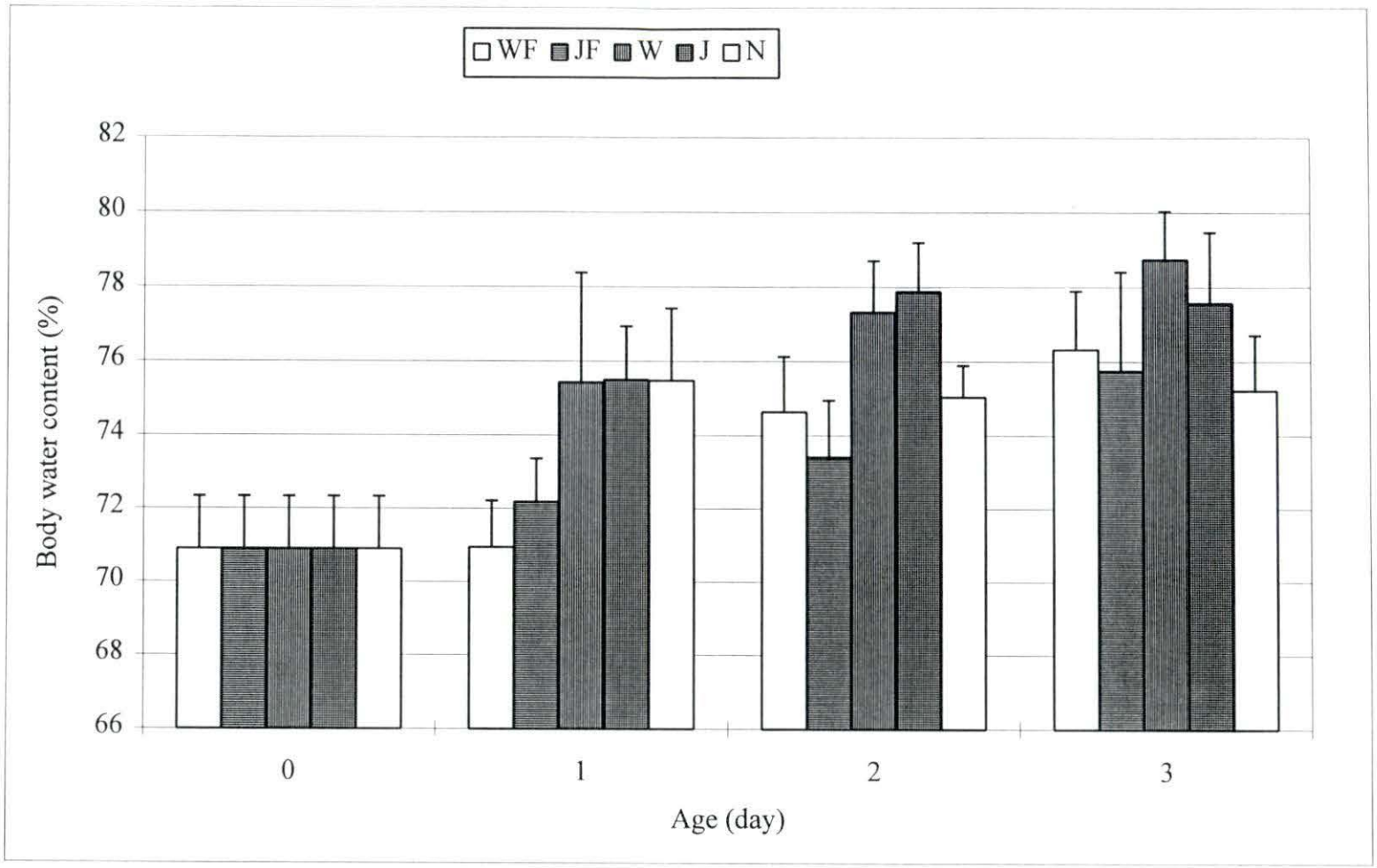


Figure 18: Total body water content of chicks (10 chicks from each group in each day).
* WF is water and feed, JF is Aqua Jel and feed, W is water only, J is Aqua Jel only, and is neither water nor feed. (The vertical bars stand for standard deviation).

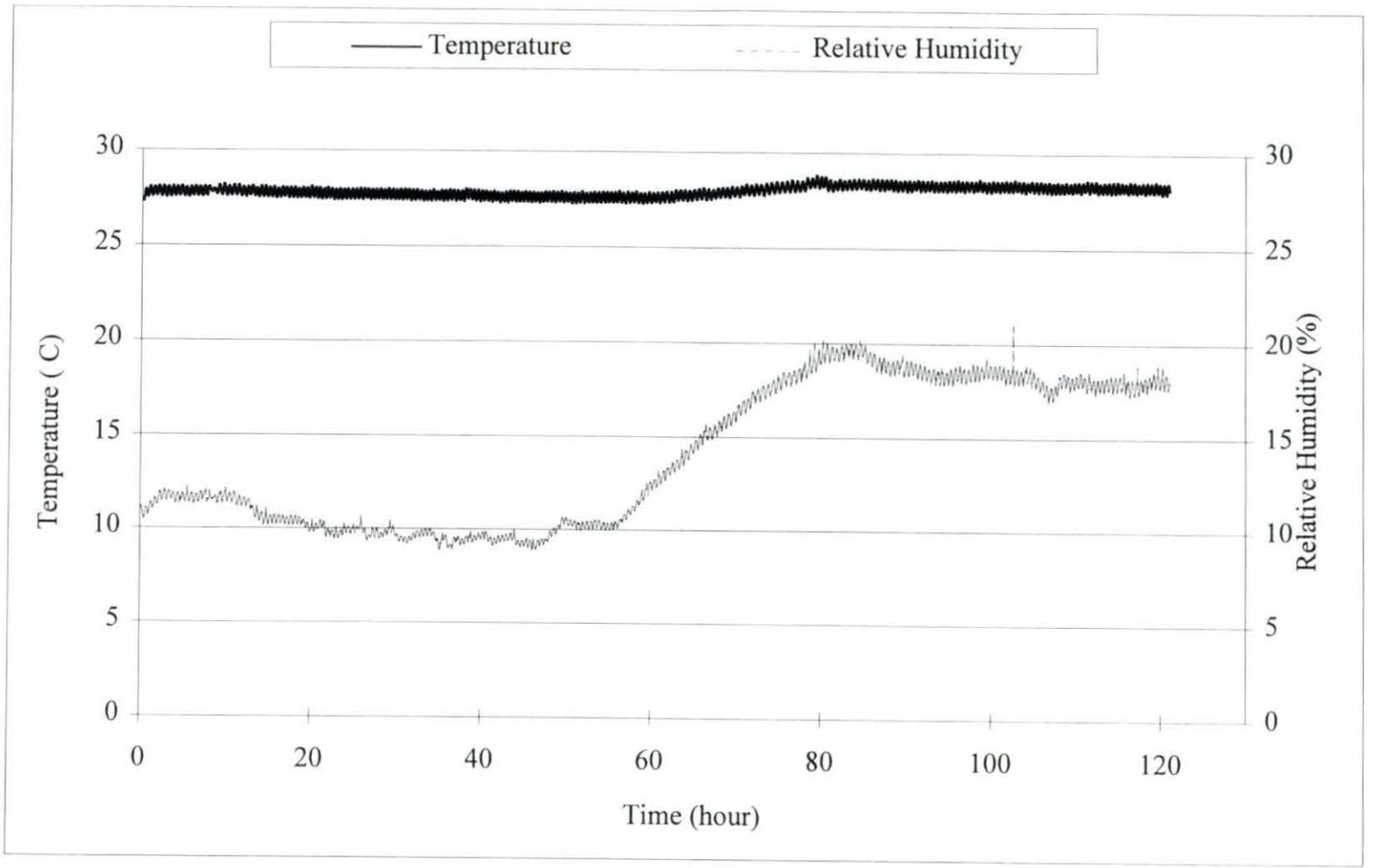


Figure 19: Environmental conditions for *Experiment XI* of body water content and PCV test.

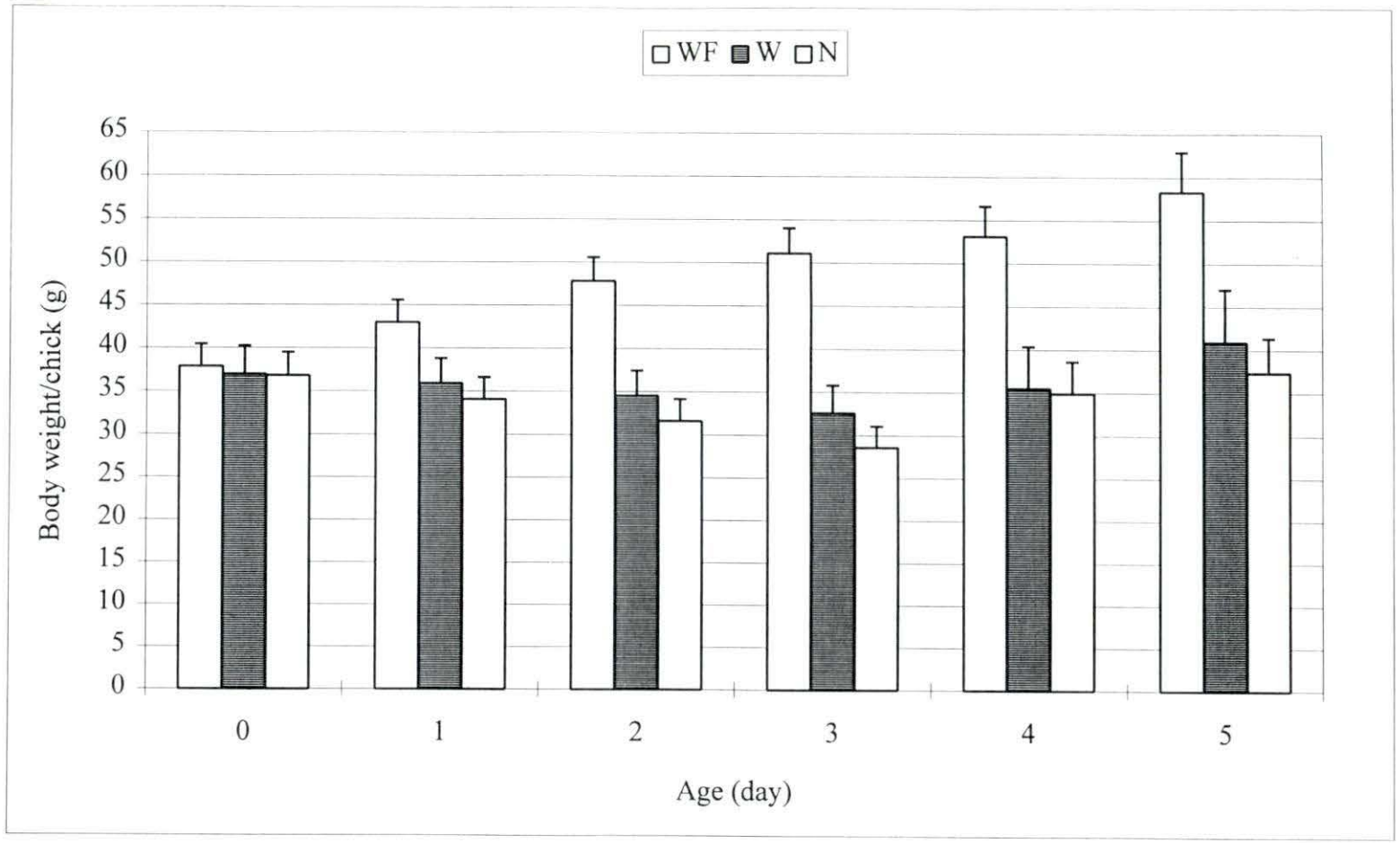


Figure 20: Total body weight change over time with different nutritional treatments (60 chicks on 0th day) from each group).

* WF is water and feed, W is water only, and N is neither water nor feed.
(from the fourth, feed and water were also provided to chicks in W and N groups.)

** The vertical bars stand for standard deviation.

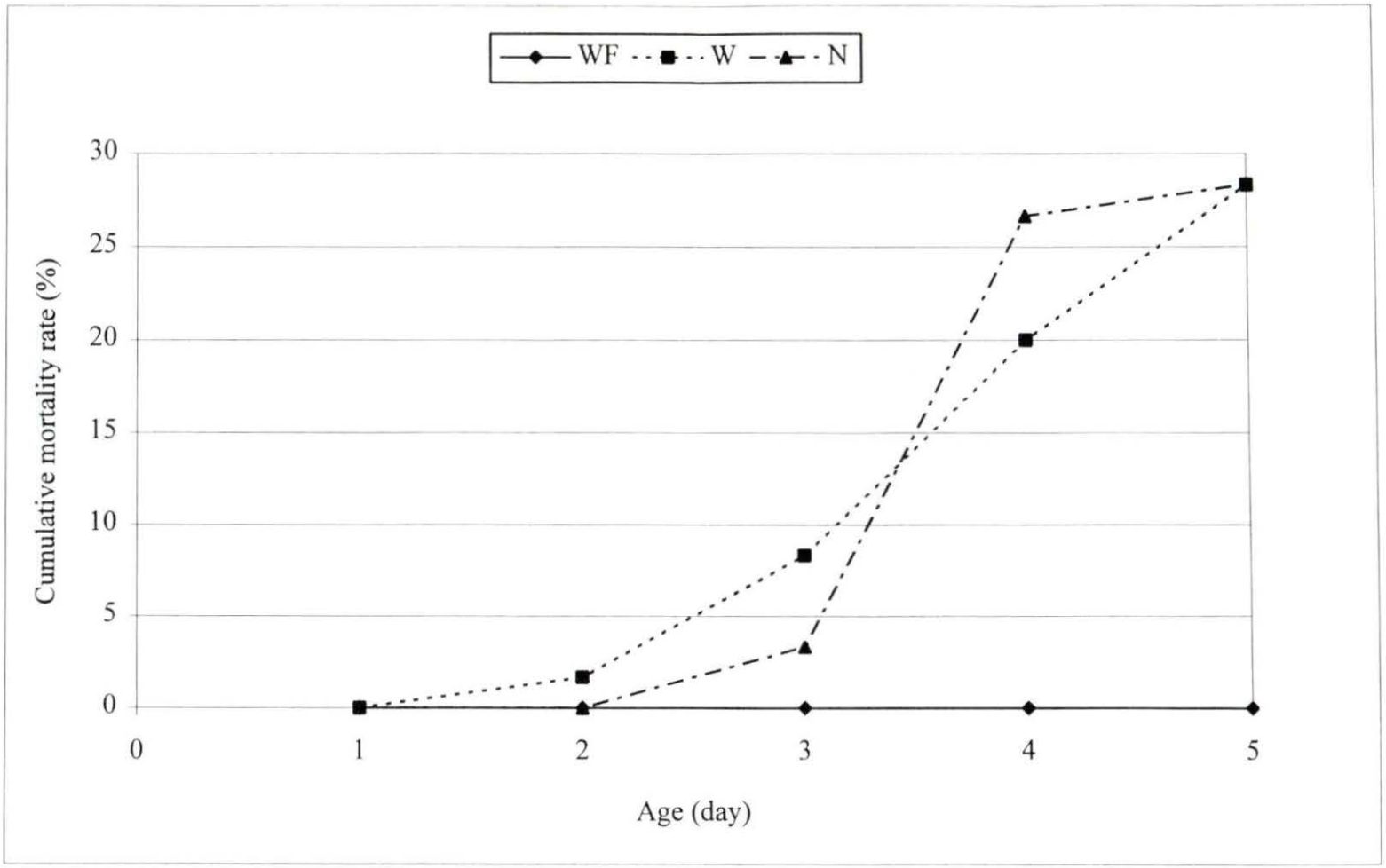


Figure 21: Cumulative mortality rate of chicks with different nutritional treatments.

* WF is water and feed, W is water only, and N is neither water nor feed.
(from the fourth, feed and water were also provided to chicks in W and N groups.)

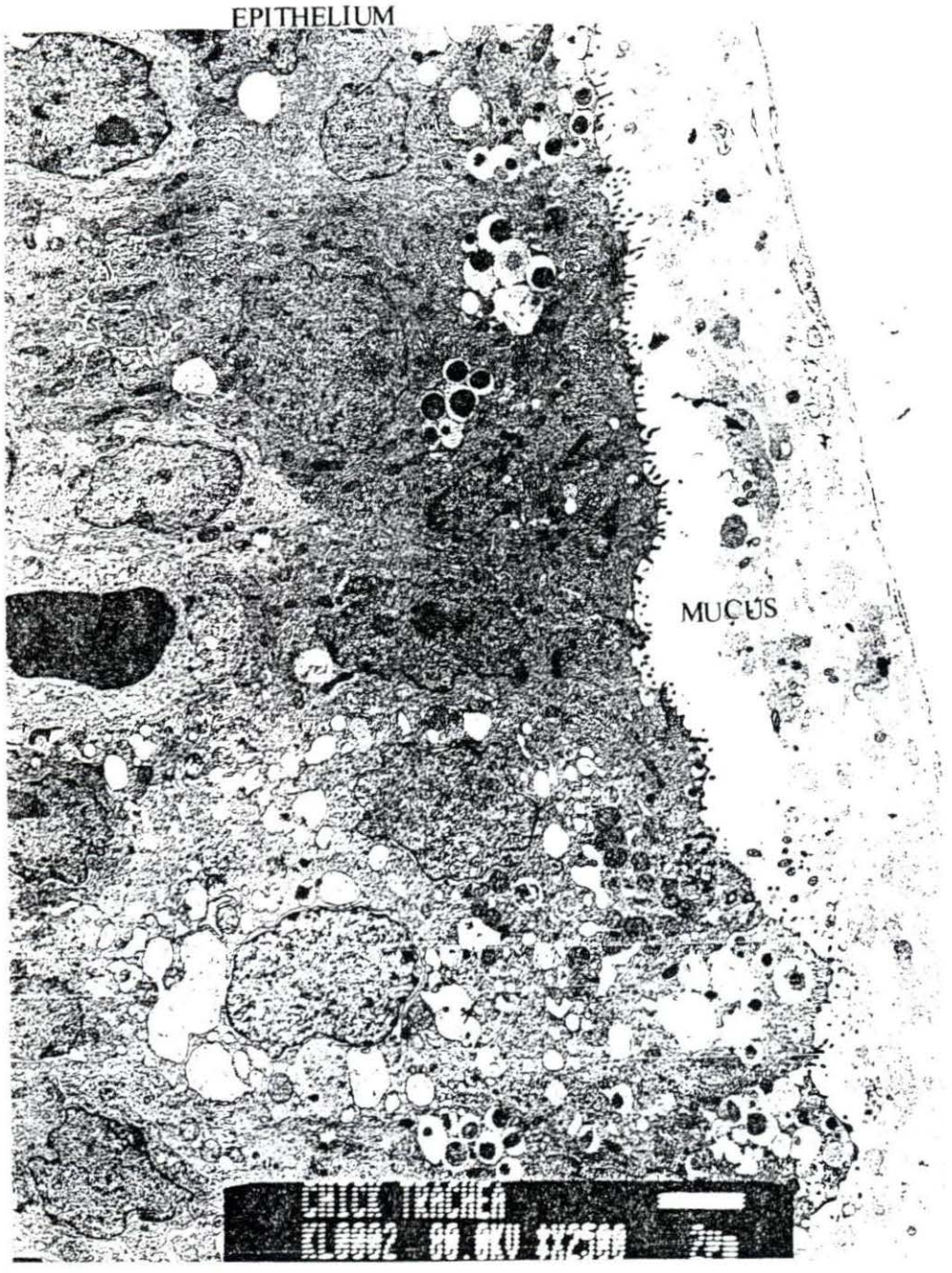


Figure 22: Trachea surface of a normal chick (using transmission electron microscope with 2500 magnitude). It shows mucus layer over epithelium of the trachea.

Figure 23: Trachea surface of a dehydrated chick (using transmission electron microscope with 2000 magnitude). It shows epithelium of trachea, but lack of mucus.

CHAPTER 5. DISCUSSION

For the convenience of comparison, the performance, physiological, and energetic parameters of the chicks are summarized in Tables 15 and 16.

Mortality Rate as Affected by Nutritional Regimes

The chick mortality rate during the first three-day treatment period was not significantly different among the treatments ($p>0.05$) except for W^C treatment which showed higher mortality ($p<0.05$). Chicks in water-and-feed (WF) and water substitute (Aqua Jel ®)-and-feed (JF) treatments had remarkably low mortality rate during the subsequent trial period. However, chicks subjected to the treatments of water (W) and water substitute (J) had excessive mortality during the subsequent period. Specially, the mortality rate on the fourth day (i.e., one day after the treatment) was the highest for these groups. The result that availability of water failed to alleviate early mortality was opposite to the previous speculation that dehydration caused the death of the chicks. The results of body water content and PCV changes were also inconsistent with previous speculation. In fact, a supply of water or Jel only tended to overhydrate the chicks as characterized by the higher body water content and lower PCV values (Table 16). Post-mortem examination of the mortalities in the W and J treatment groups following introduction of feed and water revealed empty crops and intestines. The results thus suggest that the main cause of early chick mortality was lack of nutrition as opposed to dehydration.

Table 15: Means(± S.D.) of mortality, body weight, and heat and moisture production of TK male chicks as influenced by nutritional and lighting regimes during 60 to 72 hours posthatch periods.

Performance	age (day)	Treatments							
		WF^{C}	$WF^{1:5}$	JF^{C}	$JF^{1:5}$	W^{C}	$J^{1:5}$	N^{C}	N^{D}
Cumulative mortality	3	$0.83(1.07)^{b}_{1}$	$0.5(0.64)^{b}_{1}$	$0.50(0.64)^{b}_{1}$	$0.81(0.57)^{b}_{1}$	$4.67(2.83)^{a}_{2}$	$1.00(0.86)^{b}_{2}$	$0.99(0.81)^{b}$	$0.71(0.29)^{b}$
(% placement)	7	$1.17(1.41)^{c}_{1}$	$1.33(1.22)^{c}_{1}$	$0.50(0.64)^{c}_{1}$	$1.63(1.08)^{c}_{1}$	$26.0(3.77)^{a}_{1}$*	$13.89(2.17)^{b}_{1}$		
Body weight (BW)	3	$133.4(0.9)^{a}_{2}$	$134.2(0.8)^{a}_{2}$	$130.5(2.7)^{b}_{2}$	$97.1(1.8)^{c}_{2}$	$87.8(0.1)^{d}_{2}$	$82.3(0.8)^{e}_{2}$	$74.2(2.5)^{g}_{2}$	$79.2(0.2)^{f}_{2}$
(% initial BW)	7	$184.3(3.9)^{a}_{1}$	$177.4(1.1)^{b}_{1}$	$183.2(3.0)^{a}_{1}$	$160.0(3.2)^{c}_{1}$	$131.0(3.1)^{d}_{1}$	$130.2(3.6)^{d}_{1}$	N/A	N/A
Metabolic rate	3				$10.29(2.60)^{a}_{2}$		$8.02(2.09)^{b}_{2}$	$6.64(0.73)^{c}$	$5.83(0.65)^{d}$
(kcal/kg-hour)	7				$13.66(2.75)^{a}_{1}$		$14.22(2.38)^{a}_{1}$		
Moisture production	3				$10.75(1.70)^{b}_{2}$		$12.11(1.71)^{a}_{2}$	$4.47(0.57)^{c}$	$3.66(0.72)^{d}$
(g/kg-hour) x 10^{3}	7				$16.42(2.49)^{b}_{1}$		$20.12(2.59)^{a}_{1}$		
Number of replication		8	4	4	8	2	4	4	4

Nutritional treatment: WF = water and feed; JF = Aqua Jel® and feed; W = water; J = Aqua Jel®; N = neither water nor feed
Lighting treatment: ^{C} = continuous light; $^{1:5}$ = 1L:5D; ^{D} = continuous dark;* = 6 days of age.
Column means with the same subscripts are not significantly different ($p>0.05$)
Row means with the same superscript are not significantly different ($p>0.05$).

Table 16: Means(± S.D.) of total body weight, dry body weight, body water content, ratio of body water todry matter content, and packed cell volume of TK male chicks as influenced by nutritional regimes.

Performance	age (day)	Treatments				
		WF	JF	W	J	N
Total body weight (g/chick)	0	$41.58(3.76)^{a}_{2}$	$41.58(3.76)^{a}_{2}$	$41.58(3.76)^{a}_{1}$	$41.58(3.76)^{a}_{1}$	$41.58(3.76)^{a}_{1}$
	3	$49.66(3.95)^{a}_{1}$	$47.21(3.18)^{a}_{1}$	$31.81(2.38)^{b,c}_{2}$	$32.58(2.10)^{b}_{2}$	$29.22(3.52)^{c}_{2}$
Dry body weight (g/chick)	0	$12.11(1.26)^{a}_{1}$	$12.11(1.26)^{a}_{1}$	$12.11(1.26)^{a}_{1}$	$12.11(1.26)^{a}_{1}$	$12.11(1.26)^{a}_{1}$
	3	$11.76(1.25)^{a}_{1}$	$11.46(1.60)^{a}_{1}$	$6.76(0.61)^{b}_{2}$	$7.29(0.53)^{b}_{2}$	$7.23(0.92)^{b}_{2}$
Body water content (% total body weight)	0	$70.90(1.44)^{a}_{2}$	$70.90(1.44)^{a}_{2}$	$70.90(1.44)^{a}_{2}$	$70.90(1.44)^{a}_{2}$	$70.90(1.44)^{a}_{2}$
	3	$76.32(1.58)^{b,c}_{1}$	$75.75(2.66)^{c}_{1}$	$78.74(1.30)^{a}_{1}$	$77.57(1.90)^{a,b}_{1}$	$75.22(1.49)^{c}_{1}$
Ratio of body water to dry matter content	0	$2.44(0.17)^{a}_{2}$	$2.44(0.17)^{a}_{2}$	$2.44(0.17)^{a}_{2}$	$2.44(0.17)^{a}_{2}$	$2.44(0.17)^{a}_{2}$
	3	$3.24(0.29)^{b,c}_{1}$	$3.18(0.59)^{b,c}_{1}$	$3.72(0.29)^{a}_{1}$	$3.48(0.35)^{a,b}_{1}$	$3.05(0.24)^{c}_{1}$
Number of replication		10	10	10	10	10
Packed cell volume (%)	0	$25.67(2.37)^{a}_{1}$	$25.67(2.37)^{a}_{1}$	$25.67(2.37)^{a}_{1}$	$25.67(2.37)^{a}_{1}$	$25.67(2.37)^{a}_{1}$
	3	$26.88(1.54)^{a}_{1}$	$25.24(2.37)^{a,b}_{1}$	$20.29(6.58)^{b}_{2}$	$22.52(7.53)^{a,b}_{1}$	$27.14(3.63)^{a}_{1}$
Number of replication		7	7	7	7	7

Nutritional treatment: WF = water and feed; JF = Aqua Jel® and feed; W = water; J = Aqua Jel®; N = neither water nor feed
Column means with the same subscripts are not significantly different ($p>0.05$)
Row means with the same superscript are not significantly different ($p>0.05$).

Lighting condition also played an important role in the mortality rate of fasting chicks. The mortality rate for the continuous dark was lower than that for the continuous light. The lower mortality for the dark treatment could be attributed to the lower metabolic rate and thus conservation of body energy. The intermittent lighting treatment of 1L:5D showed remarkably low early mortality rates. However, the lighting regime of 1L:11D or 0.5L:11.5D showed much higher mortality rates (7.18 and 7.03 %, respectively) compared to 1L:5D (1.03 %) for the 7-day trial period. This result suggests that chicks were unable to ingest enough nutrients under the 1L:11D or 0.5L:11.5D lighting regime.

Body Weight Change

The chicks lost or gained body weight (BW) at different rates, depending on the nutritional and lighting treatment. Under continuous lighting, the body weight gain averaged 33.4 % of the initial body weight (IBW) for chicks in the WF treatment and 30.5 % for chicks in the JF treatment during a 60- to 64-hour treatment period. This result indicates that Aqua Jel® can serve as a water substitute, although body weight gain was somewhat smaller than in chicks having water ($P<0.05$). Body weight loss of fasting chicks between Light and Dark treatments over 72 hours showed significant difference (averaged 25.79 % IBW for continuous light and 20.64 % IBW for continuous darkness) ($P<0.05$). This result was in agreement with the previous findings of 20 % IBW loss after a 72 hours posthatch holding period (Xin and Rieger, 1995) and over 10 % IBW loss after a 48 hours posthatch holding period (Pinchasov and Noy, 1993). Furthermore, body weight loss during 72 hours under the

1L:5D photoperiod was 17.7 % IBW for the J treatment and 2.9 % IBW for the JF treatment. In comparison, body weight loss of chicks in the JF treatment was 10.14 % IBW for 1L:11D photoperiod and 15.48 % IBW for 0.5L:11.5D photoperiod. Even though the chicks in the 1L:5D treatment regained their body weight almost to the degree of the WF treatment (177.4 % as compared to 184.3 %) within 4 days of normal light and nutrition condition, it still shows significant difference ($P<0.05$). However, the chicks in intermittent lighting regimes of 1L:11D and 0.5L:11.5D did not regain body weight to the same degree as the JF treatment. The results thus show that body weight loss of fasting chicks increased as the lighting period increased, presumably due to higher activity levels. A photoperiod of 1L:5D seemed to be sufficient for the chicks to ingest enough feed and water to maintain their body weight. In contrast, the photoperiod of 1L:11D or 0.5L:11.5D was not adequate. The reduction in body weight during the three-day fasting or insufficient lighting was not compensated during the subsequent four-day normal feeding treatment or even after twelve days of normal treatment.

Effects of Water Supplement

Despite the lower body weight gain of chicks treated with FJ compared to chicks treated with FW ($p<0.05$), Aqua Jel ® proved to be an effective water substitute. Specifically, under continuous lighting treatment, supply of Aqua Jel ® and feed led to a 30.5 % increase in body weight in 72 hours, as compared with a 33.4 % of body weight increase for chicks with water and feed. Even though the water substitute barely maintained the body weight

(-2.9 % body weight gain) with a 1L:5D photoperiod over 72 hours, chicks' early mortality rate in Aqua Jel ® and feed treatment group was significantly not different compared to that in water and feed treatment ($p>0.05$).

Honey Comb Bedding as a Feed Supply Medium

The honey comb bedding proved to be adequate for feed supply. In fact, it decreased the mortality rate of the chicks compared to trough feeding (3.5 % versus 7.29 %). The reduced mortality rate can presumably be attributed to easier accessibility of feed to the chicks.

Metabolic Rate

The metabolic heat production rate measured for the chicks agreed with the values in the literature. Specifically, the mean heat production rate of the one-day old fasting chick measured in this study was 6.30 (kcal/kg-hour) or 7.3 (W/kg) at 29 ^{o}C, compared with 7.4 W/kg at 30 ^{o}C by Misson (1977). Lighting and nutritional conditions greatly influenced the metabolic rate of the chicks. Specifically, the metabolic rate of chicks in the feed groups was higher than those in the fasting groups (including both J and N groups). However, the difference in heat production between the two groups narrowed with time (from 20 % for the first day to 5 % for the fourth day). The existence of light in the nutritional groups significantly increased the metabolic rate because the chicks consumed more oxygen and produced more carbon dioxide by moving or digesting feed ($p<0.05$) (Figure 11).

Conversely, the metabolic rate of fasting chicks in the dark condition maintained the basal rate but slightly increased over time (Figure 13).

Body Water Content and PCV Test

At the beginning of the experiment, the chicks had a 70.9 % body water content and 25.67 % hematocrit. These values were consistent with those reported by Medway and Kare (1959) and Sturkie (1986). The relative body water content increased in all groups and the values were higher compared with 72 % by Sturkie (1986). This result indicated that the chicks did not suffer from dehydration. The PCV test results also support that the chicks in N group were not in a dehydration state. The PCV of chicks in the N group increased by 1.47 %, as compared to the observation by Koike et al., (1983) that dehydration caused significant decrease in PCV. Therefore, dehydration would not be the main reason for chick death due to water deprivation, at least for the comfortable temperature range. In comparison, PCV results of the weak chicks revealed that they were actually in a overhydration state as characterized by the low PCV values, 8.8 % for the 3rd day of the W group, 7.7 % for the 4th day of the W group, and 13.9 % for the 4th day of the N group. These results implied that blood was diluted, which could have caused malfunctions of the body system, i.e., higher volume of blood return to heart, increased blood pressure, lack of gas delivery ability, and ultimately death.

CHAPTER 6. CONCLUSIONS

The results of this study support the following conclusions:

1. Dehydration did not seem to be the reason for elevated early chick mortality. Supply of only water to the chicks in-transit may actually be more detrimental than no water.
2. Availability of both feed and water/water substitute was most conducive to reducing chick mortality.
3. The commercially available Aqua Jel® proved to be an effective water substitute for the chick while eliminating in-transit water leakage.
4. No adverse effects on chicks were observed by supplying feed directly on the honey comb bedding. In fact, the practice tended to improve feed accessibility to the chicks.
5. Intermittent lighting of 1-hour light and 5-hour dark (1L:5D) would provide adequate lighting for the chicks to consume enough feed and water to maintain their body weight, and therefore recommended for use in chick shipment. However, intermittent lighting of 1L:11D or 0.5L:11.5D was not enough to reduce early chick mortality.
6. The metabolic rate of the chicks was largely influenced by nutritional and lighting regimes. Specifically, the metabolic rate during the first 72-hour treatment averaged 10.29 kcal/kg-hr for chicks provided with feed and Aqua Jel®; 6.62 kcal/kg-hr for fasting chicks in the continuous lighting treatment, and 5.70 kcal/kg-hr for fasting chicks in the continuous dark treatment. The respective moisture production was 10.75×10^{-3} g/kg-hr for JF treatment and 4.45×10^{-3} g/kg-hr for N treatment in continuous lighting, and 3.64×10^{-3} g/kg-hr for N treatment in continuous darkness.

CHAPTER 7. FUTURE RESEARCH NEEDS

Work is needed to implement supply of in-transit, intermittent lighting. The effects of fluctuating thermal conditions, particularly temperature, on neonate chicks need to be evaluated.

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