NUTRITIONAL MEANS OF OVERCOMING HEAT STRESS IN LAYING HENS AND BROILERS

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DECLARATION

The experimental work described in this research thesis was carried out in the Discipline of Animal and Poultry Science, School of Agricultural Sciences and Agribusiness, Faculty of Science and Agriculture, University of Natal, Pietermaritzburg, under the supervision of Prof. R. M. Gous.

The research contained in this thesis represents the original product of the author and has not otherwise been submitted in any form for any degree or diploma to any other University. Where use has been made of the work of others, it has been duly acknowledged in the text.

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CONTENTS

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GLOSSARY OF TERMS

ADG	Average daily body weight gain
AME	Apparent metabolizable energy
BHP	Basal heat production
Cat.Pr	Catabolised protein
CERU	Controlled environment research unit
CHO	Carbohydrate
CP	Crude protein
DCHO	Digestible carbohydrate
DE	Digestible energy
DLIP	Digestible lipid
DOM	Digestible organic matter
DP	Digestible protein
EE	Effective energy
EEL	Endogenous energy loss
EERQ	Effective energy requirement
EHL	Evaporative heat loss
EO	Egg output
ER	Energy retention
ER(L)	Energy retained as lipid
ER(P)	Energy retained as protein
EW	Egg weight
FCE	Feed conversion efficiency
FE	Faecal energy
FE	Faecal energy
FHP	Fasting heat production
FI	Feed intake
FOM	Faecal organic matter
FUN	Fasting urinary nitrogen
GE	Gross energy
HEX	Heat of excretion
HI	Heat increment

HIF	Heat increment of feeding
HIM	Heat increment of maintenance
HL	Heat loss
HP	Heat production
ME	Metabolizable energy
ME_c	Metabolizable energy classical
MEM	Maintenance metabolizable energy requirement
ME_n	Metabolizable energy, nitrogen corrected
MERQ	Metabolizable energy requirement
MFE	Metabolic faecal energy
MH	Maintenance heat
MHP	Metabolic heat production
MTHE	Methane energy
NE	Net energy
NR	Nitrogen retention
NRC	National Research Council
OM	Organic matter
PR	Protein retention
ROL	Rate of lay
SDE	Specific dynamic action
SHL	Sensible heat loss
TA	Ambient temperature
TB	Body temperature
Tc	Lower critical temperature
TDE	True digestible energy
THL	Total heat loss
T_{in}	Control thermal input
TME	True metabolizable energy
T_{ref}	Hypothetical reference
UE	Urinary energy
UN	Urinary nitrogen

ABSTRACT

The relationship existing between the broiler and the laying hen, the methods applied for estimating the effective energy using linear coefficients to five measurable components of interactions and their diet used in adjusting ME for heat increment of feeding, the environment in which they live, the physiological, metabolic, behavioural, and the productive changes that occur when birds are exposed to heat stress, were studied. Nutrition and temperature were of particular interest. Each different aspect studied was found to have an influence on the final performance of the broiler and the laying hen.

Two major experiments were conducted. The objective of the first experiment was determine if performance can be improved during hot weather by reducing the heat increment of the feed. Two EE: ME ratios were used, the low ratio being based on the least – cost feed and the high ratio being the maximum possible with the available raw materials, and three ND's were used to determine whether there was an interaction between the ME content of the diet and the EE:ME ratio. 360, 46-week old Hy-Line Brown layers were housed for ten weeks and each of these 2 x 3 feeds was replicated four times using 15 hens per replication (three cages of five hens per cage), making a random allocation of 60 birds per feeding treatment. Treatment means were calculated for the last seven weeks of the trial. Egg prices (c/egg), income generated and profit, under normal, 15% increase and 15% decrease for all egg grades were calculated. It was found that neither the EE nor the ME contents of the feeds had significant effects on ROL, EW, EO, ADG, or ME intake, though there were some variations in the response of these variables. Both the EE and the ME of the diet had strong significant effect (EE at $P<0.01$, ME at $P<0.001$) on FI, but their interaction had no significant influence on either the EE intake or FI. The EE intake was highly influenced by both the EE ($P<0.001$) and ME ($P<0.01$) content of the diet. The amount of feed and energy consumed was primarily dependent on the dietary energy content of the particular feed, being low at low EE: ME ratio and high at high EE:ME ratio, respectively, for FI; while energy intake increased positively with increasing ME content of the diet. While ADG increased positively at high EE: ME ratio, it increased and then decreased at low EE:ME ratio. Feeding cost for the combinations of dietary EE and ME was found to be linearly increasing and more expensive in treatments with low EE than in treatments with high EE. Under all circumstances income was positively related to dietary EE and ME. The highest profit was obtained from diets having high EE: ME ratios under all egg prices.

The objective of the second trial was to determine the extent to which broilers are able to lose heat to the environment when forced with conditions that would require them to lose more heat to the environment than would be possible for them to grow at their potential. The responses in three lysine-limiting trials were measured at three temperatures, with six diets and two sexes, and over two growth periods. The first two trials, one being a pilot trial, were conducted on broilers between 1 and 3 weeks of age, and the third trial was a finisher trial and was conducted using broilers from 3 to 5 weeks.

Dietary lysine, sex and temperature were found to have a significant effect on ADG (lysine at $P<0.001$, sex and temperature at $P<0.05$) in trial 1, and on FI (lysine and temperature at $P<0.001$, sex at $P<0.05$) and ADG (lysine and temperature at $P< 0.001$, sex at $P<0.01$) in trial 2. While dietary lysine, temperature and lysine *vs.* temperature had significant effect on FCE in trial 1 (lysine at $P<0.001$, temperature and lysine *vs.* temperature at 0.01), only dietary lysine and temperature had significant effect on FCE ($P<0.001$) in the pilot trial and trial 2. While ADG in the pilot trial was significantly affected by dietary lysine and temperature (lysine at $P<0.001$, temperature at $P<0.05$), FI in the pilot trial and trial 2 were significantly influenced by dietary lysine only ($P<0.001$). While no interaction had significant effect on FI, or ADG in both trials 1 and 2, neither sex nor any of the interactions had significant effect on FCE, FI, or ADG in the pilot trial. In all trials responses in FCE, ADG, and FI showed an increasing trend with the addition of synthetic lysine (treatment 6 *vs.* 5), irrespective of temperature, confirming that lysine was the first limiting nutrient in the summit diets.

Dietary lysine, and temperature were found to have significant effect on protein (dietary lysine at $P<0.001$, temperature at $P = 0.01$), lipid (dietary lysine and temperature at $P<0.001$) and gross energy gain (dietary lysine at $P<0.001$ and temperature at $P<0.01$) in trial 1, and on protein gain ($P<0.001$) and lipid gain (dietary lysine at $P<0.001$ and temperature at $P<0.01$) in trial 2. All the main effects had significant effect on lipid and gross energy gain (lysine at $P<0.001$, temperature at $P<0.01$ and sex at $P<0.05$) in the pilot trial, and on gross energy (lysine and temperature at $P<0.001$, and sex at $P<0.01$) in trial 2. While the interaction between temperature and dietary lysine, and temperature and sex had significant effect on lipid and gross energy gain ($P<0.05$) in the pilot trial, the interaction between sex and temperature was found to have a significant effect on protein and gross energy gain ($P<0.05$) in trial 1, the interactions between diet and temperature had a significant effect on protein and

gross energy gain ($P<0.05$) in trial 2. No other interaction had any effect on lipid, gross energy, or protein gain in any of the trials.

While all the main effects had a significant effect on HL in trial 1 (lysine at $P<0.001$, temperature at $P<0.01$, and sex at $P<0.05$), and when the data were combined (lysine and temperature at $P<0.001$, and sex at $P<0.01$), in trial 2 all the main effects and lysine x sex (lysine, temperature, and sex *vs.* lysine at $P < 0.001$, sex at $P < 0.05$), in the pilot trial only temperature and dietary lysine had significant effect (temperature at $P <0.001$, lysine at $P<0.05$)

The constant term and FI were found to have positive relationship with HL, while feather weight, degree of maturity and temperature were found to have a negative relationship with HL in both the pilot trial and trial 1. In trial 2, HL showed a positive relationship with FI and degree of maturity, and a negative relationship with feather weight and temperature. When the data were combined, HL showed a positive relationship with FI and the constant term and a negative relationship with feather weight, temperature and degree of maturity. While FI, temperature, degree of maturity and feather weight were found to have a significant relationship with HL ($P<0.001$) in the pilot trial and trial 1, as well as in the combined data, in trial 2, feather weight and feed intake had a significant relationship with HL.

INTRODUCTION

The ultimate aim of any farming business is to produce outputs that will generate income. In animal production systems, the cost of inputs account for about 70 to 80 percent of the income generated from the sale of the products. The cost of these products, for example in poultry, egg and meat, and the cost of the resources used in the production process determines the profitability of the farming business. In order for the profitability of the farming business to increase or be as desired, poultry producers must ensure that their products are of the best quality and value, and that the income derived exceeds the cost of producing the products.

Many factors, including the prevailing environmental conditions, feeding, watering, and housing system, may reduce the production capability of the laying hen and the potential growth rate of the broiler, and hence, prevent the producer from maximising profitability from this enterprise. Feed is the most expensive component in poultry production, so this should be used as efficiently as possible. Keeping poultry houses at optimum or nearly optimum temperatures reduce feed cost but may increase the cost of water and electricity, both of which are required for ventilation and evaporative cooling during hot weather. Poultry farmers usually attempt to balance these costs and benefits. However, decisions concerning ventilations and evaporative cooling could only be empirical, as the actual value of an incremental change in temperature is not known (May *et al.*, 1998).

The feed given to a bird or an animal causes the animal to produce heat, which is a problem at high temperatures. But different feeds produce different amounts of heat, and it is possible to use a feed with low heat increment to overcome heat stress (the alternative being to cool the building – but if water and electricity is scarce, it may be justified). So the first part of this research work was directed at finding out to what extent the effect of heat stress on performance of the laying hen can be reduced by manipulating the heat increment of the feed. The pros and cons of using high EE: ME diets during hot weather, together with an economic analysis indicating how such feed manipulation would affect the profitability of a poultry enterprise has been dealt with in this research project.

The content of amino acids in the diet required by growing broilers will vary depending on the amount of dietary energy and the existing environmental temperature because these factors influence food intake. If the incorrect amounts of amino acids are included in the

feed, then birds will either not grow to their potential, as they will be unable to lose sufficient heat to the environment at high temperatures, or the amino acids will be wasted, thus raising the cost. It is useful to know to what extent food intake, growth rate and carcass composition are influenced by different dietary amino acid concentrations at various environmental temperatures, as decisions may then be made as to the most efficient way of feeding broilers at different times of the year. As the relative growth rates of broilers have been improved by genetic selection, so the environmental temperature at which they would be comfortable has been reduced. Because the maximum heat loss is likely to be a function of the mature body and feather protein, feather cover weight, the degree of maturity and environmental temperature, these variables were the subject of the second series of trials conducted in this investigation of the nutritional methods of overcoming heat stress in laying hens and broilers.

CHAPTER ONE

LITERATURE REVIEW

1.1. INTRODUCTION

Poultry are increasingly being kept in areas that experience high temperatures for various lengths of time each year. Also, the potential growth rate of broilers continues to increase, resulting in more heat being generated by these birds when they grow at their potential. This means that it is increasingly likely that broilers especially will not achieve their potential because the high temperature to which they are subjected will constrain their food intake and hence growth rate. It is therefore becoming more important that methods are investigated to reduce the heat load on these birds. These could be genetic (reducing the potential growth rate), environmental (insulation, cooling mechanisms etc.) or nutritional (reducing the heat increment of feeding).

This review explores the physiological, metabolic, behavioural and productive changes that occur when birds (broilers and laying hens) are exposed to heat stress. However, there are a number of factors that can influence the response of birds to heat stress and so neither absolute, quantitative functions nor preventive measures are easily defined. Despite this, current information on the nutrient and energy needs of poultry, as influenced by heat stress, with some methods of alleviating heat stress will, be discussed. In addition, the methods applied for estimating the effective energy of the diet, using linear coefficients applied to five measurable components of the feed and the animal, used in adjusting ME for heat increment of feeding, will be discussed. But more emphasis will be given to the relationship between poultry and the feed ingredients used in the feeds formulated for them.

1.2. AN OVERVIEW OF THE PHYSIOLOGICAL EFFECTS OF HIGH AMBIENT TEMPERATURE

Both birds and mammals are able to control their heat production, to maintain a stable deep body temperature within relatively narrow limits (at the optimum) in spite of environmental temperature fluctuations and variation in activity. Such a characteristic is known as homeothermy or warm – bloodedness. Birds, being homeotherms, are less vulnerable to environmental temperature changes than poikilotherms with respect to both functional efficiency and danger of tissue damage (Smith & Oliver, 1971). Homeothermic animals are

always able to function at their optimum. But the price that they have to pay for the benefits conferred from being homeothermic is that body temperature cannot be allowed to fluctuate beyond relatively narrow limits without causing detrimental deterioration in normal functional efficiency.

The principles of thermal physiology and energy exchange provide a foundational background while considering a specific thermal environment on the production and growth of poultry. Therefore, the aim of this sub-chapter is to develop the concepts, which form the basic criteria for specifying the optimal environmental temperature to maximize the performance of poultry.

1.2.1.The mechanisms of thermoregulation

1.2.1.1 Thermoregulatory feed back system

The complexity and efficiency of thermoregulation in homeotherms is impressive. For simplifying the complex presentation of physiological thermoregulatory response, Hillman *et al.* (1985). suggested the concept of negative feed back control. The basis of regulation, described by this system, is the temperature difference between the hypothetical reference signal (T_{ref}) and the actual, controlled thermal inputs (T_{in}). Changes in the thermal environment are sensed by multiple thermoreceptors (hypothalamus, midbrain, spinal cord, abdomen and skin). This thermal sensory input, which is composed of multiple feed back signals originating from numerous points throughout the body, are fed to the comparator (hypothalamus), where temperature difference ($T_{ref} - T_{in}$) is measured. If this difference yields an "error" signal, multiple thermoregulatory controllers (hypothalamus, lower brain stem, spinal cord) will be activated to adjust the metabolic heat production (MHP) or heat loss (HL) mechanisms by affecting the actuators – panting, shivering, vasomotion, ptiloerection, and behavioural responses or both MHP and HL to reduce the temperature diffidence ($T_{ref} - T_{in}$).

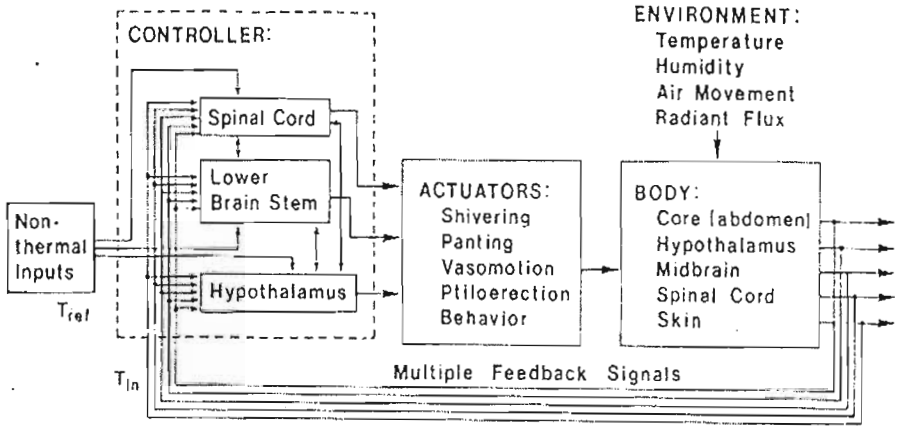


Figure 1.1. *Schematic diagram describing the temperature regulation system with multiple sensors, controllers and effectors in poultry* (After Hillman *et al.*, 1985)

Figure 1.1. Illustrates the complex thermoregulatory control indicating multiple thermal control and sensors. Evidence indicates that in an experiment in which diffident controllers are sequentially inactivated or destroyed, the highest precision in temperature regulation is obtained when the hypothalamus is intact, and thus the hypothalamus is considered to perform the role of over all co-ordination (Mount, 1979; Hillman *et al.*, 1985). In the figure, a double arrow connecting the spinal cord and lower brain stem with the hypothalamus while also showing their parallel, independent action indicates this hierarchical role.

1.2.1.2 Body temperature

The deep body temperature of chickens lies in the range of 41.2 to 42.2 ^{0}C (Mount, 1979). This variation is correlated with body size since body temperature increases as body mass decreases. However, there is a decline in this trend in smaller birds, where body temperature becomes fairly constant, irrespective of body weight (Mount, 1979; Freeman, 1983). A newly hatched chick has a deep body temperature that is 2.5 ^{0}C below that of the adult bird and the adult temperature is reached by six to ten days of age (Mount, 1979). Thus, thermoregulation is limited shortly after hatching and improves with age to become fully efficient after two weeks of age (Mount, 1979; Van Kampen, 1981).

1.2.2. Metabolic heat loss

The law governing temperature in the body of homeotherms is that the heat produced by the body has to be exactly balanced by the heat lost from the body so that body temperature will be kept constant (at equilibrium). That means a complete uniformity of body temperature is

possible only if heat exchange occurs between the body and its environment. Birds, however, constantly produce heat and lose it to the environment so that there is a thermal gradient from the warm interior (core) to the cooler surface (shell) (Smith & Oliver, 1971; Blaxter, 1977).

1.2.2.1. Evaporative (EHL) and non-evaporative (SHL) heat loss

Heat is transferred between the bird and its environment through evaporative and non-evaporative means.

The non – evaporative, also called the sensible, heat constantly flows from the deep body centre to the outside in two stages (Smith & Oliver, 1971):

Stage 1: transfer from the core to the surface to the ambient air. The rate of flow in this stage depends upon the temperature gradient between the deep body centre and the skin, the conductance of tissue, and the degree of subcutaneous vasodilatation.

Stage 2: involves both the transfer of sensible heat from the skin surface, through the feathers and boundary layer of still air to the air to the outside environment by conduction, convection or radiation, or the loss of insensible heat, by evaporation from the lungs.

Diagrammatically these two stages are more clearly defined by Blaxter (1977). His diagram (Figure 1.2) indicates that heat is produced centrally and is convicted to the surface of the body in the blood. The animal controls this flow by opening and closing capillary networks in the skin. At the skin surface some heat is used to vaporize moisture while the rest is transferred through the coat surface where it is transferred to the environment by radiation and by convection.

The unfeathered extremities of the bird, including the comb, wattle, and the legs are the major sites of SHL. The comb and the wattle are extremely vascularized and may make up to 0.7% of the total body surface area. The comb, wattle and legs are well adapted, as, in addition to lack of insulation, they also have a large surface to volume ratio (Freeman, 1983).

When the environmental temperature increases, the arterial blood pressure and the total peripheral vascular resistance to blood flow decreases. These result in vasodilation in the extremities and an increase in heat flow from the body core to these regions. This is accompanied by an increase in cardiac output of 20 to 27%, which further improves the rate of flow through the bird's extremities (Hillman *et al.*, 1985).

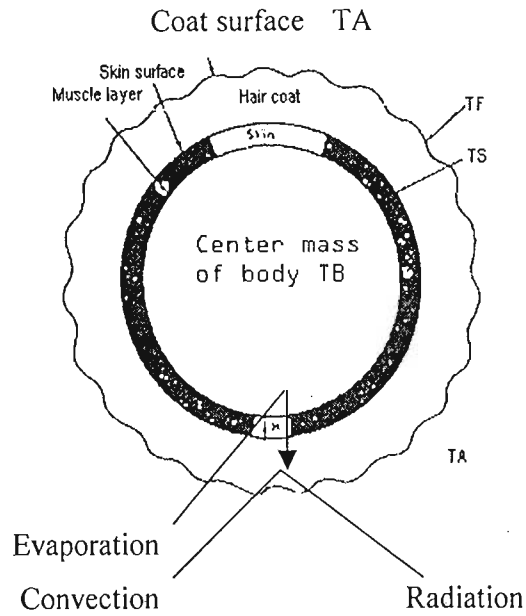


Figure 1.2. *Diagrammatic representation of heat flow in an animal* (After Blaxter, 1977)

In order to facilitate SHL during heat stress the bird shows some behavioural thermoregulatory responses including postural adjustments, change in food and water intake, and reduced movement. The bird becomes lethargic and often lies in a prone position with its head, neck and legs extended. Wings are held away from the body, thereby exposing more of the unfeathered portion of the body to the environment, which increases evaporation from the body surface. In addition, the bird will splash water on its comb and wattle to facilitate cooling via evaporation (Freeman, 1983; Hillman *et al.*, 1985), consume more water and less feed (Meltzer, 1987; Peguri & Coon, 1993). Adaptations also exist in feather loss or gain, and the accumulation of fat reserves as insulation (Peguri & Coon, 1993).

Radiation is the most important source of heat loss at temperatures below 30^0C (Peguri & Coon, 1993), with evaporation or conduction predominant above 35^0C (Wilson, 1982). According to Peguri & Coon (1993) the amount of radiation depends on:

1) The temperature of the two radiant surfaces
2) The relative position of two surfaces
3) The radiating quality of the surface

Conduction is the direct transfer of heat energy from molecule to molecule, the rate depending on the thickness of the tissue and its thermal conductivity being 0.75 for fat, 1.21 for Skin and 1.80 kJ/m.hour.^{0}C for muscle (Blaxter, 1977).

Convection is important in the thermoneutral zone where heat production (HP) is constant, and the blood acquires heat and transports it to the extremities where heat is exchanged directly with the environment. Heat loss through convection above 30^{0}C is helped by higher air velocities, increasing in proportion to the square root of air velocity (McDonald, 1978). The mechanism of heat loss is constant, such that no further increase in SHL can occur through an increase in ambient temperature.

Since SHL dependents on the temperature gradient between the animal and its environment, under colder conditions, either heat production has to be increased or the actual rate of loss has to be decreased (Freeman, 1966). This results in further sub division of heat regulation into two types (Freeman, 1966):

A) Physical control – the control of heat loss

B) Chemical control – the control of heat production.

Table 1.1.*The methods of controlling heat loss and heat of production* (After Freeman, 1966)

Physical	Chemical
Surface area	Feed intake
Feathering	Muscle activity
Breathing rate	
Blood flow	

Within the zone of thermoneutrality, birds control their heat loss by physical means. When the ambient temperature falls below this zone, birds maintain their body temperature by increasing HP, mainly by chemical means (Balnave, 1974). At temperature beyond this zone, metabolism increases as birds become incapable of controlling heat loss by sensible means except by evaporating water from the respiratory tract.

EHL, on the other hand, increases with ambient temperatures. EHL, like SHL is dependent on the gradient, but rely in this case on humidity differentials. Moisture is evaporated more

effectively off the surface by panting from the skin or mouth when the ambient environment is dry and can facilitate evaporation. Respiratory evaporation is a very important source of heat dissipation above 30^{0}C (Mount, 1979), and contributes up to 0.85% of the total heat loss at 35^{0}C (Farrel & Swain, 1977a). Figure 1.3 exhibits this curvilinear increase in EHL with increasing temperature. Farrell & Swain (1977b) noted that previous acclimatization had no effect on EHL, as a proportion of heat loss, when birds were exposed to a wide range of environmental temperatures (2 to 35^{0}C).

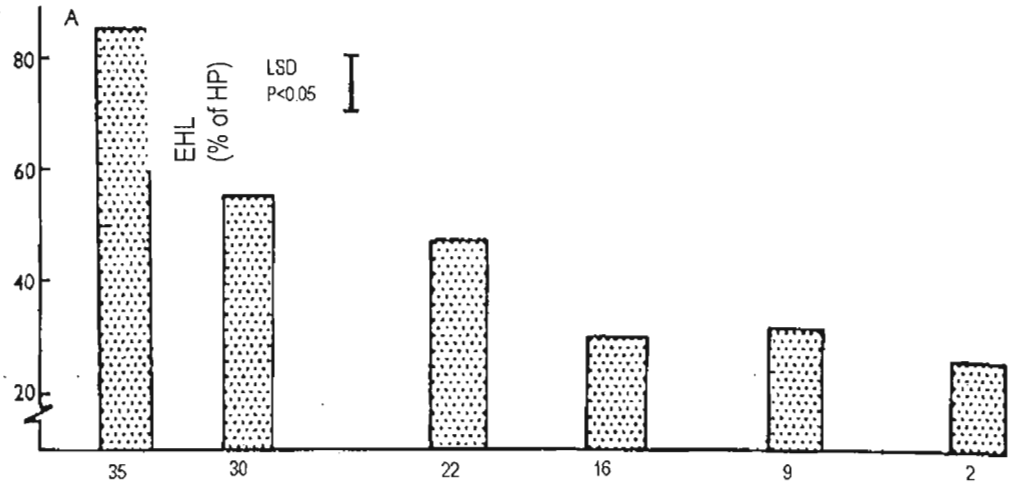


Figure 1.3. *The mean EHL of fasting chickens at each of the six temperatures* (Farrell & Swain, 1977a)

1.2.2.2 Cutaneous and respiratory evaporation

Since birds do not have sweat glands, they cannot actively lose heat by sweating. Despite this EHL does occur due to passive diffusion of water through the skin. Little control can thus be exerted except possibly by behaviour and postural changes (Reece *et al.*, 1972).

Panting initiates EHL from the respiratory tract as environmental temperature is increased above 30^{0}C (Mount, 1979). While the normal respiration rate of poultry is 37 respirations per minute with a tidal volume (amount of air inspired/expired) of 15.4 ml, the maximum respiratory rate that poultry can reach when deep body temperature is increased to 44^{0}C, is approximately 150 to 260 respirations per minute (Hillman *et al.*, 1985).

Panting is a heat producing mechanism; it increases heat loss but also generates heat as a result of the panting activity (Peguri & Coon, 1993). The process of panting can be divided into two phases. Phase I is triggered by an increase in body temperature under heat load and

rate of panting increases with increasing body temperature. The bird's tidal volume decreases when the bird attempts to maintain a normal level of alveolar ventilation. Despite this the bird falls short of preventing minute volume (volume respired per minute) from increasing. When temperature exceeds hyperthermia further, phase II will begin as body temperature exceeds 44^0C (Hillman *et al.*, 1985). At phase II, respiration rate slows and becomes deeper, resulting in an increase in tidal volume. Minute volume remains at high plateau between body temperatures of approximately 44 to 46^0C, where after, the minute volume declines (El-Haid & Sykes, 1982). Birds have the ability to increase the effect of panting by the initiation of gular flutter, which is a rapid fluttering of the gular area in the throat by flexing of the hyoid apparatus. Since gular flutter increases EHL and convective heat loss by forced convection at lower energy cost, it is more effective than panting (Mount, 1979; Hillman *et al.*, 1985).

1.2.2.3 Respiratory alkalosis

In spite of respiratory EHL being an effective mechanism for dissipating metabolic heat it causes blood acid-base imbalances within the bird. This acid-base imbalance reduces feed intake, growth rate and egg production. In addition, increasing respiration rate lowers partial pressure of CO_2 (PCO_2) in the lungs and air sacs. This in turn lowers the concentration of blood plasma CO_2 in the form of carbonic acid (H_2CO_3) or bicarbonate ions (HCO_3^-), and thus, the availability of hydrogen ion (H^+), resulting in an increase in blood pH from normal level of approximately pH 7.5 (Leeson, 1986).

In addition to the acid-base imbalance is a shift in the balance of plasma electrolytes. The elements that are predominant in satisfying the electrolyte balance within the body are sodium (Na), potassium (K), chloride (Cl), and bicarbonate ion. The shift in the electrolyte imbalance results in an increased renal loss of these ions, a process necessary to limit the effect of alkalosis. The imbalances in the ratios of these electrolytes may play a role in the reduced growth rate associated with heat stress (Teeter *et al.*, 1985; Teeter & Smith, 1986, Balnave & Muheereza, 1997; 1998).

Although alkalosis was observed at both ambient temperature of 35 and 41 ^{0}C the regular fluctuations seen in the former climate suggest a conflict between the demand of thermoregulation, tending to increase respiration, and the demand of pH homeostasis, tending to reduce it (El-Hadi & Sykes, 1982). Therefore, poultry, like other birds, have to deal with the conflict which arises where hyperthermia tends to increase respiratory rate to facilitate

EHL, while on the other hand, the demand for pH homeostasis tends to reduce it, as the main elements, the H^+ ions and PCO_2, are the factors affecting respiration rate.

1.2.3.The thermoneutral zone and regulation of body temperature

According to conventional concepts there is a narrow range of temperatures within which basal heat production (BHP) by the bird is minimal and body temperature is controlled by variations in heat loss (physical temperature regulation) (Romijn & Lokhorst, 1966). This range, which lies between that called the upper and lower critical temperature, is known as the zone of thermoneutrality. In this zone, BHP by the bird is minimal and body temperature is controlled by variation in heat loss (Khajarern & Khajarern, 1998). The lower limit of this zone is the lower critical temperature (T_C), below which the metabolic rate must be increased if the deep body temperature is to be maintained (Mount, 1974). The upper limit of this zone is defined as the region beyond which there is an increase in metabolic rate or increase in EHL (Mount, 1974). The relative bounds of this zone are important so far as they define a "region" where the animal's HP is minimal and where its body temperature is normal while sweating and panting do not occur. Because of this, it is often referred as the zone of minimal thermoregulatory effort (Mount, 1974). This region provides the sensation of maximum comfort and is the animal's preferred thermal environment, within which it is expected to exhibit the prescribed physiological and behavioural response indicating that it is within its preferred environment. Therefore, within this region, it should be able to be productive and unstressed. In addition, in this zone, feed requirements for growth and egg production are minimized (Ernst, 1995). The zone may vary depending upon age of the bird and the relative humidity of the air (Khajarern & Khajarern, 1998). Similarly the critical temperature varies with age, previous temperature experiences of the bird and, to a lesser extent, by other factors such as diet, feathering etc. (Ernst, 1995).

Meltzer (1983) calculated the upper and lower critical temperatures, and thus the thermoneutral zone, of broilers at different ages, in order to determine environmental temperatures that would ensure optimum growth rate as is indicated in Table 1.2. The table shows that the thermoneutral temperature range is reduced as body weight increases.

Table 1.2. *Thermoneutral zone of broilers at different ages* (After Meltzer, 1983)

Age (days)	Weight (g)		Husbandry temperature	Lower critical temperature	Upper critical temperature
	Male	Female			
0	40	40	31.0	35.0	37.0
7	130	120	30.0	32.0	25.0
14	290	250	29.0	29.5	33.0
21	490	430	28.0	27.5	31.5
28	730	650	27.0	26.0	30.0
35	1000	890	26.0	24.5	29.0
42	1310	1150	25.5	23.5	28.5
49	1660	1400	25.5	23.0	28.0
56	1990	1640	25.5	23.0	28.0

The general considerations of the effect of environmental temperature have been put into terminology which is used in the discussion about the response of strict homoetherms to the change in environmental temperature as is represented in Figure 1.4. The representation of Figure 1.4 was originally based on the proposal of Mount (1974), but in this discussion, the proposal of Hillman *et al.* (1985) is followed to illustrate the relationship between MHP, SHL, EHL, and body temperature as a function of ambient temperature (Ta). In the figure three important zones described are: (1) the *zone of minimum metabolism* (ZMM), a zone accompanied by increasing MHP at each end, is bounded by the critical temperature where MHP starts rising with a decease in Ta (point [b]) to provide energy to maintain body temperature, and by the upper critical temperature where MHP starts to increase with increase in Ta (point [d]) to provide energy for panting. (2) *The thermoregulatory zone* is bounded by the lower critical temperature at point [a], the temperature of peak MHP and incipient hypothermia and above by the critical thermal maximum at point [g], a point at which thermoregulatory function begins to break down. In this region [a][g], there is an increase in metabolic rate on either side but within the range there is minimum metabolism (3) *the zone of least thermoregulatory effort* (ZLTE), usually known as zone of thermal comfort, is bounded by increasing metabolic rate (cold) at point [b] at its lower limit and by increasing EHL point [c] at its upper limit. In the region [b][c], the bird's HP is minimal. Marsden & Morris (1987) identified this region in relation to cubic model of HP per unit of metabolic body size with increasing temperature. The cubic equation in Marsden & Morris (1987) implies that the slope of the heat output curve is lower in the "comfort zone" than at

temperatures much higher or lower. This is consistent with the idea of a minimum thermoregulatory zone, which was defined by Mount (1974).

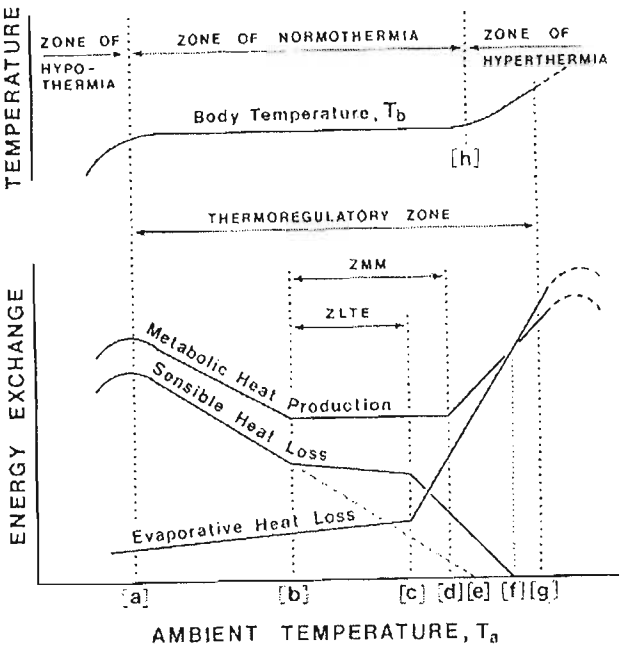


Figure 1.4. *Relationship between HP, EHL, SHL, and deep body temperature in the homeotherm* (From Hillman et al., 1985)

1.2.4.The environment

A bird is a thermally active animal in which it undertakes direct and indirect heat exchanges with its environment (Blaxter, 1977). The environment to which a bird is exposed encompasses many physical factors including ventilation rate, ammonia levels, relative humidity and ambient temperature. The thermal environment has a strong influence on farm animals with air temperature having the primary effect, but altered by wind, precipitation, humidity, and radiation (NRC, 1981). The main aspect of the environment considered here, the temperature, is but a small part of the effective temperature concept. The temperature of the environment can be seen both as a resource and a constraint on the bird's performance. This is due to the physical nature of heat transfer and temperature gradients (Emmans, 1995). Even though the term environmental temperature is used for comparative purposes, it is necessary to define the effective ambient temperature. Effective ambient temperature is one such index described in terms of environmental heat demand: the temperature of an isothermal environment without appreciable air movement or radiation gain that results in the same heat demand as the environment in question (NRC, 1981). Payne (1967) defined the

concept of air temperature by noting that the experience that bird has on the environmental temperature depends on stocking density, ventilation rate and structural insulation. Emmans (1981) defined the effective temperature as dry bulb temperature, radiant temperature (if different), air speed, and wet bulb temperature (only at high dry bulb temperatures), in order of importance.

If birds were to be placed in an environment where hotness varies spatially, it would be expected that the bird would move to an area where it is most comfortable, provided that such an area exists. In an environment that does not provide such pockets of comfort, it is the environment that largely determines the rate at which the bird can lose and therefore, produce heat (Emmans, 1989). For a given bird, in a given state the relationship between heat loss and environmental temperature may be shown as in the Figure 1.5 of Emmans (1989). According to this, for a given bird the total heat loss (THL) is the sum of the SHL and EHL. Based on the explanation of Emmans (1989) the broiler and the laying hen can be seen as having some measure of control over both SHL and EHL. This control is effected by many physiological and behavioural patterns. While the loss of heat to the environment by EHL is independent of the environment, the SHL is dependent (Emmans, 1989), and decreases as a proportion of the total heat loss as the environmental temperature increases. The value of the evaporative heat loss is constant at 20 – 30% of the total heat loss (Romijn & Lokhorst, 1966) below the environmental temperature of about 32^0C (Gous, 2002).

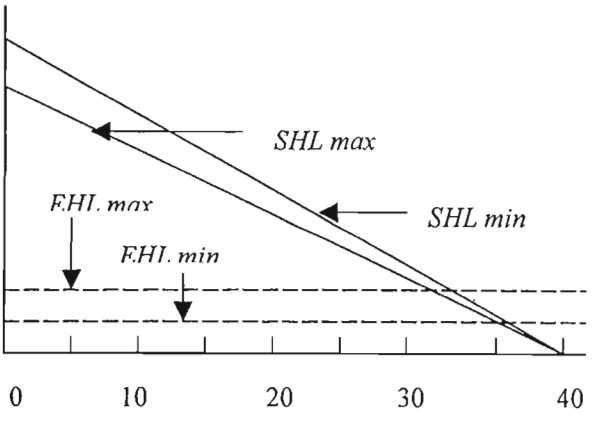


Figure 1.5. *SHL and EHL at different temperatures* (Emmans, 1989)

The representation in figure 1.5 poses several questions (Emmans, 1989):

1. At what temperature is SHL zero?
2. What is the value of the slope relating SHL_{min} to temperature, and in what ways does this vary between birds?
3. What is the value of EHL_{min} and to what extent does this vary between birds?
4. What is the ratio of SHL_{Max} to SHL_{min}?
5. What is the ratio of EHL_{max} to EHL_{min}?

1.2.5. Heat loss, heat production and feed balance

1.2.5.1 Feed intake regulation

The decrease in feed intake with increasing ambient temperature is not questionable. The most likely series of events leading to the decease in feed intake as ambient temperature increases are the following:

Energy requirements for maintenance of body temperature decrease with increasing environmental temperature until the region of the upper critical temperature is reached, as is evidenced by a decease in MHP and oxygen consumption (Hillman *et al.*, 1985). Below the lower critical temperature birds become more active, generate more heat, and consume more food than at temperatures within the thermoneutral zone (i.e. chemical control of the body temperature) (Freeman, 1966). This is known as the heat increment (HI) or specific dynamic action of food (SDE). Under such conditions the bird is more likely to meet its requirements for the first limiting nutrient in a marginally limiting feed than at high temperatures. As the environmental temperature falls, body heat production rises until it reaches a maximum (summit metabolism) (Figure 1.6). If environmental temperature falls further, HP can no longer balance HL, body temperature decreases and metabolic intensity declines in accordance with Van't Hoff relationship (Smith & Oliver, 1971). Death might eventually result at lower lethal temperature.

As the average environmental temperature rises above the critical temperature, where thermoregulatory functions becomes increasingly inefficient, the HI of the food becomes increasingly detrimental to the maintenance of body temperature, the bird becomes stressed and decreases feed intake such that its heat production falls to that permissible at that high temperature. Growth rate declines progressively as the temperature rises above 20^0C and above 27^0C feed conversion declines (Howlider & Rose, 1987) and bird starts using more

energy in an attempt to stay cool by dilating certain blood vessels in order to get more blood to the comb, wattles, feet etc and by panting and wing drooping (Leeson & Summers, 1997). The exact temperature rise above normal depends on the relative humidity of the atmosphere and the degree of acclimatization of the bird (Smith & Oliver, 1971). Thus regulation of feed intake is a major mechanism of thermoregulation.

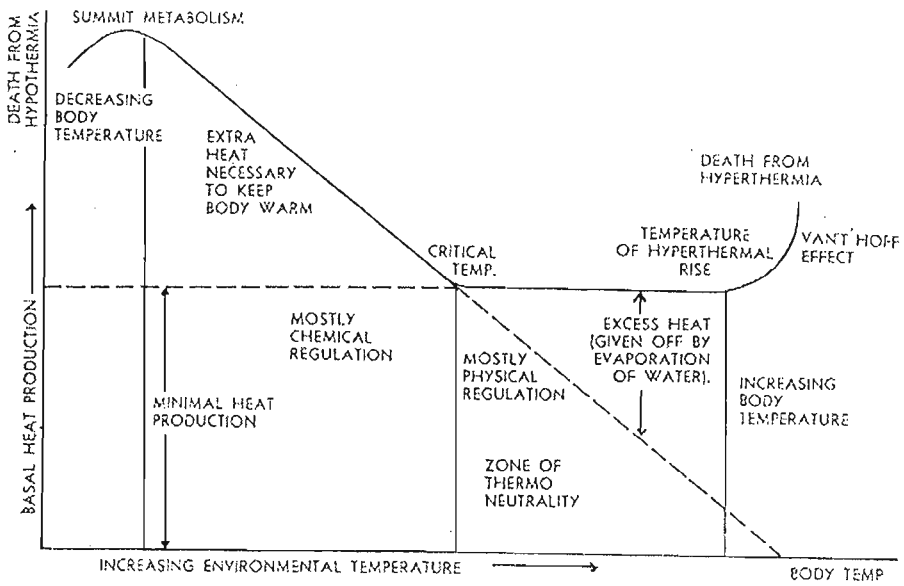


Figure 1.6. *BHP in adult birds as affected by changes in environmental temperature* (After Smith & Oliver, 1971)

1.2.5.2. Heat loss and feed balance

It is has been indicated that the rate of intake of a given feed by a given bird in a given state will depend on the temperature of the environment in which it is kept (Emmans, 1995). The simplest explanation for this is that heat produced by the bird with association to eating of the food, must be completely lost to the environment except in the short term. Emmans (1995) suggested that as the ability of the bird to store heat, other than in the short run, is effectively zero, its rate of heat loss must be equal to its rate of heat production. It now become evident that that there is a relationship between the bird and its environment, which was quantified by Emmans (1995) and is extremely helpful to the validity of this research (see Chapter four).

The environmental constraint on the rate of heat loss can thus become a constraint on the rate of heat production. Since heat production is related to the rate of food intake of a given food,

the temperature of the environment sets the upper limit to the rate of food intake of a given food by imposing a limit on the heat production of the bird (Emmans, 1995).

Source of heat production by birds in thermally neutral environment as indicated by Emmans (1995) are:

- Maintenance heat, including activity
- Direct heat increment of the feeding (the temperature of the feed itself)
- Heat increment of feeding
- Heat increment of protein retention
- Heat increment of lipid retention
- Cold thermogenesis (when temperature is very cold)

Emmans (1995) suggested that on a feed of a given composition heat production would increase as food intake increased providing that, at all intakes, the environment is thermally neutral. This implies that the thermoneutral temperature of the environment will decrease as the rate of food intake decreases. Thus the problem of predicting the potential growth of an animal depends on whether the birds are capable of consuming the balanced food at their potential rate of intake in the given environment.

1.3.EFFECT OF HIGH TEMPERATURE ON PERFORMANCE AND ENERGY BALANCE

The physiological changes that occur when chickens are exposed to high temperature have been discussed. It is now important to discuss to what extent these changes will affect the performance, efficiency and profitability of poultry.

1.3.1. Feed intake and energy balance

Many attempts have been made to relate the decline in feed intake with performance. In early studies, Payne (1966a) recognized that a drop in egg production by laying hens in hot environments was due to lower essential nutrient intake. Payne (1966 a, b) and Smith (1972) proved that feed intake decreased substantially as environmental temperature increased. Yet Payne (1966 b) and Mowbray & Sykes (1971) showed that the reduction in feed intake at temperatures of about 30^0C was not associated with a depression of egg production if the intake of the nutrients other than those needed for energy was maintained at the required levels. It was because of the reduced intake of the essential nutrients at high temperature, that rate of lay, egg weight, growth and average body weight decreased.

Because many factors, both managerial and environmental play a role, feed intake may be difficult to predict accurately. Despite this, a number of authors have attempted to quantify the change in feed intake in relation to environmental temperature. Van Kampen (1981) and Daghir (1995) from work of several authors on laying hens, calculated the decrease in feed intake per degree Celsius in temperature as shown in Table 1.3.

Table 1.3. *Effect of temperature on feed intake of laying hens* (After Van Kampen, 1981; Daghir, 1995)

Van Kampen (1981)		Daghir (1995)	
Temperature (^{0}C)	Decrease per 1^{0}C rise (g/d)	Temperature (^{0}C)	% Decrease per 1^{0}C rise
<20	1.0	20	-
20 – 25	1.3	25	1.4
25 – 30	2.3	30	1.6
30 – 35	4.0	35	2.3
		40	4.8

The decline in feed intake in laying hens was found to be either linear (Payne, 1966 b) or curvilinear (Smith, 1973; Marsden *et al.*, 1987; Marsden & Morris, 1987; Lesson & Summers, 1997), with a progressive decline in ME intake as the ambient temperature approaches body temperature. The work of 14 and 30 published experiments was summarized by Emmans (1974) and Marsden & Morris (1987) respectively. These experiments showed the progressive nature of the decline in ME intake. Figure 1.7 shows the variables commonly used to partition equations to predict ME intake indicating that the rate of egg deposition and body energy change are almost constant between 10 and 25^{0}C. Above 25^{0}C, energy intake falls more quickly than heat loss, the difference being for reductions in egg and body energy. Around 37^{0}C the curves of energy intake and HP cross over, indicating an unstable situation, which implies a continuous loss of body energy

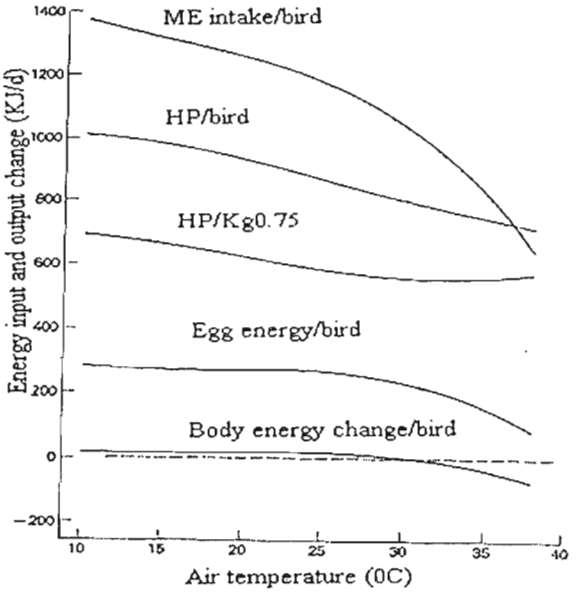


Figure 1.7. *ME intake, heat out put, egg energy and change in body energy* (After Marsden & Morris, 1987).

The resulting equation for ME intake which describes this curve (Marsden & Morris, 1987) is:

$$Y = 1606 - 35.283\ T + 16469\ T^2 - 0.0362\ T^3$$

Where:

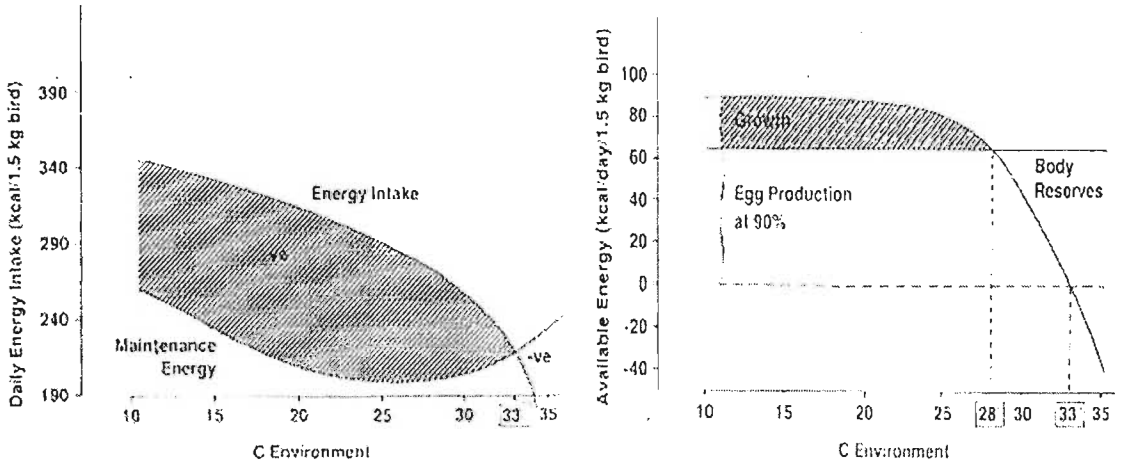
Y = ME intake (KJ/bird day)

T = ambient temperature (^{0}C)

The relationship can further be elaborated by using Leeson & Summers (1997) diagram (Figure 1.8A). The upper line in the figure indicates energy intake for 1.5 kg laying hen. The shaded area in Figure 1.8A represents the energy available for production. According to this figure, as the critical temperature is approached and exceeded, the energy available for production drops dramatically and becomes negative when the temperature reaches 33^{0}C.

Leeson and Summers (1997) plotted the shaded area (available energy) against temperature to see the pattern with respect to potential for egg production (Figure 1.8B). These authors assumed that an average egg contained the equivalent of 335 KJ ME, thereby calculating the ME needed for production at 90% production. According to their calculation there is a daily need for 293 KJ to meet the needs for production only. The total available energy that they indicated was 377 KJ per day, which shows that there is only a small pocket of energy that will go for growth or increased body weight. At 28^{0}C, there is energy available only for egg

production and none for growth and above 28^{0}C energy available cannot meet energy demands for 90% production. Thus, either egg production has to be decreased or another energy source has to be used. At this stage the bird's body reserve will be used as a source of energy, but it will only support the relatively high production temporarily and eventually will result in lowered body weight. At 33^{0}C the bird is in negative energy balance (Figure 1.8B).



A B

Figure 1.8. Environmental temperature and energy balance (After Leeson & Summers)

1.3.2. Feed intake and Heat production

Like all other animals, the constantly occurring energy transformations in the bird are never completely efficient and are accompanied by loss of energy, in the form of heat. Even under the ideal environmental temperature conditions, MHP occurs at some minimal rate. This MHP exhibits a diurnal rhythm and is lower at night when ambient temperature, activity, and feed intake are at a minimum. The difference between the minimum and maximum daily MHP is approximately 11% in the adult fowl (Hillman *et al.*, 1985).

Mean HP in relation to environmental temperature and feed intake is shown in Figure 1.9. HP decreases with increases in environmental temperature and decrease in food intake (Li *et al.*, 1992). The decrease in HP with increase in temperature results in a decrease in energy requirement for maintenance (Sykes, 1977). HP may be influenced by either net energy requirement for maintenance or HP associated with feed intake or both. According to Blaxter (1989) in mammals, the lower critical temperature is noted to decease as feed intake increases

and the HP associated with FI to contribute to the thermoregulation at low temperature. But the fact that HP deceases continuously with increasing temperature at any FI suggests that the HP associated in laying hens is not directly linked with thermoregulation (Li *et al.*, 1992). The increase in HP with food intake amounted to 16% of ME intake (Li *et al.*, 1992).

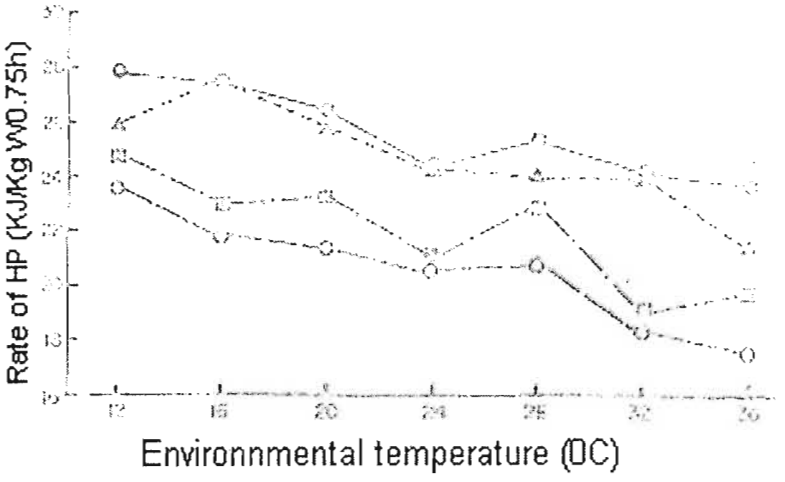


Figure 1.9. *HP of hens in relation to environmental temperature and food intake (●, 90; ▲, 60; ■, 30; O, 0 g/24 h)* (After Li et al., 1992)

1.3.3. Egg production and growth rate

In the laying hen, heat stress depresses egg production (de Andrade *et al.*, 1977; Marsden *et al.*, 1987) and egg weight (Smith, 1974; Peguri & Coon, 1991), egg size and egg shell strength (de Andrade *et al.*, 1977), and in broilers, growth rate is depressed above 20^0C and feed conversion above 27^0C (Howlider & Rose, 1987). The negative effect of temperature on growth and production is probably due to reduced feed intake for broilers (Hurwitz *et al.*, 1980) and laying hens (Savory, 1986). Egg composition and egg weight are unlikely to be affected over a wide range of temperatures (Emmans, 1974). For birds at different temperature having the same nutrient intake, egg weight depresses at temperature above 25^0C (Bray & Gesell, 1961; Payne, 1966 b; Marsden *et al.*, 1973), and above 26^0C 80% of the decrease in egg weight was due to heat stress and only 20% due to inadequate energy in the diet (Smith, 1974).

Marsden *et al.* (1987) found that the rate of egg production did not vary significantly in birds under temperatures ranging from 15 to 27^0C, although there was a tendency to increase to maximum at 27^0C. There was a little difference in egg weight at temperature of between 15

and 24^{0}C but a rapid decrease between 24 and 32^{0}C (Figure 1.10). It was possible also to measure the long-term effect of high temperature of 27 to 30^{0}C on egg weight when temperature treatments were applied over a number of months (34 weeks). It was indicated that the decline in egg weight curvilinear, and that the effect can be progressive with time. Thus, those authors concluded that since egg weight was reduced at 27^{0}C, while egg output reached its maximum level at this temperature, egg mass and rate of lay may be controlled by different mechanisms, if an adequate supply of nutrients to both is provided. Age of the hen is one factor (Smith & Oliver, 1972b; Marsden *et al.*, 1987).

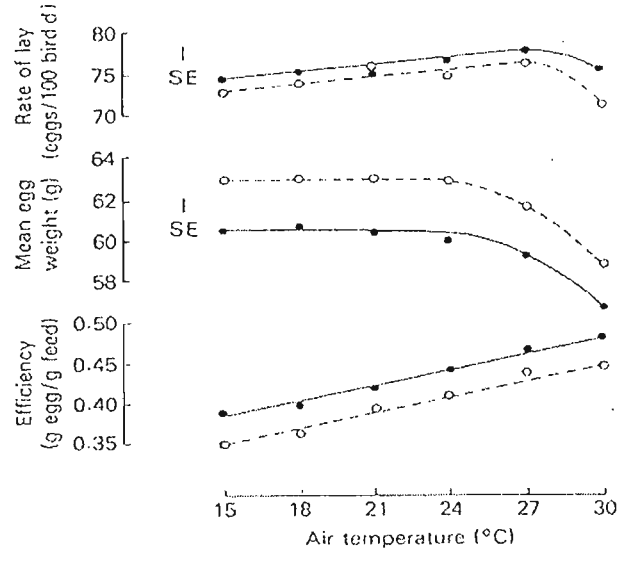


Figure 1.10. *The effect of air temperature on rate of lay, mean egg weight and efficiency of food utilization from 32 to 66 weeks of age for Warren pullets (-0-0-0-) and Babcock pullets (-●-●-●-)* (After Marsden *et al.*, 1987)

Despite the gradual decline with increasing temperature, maintenance of egg output and egg size for a short period at temperatures between 10 and 25^{0}C indicates that the hen was using its body energy reserves. This could be the cause for the decline in body weight gain at increasing environmental temperature (Van Kampen, 1981; Marsden & Morris, 1987). Therefore, the decline in egg production, egg weight and rate of lay, at high Ta's may possibly be the direct result of inadequate body reserves associated with insufficiency of certain nutrients in the daily intake and/or heat stress. However, using the techniques of paired feeding Smith & Oliver (1972a) showed that the main cause of production loss was the reduction in energy intake caused by high temperatures.

Factors other than feed intake have been suggested to play a role in the adverse effects of high temperature on growth rate (Deaton *et al.*, 1972; Leeson, 1986). Dale & Fuller (1980) demonstrated that when broiler chicks, reared in a cool environment (14^0C), were pair fed according to the feed intake of those maintained under hot summer conditions (31^0C), growth rate was significantly better than that of the heat stressed chicks. They concluded that only 63% of the growth depression due to heat stress was directly related to reduced feed intake and 37% to factors not directly related to the quantity of feed consumed.

A number of experiments have been conducted on the effect of cycling temperature regimes on egg production. Wilson *et al.* (1972) showed that a diurnally cycling regime between 10 and 30^0C depressed egg production as much as did a constant 34^0C. On the other hand, work done by Mowbray and Sykes (1971) indicated that the egg production from hens in a diurnal cycle regime from 13 to 35^0C was similar to that from hens in a regime varying between 10 and 15^0C. Muller (1967) found that egg production of hens held in a 13 - 32^0C regime was similar to that of hens held at constant 13^0C, while Smith & Oliver (1972a) indicated that pullets kept at temperatures of 32^0C and 38^0C produced eggs with mean egg weights in the experimental period that were 4, 6, and 20% less respectively than those of egg produced by pullets kept at 21^0C. The mean shell weight was markedly reduced in eggs produced by pullets kept at 38^0C as compared with the control, but shell weight was not affected by the corresponding rationing treatment (Smith & Oliver, 1972a). It seems apparent that as temperature increases above the threshold point somewhere between 26 and 29^0C, a diurnally cycling temperature is needed to maintain "normal" egg production (Smith, 1981). Thus, when the daily maximum increases, the amount of the diurnal variation must increase to prevent a decrease in egg production.

At in increasing constant high temperature, egg production was progressively reduced. Howes *et al.* (1965), cited by Smith (1981) found that depression of production, as measured by number of days taken for egg production to decline to 50%, was accelerated as temperatures increased.

1.3.4.Body composition

It has been demonstrated that environmental temperature affects the body composition of birds. These changes primarily existed in fat and moisture content, with protein being relatively unaffected. The demonstration by Kubena *et al.* (1972) indicated that fat content in

birds reared at 10^0C was lower than those at 32^0C, while the moisture content followed an opposite trend to fat content. Farrell & Swain (1977 b) found an increase in body fat content as ambient temperature increased from 2 to 22^0C and maximum fat balance was observed between 16 to 22^0C. These authors further noted that an increase in ambient environmental temperature resulted in rapid decline in body fat between 22 and 35^0C (Figure 1.11). Kubena *et al.* (1972) and Farrell & Swain (1977 b) reported carcass protein gain was not affected by temperature and it was thus assumed that protein anabolism is relatively independent of environmental temperature. This was the case of investigation in an experiment by Farrell & Swain (1977a, b), with the assumption that energy retention as protein was relatively constant from 2 to 22^0C. A marked decline in protein retention was observed between 30 and 35^0C, with a maximum retention occurring between 16 and 22^0C. Thus a quadratic relationship was evident between energy retained as protein, and temperature. The reason might be that endogenous nitrogen excretion increased when broiler chickens were exposed to constant high temperatures (30 and 35^0C) probably due to an increased contribution from protein tissue to maintenance energy requirement (Farrell & Swain, 1977 a).

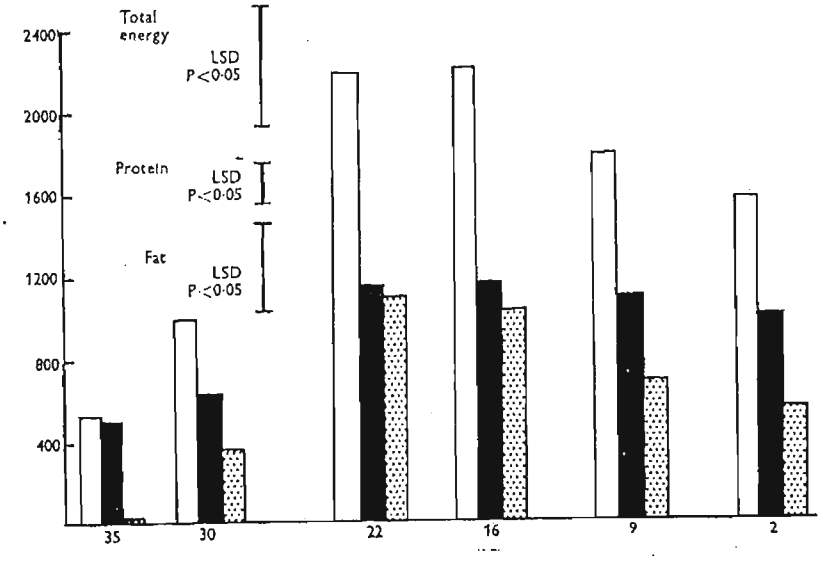


Figure 1.11. *Energy balance, protein energy, fat energy retained by individual broiler chickens at six temperatures* (Farrell & Swain, 1977 b)

1.4. ENVIRONMENT – NUTRITION INTERACTION IN POULTRY

Animals require major organic nutrients as materials for the construction of body tissue, synthesis of expelled products such as eggs and as a source of energy for work done. A unifying characteristics of these functions is that all of them involve energy transfer which applies the conversion of chemical energy into mechanical or heat energy, as for example, when nutrients are oxidised, and when chemical energy is converted from one form to another, like when body fat is synthesised from carbohydrate (MacDonald *et al.*, 1995).

Animals deprived of food continue to require energy for essential functions such as body movement – including chemical work (movement of substance against gradient), mechanical work (essential muscular activity), and for the synthesis of expended body constituents (enzyme and hormones). Thus, in starving animal the energy required for these purposes is obtained by catabolism of body reserves, first glycogen then fat and protein (MacDonald *et al.*, 1995). In the fed animal, however, the primary demand of the food energy is in meeting the requirement for body maintenance so as to prevent this catabolism. When the food chemical energy is converted into muscular or chemical work, the animal is doing no work on it's surrounding, and the energy used for such purpose is converted into heat energy. This is regarded as the energy that is expended as heat energy, and is useful only in maintenance of body temperature (MacDonald *et al.*, 1995).

1.4.1 Energy partition of the feed and the effective energy system (EE)

All energy-feeding systems begin with gross energy (GE). GE or intake energy is the total feed energy provided to the animal. Feed ingested is not completely digested or absorbed. The unabsorbed fraction is excreted as faeces and its combustible energy is called faecal energy (FE). Apparently digestible energy (DE) is calculated as GE – FE. This is distinguished from the true digested energy (TDE), which accounts for metabolic faecal energy (MFE) and heat of fermentation (HFE) (Reynolds, 2000). Similarly, the metabolizable energy (ME) intake is calculated by subtracting the gross energy lost in FE, urine (UE) and the gaseous products of digestion (largely methane) (MTHE). Net energy (NE) is metabolizable energy less heat increment (Figure 1.12).

ME for production is available after the maintenance needs of the animal are met. But because of the HP (inefficiencies of product synthesis), energy available for production is not entirely

incorporated into the animal products, be it retained in tissue growth, or expelled product, such as milk, egg, pelage, or offspring (NRC, 1981). The latter include inefficiencies of product synthesis and cost of retaining or expelling the product.

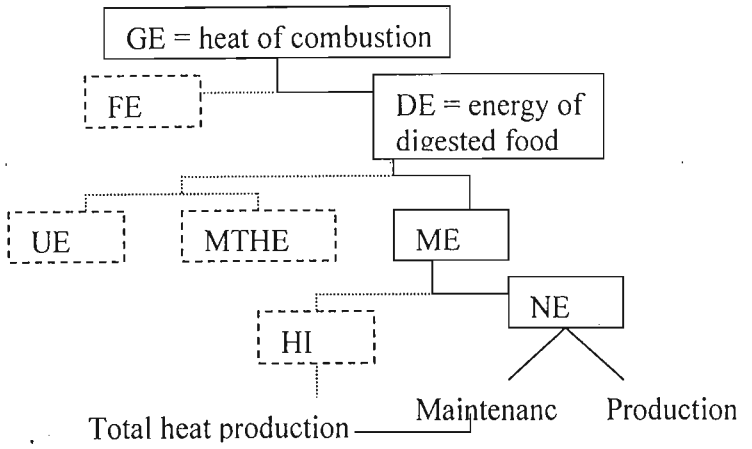


Figure 1.12. *Partition of food energy in the animal. Losses of energy are shown as dashed boxed items on the left* (After MacDonald et al., 1995)

The NE is the energy that is available to the animal for useful purposes, i.e. for body maintenance and for various forms of production. NE used for maintenance is mainly used to perform work within the body, and leaves the animal as heat, while that used for growth, fattening, milk, egg, or wool production is either stored in the body or leaves it as chemical energy, and the quantity so used is referred to as the *animal's energy retention* (MacDonald *et al.*, 1995).

The NE system termed as the effective energy system by Emmans (1994) estimates the effective energy of the diet (ingredients) by applying linear coefficients to five measurable components of interaction between the animal and its diet when adjusting ME for heat increment of feeding.

The starting point for the calculation of effective energy in Emmans (1994) is chosen to be ME classical (ME_C), which its rate of supply was seen by Armsby (1903), cited by Emmans (1994) as the difference between the GE ingested and that lost in excretions and combustible gaseous products (largely methane).

$$ME_C\,(KJ/d) = GE - (FE + UE + MTHE)$$

The production of GE (largely methane) by poultry is negligible and can be ignored (Emmans & Fisher, 1986; MacLeod, 2000). The ME_C value is 'apparent.' It is usually adjusted to a 'TME' (true metabolizable energy) value by the correction factor that takes into account fasting energy loss, or endogenous energy loss (EEL), which vary with fatness of the bird and environmental temperature.

$$TME\,(KJ/d) = GE - (FE + UE - EEL)$$

ME of the diet is produced from its digestible components – the carbohydrate, protein, and fat. Apparently digested protein is either catabolized or retained & its nitrogen appears as various urinary compounds. Catabolized protein produces less energy than that burnt in a bomb calorimeter. Therefore, for appropriate correction one should subtract from both the diet and the protein retained the urinary energy that would have resulted had all the digestion been catabolized (Emmans & Fisher, 1986).

Correcting the classical ME to zero nitrogen retention (NR), to give ME_n (N – corrected ME or catabolizable energy), is estimated by the formula:

$$ME_n\,(KJ/d) = ME_C - a(6.25NR)$$
$$= (h_p - a)xPR + h_lxLR + H$$

Where the value of 'a' is assumed to be constant having a value close to 5.63 KJ/d, LR and PR are the rates of retention of lipid and protein (g/d), h_p and h_l are the heats of combustion of protein (23.8 KJ/d) and lipid (39.6 KJ/d), and H is heat of production (KJ/d). From the principles of energy conservation, in the above formula for ME_n, the ME yielded to the animal by its diet must either be retained in the animal or lost as heat.

Heat of production is considered to have two components, i.e. fasting heat production (FHP) - the rate at which the animal would produce heat when given no feed, and heat increment of feeding (HIF) which depends on the animal; it can be seen as some function only of the kind of animal and its current state, providing that the environment is thermally neutral and the activity level of the animal is set at, or adjusted to, some constant level (Armaby, 1903; cite by Emmans, 1994). Most of Emmans (1994) work deals with the heat produced by fasting animal, which comes only from metabolism lipid protein and lipid of the body, once the small stock of carbohydrate has been exhausted. The heat produced by the catabolism of lipid is its heat of combustion, and protein catabolism leads to some energy appearance in the urine as N – containing compounds, so that the HP of protein catabolism is less than its heat of combustion. The FHP is give by (Emmans, 1994):

$$FHP\ (KJ/d) = (h_p - a)xPR + h_lxLR,$$

Where PR and LR (g/d) are protein and lipid retention (which are negative in the fasted bird, MacLeod, 2000). As some part of the FHP comes from the synthesis and excretion of N-containing compounds in the urine, the rate of heat excretions (HEX, KJ/d) is assumed to be given at W_u (KJ/gN) in the urine. Thus, FHP other than HEX is the MHP, which is calculated by assuming that the fasted bird is catabolizing only lipid. The relationships are:

$$HEX\ (KJ/d) = W_u \times FUN$$

$$MH\ (KJ/d) = FHP - w_u \times FUN$$

Where FUN (g/d) is the rate of excretion of N during the fast and W_u (KJ/d of N) is the HP associated with the synthesis and excretion of urinary N. When MH taken as base, instead of FHP, the FHP includes HI due to the fasting excretion. Heat increment for maintenance (HIM, KJ/d), ignoring methane production for poultry is given by the equation:

$$HIM = W_d \times FOM + w_u \times UN$$

Where W_d (KJ/d) is HP associated with the production of FOM. Maintenance ME requirement (MEM, KJ/d) is given by

$$MEM = MH + HIM$$

Considering a diet leading to positive retentions of both protein and lipid and ignoring methane production for poultry, HIF (heat increment of feeding) is given by

$$HIF\ (KJ/d) = W_d\ FOM + w_u\ UN + W_p PR + W_l\ LR$$

Where W_d and W_l are the production of heat associated with protein and lipid deposition respectively. Thus the ME needed by the bird is given by:

$$ME\ (KJ/d) = ER + MH + HIF,$$ where ER is energy retention.

By considering the energy content of protein and lipid, the energy cost expended in depositing them (protein and lipid) and in excreting the nitrogenous waste products, is shown as the effective energy requirement, which is given by the formula:

$$EERQ\ (KJ/d) = MH + 50 \times PR + 56 \times LR$$

Emmans (1994) suggests ME derived from protein has a significantly lower efficiency of utilization and that an increasing proportion of digestible crude protein reduced the effective energy. His model also proposed that high faecal energy losses are associated with high HI of digestion. A reduction of the determined ME for the amount of excreted FOM, henceforth, gave an improved estimate of the effective energy. According to the model, if dietary fat was used directly for lipid growth, there was a reduction in HI of 12 MJ/Kg. He has also shown that 30% of body fat to come directly from feed lipid. Therefore, an increase of 4 MJ/Kg (12 X 0.3) for the effect of direct transfer of dietary lipid was proposed. Thus, weighing the result

of the difference by Z (the proportion of the lipid retained that apparently came from feed lipid), and setting MTHE at zero, the EE value of an ingredient for monogastric animals can be expressed as:

$$EE\ (KJ/g) = Me_n - w_d \times FOM - 0.16\ w_u \times DCP + 12 \times DCL$$

Where DCL is the digestible crude lipid (g/g) and Z has a value between 0 and 1. For poultry the value of Z is 0.3 (Emmans, 1994).

As ER is the consequence of the performance of the animal, as rates of protein and lipid retention, and as MH depends only on the kind of animal and its state, it follows that the estimation of ME needed by an animal in a given state to attain some particular level of performance depends on the HIF (Emmans, 1994). For ruminants and monogastric animals, HIF can be estimated from the quantities FOM, UN, MTHE, PR and LR providing that the assumptions made are correct and the value of W_d, W_u, W_m (is the heat associated with the production of methane (KJ/d), W_p, and W_l are known. The scheme for predicting HIF can be show by the diagram below.

The feed (OM) ingested by the animal follows several paths within the body, and is either catabolized (given off as heat) or is retained in the body tissue.

The figure below, a flow of OM in animals, illustrate several areas by which heat produced contributes to the heat increment (HI).

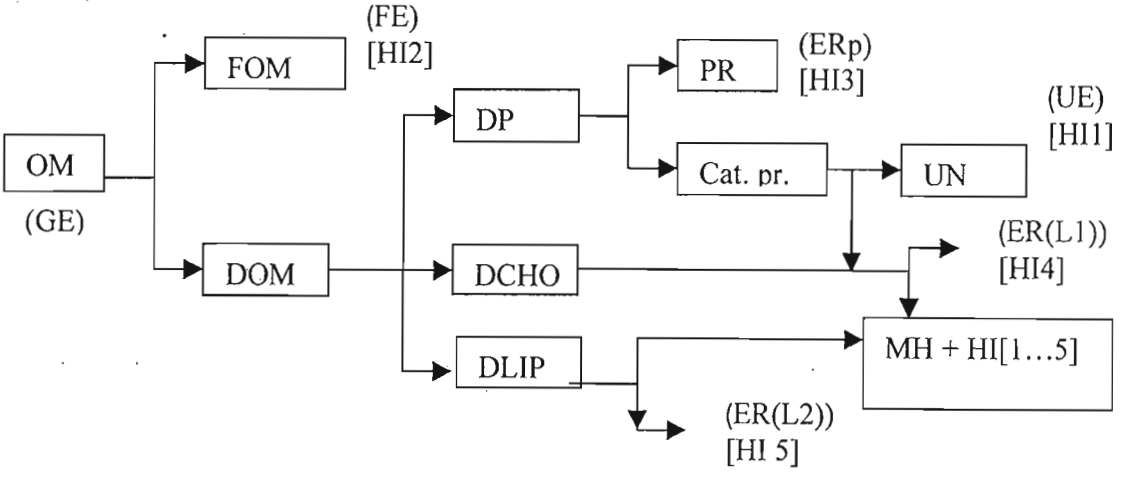


Figure 1.13. *Scheme for predicting the heat increment of feeding in monogastric* (After Emmans, 1994)

The material components (g/d) are OM, organic matter; FOM, organic matter in faeces; DOM, digestible organic matter; DP, digestible protein; DCHO, digestible carbohydrate;

DLIP, digestible lipid; PR positive protein retention; Cat. Pr., catabolized protein; and UN, Urine. The energy components (MJ/d) are GE, gross energy eaten; FE, faecal energy; UE, urinary energy; ER(P), energy retained as protein; ER(L), the energy retained as lipid. The components (MJ/d) are: MH, maintenance heat increment; HI, heat increment of feeding which has the components due to separate heat increments of FOM, UN, PR and LR (Emmans, 1994).

The OM eaten by the animal could be either digestible or not. The indigestible OM is excreted as FOM, on which additional heat is produced in the formation and excretion of the product. The digestible OM (0.9 efficiency, Emmans, 1994) yielded as protein, lipid, and carbohydrate are partitioned to meet the various body requirement of the animal. The protein component is either catabolized or retained. The catabolized protein contributes to the urine, where the nitrogen products appear in the urine, or used as precursors in the synthesis of body lipid by deamination of the catabolized protein products. Digestible carbohydrate contributes to the energy retained as lipid. Lipid retention in the body can be due to the digestible lipid being deposited directly as fat, or due to the digestible lipid being broken down and re-synthesised along with precursors from carbohydrate and protein components, to body lipid. Because more energy is required for catabolism and anabolism in the latter route as compared to the former one, the latter path is less efficient.

The heats of production, illustrated in Figure 1.13, as heat increments associated with positive retention of lipid and protein, and with the production of faecal organic matter and urinary products, are added to a maintenance heat quantity, reflecting the total heat produced in monogastrics above maintenance. Because these heats of production reflect energy that is given off and usurped from that supplied in the ME of the diet, it would make energetic sense that these losses be accounted in an energy system.

1.4.2. Heat increment as influenced by dietary protein

Any source of feed provided to the animal causes an increase in HP but the increase varies with the type of nutrient. Earlier studies with dogs showed that protein caused a larger increase in HP than either CHO or fat (Rubner, 1902 cited by Musharaf & Latshaw, 1999). SDE, a term used by Rubner in Musharaf & Latshaw (1999), was higher than that of CHO or fat on which more energy was required from protein to yield an equivalent amount of useful

energy to the animal. In addition, the increase in HP was thought to be wasteful to cause problem for an animal at high temperatures (Musharaf & Latshaw, 1999).

According to NRC (1981) all HP in the animal is accounted for in maintenance or HI. Table 1.4 describes the relation of HP with the net energy scheme. In the table F1 is the fasting HP resulting from the metabolism of GE to DE. During fasting, since no food is provided to the animal, logically there should be no F1. The probable reason why F1 exists is that the metabolism required to cause the endogenous faecal loss would be expected to result in HP. Moreover, the catabolism of body protein results in the production of amino acids that would be expected to produce SDA similar to that from feeding amino acids to fasting animal (Richardson & Mason, 1923, cited by Musharaf & Latshaw, 1999). F2 shows HP associated with the production and excretion of urinary nitrogenous products. The cost of nitrogen excretion was allocated to F2, while any additional HP resulting from the SDA were included under F3 and all other HP during fasting but not accounted in F1 & F2 were assigned to F3 (Musharaf & Latshaw 1999).

Table 1.4. Components of HP related to the net energy scheme by physiological function (After Musharaf & Latshaw, 1999).

Physiological function	Designation of HP associated with each component of the net energy scheme		
Production	P1	P2	P3
Maintenance	M1	M2	M3
Fasting	F1	F2	F3

Food energy is needed to maintain an animal, and food intake triggers many reactions that enhance HP within the animal. In Table 1.4 maintenance functions are designated by M1, M2 and M3. M1 has been calculated to be only a small component of total HP (Webster *et al.*, 1975). M2 represents the increase in HP resulting from the excretion of the nitrogen from the amino acids in the food. Any additional effects of dietary amino acids on HP beyond those of additional nitrogen excretion would be in found in M3 (Musharaf & Latshaw, 1999).

Amino acids occupy a unique position among the energy sources when determining maintenance energy requirements (Musharaf & Latshaw, 1999). They cause larger increase in

HP than CHO or fat; however, neither CHO nor fat is a suitable source for long term maintenance need (Musharaf & Latshaw, 1999). This, therefore, shows that they are essential part of any nutritionally complete diet. Blending food energy source has presented an important effect on the HP of the animal. Diets with large excesses of one or more amino acids cause large increase in HP (Musharaf & Latshaw, 1999). When protein was combined with CHO and fat the HI was less than that predicted from the value for the individual energy sources showing a response known as associative dynamic effect (Musharaf & Latshaw, 1999).

Energy for product is available when the animal's energy intake becomes more than maintenance requirement. Components of HP in relation to production are designated as P1, P2 and P3 (Table 1.4). These components are essentially the same as their counterparts for maintenance, except that P1, P2 and P3 originate from metabolism that causes a net synthesis of product, whereas maintenance is required to prevent the loss of body tissue.

Protein, like CHO and fat is more efficiently used for maintenance than for making product (Blaxter, 1989). An average value for CHO, fat and protein conversion to product, as is given by Musharaf & Latshaw (1999), is approximately 75%, 80% & 55% respectively. But when used for maintenance their conversion efficiencies are all 15 – 20% higher.

When protein is fed in high proportion of the diet, it is a less efficient source of energy as compared to CHO and fat (Blaxter, 1989). A partial vindication for this would be the use of protein as an energy source for maintenance or production resulting in nitrogen excretion. Compared with other energy sources protein increases in M2 & P2 in Table 1.4. M3 & P3 would also be increased by protein because dietary protein stimulates protein turn over (Reeds *et al.*, 1982; Reeds & Fuller, 1983).

The use of more synthetic amino acids and less intact protein permits essential amino acid requirements to be met at lower concentrations of dietary protein (Keshavarz & Jackson, 1992). The ability to produce synthetic amino acids at relatively low cost provides the opportunity to lower the dietary crude protein content while still meeting the essential amino acid needs (Wang & Fuller, 1989). Based on considerations of HI, diets with lower protein contents would be expected to improve food efficiency by reducing P2 & P3 in Table 1.4 (Musharaf & Latshaw, 1999).

1.4.3. Nutritional considerations of poultry during heat stress

1.4.3.1. Energy requirements and the addition of fat

Several experiments have proven that hens have the capacity to adjust feed intake to supply ME needed for production and maintenance at high temperature (Davis *et al.*, 1972, 1973; Smith & Oliver, 1972b). The idea was reviewed by Morris (1968), for which, he concluded that groups of pullets offered different diets tend to adjust consumption so as to maintain the same energy intake, although the adjustment by pullets was imperfect in the majority of the cases, since birds fed high energy diets over consumed energy. Peguri & Coon (1991) verified that feed intake declined with increasing dietary energy level at high temperature. They also noted that the increase in dietary energy from 11.1 to 12.5 MJ ME_n/Kg was accompanied by a decrease of 5.9 g in feed intake as the temperature increased from 16.1 to 31.1^{0}C. At this temperature, the ME intake of hens was 0.26 MJ/hen per day lower in treatments with higher temperatures. More over, while egg weight increased (0.78g) with increase in dietary energy density from 11.1 to 12.5 MJ ME_n/Kg and decreased (3.18g) with an increase in temperature from 16.1 to 31.1^{0}C, egg production was not affected by either temperature or dietary energy density. Mean body weights and body weight gain were significantly higher when treatments with higher energy density and lower in treatments higher environmental temperature.

Dietary energy content, in Marsden *et al.,* (1987) had small but significant effects on egg weight and egg output but didn't interact with temperature. In addition, it was not possible to maintain egg weight, egg output or rate of lay at 30^{0}C by feeding a high-energy diet (Marsden *et al.*, 1987). This agreed with the idea of Smith & Oliver (1972b) who noted that energy intake at high temperatures was inadequate to support maximum egg output but were unable to correct it by feeding high-energy diets. Henceforth, it seems that increasing dietary energy concentration increases the energy intake (between 15 – 27^{0}C, Payne 1966 b; Marsden *et al.*, 1987), but the effect become smaller as the temperature increases. Further, although egg output remained fairly high at 27^{0}C, egg weight was reduced (Marsden *et al.*, 1987).

As was noted earlier, birds consume less feed with increasing energy level. With increase in environmental temperature the situation seems to worsen. The following results were observed when diet energy levels were increased from 11.97 to 14.44 MJ ME/Kg (Payne, 1967).

Table 1.5. *Effect of diet energy level on ME intake* (After Payne, 1967)

	At 18^0C		At 30^0C	
Energy level (MJ/Kg)	Feed intake per day (g)	Caloric intake (MJ/b d)	Feed intake per day (g)	Caloric intake (MJ/b d)
11.97	127	1.52	107	1.28
12.80	118	1.51	104	1.34
13.60	112	1.52	102	1.38
14.44	106	1.53	101	1.46

The table shows that while at high temperature (30^0C), layers adjusted less perfectly, such that they consumed more energy, at low temperature (18^0C) their energy adjustment was fair though accompanied by marked drop in feed intake.

The approach of adding fat to the layer diets during thermal stress has not been consistently successful. Reid (1979), cited by NRC (1981) was able to achieve some success by adding up to 9 percent tallow in the feed of laying hen reared at 29^0C. Daghir (1987), cited by Daghir, (1995) observed that added fat at 31^0C improved feed consumption in laying hens to a greater extent than at lower temperature. Table 1.6 shows the addition of fat to laying rations increased feed intake by 17.2% at 31^0C and only 4.5% at lower temperatures (10 – 18^0C). Supplemental fat increases energy of the diet fed during hot weather by increasing energy density of the diet, reducing heat increment, improving palatability, lowering rate of food passage and thus improving nutrient digestibility (Mateos, *et al.,* 1982). Supplementation of 2.5 percent fat to the diet increased energy intake (Dale & Fuller, 1980) and appeared to increase the energy value of other feed constituents (Mateos & Sell, 1981). An increase in ME per day improved the hen's energy balance when both egg production and body weight gain was considered (Reid, 1979, in NRC, 1981). Sell (1979), cited by NRC (1981) on the other hand, reported that an increased energy efficiency in the laying hens fed added dietary fat during heat stress was due to tissue deposits of the fat while egg energy per unit of ME consumed declined. Similarly, Smith (1972) indicated that in pullets that lost body weight due to high environmental temperature, proportionally more fat was lost from carcass than protein or ash. Polin & Wolford (1976) also indicated a lower heat increment of hen's diet as greater percentage of ME was in the form of lipid than as carbohydrate or protein dominated the energy supply.

Table 1.6. *Interaction of temperature and added fat on feed consumption* (g hen $^{-1}$ day $^{-1}$)

	Added fat (%)		
Temperature (^{0}C)	0	5	% Increase in feed intake
31	93	109	17.2
10 – 18	127	133	4.5

(After Daghir, 1987 cited by Daghir, 1995)

While some researchers have reported that the performance of broilers kept at high temperatures can be improved by increasing the proportion of dietary fat (Dale & Fuller, 1980; McNaughton & Reece, 1984), others have found no response to the inclusion of dietary fat of up to 10%, or at the most an improvement in growth that is not better than that obtained by supplementation at lower temperature (Reece & McNaughton, 1982). This may be because fat absorption, particularly of saturated fats, is limited in young chicks. Moreover, since the growth of young chicks is less affected by dietary energy than by protein level as opposed to that of older birds, their growth is therefore less sensitive to the changes in the dietary energy level.

1.4.3.2. The addition of energy and amino acids (Nutrient density)

Several attempts, which have been made to overcome the adverse effects of temperature on the declining feed intake and the resulting effect to correct it by using high nutrient density of the diet, have only been met with partial success (Payne, 1966 b; Mowbray & Sykes 1971). de Andrade *et al.* (1976, 1977) compared diets whose nutrients were increased 20 – 25 percent and energy density by 10 percent over that of a diet typically used at thermoneutrality. All three diets were fed to laying chicken in three different environments. The higher nutrient densities prevented a major decline in egg production, moderated a decline in egg weight, markedly improved efficiency of feed conversion to egg, but were unable to prevent the loss in shell quality, which reinforces the belief that this variable is affected by acid-base balance.

Similar trials conducted with broilers showed less promising results than with layers (Reece & McNaughton, 1982). The apparent lack of response to an increase in the energy content of broiler diets could be related to the amino acid profile of the diets, since the growth depressing effect is seen with an amino acid imbalance, a condition that is aggravated by an increase in energy supply originating from either the diet or a situation of heat stress (March & Biely, 1972). Dale & Fuller (1980) improved growth with heat stressed broilers by offering

diets of higher energy content, used much higher lysine levels compared to those reported by Reece & McNaughton (1982). A report by Sinurat & Balnave (1986) indicated that the feed intake and growth rate of broilers in a diurnally cyclic temperature of 25 to 35^{0}C were improved by increasing the dietary ME and reducing the amino acid to ME ratio during the finishing period. They suggested that the concept that dietary amino acid concentrations should increase at high temperature might be erroneous.

1.4.3.3.The addition of protein and amino acids

The relationship between dietary nutrient concentration and temperature has been reported in Emmans & Charles (1977) to occur through the influence of temperature on feed, and therefore, nutrient intake. Because high temperature depress feed intake, and hence, nutrient intake, it has been common practice to adjust the dietary concentrations of protein and amino acid in order to obtain a constant intake of these nutrients at all environmental temperatures. The adjustment is usually linear in nature. However, allowances of certain percentage change in the concentration of protein and amino acids for each unit change in temperature above or below a specified temperature would be important in the optimum range of performance. Although this adjustment gives approximate estimates of the dietary requirements of birds under these conditions, it might not be an accurate measure of the requirements at extremely high temperatures. A more accurate estimation of protein and amino acid requirements should probably need to take into account the reduction in production at these high temperatures and perhaps a more rapid decline in feed intake (Hillman *et al.*, 1985; Leeson, 1986; Marsden & Morris, 1987).

Generally, no significant benefits have been conferred by increasing the overall protein level from 16 to 30.8% in the diets of broilers under high temperatures. This implies that the efficiency of protein utilization decreases as protein concentration of the diet increases, indicating that protein requirements are not increased with increasing environmental temperature. It is, therefore, suggested that minimizing the protein levels and improving the balance of amino acids could minimize the SDE or heat increment of the diet (Sinurat & Balnave, 1986; Dale & Fuller, 1979). Sinurat & Balnave (1986) however suggested that because an amino acid deficiency stimulates an increase in feed intake, dietary energy concentration should be increased at a slight amino acid deficiency, which would thus increase energy intake.

In contrast to broilers, increasing the protein concentration of feeds for laying hens appears to partially overcome the effect of high temperature on performance. Despite the demonstration by Bray & Gesell (1961) that egg production could be maintained at 30^0C provided a daily protein intake of about 15g is ensured by appropriate dietary formulation, increasing dietary protein level under heat stress didn't improve egg production (Reid & Weber, 1973), increased egg weight as protein level increased at 32.2 ^{0}C (Reid & Weber, 1973, Valencia, *et al.*, 1980), but not at 35^0C (Reid & Weber, 1973). Although not conclusive, results suggest that ambient temperatures does not increase or decrease the requirement for protein (Sykes, 1977). Figure 1.14 shows the birds protein requirement does not change very much with temperature. Egg production can be maintained even at 30^0C as long as 15g-protein intake per day is maintained (Figure 1.14). Since feed intake reduced with increase in temperature, it requires a high protein diet if protein intake is to be maintained. Reid & Weber (1973) showed that though methionine supplementation significantly improved egg production and egg weight at 21.1 and 32.2^0C, no improvements in performance were obtained above the concentration of 0.289% methionine or 0.549% methionine plus cystine (at 21.1^0C). In other experiments, however, egg mass output of hen at cyclic 25^0C to 35^0C significantly increased with increasing dietary protein from 15% and 12 MJ of ME/ kg 23.6% and 10.79 MJ/ kg (Balnave & Murtsari Abdellah, 1990) and also with increasing intake of ME, lysine, methionine and methionine plus cystine (Balnave, 1996; cited by Khajarern & Khajarern 1998). Marsden *et al.* (1987) however, found the response was quite different. They found that by increasing the protein concentration, it was possible to maintain egg output up to 27^0C, but at 30^0C egg output depressed even though protein intake was maintained at 18g/d. Body weight increased as protein concentration in the diet was increased. This pattern was not changed by temperature even at 30^0C.

1.4.3.4.The Addition of electrolytes

Electrolytes are compounds that dissolve into positive (cation) and negative (anion) particles in solution and have the inherent ability to conduct electrical current. The elements that are predominant in satisfying the electrolyte balance within the body are sodium, potassium and chloride. Broilers and laying hens have definite requirements for these elements in the correct amounts for homeostasis know as dietary electrolyte balance.

During times of heat stress, feed consumption may severely decline, initiating a drop in egg production and size that may be related to sodium. Under these conditions (heat stress), it is

the availability of bicarbonate *per se* which seems to be the major factor influencing egg shell thickness, which in turn, is governed by acid-base balance, kidney function and respiration rate (Leeson & Summers, 1997). Dietary supplementation of $NaHCO_3$ is found to improve shell breaking strength (Balnave & Muheereza, 1997; 1998).

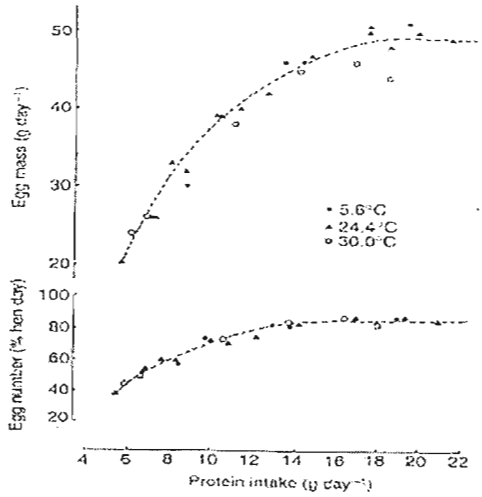


Figure 1.14. *Eggs mass output (upper curve) and number (lower curve) at different Ta's, as affected by protein intake (After Daghir, 1995, adapted from Bray & Gesell, 1961)*

The electrolyte balance of potassium in laying hens has been shown to have a substantial effect on shell quality and egg production. The formation of hydrogen ion generated by the syntheses of calcium carbonate creates an acidosis condition during shell formation within the laying hen. Deficiency in dietary potassium results in reduced production, egg weight, shell thickness and albumen content.

To alleviate the drop in blood plasma bicarbonate (HCO_3) levels during alkalosis, Bottjie & Harrison (1985) indicated that the addition of 1% $NaHCO_3$ had no effect on the growth performance of heat stressed cockerels between 8 and 11 weeks of age. On the other hand, Teeter *et al.* (1985) found that the addition of just 0.5%NaHCO3 to the diet of broilers under heat stress enhanced body weight gain by 9%.

1.4.3.5. Methods of alleviating heat stress

Heat stress can be alleviated in to general ways: altering the environment, and altering the nutrient content of the feed.

Environmental approaches include: keeping the bird in an open sided house with an open sided cage, increasing the ventilation rates (using evaporative cooling systems in closed house), increasing the airflow over the bird to increase heat loss, lowering densities, shading, roof insulation, and painting the roof's upper surface white to reflect some of the heat. With enclosed houses evaporative cooling of the entire house could provide an air movement in addition to reducing room temperature. If water is available, the use of roof sprinklers and mist sprayer (foggers) can be an effective means of cooling birds during heat waves (Wilson *et al.*, 1957).

Another means of alleviating heat stress is the provision of cold water. Cold water consumption, in chicken maintained at high temperature, was important to decease body temperature, HP and respiration rate, increase in feed intake, egg production and decrease in mortality (Van Kampen, 1981; Degen *et al.*, 1992). Wilson (1948) noted that water consumption for hens at 35^0C was double than for hens at 21^0C. Fox (1951) demonstrated that when laying hens were exposed to a temperature of 42^0C, a longer survival rate was associated with the persistency with which a bird continued to drink. When water is not available to a bird held at 30^0C, death follows from prostration rather than desiccation (Jones & Huston, 1967). Wilson & Hillerman (1952) maintained laying hens in an ambient temperature of 32^0C and found that body temperature of the bird could be lowered by immersion in water at 23^0C, by mist spraying, or by head wetting. Furthermore, experiment conducted by Vo and Boone (1977) showed that hens survived longer under heat stress conditions if the waterer type allowed them to immerse their heads. Even if the waterer allowed only immersion of wattles, hens survived longer than those that were allowed no immersion at all.

Nutritional modification include optimising the diet to meet the altered needs of heat stressed bird's energy and protein and providing certain additional nutrients which have been shown to have beneficial effects. Adding vitamin C and vitamin E to the diet of the laying hen is one of the methods used. Vitamin C was reported to improve the performance of poultry in hot environments. It increased egg production, improved hatchability and fertility and reduced egg breakage and mortality (Thornton & Moreng, 1959). Ultomo *et al.* (1994), Whitehead & Mitchell (1997 and 1998) and Bollengier-Lee *et al.* (1998) have reported that adding extra vitamin E to the diet of laying hens can help to minimize the depression in egg production caused by heat stress. The results of Whitehead & Mitchell (1997; 1998) suggest that the

optimum dietary concentration of vitamin E associated with minimising the adverse effect of heat stress is about 250 mg vitamin E/kg. According to their suggestion, however, it is important to start feeding the diet before the onset of stress in order to build up tissue levels. Otherwise, it will be too late to wait until the hot periods, by which time feed intake and egg production will have started to fall, and it would be more difficult to repair the defects. An alternative to dietary supplementation would be adding vitamin E to the drinking water (Whitehead & Mitchell, 1997; 1998), which might be a faster way of increasing vitamin E intake of hens at the approach of a period of hot weather.

Another but important means of alleviating the heat stress is to have breeding hens with less feather coverage. A research report by Peguri & Coon (1993) showed that lower feather coverage would increase feed consumption as a consequence of greater heat dissipation at high temperatures. The removal of feathers from neck, back, and breast from layers resulted in an average of 25 % increase in feed consumption or 0.29 MJ ME/day across all temperatures (Peguri & Coon, 1993). Emmans & Charles (1977) showed that maintenance energy requirement for layers could be increased 40 % by lack of feathers. Hens with poor feather condition at temperatures above 18^0C required an average of 2.19 MJ less ME/Kg of egg weight or 0.13 MJ less ME per egg than hens with good feather condition (Hagger *et al.*, 1989). Layers housed at high temperature have been shown to expend less of their ME for maintaining a constant body temperature and appear to have the option of shifting the energy savings to production or improved feed efficiency (NRC, 1981). Decease in the percentage of feather from 100% to 0% is shown to have increased egg weight at 12.8 ^{0}C from 57.9 to 61g, and from 56.4 to 58 g for hens with 50% feather coverage at 33.9^0C (Peguri & Coon, 1993).

Acclimatization is another important factor affecting the performance of the poultry at high temperature. During acclimatization heat tolerance (i.e. the rise in Tb when challenged in a standard hot environment) is increased (Meltzer, 1987). The length of time that heat tolerance will persist following high temperature exposure is not well defined (Ernst, 1995). Miller and Sunde (1975) noted that laying hens took 7 days to a shift of cyclic cold to hot. Jones *et al.* (1976) observed that chickens stabilised at lower values of feed intake within 24 hours when shifted to warmer temperatures. However, other investigators noted that as much as three days (Reece *et al.*, 1972), or 21 to 28 days (Shannon and Brown, 1969; Davis *et al.*, 1972) was required for acclimatization to occur. Deaton *et al.* (1982) showed that laying hens subjected to heat stress of 39^0C laid fewer eggs having decreased shell quality when acclimatized to

constant rather than to cyclic temperatures. Moreover, Van Kampen (1981) indicated that acclimatized chickens developed enlarged combs and wattles, contained less fat and their feather cover was less than that of the controls; and their HP was lower than that of the unacclimatized, with a shift to the right of the thermoneutral zone.

Discussion

In the topical and subtropical regions of the world, there has been a rapid expansion of poultry due to the increasing demand of meat and egg production. Related to this has been the increasing awareness of the extent to which high environmental temperatures negatively affect the performance of poultry. This in turn favours the need to investigate more favourable and intensive production methods when trying to achieve the genetic potential of the flock.

Indeed, the physiological responses and adaptations of broilers and laying hens to high temperature and the overall effect on their energy balance provided an understanding for the reasons why growth rate and egg production are lowered, and thus enabled different suggestions for possible means of alleviating the problem. The physiological responses, however, are complex as they are affected by interacting nutritional, genetic or environmental factors. Hence, evaluating the effect of environmental temperature on production parameters can be difficult to compare, as there are no two experiments conducted under the same environmental conditions.

Practically, maximum energetic efficiency of the bird is said to be achieved when feed is converted into the highest possible mass of marketable product. What makes this interesting is that feed conversion increases with an increase in environmental temperature. But it is still important to define the narrow range of environmental temperature where feed conversion efficiency (FCE), and hence, outcome is optimised, without compromising with a reduction in production level. The region occurs where the difference between feed intake and ME requirements is maximum resulting in a large proportion of feed intake available for production. Generally, however, there is disagreement as to what this ideal temperature for broilers and laying hen is, and what is ideal for growth is not ideal for feed efficiency, and what is ideal for feed efficiency is not ideal for egg weight, egg production or rate of lay. The range of temperatures for laying hens seems to be much wider as compared to that of broilers, since egg production remains fairly constant between 15 and 27^0C. The fact that feed intake

declines between these temperatures indicates, however, that the optimum temperature may be somewhere between 25 and 27^0C, whereafter the decline in output could be economically unfavourable.

The key factor for efficient production both broilers and layers is optimum nutrient intake. Because temperature is but the most important single factor affecting feed intake, and because feed constitutes for about 65 to 70% of the total production cost in poultry production enterprises, prediction and quantification of the decrease in feed intake with increasing temperature would be necessary when determining the nutrient composition of the diet. Besides, identifying the first limiting nutrients under conditions of high temperature would be important. It is suggested that dietary energy supply could be the most important factor to consider under hot whether conditions. Under such conditions, the addition of fat to increase the energy concentration while reducing the heat increment of the feed has been used to assist the bird to consume more energy, as the dietary energy concentration is increased. Moreover, defining the heat increment of feeding could result in a more accurate prediction of the energy requirement while formulating poultry diets. When describing the energy content of the diet it is suggested that the effective energy scale be used rather than the ME scale, as EE differentiates between the efficiency of utilization of the energy originating from digestible components of protein, carbohydrate, and lipid and it considers the effect of indigestible organic matter on the energy available to the animal from the diet, none of which are taken into account when the ME scale used.

There is some evidence supporting the view that the efficiency of protein deposition is reduced by environmental temperature, and consequently protein supplementation has generally failed to improve growth rate. Hence, the increase in the energy content of the diet by using fat supplementation may result in a decrease in the amino acid concentration in proportion to energy, since feed intake on a high-energy diet may usually be higher than expected, and thus a fixed ratio of amino acid to energy may not seem to apply in this situation.

It is likely that the maximum amount of heat that a broiler could lose to the environment is probably a function of its feather cover, its degree of maturity and of the environmental temperature, and there is insufficient evidence in the literature to enable this to be predicted satisfactorily. Besides, there are few theories that enable heat loss to be predicted at high

temperatures, which occur for most part of the world, and which need to be investigated for an accurate prediction of the growth of the broiler and egg production of the laying hen.

Nutritional modifications, including the addition of vitamins and minerals, may play a vital role in hot climatic environments. However, the addition of ionised salts such as potassium, chloride, and sodium bicarbonate, should follow with great caution. Though, these ionised salts could alleviate the effect of heat stress by replacing the excreted electrolytes, if used in excess quantities, they can be a cause of abnormal metabolic acid-base balance, which in turn may worsen the situation. Improvement of production through increasing nutrient density of the diet seems to alleviate the problem to a limited extent. Concurrently to the nutritional modification, environmental and management modification would improve the degree of heat stress of birds and attempt to provide an optimum environment at an economical cost.

CHAPTER TWO

DIET MANIPULATION AS A MEANS OF OVERCOMING HEAT STRESS IN LAYING HENS

2.1 INTRODUCTION

The gross energy contained in the feed is transformed by animals into other forms, some of which are valuable to the animal while others must be lost to the environment. The losses are of two general classes – those that leave the body as chemical energy in the visible excreta and combustible gases, and those that result in heat production. During periods of heat stress it would be useful to be able to reduce the amount of heat produced by the animal from the feed consumed.

Food energy remains a major component in the feeding of the laying hen. Thus, quantifying the food energy available for maintenance, growth, egg production and conversion efficiency is vital in being able to predict the energy requirements of the laying hen. It has been illustrated that under hot weather conditions, optimum egg production cannot be achieved with low energy diets, and hence, high-energy diets containing fat are essential (Leeson & Summers, 1997). These observations suggest that environmental temperature has a significant effect on the performance of the laying hen, and thus the food intake and hence performance of laying hens may react differently to the energy concentration depending on the environmental temperature.

The concept of heat increment introduced by Armsby (1903), cited by Emmans (1994) provided useful information for the construction of the effective energy system by Emmans (1994). The heat increment of feeding needs to be predicted to be able to estimate the net energy available for growth, maintenance and egg production. Emmans (1994) proposed the heat increment to be measured using values from the quantities of faecal organic matter (FOM), urinary nitrogen (UN), positive protein retention (PR) and positive lipid retention (LR). The energy required for the performance of these work functions are given by Emmans (1994) as:

$$W_u = 29.2 \text{ MJ/kg UN}$$

$$W_d = 3.80 \text{ MJ/kg FOM}$$

W_p = 36.5 MJ/kg PR

W_I = 16.4 MJ/kg LR (non-lipid sources)

W_{II} = 4.4 MJ/kg LR (lipid sources)

The equation derived for calculating the effective energy using these work functions is given as:

$$EE\ (MJ/kg) = AMEn - (3.8 \times FOM) - (4.67 \times DCP) + (12 \times z \times DCL)$$

Where:

FOM = Faecal organic matter

DCP = Digestible crude protein

DCL = digestible crude fat

z = proportion of body fat derived directly from dietary fat.

Emmans (1994) quantified the value of z to be between 0 and 1, of which 0.3 could be used for calculating the effective energy of poultry.

In mature laying hens the energy consumed is directed to functions other than growth and fattening. Thus, the laying hen fulfils its production capabilities from the energy supplied but is utilized in the formation of the product. Lipid and protein retentions are redirected in that these are incorporated as components of product.

Clearly the energy requirements, which are defined in terms of ME for maintenance, growth, and egg production, are influenced by environmental temperature. Armsby (1903) defined the metabolizable energy (ME) of food as heat of combustion of the diet minus the combined heats of the combustion of the excreta produced from it. However, the ME does not represent the total amount of energy available to the animal. This energy (available energy) is given by:

$$AVE\ (MJ/d) = ME - W_m.MTHE - W_d.FOM$$

Metabolizable energy for production is available after the maintenance needs of the animal are met. Animals always attempt to utilize all the chemical energy supplied by the feed. But because of the heat production – inefficiencies of product synthesis, energy available for production is not entirely incorporated into the animal products, be it retained in tissue growth, or expelled product, such as milk, egg, pelage, or offspring (NRC, 1981). The latter include inefficiencies of product synthesis and cost of retaining or expelling the product. The energy available for productive purposes is further reduced by digestion, absorption and

excretion of feed ingredients. The energy associated with the heat increment of feeding defined by Emmans (1994) is given as:

$$HIF\ (MJ/d) = W_d.FOM + W_u.UN + W_m.MTHE + W_p.PR + W_l.LR$$

The maintenance energy requirement (MERQ MJ/d) of the animal is a function of the energy retention, maintenance heat and heat increment of feeding. While the maintenance energy depends on the state of the animal, energy retention is a consequence of the animal in terms of protein and lipid retention. This implies that the MERQ of the animal being influenced by the given state of the animal will depend on the heat increment of feeding. The basic principle of the EERQ is that it considers the energy cost expended in depositing body protein and lipid and in excreting the nitrogenous waste products. This means that it also predicts the energy that is not utilised by the animal, i.e. the heat produced, otherwise known as the heat increment of feeding.

$$EERQ\ (MJ/d) = MH + 50PR + 56LR$$

Emmans (1994) has corrected the ME values of the feed ingredients to take account of the heat increment, and called this the effective energy. Accordingly, the ME derived from protein results in a significantly lower efficiency of utilization, which would mean that an increasing proportion of the digestible crude protein would reduce the effective energy of the food. Also the inefficiencies due to faecal loss could bring about an increase in heat increment thereby reducing the energy available to laying hens. Therefore, defining the heat increment of feeding could result in a more accurate prediction of the energy requirement over a wide range of dietary situations. Further, the effective energy could increase when the source of energy is fat rather than carbohydrate, as fat costs less in terms of chemical energy for digestion and metabolism of the feed as compared to protein or CHO.

The objective of this experiment was to produce feeds varying in heat increment and to feed them to laying hens in cages in an open house to determine whether performance can be improved during hot weather by reducing the heat increment of the feed.

2.2. Materials and methods

2.2.1.Materials

2.2.1.1. Housing

The experiment was conducted in layer house 1 at the Ukulinga Research Farm, belonging to the University of Natal. The house used was an open-sided house with 520 cages, in four rows, facing back to back, with each row having two levels of 65 cages, of which 72 cages (2 rows of 36 cages each) were used for the trial. Each cage, measuring 50 x 36 x 45 cm in length, height and width respectively, has two nipple drinkers and a feed trough. Five birds were housed in each cage.

2.2.1.2. Animals

Hy-Line Brown layers were raised at Ukulinga Research Farm. They were fed a commercial layer mash *ad libitum* before they were used for the experiment.

360 hens, 46 weeks of age were randomly selected and used for the experiment. Fifteen birds were used for each replication in the trial, being placed in three cages of five birds each. The hens had *ad libitum* access to feed and water, and 14h light throughout the experiment. The hens were acclimatized for three weeks. The experiment was conducted during the period January 17 – March 28. These months were expected to be the hottest months in the area.

2.2.1.3. Experimental design

Two EE: ME ratios were used in the trial, the low ratio being based on a least – cost feed and the high ratio being the maximum possible with the available raw materials. Three nutrient densities were used to determine whether there was an interaction between ME content and the EE: ME ratio.

Each of these 2 x 3 feeds was replicated four times using 15 hens per replication (Three cages of five hens per cage), making a random allocation of 60 birds per feeding treatment.

Treatment means were calculated for the last seven weeks of the trial. The General linear model (GLM) Minitab release 13.1 was used for analysing the data.

2.2.1.4. Feeds

Four feeds were formulated using Winfeed 1.1 (Windows Feed Formulation Software) – two at high nutrient density (ND) and two at low ND. At each ND, one of the feeds was formulated at least cost (LC), to reflect commercial practice, while the other was formulated to minimize the heat increment. This was achieved by maximizing the EE content of the feed whilst maintaining the ME content at the same level as in the equivalent least-cost feed. A minimum amount of oil was used when formulating the LC feeds so as to obtain a maximum benefit from the substitution of oil for CHO in the diet. Amino acid minima were specified to be the same as in the equivalent least-cost feed. The result was that some of the carbohydrate energy was replaced with lipid energy, and the excess dietary crude protein content was reduced. These high EE feed were more costly than the equivalent least cost feeds. These four feeds (Table 2.1) were blended to produce feeds with an intermediate ND. Each of the six diets was replicated four times and each of these replicates was randomly allocated to three cages, with five birds per cage, making a total of fifteen birds.

2.2.1.5. Feed Mixing procedure

The amount of food that a laying hen of the age 46 to 56 would eat was estimated to be 1.5 kg per week. The individual bird consumption value was multiplied by the number of experimental birds, the number of experimental weeks, and 5% was added for food wastage considerations. This enabled the total amount of food needed for the entire experiment to be calculated. The total food was divided by six to find the amount of food required per treatment and the value was multiplied by the ingredient inclusion percentage to find the value of each ingredient to be included in each treatment. This value (the value of each ingredient inclusion in the formulated diet) in treatment one, two, five and six were multiplied by 1.5 and was mixed thoroughly for 25 minutes using the horizontal feed mixing facility at the Ukulinga Research Farm. $2/3^{rd}$ of each of these treatments feeds were then weighed and placed in a pre – labelled bags, and the remaining, $1/3^{rd}$ of treatment one was blended for about 20 minutes with $1/3^{rd}$ of treatment five, and $1/3^{rd}$ of treatment two with $1/3^{rd}$ of treatment six to produce treatment three and four, respectively.

Table 2.1. *Composition (g/kg) of the feeds used in the experiment*

	Diet					
	Low ND (Formulated)		Medium ND (Blended)		High ND (Formulated)	
	[1]LC	[2]HC	LC	HC	LC	HC
Treatment	1	2	3	4	5	6
Maize	664	383	656	392	649	401
Wheat bran		262		203		145
Soybean 46	47.6	168	69.6	193	91.5	219
Sunflower 37	134		70.4		6.40	
Fish meal 65	54.4		86.9		119	
L-lysine HCL		0.10		0.10		0.10
DL methionine	0.30	1.70	0.55	2.00	0.80	2.30
Vit + min premix	1.50	1.50	1.50	1.50	1.50	1.50
Limestone	86.7	92.0	84.6	91.5	82.4	91.0
Salt	0.80	1.90	0.45	1.95	0.10	2.00
Monocal. Phosphate	6.10	5.80	3.65	7.05	1.20	8.30
Sodium bicarbonate	4.40	4.40	3.30	4.45	2.20	4.50
Oil-sunflower		8.00	2.30	10.3	4.60	12.6
Total	1000	1000	1000	1000	1000	1000

[1]LC = least cost formulation

[2]HC = low heat increment formulation

Included in Table 2.2 below are the calculated and actual chemical compositions of the feeds used in the experiment.

Table 2.2. *Chemical (calculated) composition (g/kg) of the feeds used in the experiment*

[3] Calculated analysis

	EE (MJ/kg)					
	High	Low	High	Low	High	Low
	10.03	9.97	10.99	10.94	11.95	11.91
AMEn (MJ/kg)	11.3	11.3	12.15	12.15	13	13
EE: ME ratio	0.888	0.882	0.905	0.900	0919	0.916
Calcium	35.0	35.0	35.0	35.0	35.0	35.0
Available phosphorus	3.5	3.5	3.5	3.5	3.5	3.5
Sodium	2.2	2.2	2.2	2.2	2.2	1.8
Chlorine	1.8	1.8	1.9	2.0	2.0	2.2
Crude protein	164	152	171	163	179	173
Cost (R/ton)	1432	1522	1645	1625	1859	1728

[3]Chemical compositions were calculated by using Winfeed (Windows Feed Formulation Programme) on as is basis at the University of Natal, 2002.

[4]Analysed composition (g/kg)

	Treatment					
Chemical composition	1	2	3	4	5	6
AMEn (MJ/kg)	11.57	12.09	12.27	12.84	12.40	13.54
EE (MJ/kg)	9.67	11.5	10.80	12.54	11.43	13.59
EE/ME ratio	0.84	0.95	0.88	0.98	0.92	1.00
TMEn (MJ/kg)	11.98	12.50	12.68	13.25	12.81	13.95
Moisture	109	107	110	107	108	107
Ash	127	131	114	123	112	125
Fat	29	106	54	119	81	138
Protein	165	141	169	163	177	160
Digestible amino acids						
Threonine	5.7	4.3	5.9	5.1	6.6	4.7
Valine	8.2	6.3	8.1	7.2	8.3	7.0
Methionine	3.1	2.9	3.1	3.3	4.0	4.8
Isoleucine	6.6	5.1	6.7	6.3	7.0	5.9
Leucine	13.7	10.6	14.1	12.5	15.2	12.1
Tyrosine	4.2	3.6	4.3	4.4	5.5	4.2
Phenylalanine	7.5	6.2	7.3	7.3	8.1	7.0
Histidine	4.6	3.5	4.6	4.0	5.2	3.9
Lysine	7.8	6.6	8.5	8.4	10.2	7.8
Arginine	10.3	8.4	9.6	10.3	10.0	9.3

[4]Chemical composition as determined on as is basis by the Feed Evaluation Unit, Animal and Poultry Science, University Of Natal, 2002.

2.2.2. Methods

2.2.2.1. Body weight

All birds were weighed at the start of the experiment, on the fifth week and at the end of the experiment. All the five birds in each cage were weighed together.

2.2.2.2. Feed intake

Birds were given *ad libitum* access to the feed allocated to them. A feed bin was assigned to each replication. These were filled and weighed at the start of the experiment, and feed was transferred to the feed troughs from these bins when necessary. At the end of each week, the feed remaining was returned to the bin, which was weighed to determine the amount of feed consumed during the week. The bins were then refilled and weighed again, and the process repeated.

2.2.2.3. Rate of lay

Eggs were collected daily at 07h30. All eggs in each replication were collected together and recorded. The numbers of eggs produced by laying hens in each replication were divided by the number of birds in the replication to give an average rate of lay per bird day.

2.2.2.4. Egg Weight

Eggs that were collected at 07h30 every Monday, Tuesday and Wednesday were counted and weighed immediately after collection to determine the average egg weight for each replication.

2.2.2.5. Data recording

All measurements were recorded on pre-prepared forms, and these data were captured on spreadsheet. The data were then re-arranged so that it would be suitable for further analysis. Any irregularities were also noted and corrected where necessary.

2.3. Temperature

Daily temperatures were obtained using a maximum and minimum thermometer. The thermometer was suspended in the middle of the house just above the cages. Minimum and

maximum temperatures readings were carefully noted and recorded daily. The mean for minimum and maximum temperatures and the mean daily temperatures were then calculated. The number of experimental days during which the maximum temperature equalled or exceeded 27^0C was calculated, as these were regarded as being days that were hot for the laying hens.

2.4. Economic analysis

Mean responses of the variables contributing to profit including feed intake, egg weight and rate of lay for dietary effective energy and ME are given in Table 2.3. Feeding costs were calculated by multiplying the ingredients price (Table 2.4) with their inclusion rates (Table 2.1) for each treatment, and then multiplying this by the amount food consumed (Table 2.3). A table of proportion of egg grades (small, medium, large, extra large and jumbo) for each mean egg weight developed by Gous (2002) was used (Appendix 1). The price, R 4.00, for a dozen large eggs from Ukulinga Research Farm was used. Egg prices (c/egg) under normal, 15% increase and 15% decrease were calculated (Table 2.5). Relative egg prices for other grades was calculated by using -30, - 15, 15 and 30% change in large eggs price for small, medium, extra large and jumbo eggs, respectively (Table 2.5). Income generated by specific treatment combinations was calculated on Excel spreadsheet. Income per 100 hens was calculated by multiplying income per hen with rate of lay for that treatment.

Table 2.3. *Mean response in feed intake (g/bird d), egg weight (g) and rate of lay (%) for dietary EE and ME*

		ME (MJ/kg)		
Response variable	EE (MJ/kg)	11.30	12.15	13.00
Feed intake (g/bird d)	High	111	108	101
	Low	108	101	98.0
Rate of lay (%)	High	87.0	86.5	84.5
	Low	83.3	83.0	83.3
Egg weight (g)	High	58.5	60.1	59.6
	Low	58.4	58.9	58.9

Table 2.4. *Prices for different ingredients used for calculating feed cost*

Ingredients	Quantity (kg or l)	Price (R)	Unit price (R/kg or l)
Maize (%)	50	56.70	1.10
Wheat bran	25	36.00	1.40
Soybean 46	40	161.00	4.00
Sunflower 37	50	115.75	2.30
Fish meal 65	50	263.85	5.30
L-lysine HCL	1	28.89	28.90
DL methionine	1	38.48	38.50
Vit + min premix	1	24.20	24.20
Limestone	50	30.50	0.60
Salt	50	29.02	0.60
Mono cal. Phosphate	1	3.59	3.60
Sodium bicarbonate	1	3.32	3.30
Oil-sunflower	5	40.00	8.00

Table 2.5. *Prices for different egg grades under normal, 15% decrease and 15 increase in egg price*

Egg grade	Weight range (g)	Relative price increase (%)**	Price (c/egg)		
			Normal	-15%	+15%
Small	<45	-30	0.2333	0.1983	0.2683
Medium	45 – 50	-15	0.2833	0.2408	0.3258
Large*	50 – 60	0	0.3333	0.2831	0.3833
Extra large	60 – 65	15	0.5333	0.4533	0.6133
Jumbo	>65	30	0.7333	0.6233	0.8433

*Normal egg price was assumed to be R 4.00 per dozen of large eggs

**Approximate price change relative to the price of large eggs

2.5. RESULTS

2.5.1. Rate of lay, egg weight, egg output and ME intake

The mean responses in rate of lay, egg weight, egg output, and ME intakes measured over the seven – week trial periods are given in Table 2.6. Although there were some variations in the response of these variables, neither the EE nor the ME contents of the feeds had significant effects on rate of lay, egg weight, egg output or ME intake.

2.5.2. Feed intake, body weight gain, effective energy (EE) intake

The mean responses in feed intake, body weight gain and effective energy intake to dietary energy contents are given in Table 2.6. Both the EE and the ME of the diet had strong significant effect (EE at $P<0.01$, ME at $P<0.001$) on feed intake, but their interaction had no significant influence on either the EE intake or feed intake. The effective energy intake was highly influenced by both the EE ($P<0.001$) and ME ($P<0.01$) content of the diet. Neither the EE nor the ME content of the diet had any significant effect on body weight gain. However, body weight was slightly (not statistically significant, $P = 0.059$) influenced by the EE as compared to the ME content of the diet.

2.5.3. Temperature (^{0}C)

The minimum temperature was found to be 19.7, the maximum temperature was 26.5 and the overall mean temperature was 23.1.

The number of days, which were above and below the maximum temperature (26.5^{0}C) were counted, and days, which were equal or over 27^{0}C, were considered to be hot to laying hens. Accordingly, 29 days were found to be equal and/or over 27^{0}C.

2.5.4 Economic analysis

Feeding costs for the combinations of dietary EE and ME was found to be linearly increasing and more expensive in treatments with low effective energy than in treatments with high effective energy (Table 2.7).

The income for the combinations of dietary EE and ME under normal, 15% reduction and 15% increase in price for all egg grades and 15% increase only for X-large and Jumbo eggs is shown in Table 2.8. Under all circumstances income was positively related to dietary EE and ME.

Table 2.9 shows the calculated profit. Profit was calculated by subtracting feeding cost from income obtained by the combinations of dietary EE and ME under normal, 15% reduction and 15% increase in price for all egg grades, and 15% increase only for X-large and Jumbo eggs. The highest profit was obtained from diets having high EE: ME ratios under all egg prices.

Table 2.6. *Effect of dietary energy content on rate of lay (%), egg output (g/bird d), egg weight (g), weight gain (g/ bird d), feed intake (g/ bird d), ME and EE intake (MJ/d) of Hy-line Brown birds*

Response variable	EE (MJ/kg)	ME (MJ/kg) 11.30	12.15	13.00	Means (EE)
Rate of lay (%)	High	87.0	86.5	84.5	86.0
	Low	83.3	83.0	83.3	83.2
	Means (ME)	85.1	84.8	83.9	
	S.E.M: EE = 1.37 ^{NS}		ME = 1.68 ^{NS}	EE X ME = 2.38 ^{NS}	
Egg output (g/bird d)	High	51.0	52.0	50.1	51.2
	Low	48.6	48.8	48.9	48.8
	Means (ME)	49.8	50.4	49.7	
	S.E.M: EE = 1.015 ^{NS}		ME = 1.244 ^{NS}	EE X ME = 1.759 ^{NS}	
Egg weight (g)	High	58.5	60.1	59.6	59.4
	Low	58.4	58.8	58.9	58.7
	Means (ME)	58.4	59.4	59.2	
	S.E.M: EE = 0.442 ^{NS}		ME = 0.541 ^{NS}	EE X ME = 0.765 ^{NS}	
Weight gain (g/ bird d)	High	0.67	1.24	2.11	1.34
	Low	1.09	1.44	0.91	1.15
	Means (ME)	0.88	1.34	1.51	
	S.E.M: EE = 0.15 ^{NS}		ME = 0.18 ^{NS}	EE X ME = 0.25*	
Feed intake (g/ bird d)	High	111	108	101	106
	Low	108	101	98.0	102
	Means (ME)	109	105	99.4	
	S.E.M: EE = 0.86*		ME = 1.06**	EE X ME = 1.50 ^{NS}	
ME intake (MJ/bird d)	High	1.28	1.33	1.28	1.29
	Low	1.33	1.30	1.30	1.31
	Means (ME)	1.30	1.31	1.29	
	S.E.M: EE = 0.01 ^{NS}		ME = 0.01 ^{NS}	EE X ME = 0.02 ^{NS}	
EE intake (MJ/bird d)	High	1.08	1.15	1.18	1.13
	Low	1.23	1.28	1.30	1.27
	Means (ME)	1.15	1.21	1.24	
	S.E.M: EE = 0.01**		ME = 0.02*	EE X ME = 0.02 ^{NS}	

*P <0.01 **P<0.001 NS = not significant

Table 2.7. *Calculated feeding costs (R/100 birds d) for different combinations dietary ME and effective energy for Hy-line Brown birds*

EE (MJ/kg)	ME (MJ/kg)		
	11.30	12.15	13.00
High	18.65	21.20	22.68
Low	25.10	25.78	27.25

Table 2.8. *Calculated income (R/100 birds d) under different dietary ME and EE treatments for Hy-line Brown birds at normal, 15% reduction and 15% increase in price for all egg grades and 15% increase only for x-large and Jumbo eggs*

Price situation	EE (MJ/kg)	ME (MJ/kg)		
		11.30	12.15	13.00
Normal egg price	High	50.07	52.14	50.94
fro all grades	Low	45.47	47.77	47.94
15% reduction for	High	42.60	44.30	43.30
all grades	Low	38.60	40.60	40.70
15% increase for	High	57.58	59.96	58.58
all grades	Low	52.29	54.93	55.13
15% increase for X	High	51.12	55.15	53.87
– large and Jumbo	Low	44.24	48.77	48.94

Table 2.9. *Calculated profit (R/100 birds d) under different dietary ME and EE treatments for Hy-line Brown birds at normal, 15% reduction and 15% increase in price for all egg grades, and 15% increase only for X-large and Jumbo eggs*

Price situation	EE (MJ/kg)	ME (MJ/kg)		
		11.30	12.15	13.00
Normal egg price	High	31.42	30.94	28.26
	Low	20.37	21.99	20.69
15% reduction	High	23.90	23.10	20.60
	Low	13.50	14.80	13.50
15% increase	High	38.93	38.76	35.90
	Low	27.19	29.15	27.88
15% increase for X	High	32.47	33.95	31.19
– large and Jumbo	Low	19.14	22.99	21.69

Discussion

The objective of the study was to overcome heat stress in laying hens by designing feeds that had a lower heat increment than the conventional diets, and to determine whether these were more beneficial at high than at low nutrient densities. Accordingly feeds were designed (Table 2.1), and fed laying hens housed in an open – sided house during hot weather.

The results of this experiment showed that the experimental days having temperatures over 27^0C were more than half of the entire experimental days. This implies that the environment was really hot to laying hens and critical to egg production. Marsden & Morris (1987) and Leeson & Summers (1997) showed that above 27^0C energy intake falls more quickly than heat loss, the difference being for reductions in egg and body energy, and change in metabolic rate in response to activities such as panting and heat load. During hot weather, feed intake is expected to decline, as the amount of heat that can be lost to the environment is reduced, and in order to stay in thermal balance the heat produced by the hen must also be reduced. This may result in one or more essential nutrients becoming limiting with the result that egg production will decrease. In order to reduce the heat increment whilst maintaining an adequate intake of essential nutrients, a higher EE: ME ratio in the feed is required.

The results indicate that feed intake remains higher on diets with high EE: ME ratio resulting in a numerically higher egg output. At higher environmental temperatures the differences in feed intake could be expected to be even higher (Gous, 2002).

Emmans (1994) noted that the benefits of the effective energy would depend on nutritional restriction, such that during cold weather, little energy benefits would be achieved from the system, as nutritional inadequacies would simply be compensated by an increase in nutrient intake with the environment placing no limitations on the amount of heat the bird could lose. During hot weather conditions, however, the situation becomes critical that nutrient density should be maximized, or rather; the supply of effective energy should be maximized in order to maintain egg production. Bray & Gesell (1961) showed that egg output at high temperature could be sustained by increasing the concentration of nutrients in the feed. However, it is likely that egg output would be restricted by inadequate energy intake at high temperature (Smith & Oliver, 1972). Figure 2.1 shows at an increasing energy content of the diet, feed intake decreases at both levels of the effective energy. This occurred because, in hot weather

conditions, nutrient intake decreases so that heat production as a result of heat increment will decrease to cope with the elevated environmental constraint to the heat loss, and since the high EE level has lower heat increment as compared to the low level, birds were able to eat more of this food. A decrease in feed intake in hot weather is usually accompanied by the depletion of body reserves, which were accumulated during the cool periods to curtail the loss in egg production (Swain & Farrell, 1975; El Husseiny & Creger, 1980).

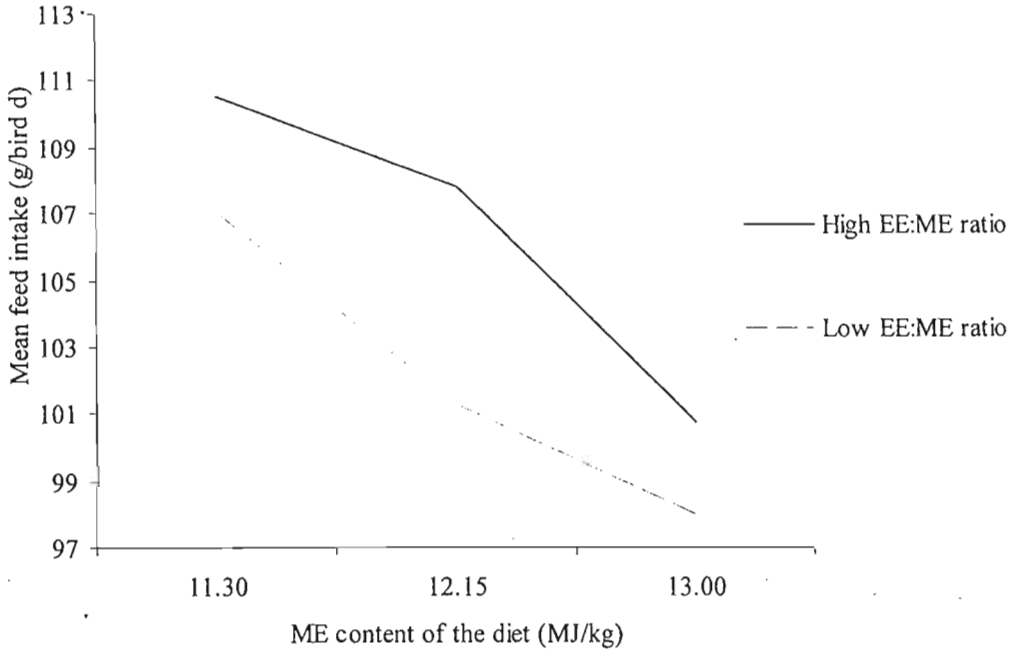


Figure 2.1. *Feed intake response of Hy-Line Brown laying hens to dietary energy content of the diet*

The result of the present study shows birds that were eating high-energy diets (Figure 2.3) were able to prevent the loss of body weight (Figure 2.2). Figure 2.2 also shows that when birds were given a feed high in EE, body weight increased linearly with the increase in the energy content of the diet. The substitution of oil for carbohydrate in these diets would have resulted in low heat increment of feeding (High EE/ME), reducing heat load to layers, and thus, allowing hens to eat sufficient to prevent loss in body weight. The result was similar to the result obtained by Gous *et al.* (1987) who showed body weight gain was positively correlated to energy concentration of the diet: as the energy concentration of the diet increases, birds tend to gain more weight.

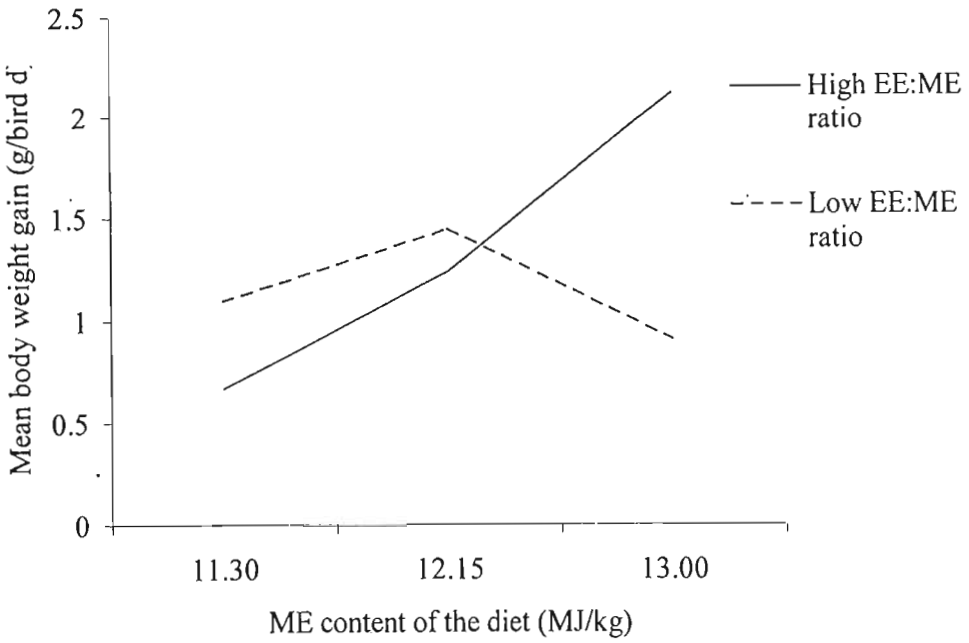


Figure 2.2. *Mean body weight gain of Hy-Line Brown laying hens from 46 to 56 weeks of age*

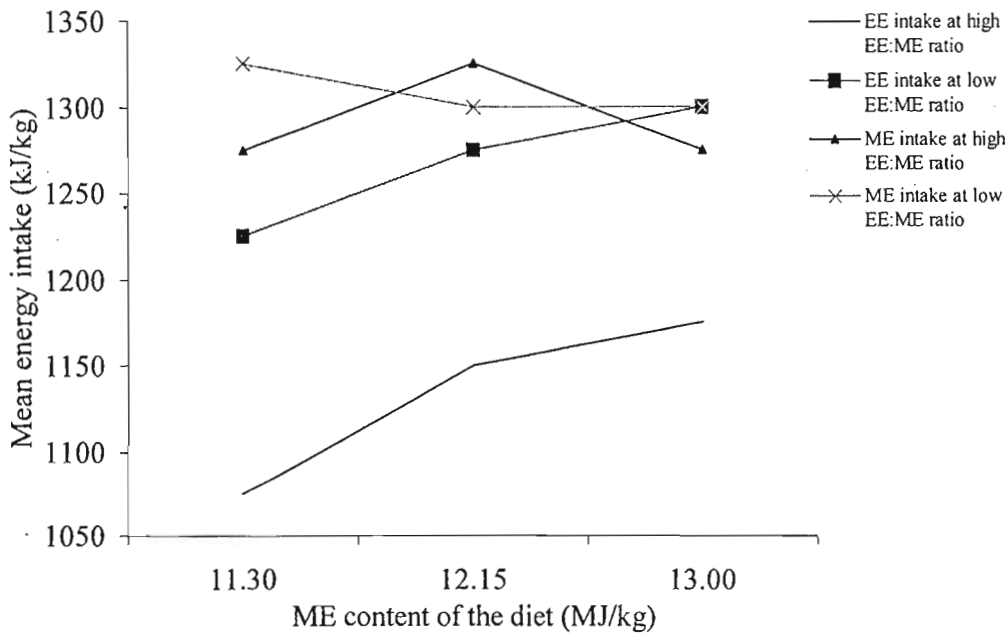


Figure 2.3. *Responses to energy intake of Hy-Line Brown laying hens*

Decrease in feed intake may not, in general, prevent the increase in feed cost with an increase in energy content of the diet. As predicted, the result of the present study showed a linear increase in feeding costs as the energy content of the diet was increasing (Figure 2.4). Comparatively, however, feeding cost was lower at high effective energy than at low effective energy.

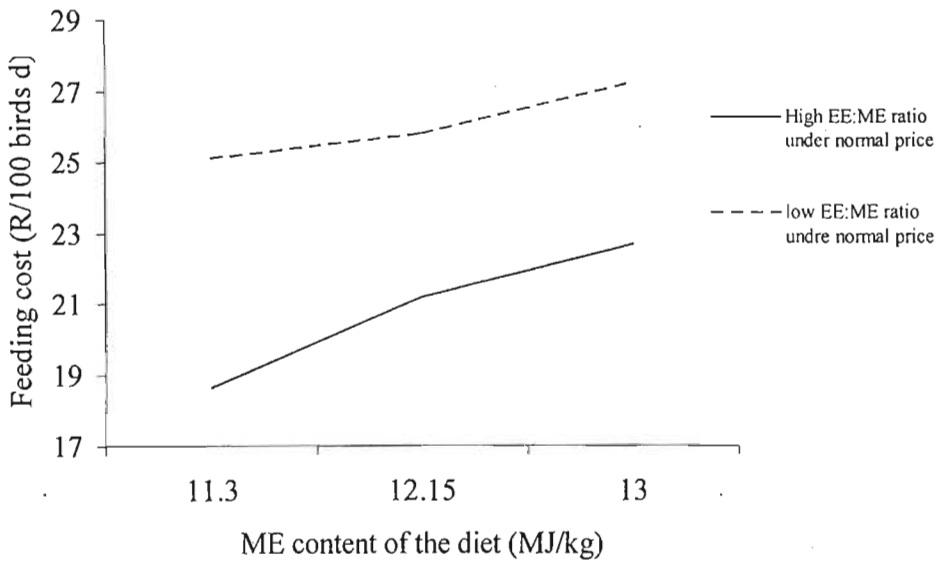


Figure 2.4. *Feeding cost (R/100 birds d) for different combinations of dietary ME and EE for Hy-Line Brown laying hens*

From the result of the present study, it was evident that the income generated from the sale of eggs was dependent on the performance of the laying hen, i.e., the rate and the weight of the eggs laid. The mean weight of eggs laid during this experiment ranged between 58.4 and 60.1g, and thus, the price gained was based on the relative equivalency to the price of large eggs sold from commercial production markets. Accordingly, highest incomes were obtained at high EE:ME ratio than at low EE:ME ratio under all egg prices (Figure 2.5). This was because of the fact that the performance of the laying hens were improved, and thus, birds were being able to increase their rate of lay with the used of high EE:ME ratio diet as compared to the low EE:ME ratio diets.

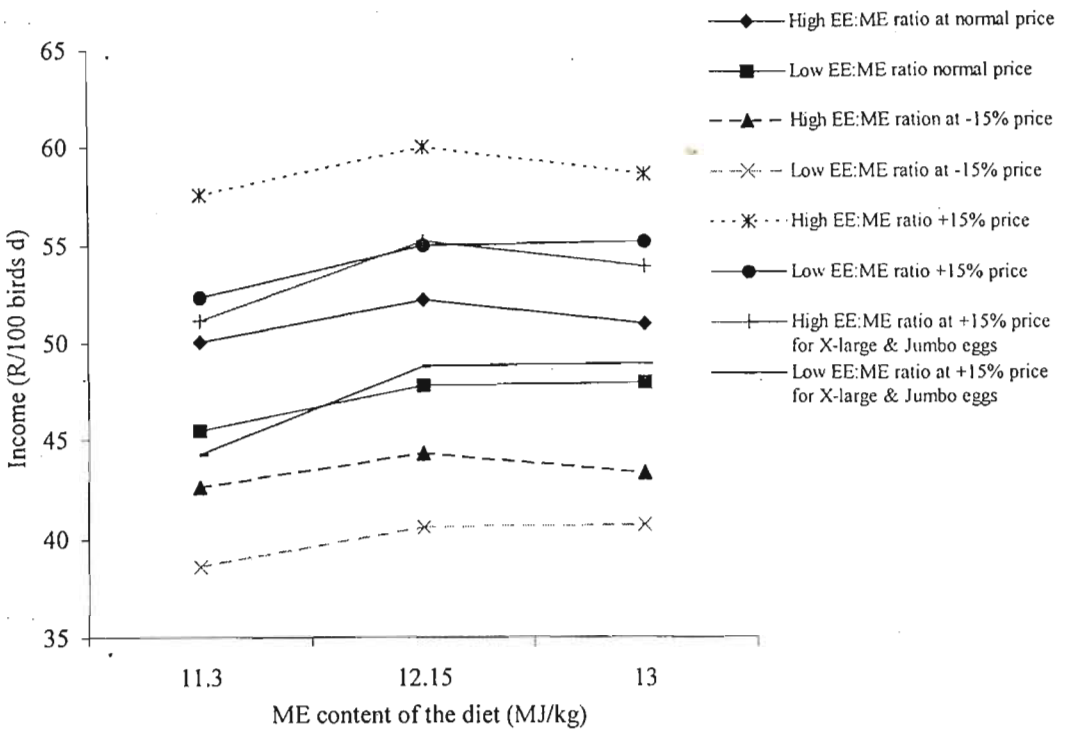


Figure 2.5. *Income (R/100 hens d) for different combinations of dietary ME and EE for Hy-Line Brown laying hens*

The profit derived (income – feeding cost) was a function of both performance (egg production) and feeding cost expended in achieving the performance. Highest profits were realized from treatments that were formulated with high EE:ME ratio (Figure 2.6), i.e. form dietary combinations where the margins between income (egg sales) and feeing cost (cost of feed for producing the egg) were highest. The lowest profit was obtained when the price of eggs was reduced by 15% for all egg grades below the normal egg price, at low EE:ME ratio diets. This was because the highest feeding cost associated with production of these egg was not being able to be offset by the revenue generated. In the present study, the effect of a 15% increase for all egg price, and for extra large and Jumbo egg grades on profit obtained was highest at high EE:ME ratio diets. This improvement was due to the fact that the use of high EE:ME ratio diets would have improved the performance of the laying hen in general, and that of egg weight and rate of lay in particular. Since at all times, and for all egg grades, the profit obtained was higher with the use of high EE:ME ratio diets, it makes advantageous and cost effective to use high EE:ME ratio diets during hot weather as compared to low EE:ME ratio diets.

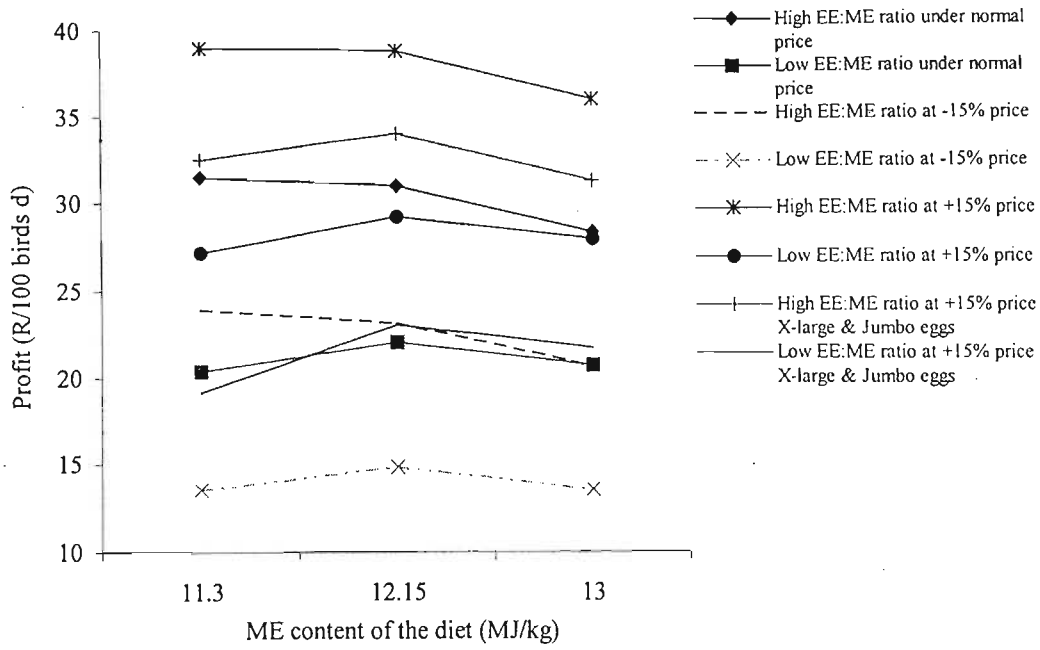


Figure 2.6. *Profit (R/100 hens d) for different combinations of dietary ME and EE for Hy-Line Brown laying hens, under normal price and 15% price increase above normal*

The present finding may be summarized as, while dietary energy content of the diet had no significant effect on egg production (rate of lay, egg weight, and egg output) and ME intake, it had significant effect on effective energy intake, change in body weight gain, and feed intake. Though the ME's of the diet that were supposed to be the same actually differed, that the EE:ME ratios was not necessarily increased, the amount of feed and energy consumed was dependent primarily on the dietary energy content of the particular feed, being low at low and high at high effective energy content, respectively, for feed intake; while energy intake increased positively with increasing dietary energy content of the diet. While body weight gain increased positively when effective energy is high, it increased and then decreased when the effective energy was reduced, and thus depletion of body weight reserve can be conserved at hot environments by using high EE/ME rather than low EE/ME ratio of the diet. Hence forth, the high EE:ME ratio diets were cost effective, and were being able to improve performance as compared to the low EE:ME ratio diets.

As the EE:ME ratio deals with factors that would cause differences in heat production, it would be expected to be as good as the ME system in formulating diets for laying hens and/or broilers at high temperature. The next experimental procedure is designed especially to

evaluate the extent to which broilers will be able to lose heat to the environment when forced with conditions that would require them to lose heat to the environment than would be possible for them to grow at their potential.

CHAPTER THREE

MEASUREMENT OF THE RESPONSE OF BROILERS TO DIETARY LYSINE AS MEASURED IN CAGES IN ENVIRONMENTALLY CONTROLLED CHAMBERS

3.1. INTRODUCTION

As with all living species, the broiler hen lives in a very complex environment encompassing a multitude of factors both physical and physiological. Generally, environmental factors, including temperature, humidity, light (intensity and day length), ventilation and wind velocity, altitude (partial pressure of oxygen and carbon dioxide and air pressure), radiation (solar energy), stocking density (population density), quality of water and air have a major effect on egg and meat production. In spite of the strong effect of all these factors, temperature has a primary effect. Recently, commercial producers have been providing poultry with a preventive shield from the adverse effects of the microenvironments, and thereby increasing productivity. The structure, however, creates both meso and macro environments around the bird, which moderate but do not alleviate the environmental impact (Charles, 1974).

Birds, being homeothermic, maintain a relatively constant core (body) temperature, and they accomplish this by balancing the heat gained from metabolism against that gained from or given up to the environment. Within the thermo-neutral zone, when the environmental factors are ideal, the broiler hen is in thermal equilibrium. However, if one of the environmental factors is modified or altered, the outcome could be different. Thus, the broiler hen needs to modify its thermoregulatory mechanism in order to maintain its heat balance and body (core) temperature within the normal comfort range. Heat balance is achieved through the concerted effects of physiological, morphological, and behavioural thermoregulatory mechanisms (Monteith, 1974). In extreme situations and over prolonged periods of time, where the chicken adaptability to the adverse environment is exceeded, too rapid rate of heat loss leads to hypothermia, too slow to hypothermia. Neither the former nor the latter is tolerated, and both of them lead to the bird's death.

Dissipation of excess heat from the bird's body can be accomplished by evaporative and non-evaporative (sensible) means. Sensible heat loss involves dissipation of heat from the bird's

body to the moving air around the bird. This loss is then dependent on the velocity of air movement. Ventilating measures enabling the air movement at the bird level of 100 to 120 m/minute is optimum for sensible heat loss (Khajarern and Khajarern, 1998). Thus sensible heat loss is the main source of heat dissipation in the thermoneutral zone. Evaporative heat loss, which is of particular importance under warm condition, dissipates warm air by expiration and water evaporation from the air sacs and lungs.

A growing broiler produces heat in a number of different ways – including in maintaining the body, in moving about, in processing (digestion and metabolism) food, in depositing body protein and body lipid, and in excreting unwanted substances from the body (Emmans, 1995). Heat loss from the bird varies proportionally to the environmental temperature. Thus, the bird reaches a state of equilibrium when its ability to store heat is effectively zero, i.e., when its rate of heat loss is equal to heat production. When this is not true, the bird may be unable to lose sufficient heat, generated by both the growth process and the processing of ingested food, to its surroundings. Consequently, the environmental temperature becomes a major constraint in achieving its potential. This may occur at high temperatures where the bird has no alternative but to reduce the amount of feed required to meet its potential, and the actual feed intake and growth rates both fall below the potential. Under such conditions a general decline in growth rate occurs. It is clear from this that the environment imposes an upper limit on the amount of heat that a bird can lose to its environment, and that this has an important consequence in predicting whether or not the bird will be able to grow at its potential, and in predicting the dietary amino acid content required by the bird.

The responses to lysine limiting feeds were measured at three temperatures and over two growth periods, making a total of three trials that were conducted. The first trial was a pilot trial for the second trial, both of these trials being conducted on broilers from 1 to 3 weeks of age. The third trial was a finisher trial and was conducted using broilers from 3 to 5 weeks of age.

There were two objectives to the research reported here. The first was to determine the extent to which broilers are able to lose heat to the environment when faced with conditions that would require them to lose more heat to the environment than would be possible for them to grow at their potential. This was based on the theory that the maximum amount of heat that the bird would lose to the environment is a function of its feather cover, its degree of maturity

and of the prevailing environmental temperature. The second, and major, objective was to determine whether the constraining effects of high temperatures on food intake and growth of broilers could be overcome by feeding high protein diets at these high temperatures.

3.2. Materials and methods

The amount of heat a broiler produces can be measured in a number of different ways. The method used in this experiment was to measure the ME consumed by individually caged broilers over a two-week period, and the amount of energy retained by the bird in that time. The difference between the energy consumed (ME) and retained (GE) is the amount of heat that has been produced over the period.

$$\text{HL (kJ/bird d)} = \text{ME intake (kJ/bird d)} - \text{GE (kJ/bird d)}$$

In order to ensure that broilers were not constrained in their ability to lose heat (i.e., their maximum rate of heat production could then be measured), the feeds offered were imbalanced, thereby encouraging the birds to over-consume other nutrients and energy. In addition, different environmental temperatures were used, as the total heat loss would be expected to be a function of the environmental temperature.

3.2.1. Housing

Six environmentally controlled chambers at Ukulinga Research Farm, belonging to the University of Natal, were used in each trial (Plate 3.1). The controlled environment research unit (CERU) was founded in 1994, consisting of five insulated marine shipping containers, the sixth being installed in 1998. Each chamber is individually and independently controlled with respect to temperature, humidity, lighting, and internal air exchange and has its own water supply and measures 2.26m wide, 5.75m long and 2.24m heights, making up 29m^3 in volume and 13m^2 in surface area (Paton, 1994).

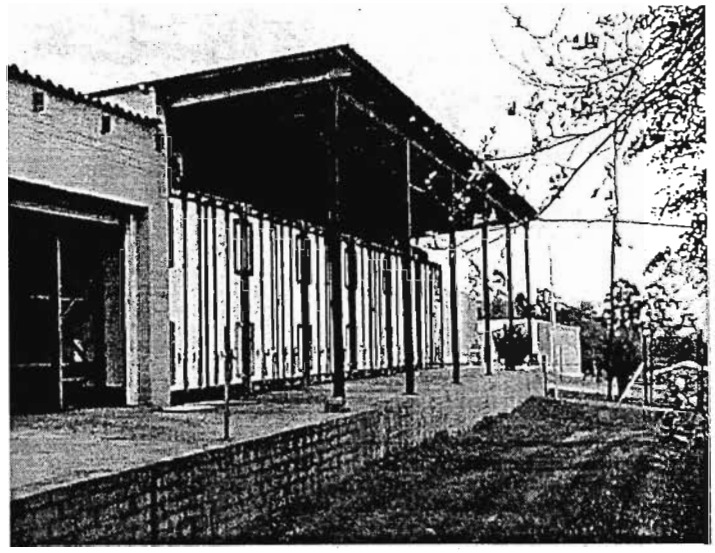


Plate 3.1. *Controlled environmental chambers at Ukulinga Research Farm, University of Natal*

Inside the chambers, individual broiler cages run along the length of each chamber (Plate 3.2). Each chamber has three tiers of 16 cages, making 48 individual cages per chamber.

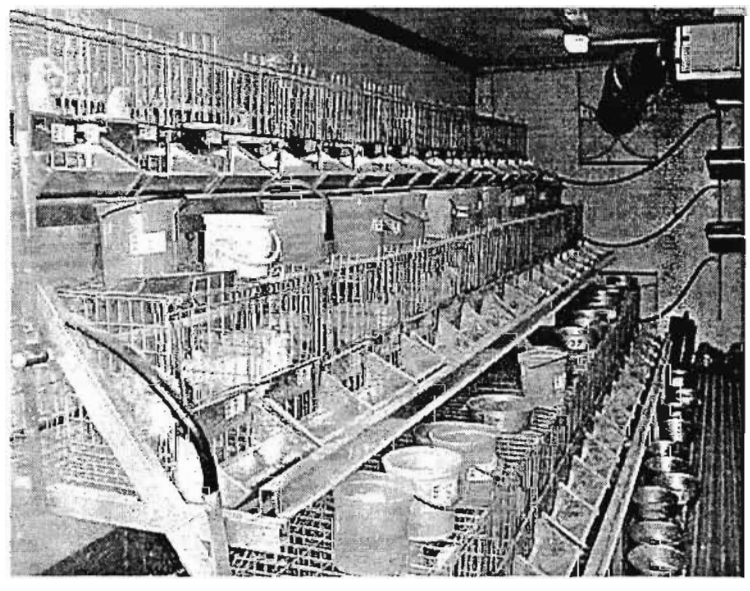


Plate 3.2. *A longitudinal view of broiler cages from the door of the chamber*

Each chamber is equipped with nipple (cistern) drinkers and a trough that runs along the length of the cage holding individual feeders, in such way that each bird has *ad libitum* access to its own feed and water (Plate 3.3). Since birds in the first two trials (trial one and two) were very young, during the first week of each trial, a small feeder was placed inside each

cage, such that each bird was able to reach the food through the holes of the feeder. During the second week of these trials and throughout the third trial, individual feeders that run along the length of the cages were used. To prevent thieving, the cages were designed in such a way that birds are not able to reach the drinkers or the feeders of the neighbouring bird.

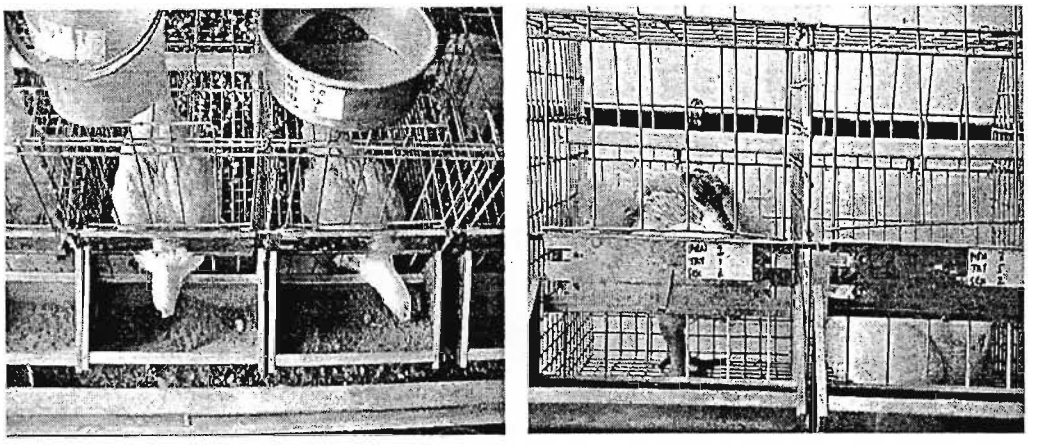
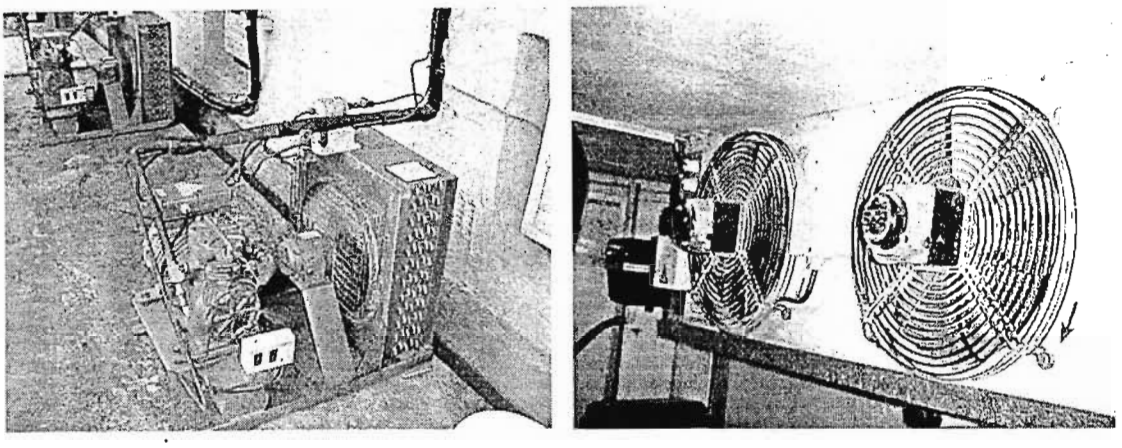


Plate 3.3. *Food and water supply to the broilers within the chamber*

A brief description of the CERU operating system is given here to highlight the heating, cooling, and ventilation features of the chambers. The chambers use a Hot Bypass Gas refrigeration system (Plate 3.4). The refrigeration unit comprises six compressor motors (Plate 3.4A), each having condenser coil and fan, blower coils and thermo-expansion valves. For refrigeration, there are two Recoil (NST 1300) blower unit in each chamber (Plate 3.4B). These blowers have 34-watt fans in each unit. Behind the blower unit, and in line with the fans, there are two Eintal^{Tm} pulsating jets. These jets operate on the pressure of the incoming water.

The heating system consists of two electric black heat type single-phase heaters, with 1500-watt heating elements mounted inside the blowing unit, just in front of the heat exchanger but behind the fan motors. These heating elements are controlled by the electronic controller unit.



A B

Plate 3.4. *Refrigeration system of the CERU unit, A: compressor, condenser coil and fan, B: blower unit, fans, and thermoexpansion valve*

The internal lighting system of each chamber is provided by two 9-watt fluorescent tubes, which are enclosed in waterproof bulkheads. These are situated at equal distance from the centre of each chamber's roof and are controlled automatically by means of an electronic controller.

The air exchange in the containers is undertaken by means of fans blowing air through the portholes of the container. The top porthole receives air for the chamber with a motor blower fan, which has single speed. Air movement is controlled by the interval at which this is operated. Exhaust air is emitted from the chamber through the bottom porthole. The incoming air is controlled by the electronic control unit situated at the back of the each chamber (Plate 3.4). Air exchange occurs at a maximum rate of $6.5m^3$ (Paton, 1994; Young, 1998).

The electronic controller controls:

- Temperature within the chamber
- Alternation of day and night temperature regimes
- Number of daylight hours
- Humidity of the environment

- The amount of air exchange between the chamber and the environment.

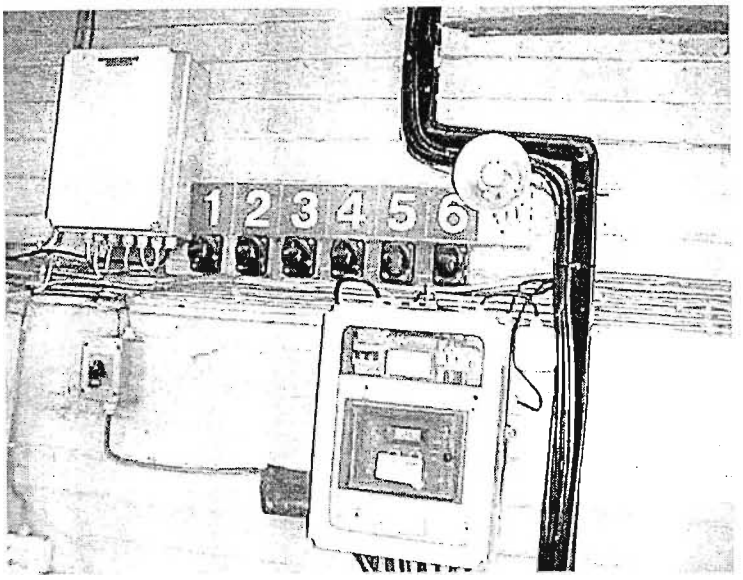


Plate 3.5. *The electronic controller unit situated at the rear of each chamber*

3.2.2. Experimental design

The experiment was a 2 x 3 x 6 factorial design, the factors being 2 sexes, 3 temperatures, and 6 lysine levels. Each temperature was replicated twice and within each room each diet was replicated four (three for lysine pilot – trial) times for each diet x sex combination.

3.2.3.Birds

Two hundred and fifty, and six hundred day old Ross broilers were obtained from the Ross breeders for the pilot trial, and trials 1 and 2, respectively. They were feather sexed, and equal number of males and females were placed in cages in the brooder room located at Ukulinga Research Farm.

While two hundred and sixteen birds were used for the pilot trial, two hundred and eighty eight birds were used for trials 1 and 2. The trials were conducted over two periods of two weeks each. Broilers between 1 – 3 weeks (starter trial) and 3 – 5 weeks (finisher trial) of age were housed in the chambers for each two-week trial. Only broilers between 1 – 3 weeks were used for pilot trial. The birds were randomly allocated individually to 216, 288, and 288 pens for pilot trial, trial one, and two, respectively. An additional 12 birds (six males and six females) were killed at the start of each trial to determine the initial body composition of the

birds remaining on the experiment. Therefore, a total of 828 birds were used for the three experiments.

3.2.4. Temperature

Three different temperatures were used in each trial (Table 3.1). The temperatures remained constant throughout each of the two-week trial periods. In order to achieve these temperatures the environment was controlled using computerized system. The computer was used to monitor and control temperature and humidity automatically.

Table 3.1. *Temperature (^0C) treatments of the experiment*

Temperature treatment	Age of birds	
	1 – 3 weeks	3 – 5 weeks
T1	24	18
T2	28	25
T3	32	32

The different temperatures were randomly assigned to each of the six environmentally controlled chambers in order to get two replications per temperature treatment. 72 (for pre-trial) and 96 (for trials 1 and 2 each) birds per temperature treatment were randomly allocated to the two chambers. Within the chambers the birds were randomly assigned to individual cages. Then diets were randomly assigned to each bird.

3.4.5. Diets

The summit dilution technique was used in formulating and mixing the dietary treatments. The summit diet was formulated to be first limiting in lysine (Table 3.2). The amino acid requirements of broilers between 1 and 3 weeks, and between 3 and 5 weeks were obtained from the EFG broiler growth model (Table 3.5). The limiting amino acid in the summit diet, lysine, was made 1.3 times the requirement, whilst all the other amino acids were made at least 1.5 times their requirement, thereby ensuring that the test amino acid was first limiting in the summit feed. Because the dilution feed contained no protein, all blends of the summit plus dilution feed had the same amino acid balance, and hence, all were limiting in the test amino acid.

The summit diet was blended in different proportions with the dilution diet to produce five levels of the test amino acid (Table 3.6). The sixth diet had the same proportions of the summit plus dilution as the fifth diet, but was supplemented with the synthetic form of the test amino acid to give the same lysine content as diet four. The objective was to test whether the amino acid under test was limiting in the series of the diets fed, and to determine whether a different amount of heat would be produced by birds on feeds with similar amino acid contents but different protein contents. A positive response in growth and/or heat loss would confirm that lysine was the most limiting nutrient. Vitamins and minerals were included at 2.5 times the rate recommended by the suppliers to ensure that these were not limiting.

The six dietary treatments were randomly assigned to the 36 (for pilot trial) and 48 (for trials one and two each) birds in each chamber making three and four replicates of each diet, respectively.

Table 3.2. *Composition (g/kg) of the basal diets used in the pilot trial, trial one and trial two*

Ingredient	Summit Lysine limiting	Dilution Lysine limiting
Yellow maize	95.5	
Maize gluten 60	120.0	
Soybean oilcake meal	490.6	
Sunflower oilcake meal	150.0	
Fish meal	15.0	
DL Methionine	1.2	
Limestone	14.6	18.3
Monocalcium phosphate	14.7	20.0
Salt	2.2	3.8
Sodium bicarbonate	1.4	1.2
Sugar/starch (50:50)		576.2
Oil	92.3	100.0
Filler (sand + husks)		278.0
Vitamin & mineral premix	2.5	2.5

Table 3.3. *Calculated composition (g/kg) of the diets used in pilot trial, trials one and two*

Ingredients	Summit Lysine limiting	Dilution Lysine limiting
AMEn (MJ/kg)	11.36	13.0
Calcium	10.0	10.0
Avai.phosphorus	5.00	5.00
Sodium	1.80	1.80
Chloride	2.30	2.30

Chemical compositions were calculated using Winfeed (Windows Feed Formulation Programme) on as is basis at the University of Natal, 2001.

Table 3.4. [4]*Analysed composition (g/kg)*

Chemical composition	Summit	Dilution
AMEn (MJ/kg)	12.45	12.15
TMEn (MJ/kg)	12.86	12.56
Moisture	96	56
Protein	390	8.2
Digestible amino acids		
Threonine	10.9	0.32
Valine	19.0	0.67
Methionine	5.6	0.08
Isoleucine	16.5	0.28
Leucine	34.7	0.53
Tyrosine	10.4	0.14
Phenylalanine	18.6	0.32
Histidine	8.8	0.19
Lysine	18.3	0.30
Arginine	23.5	0.30

[4]Chemical composition as determined on as is basis by the Feed Evaluation Unit, Animal and Poultry Science, University Of Natal, 2002.

Table 3.5. *Amino acid requirements (%) for broilers at an AMEn of 13.0 MJ/kg (EFG Growth Model)*

Age (Weeks)	Lys	Met	Tsaa	Trp	Ile	His	Arg	Thr	P+t	leu	Val
1 – 3	1.37	0.46	0.80	0.19	0.80	0.48	1.35	0.84	1.47	1.41	0.91
3 – 5	1.14	0.38	0.72	0.16	0.69	0.40	1.16	0.72	1.28	1.21	0.79

3.2.6. Feed mixing procedures

The amount of food that a growing broiler of the age 1 to 3 week and 3 to 5 week would eat in two weeks period was estimated to be 1.5 and 2.5 kg, respectively. The individual bird consumption values for two weeks was multiplied by the number of experimental birds and 5% was added for food wastage considerations. This enabled the total amount of food to be calculated for each experiment.

Each basal feed was mixed for about 25 minutes using the horizontal mixer at the Ukulinga Research Farm. Using the proportions in Table 3.6, the blends were then mixed thoroughly for about 25 minutes.

Table 3.6. *Blending proportions of the summit and dilution diets and expected lysine concentration used in pilot trial, trial one, and trial two*

Diet treatment	Proportion of Summit %	Proportion of dilution %	Added lysine %	Lysine concentration (g/kg)
1	100	0		18.30
2	85	15		15.60
3	70	30		12.90
4	55	45		10.20
5	40	60		7.50
6	40	60	0.3	10.50

3.2.7. Measurements

3.2.7.1. Body weight:

Birds were weighed individually at the start of the experiment and at the end of each week.

3.2.7.2. Feed intake:

At the beginning of the trial feed was placed in plastic buckets for each pen, and weighed. Feed was dispensed from this container to the feed trough as needed. Feed intake for each bird was recorded at the end of each week by weighing back the unconsumed feed.

3.2.8. Feather and carcass sample preparation

At the start of the experiment 12 birds, six of each sex, were weighed and killed for initial carcass analysis. At the end of the experiment half of the birds from each treatment were randomly killed for carcass analysis. Each bird was killed by cervical dislocation. After killing, each bird was placed in a pre - labelled freezer bag and immediately transported to the abattoir. In the abattoir the weight of each un – plucked bird was taken. After weighing, the birds were plucked by hand and all the feathers were placed in a pre - labelled freezer bag. The plucked birds were weighed again and the weights noted. Feather weight was calculated as the whole body weight less plucked body weight. The whole feather free carcasses were frozen at – 20^0C. After freezing, the whole feather free carcass was individually minced four times using a standard electrically powered auger mincer. Between mincing individual birds, the mincer and all utensils were completely washed and dried before starting with the mincing of the next bird. This was to ensure that there was no contamination of material with previously minced bird or contamination with water. Minced carcass was mixed thoroughly. A representative sample was taken and placed in specimen honey jars that had been pre – labelled with the bird's laboratory analysis number. The remainder of the sample was discarded.

The feather samples of birds in each trial were weighed out in brown paper bags and were put into oven for drying at 65 – 70 ^{0}C and there they remained for 168 hours. Once they were removed and cooled, they were reweighed to determine the moisture content. A representative sample of the feather samples was then milled using 2mm electrically powered milling machine. Upon milling, the feather samples tended to separate out into two different components, i.e. the milled feather from the shafts and from the vanes. The milled feather samples were then analysed in a LECO FP2000 Nitrogen Analyser using the Dumas

combustion method. The results were not as hoped. The duplicates were found to vary greatly, more than 10%, and hence, only feather samples from birds that were in dietary treatment one, three, and five were taken for analysis, and these feather samples were cut into small pieces using scissors. They were then analysed in the LECO FP2000 Nitrogen Analyser using the Dumas combustion method and in this case the duplicate samples yielded similar results. Simple linear regression analysis was then undertaken in GenStat release 6.1 to find if there existed a relationship between moisture and protein content of these feather samples. The feather protein content for the remaining samples (dietary treatments two, four and six of pilot trial, and trials one and two) was calculated by using the formula detailed below.

$$\text{Protein (\%)} = 96.1 - 0.119 \text{ Moisture (\%)}$$

18 samples were used for developing this equation. The standard errors for the constant term and the regression coefficients were 8.374 and 0.1487 respectively.

The entire feather was assumed to contain moisture and dry matter (containing protein and lipid, and the small amount of ash and carbohydrate being ignored). Deduction of moisture (%) from 100% gave the percentage dry matter content of the feathers. To find protein content on as-is basis, the protein given as percentage in the equation above, was multiplied by the dry matter content. The remaining part of the dry matter, the lipid, was obtained by difference.

The gross energy content of these feathers was calculated by multiplying the protein and lipid contents (on as is basis) with their heats of combustion, i.e., 23.8 and 39.6 kJ/g, respectively. This procedure was done to both the initial (feathers samples obtained from broilers that were killed at the start of the trial) and the final feather samples (feathers samples obtained from broilers that were killed at the end of the trial). The difference between the initial and the final gross energies was divided by the number of experimental days (14) to find the gross energy content per bird day. For birds that were slaughtered but not sampled for feather analysis in the laboratory, the only information available was their feather weight. Therefore, an equation was produced by regressing gross energy on feather weight using simple linear regression in GenStat release 6.1, and the gross energy of these birds was estimated by using this equation. The equation is:

$$\text{GE (kJ/bird d)} = - 5.02 + 0.847 \text{ dried feather weight (g)}$$

36 samples were used to produce this equation. The standard errors for the constant term and the regression coefficients were 1.452 and 0.04667 respectively.

In this manner, all gross energies for all birds that were slaughtered in all trials were calculated and the result was added to the gross energy of the entire feather-free carcass for heat loss analysis.

The carcass samples were frozen to allow prolonged storage without putrefaction. The entire content of each sample was placed in jars that fitted an electric vacuum freeze-drying machine. The carcass samples were placed in a freeze drier, where they remained for approximately 200 hours before being removed, checked for complete dryness and weighed, the difference being the carcass moisture content. The samples were then milled using centrifugal blade and gauze grinder, having 1.00 mm sieve holes. After milling for each sample the centrifugal blade and plate were completely cleaned and the sieve washed and dried before grinding the next sample. This prevented contamination of samples. The milled samples were then given to the Feed Evaluation Unit of the department of Animal and Poultry Science, University of Natal, for chemical analysis. The samples analysed and methods of analysis carried out by the feed evaluation unit are detailed in the table below.

Table 3.6. *Analysis performed and the methods used to determine the chemical composition of feather and carcass samples*

Analysis	Method used	Sample type
Moisture content	Freeze drying	Carcass
	Oven drying	Feathers
Crude protein	Dumas combustion	Carcass
		Feathers
Ash	Furnace combustion	Carcass
Gross energy	Bomb calorimeter	Carcass

To determine the moisture contents of the carcass samples they were freeze dried at 10^{-1} torr. The crude protein contents of the carcass samples were analysed in a LECO FP 2000 Nitrogen Analyser using the Dumas combustion method. The carcass ash contents analysis required that the furnace be set at 550^0C for four hours. The gross energy contents of the carcass samples from the pilot trial were analysed using the adiabatic bomb calorimeter. A duplicate

analysis of all these carcass compositions, including moisture, protein, lipid, ash and gross energy was carried out according to the Association of Analytical Chemists' (AOAC, 1990). Where the moisture, lipid, protein, ash and gross energy measurements between duplicated samples differed by more than 5%, two further analyses were performed and the means of all four analyses were used. Instead of determining the gross energy of each carcass samples for trials 1 and 2, the lipid content of the carcass was predicted from water content using the following equation:

$$\text{Lipid (\%)} = 84.9 - 1.03 \text{ carcass water (\%)}$$

In developing this equation the lipid contents of the first 146 carcasses were determined from their gross energy contents, using the following equation developed in the department:

$$\text{Lipid (g/g)} = -0.8756 + 0.04754 \text{ gross energy (kJ/g)}$$

3.2.9. Manipulation of carcass laboratory results for heat loss analysis

Results for carcass compositions, including moisture and moisture pick up since freeze-drying, protein, gross energy, and ash were obtained. Protein and gross energy were corrected for moisture pick-up since drying. Lipid content (for the pilot trial) was derived from gross energy using the model detailed above.

A spreadsheet containing pen number, temperature (^{0}C), sex, plucked and un – plucked body weight (g), feather weight (g), feed intake (g), ME in (MJ/kg), and carcass composition contents (on as is basis) was constructed. Average feather weight of birds that were killed at the start of each experiment was calculated separately. Deduction of this average feather weight from the initial un-plucked body weight of birds that were killed at the end of each experiment gave their initial plucked body weight. The initial plucked body weights were then multiplied with each of the carcass composition constituents (on as is basis) of broilers that were killed at the start of the pilot trial. In this manner, the initial protein (g), water (g), gross energy (kJ/bird) and lipid (g) contents of the birds that were killed at the end of pilot trial were calculated. The product of the final plucked body weight and the carcass composition constituents (on as is basis) gave the final weights for carcass protein (g), water (g), gross energy (kJ/bird) and lipid (g) contents of the birds that were killed at the end of trial. The difference between the final and the initial carcass constituent weights gave the

gains. The gross energy calculated from carcass in this manner was added to the gross energy of the feathers to give the total gross energy contained in each bird. The same procedure was used when calculating the initial and the final protein and water weights of birds that were killed at the end of experiments one and two except that no correction was done for moisture pick up since freeze drying and that the gross energy was calculated directly by multiplying the protein and lipid contents (on as is basis) with their heat contents, i.e., 23.8 and 39.6 kJ/g, respectively.

The energy intake per day was calculated by multiplying the ME content of the food ($ME_{in\ food}$, kJ/kg) by the average daily feed intake.

$$ME\ intake\ (kJ/day) = ME_{in\ food} \times FI$$

By definition heat loss (kJ/bird d) is the difference between the energy intake (ME intake, kJ/bird d) and the gross energy retained (GE, kJ/bird d).

$$HL\ (kJ/bird\ d) = ME\ intake\ (kJ/bird\ d) - GE\ (kJ/bird\ d)$$

The relevant data was then analysed using multiple linear regression analysis in GenStat release 6.1.

3.10. Prediction of heat loss

Heat loss is likely to be a function of one or more of the following variables: environmental temperature, weight of the feather cover of the bird, food intake and degree of maturity. In order to determine to what extent these variables were related to heat loss, a multiple regression was performed on the data from each experiment separately, and then combined, and a step – wise multiple regression analysis was performed on treatments that were non limiting (dietary treatments 1 and 2) and most limiting (dietary treatments 3 – 5), in GenStat, release 6.1. Treatment 6 was not included in the analysis because lysine may not have been the most limiting amino acid in the feed. The effect of bird size on heat production was taken into account by scaling the heat lost by each bird (kJ/b d) by dividing by (logarithmic mean body weight) $^{0.67}$ to become heat loss ($kJ/kg^{0.67}$ per day), this being the surface area of the bird, the amount of heat lost to the environment more likely being a function of the surface area of the bird than its body weight. The rate of protein, lipid, and gross energy gain was

determined as the difference between the initial and the final body weights. Degree of maturity was calculated by dividing the final protein weight (g) with the assumed mature protein weight (Pm) of 1250 and 900 (g) for males and females, respectively.

3.3. Results

3.3.1. Body weight gain, feed intake and feed conversion efficiency

The data were collected primarily for the estimation of heat loss over the trial period. However, the effect of treatments on the estimation of FCE, FI, and average daily gain (ADG) over the experimental period was also of interest. Treatment means were calculated for each of the two-week trial periods. Since one third of the birds from one of the chambers used in pilot trial died on the sixth day after the start of the experiment, the data collected from that particular chamber was discarded, and hence, the data for pilot trial was unbalanced. Therefore, the residual maximum likelihood (REML) in GenStat release 6.1 was used to estimate the treatment effects in linear model with fixed effects of temperature, sex, and lysine content for pilot trial, and ANOVA in GenStat release 6.1 was used for analysing the treatment means of trials one and two.

3.3.1.1. Pilot trial

The mean responses in FCE, ADG, and FI are shown in Table 3.7 Both dietary lysine and temperature had a significant effect on FCE ($P<0.001$), and ADG (dietary lysine at $P<0.001$, temperature at $P<0.05$). Food intake was highly influenced by dietary lysine ($P<0.001$). Neither sex of the birds alone nor its interaction with temperature or dietary lysine content of the diet or both was found to affect FCE, FI or ADG. The increased response in FCE, ADG, and FI (Table 3.7) with the addition of synthetic lysine (dietary treatment 6 *vs.* 5), irrespective of temperature, confirmed that lysine was the most limiting nutrient in the summit diets. The mean FCE decreased as the lysine content of the diet decreased from 18.3 to 7.5 g/kg (diet treatment 1 – 5), and increased when the lysine content was increased by adding synthetic lysine to dietary treatment 5. However, the mean ADG showed different pattern. It was lower when the dietary lysine content was highest (diet treatment 1 and 2), increased (diet treatment 3 and 4), and then decreased (diet treatment 5). FI showed the same pattern as ADG, with decreasing response in dietary treatment 1 and 2, increasing response in dietary treatment 3 and 4 and decreasing response at dietary treatment 5.

3.3.1.2. Trial 1

The mean responses in FCE, ADG, and feed intake are shown in Table 3.8. Dietary lysine, sex and temperature were found to have a significant effect on ADG (dietary lysine at $P<0.001$, sex and temperature at $P<0.05$), and only dietary lysine, temperature and their interaction (lysine *vs.* temperature) were found to have a significant effect on FCE (dietary lysine at $P<0.001$, temperature and lysine *vs.* temperature at 0.01). Feed intake was significantly influenced by dietary lysine ($P<0.001$), but not by temperature ($P = 0.054$). None of the interactions had significant effect on feed intake, or ADG. Similar to pilot trial, birds in this experiment also showed an increased response in FCE, ADG, and FI (Table 3.8) with the addition of synthetic lysine (diet treatment 6 *vs.* 5), irrespective of temperature, confirming that lysine was the first limiting nutrient in the summit diets. The lowest mean FCE response to dietary lysine next to treatment 5 was found to be dietary treatment 1, followed by treatments 4, 2, and 3. A similar pattern was followed with mean ADG. Minimum and maximum response in mean FI were found on dietary treatment 1and dietary treatment 4, respectively.

3.3.1.3. Trial 2

The mean responses in FCE, ADG, and FI are shown in Table 3.9. Dietary lysine, sex, and temperature were found to have a significant effect on feed intake (dietary lysine and temperature at $P<0.001$, sex at $P<0.05$), and ADG (dietary lysine and temperature at $P< 0.001$, sex at $P<0.01$), and only dietary lysine and temperature had a significant effect on FCE ($P<0.001$). There were significant interactions between the main effects, temperature and dietary lysine content, on FCE ($P<0.001$). No interaction had any significant effect on FI, or ADG. The response on FCE, ADG, and FI showed an increasing trend with the addition of synthetic lysine (diet treatment 6 *vs.* 5), irrespective of temperature, confirming that lysine was the first limiting nutrient in the summit diets. Unlike the results of the pilot trial, mean FCE, ADG, and FI showed an increasing pattern with a decrease in lysine content from 18.3 to 7.5 g/kg.

3.3.2. Carcass protein, lipid and gross energy

3.3.2.1. Pilot trial

The mean daily gain responses of birds in protein, lipid and gross energy are indicated in Table 3.10. Dietary lysine was found to have significant effect on the daily protein gain ($P<0.001$). Lipid and gross energy gains were significantly influenced by dietary lysine, sex,

and environmental temperature (dietary lysine at P<0.001, temperature at P<0.01 and sex at P<0.05). While the interaction between temperature and dietary lysine, and temperature and sex had significant effect on lipid and gross energy gain (P<0.05), the interaction between dietary lysine and sex, and dietary lysine, sex, and temperature had no effect on lipid and gross energy gain of growing broilers used in this trial. None of the interactions had any significant effect on protein gain.

3.3.2.2. Trial 1

The effect of environmental temperature, dietary lysine and sex on protein, lipid and gross energy gain of broilers between one and three weeks of age is shown in Table 3.11. Dietary lysine, and temperature were found to have a significant effect on body protein (dietary lysine at P<0.001, temperature at P = 0.01), lipid (dietary lysine and temperature at P<0.001) and gross energy gain (dietary lysine at P<0.001 and temperature at P<0.01). While the interaction between sex and temperature was found to have significant effect on body protein and gross energy gain (P<0.05), none of the other interactions had any effect on either body protein or gross energy gains. None of the interactions was found to have any significant effect on body lipid gain.

3.3.2.3 Trial 2

The response in body protein, body lipid and body gross energy gain of broilers between three and five weeks of age are shown in Table 3.12. The main effects, dietary lysine and temperature, were found to have a significant effect on body protein gain (P<0.001) and body lipid gain (dietary lysine at P<0.001 and temperature at P<0.01), and all the main effects had significant effect on gross energy (dietary lysine and temperature at P<0.001, and sex at P<0.01). Although none of the interactions had a significant effect on body lipid gain, the interactions between diet and temperature had a significant effect on body protein and gross energy gain (P<0.05).

Table 3.7. *Effect of dietary lysine and temperature on mean FCE, ADG and FI of growing male and female broilers between one and three weeks of age (Pilot trial)*

Response variable	Diet treatment	Sex						Mean (Diet)
		Females			Males			
		Temperature treatment (^{0}C)						
		24	28	32	24	28	32	
FCE (g/kg)	1	569	624	679	591	578	673	619
	2	558	608	666	516	584	648	597
	3	508	594	581	577	603	632	582
	4	574	544	569	550	594	611	573
	5	486	512	537	541	550	553	530
	6	527	604	602	598	604	628	593
Mean Temp vs. Sex		537	581	605	562	586	624	581
S.E.M: Diet = 13.96***			S.E.M: Temp = 9.730***			S.E.M: Sex = 8.060^{NS}		
S.E.M: Diet X Temp = 23.83^{NS}			S.E.M: Diet X Sex = 19.74^{NS}					
S.E.M: Temp X Sex = 13.76^{NS}			S.E.M: Temp X Sex X Diet = 33.70^{NS}					
ADG (g/bird d)	1	30.7	30.0	29.8	26.7	29.2	30.2	29.4
	2	30.3	30.7	31.0	27.0	27.3	30.3	29.4
	3	27.3	29.3	30.5	29.7	31.7	32.5	30.2
	4	29.3	30.2	28.3	28.3	32.3	31.2	29.9
	5	24.0	27.0	29.7	26.7	28.3	25.2	26.8
	6	28.7	32.5	31.2	31.3	32.5	34.2	31.7
Mean Temp vs. Sex		28.4	30.0	30.1	28.3	30.2	30.6	29.6
S.E.M: Diet = 0.782***			S.E.M: Temp = 0.545*			S.E.M: Sex = 0.452^{NS}		
S.E.M: Diet X Temp = 1.335^{NS}			S.E.M: Diet X Sex = 1.106^{NS}					
S.E.M: Temp X Sex = 0.771^{NS}			S.E.M: Temp X Sex X Diet = 1.888^{NS}					
Feed intake (g/bird d)	1	54.3	48.0	44.5	45.3	50.3	45.8	48.0
	2	54.3	50.3	47.0	52.3	46.8	47.3	49.7
	3	53.3	49.5	52.5	51.3	51.0	51.2	51.5
	4	51.0	55.5	49.8	51.3	54.5	51.2	52.2
	5	49.3	53.2	55.5	49.7	51.8	46.8	51.1
	6	55.0	53.8	52.3	52.7	53.3	54.3	53.6
Mean Temp vs. Sex		52.9	51.7	50.3	50.4	51.3	49.4	51.0
S.E.M: Diet = 1.087***			S.E.M: TEMP = 0.7578^{NS}			S.E.M: Sex = 0.628^{NS}		
S.E.M: Diet X Temp = 1.855^{NS}			S.E.M: Diet X Sex = 1.537^{NS}					
S.E.M: Temp X Sex = 1.071^{NS}			S.E.M: Temp X Sex X Diet = 2.624^{NS}					

***P < 0.001 **P < 0.01 *P < 0.05 NS = non significant

Table 3.8. *Effect of dietary lysine and temperature on mean FCE, ADG and FI of growing male and female broilers between one and three weeks of age (Trial 1)*

Response variable	Dietary treatment	Sex						Mean (Diet)
		Females			Males			
		Temperature treatment (^{0}C)						
		24	28	32	24	28	32	
FCE (g/kg)	1	588	647	628	572	577	670	614
	2	572	676	630	603	682	680	640
	3	607	652	668	654	694	692	661
	4	575	587	613	584	623	628	601
	5	622	512	494	534	486	574	537
	6	641	629	641	609	655	635	635
Mean Temp vs. Sex		601	617	612	593	619	646	615
S.E.M: Diet = 10.31***		S.E.M: Temp = 7.29**			S.E.M: Sex = 5.95^{NS}			
S.E.M: Diet X Sex = 14.59^{NS}		S.E.M: Diet X Temp = 17.86**						
S.E.M: Temp X Sex = 10.31^{NS}		S.E.M: Temp X Sex X Diet = 25.26^{NS}						
ADG (g/bird d)	1	29.2	32.2	29.4	28.9	29.4	30.7	29.9
	2	28.4	33.9	29.7	32.0	34.4	32.9	31.9
	3	30.4	37.1	32.8	36.5	37.9	37.5	35.4
	4	31.6	34.2	33.8	32.6	35.4	35.3	33.8
	5	31.5	27.8	27.8	28.3	27.8	31.9	29.2
	6	36.8	36.6	37.7	36.7	37.7	35.5	36.8
Mean Temp vs. Sex		31.3	33.6	31.9	32.5	33.7	34.0	32.8
S.E.M: Diet =.0.654***		S.E.M: Temp = 0.462*			S.E.M: Sex = 0.377*			
S.E.M: Diet X Temp = 1.132^{NS}		S.E.M: Diet X Sex = 0.924^{NS}						
S.E.M: Temp X Sex = 0.654^{NS}		S.E.M: Temp X Sex X Diet = 1.601^{NS}						
Feed intake (g/bird d)	1	49.6	49.9	46.7	50.5	50.8	46.0	48.9
	2	49.9	50.7	47.6	53.1	50.7	48.7	50.1
	3	50.3	57.0	49.1	56.0	54.8	54.2	53.6
	4	54.9	58.4	55.2	55.7	57.5	56.4	56.3
	5	50.8	54.7	56.8	53.2	57.7	56.4	54.9
	6	57.5	58.5	59.0	60.2	57.6	56.1	58.2
Mean Temp vs. Sex		52.2	54.9	52.4	54.8	54.8	53.0	53.7
S.E.M: Diet = 0.908***		S.E.M: Temp = 0.642^{NS}			S.E.M: Sex = 0.524^{NS}			
S.E.M: Diet X Temp = 1.573^{NS}		S.E.M: Diet X Sex = 1.284^{NS}						
S.E.M: Temp X Sex = 0.908^{NS}		S.E.M: Temp X Sex X Diet = 2.224^{NS}						

***P < 0.001 **P < 0.01 *P < 0.05 NS = non significant

Table 3.9. *Effect of dietary lysine and temperature on mean FCE, ADG and FI of growing male and female broilers between three to five weeks of age (Trial 2)*

Response variable	Diet treatment	Sex						Mean (Diet)
		Females			Males			
		Temperature treatment (^{0}C)						
		18	25	32	18	25	32	
FCE (g/kg)	1	405	562	511	440	469	528	486
	2	438	550	518	445	559	569	513
	3	434	571	542	442	552	581	520
	4	450	537	515	378	534	480	482
	5	388	441	414	420	464	402	421
	6	441	454	432	438	494	432	448
Mean Temp vs. Sex		426	519	489	427	512	499	479
S.E.M: Diet = 9.40***			S.E.M: Temp = 6.65***			S.E.M: Sex = 5.43^{NS}		
S.E.M: Diet X SEX = 13.29^{NS}			S.E.M: Diet X Temp = 16.28***					
S.E.M: Temp X Sex = 9.40^{NS}			S.E.M: Temp X Sex X Diet = 23.02^{NS}					
ADG (g/bird d)	1	50.6	58.0	54.3	56.0	60.9	56.8	56.1
	2	53.6	63.5	54.3	59.4	72.8	61.9	60.9
	3	56.8	64.9	56.9	59.4	68.5	67.9	62.4
	4	58.0	62.0	59.6	49.6	67.3	53.8	58.4
	5	57.1	57.3	48.0	51.0	62.8	47.4	53.9
	6	61.9	60.5	50.5	63.6	66.6	56.8	60.0
Mean Temp vs. Sex		56.3	61.0	53.9	56.5	66.5	57.4	58.6
S.E.M: Diet = 1.424***			S.E.M: Temp = 1.007***			S.E.M: Sex = 0.822**		
S.E.M: Diet X Temp = 2.467^{NS}			S.E.M: Diet X Sex = 2.014^{NS}					
S.E.M: Temp X Sex = 1.424^{NS}			S.E.M: Temp X Sex X Diet = 3.489^{NS}					
Feed intake (g/bird d)	1	124.9	104.1	106.4	127.8	108.4	107.3	113.1
	2	122.3	115.9	104.9	135.9	131.1	109.0	119.8
	3	133.5	113.9	104.9	135.3	124.4	116.9	121.5
	4	128.6	115.5	115.6	131.5	126.3	112.0	121.6
	5	147.6	131.0	115.9	122.3	135.1	115.5	127.9
	6	140.4	136.6	117.3	146.1	135.3	130.4	134.3
Mean Temp vs. Sex		132.9	119.5	110.8	133.1	126.7	115.2	123.0
S.E.M: Diet = 2.398***			S.E.M: TEMP = 1.696***			S.E.M: SEX = 1.385*		
S.E.M: Diet X TEMP = 4.154^{NS}			S.E.M: Diet X SEX = 3.392^{NS}					
S.E.M: TEMP X SEX = 2.398^{NS}			S.E.M: TEMP X SEX X Diet = 5.875^{NS}					

***P < 0.001 **P < 0.01 *P < 0.05 NS = non significant

Table 3.10. *The response in protein gain (g/bird d), lipid gain (g/bird d) and gross energy gain (kJ/bird d) of broilers between one and three weeks of age (Pilot trial)*

Response variable	Diet treatment	Sex						Mean (Diet)
		Females			Males			
		Temperature treatment (^{0}C)						
		24	28	32	24	28	32	
Protein gain	1	5.15	5.38	5.03	5.43	4.63	4.70	5.05
	2	5.28	4.63	4.98	5.38	5.28	5.25	5.13
	3	4.68	5.35	5.50	5.08	6.33	5.08	5.34
	4	5.15	5.10	4.33	4.25	5.45	5.08	4.89
	5	3.83	4.00	5.00	3.50	4.33	3.60	4.04
	6	5.18	5.75	4.83	4.13	5.43	5.33	5.11
Mean Temp vs. Sex		4.88	5.04	4.95	4.63	5.24	4.84	4.93
S.E.M: Diet = 0.1693***		S.E.M: Temp = 0.1197^{NS}			S.E.M: Sex = 0.0978^{NS}			
S.E.M: Diet X Temp = 0.2933^{NS}		S.E.M: Diet X Sex =0.2395 ^{NS}						
S.E.M: Temp X Sex = 0.1693^{NS}		S.E.M: Temp X Sex X Diet = 0.4148^{NS}						
Lipid gain	1	2.50	2.53	2.63	2.20	2.13	3.63	2.60
	2	3.00	1.50	2.93	2.50	1.70	2.75	2.40
	3	2.75	2.60	2.85	2.08	3.38	2.75	2.74
	4	3.35	3.38	3.40	1.83	3.33	3.40	3.12
	5	3.80	4.78	5.35	2.83	4.70	4.10	4.26
	6	5.20	6.60	4.50	2.70	4.73	5.10	4.81
Mean Temp vs. Sex		3.43	3.57	3.61	2.36	3.33	3.62	3.32
S.E.M: Diet = 0.2246***		S.E.M: Temp = 0.1588**			S.E.M: Sex = 0.1297*			
S.E.M: Diet X Temp = 0.3890*		S.E.M: Diet X Sex = 0.3176^{NS}						
S.E.M: Temp X Sex =0.2246*		S.E.M: Temp X Sex X Diet = 0.5501^{NS}						
GE gain	1	202	220	211	196	191	246	211
	2	231	162	230	212	178	222	206
	3	211	234	242	185	267	219	226
	4	242	257	237	163	253	255	235
	5	235	242	301	183	286	245	249
	6	324	383	324	199	296	345	312
Mean Temp vs. Sex		241	250	258	190	245	255	240
S.E.M: Diet = 9.16***		S.E.M: TEMP = 6.48***			S.E.M: Sex = 5.29*			
S.E.M: Diet X Temp = 15.87*		S.E.M: Diet X Sex = 12.96^{NS}						
S.E.M: Temp X Sex = 9.16*		S.E.M: Temp X Sex X Diet = 22.45^{NS}						

***P < 0.001 **P < 0.01 *P < 0.05 NS = non significant

Table 3.11. *The response in protein gain (g/bird d), lipid gain (g/bird d) and gross energy gain (kJ/bird d) of broilers between one and three weeks of age (Trial 1)*

Response variable	Diet treatment	Sex						Mean (Diet)
		Females			Males			
		Temperature treatment (^{0}C)						
		24	28	32	24	28	32	
Protein gain	1	4.55	5.20	4.55	5.15	4.70	4.88	4.84
	2	4.53	6.00	4.60	5.15	5.85	5.03	5.19
	3	4.98	5.98	5.30	6.28	5.60	6.73	5.81
	4	4.68	6.28	4.83	4.98	5.58	5.90	5.38
	5	4.62	3.60	4.25	4.55	4.05	4.5	4.26
	6	4.10	4.75	4.15	5.00	4.68	5.03	4.81
Mean Temp vs. Sex		4.76	5.61	4.67	4.80	5.09	5.36	5.05
S.E.M: Diet = 0.1740***		S.E.M: Temp = 0.1230^{NS}			S.E.M: Sex = 0.1005**			
S.E.M: Diet X Temp = 0.3014^{NS}		S.E.M: Diet X Sex = 0.2461^{NS}						
S.E.M: Temp X Sex = 0.1740*		S.E.M: Temp X Sex X Diet = 0.4263^{NS}						
Lipid gain	1	2.03	3.10	3.08	2.80	2.13	2.13	2.55
	2	2.48	3.60	2.83	2.68	3.13	3.00	2.95
	3	3.30	4.70	4.08	3.80	3.08	4.63	3.93
	4	3.43	5.70	5.05	3.58	4.65	4.78	4.53
	5	5.30	4.53	4.75	3.80	4.28	5.75	4.74
	6	4.00	5.68	5.60	3.40	4.98	5.33	4.83
Mean Temp vs. Sex		3.42	4.55	4.23	3.34	3.71	4.27	3.92
S.E.M: Diet = 0.2082***		S.E.M: Temp = 0.1472***			S.E.M: Sex = 0.1202^{NS}			
S.E.M: Diet X Temp = 0.3605^{NS}		S.E.M: Diet X Sex = 0.2944^{NS}						
S.E.M: Temp X Sex = 0.2082^{NS}		S.E.M: Temp X Sex X Diet = 0.5099^{NS}						
GE gain	1	214	270	263	254	202	220	237
	2	235	305	248	241	274	253	259
	3	277	354	314	319	267	359	315
	4	277	418	358	281	339	355	338
	5	350	297	324	278	284	359	315
	6	290	370	354	275	327	350	328
Mean Temp vs. Sex		274	336	310	275	282	316	299
S.E.M: Diet = 12.25***		S.E.M: TEMP = 8.066**			S.E.M: Sex = 7.07^{NS}			
S.E.M: Diet X Temp = 21.21^{NS}		S.E.M: Diet X Sex = 17.32^{NS}						
S.E.M: Temp X Sex = 12.50*		S.E.M: Temp X Sex X Diet = 30.00^{NS}						

***P < 0.001 **P < 0.01 *P < 0.05 NS = non significant

Table 3.12. *The response in protein gain (g/bird d), lipid gain (g/bird d) and gross energy gain (kJ/bird d) of broilers between one and three weeks of age (Trial two)*

Response variable	Diet treatment	Sex						Mean (Diet)
		Females			Males			
		Temperature treatment (^{0}C)						
		18	25	32	18	25	32	
Protein gain	1	5.62	6.20	5.33	5.62	6.03	5.85	5.78
	2	5.20	6.40	4.78	6.77	8.03	4.83	6.00
	3	6.55	7.20	6.80	6.02	8.15	6.90	6.94
	4	6.10	6.90	5.03	5.22	7.58	5.62	6.08
	5	5.35	6.55	3.40	4.97	5.32	1.77	4.56
	6	6.73	6.83	1.53	5.27	6.00	3.83	5.03
Mean Temp vs. Sex		5.93	6.68	4.48	5.65	6.85	4.80	5.73
S.E.M: Diet = 0.346***		S.E.M: Temp = 0.244***			S.E.M: Sex = 0.200^{NS}			
S.E.M: Diet X Temp = 0.599*		S.E.M: Diet X Sex = 0.489^{NS}						
S.E.M: Temp X Sex = 0.346^{NS}		S.E.M: Temp X Sex X Diet = 0.847^{NS}						
Lipid gain	1	4.17	4.12	4.60	5.57	5.17	4.72	4.73
	2	4.75	6.88	4.45	4.05	7.45	4.22	5.30
	3	5.60	7.15	7.73	5.85	7.70	6.35	6.73
	4	5.78	8.65	8.88	5.47	10.00	8.17	7.83
	5	6.80	8.88	9.90	8.05	8.88	8.08	8.43
	6	11.85	11.77	6.97	9.13	8.35	9.63	9.62
Mean Temp vs. Sex		6.49	7.91	7.09	6.35	7.93	6.86	7.10
S.E.M: Diet = 0.460***		S.E.M: Temp = 0.325**			S.E.M: Sex = 0.266^{NS}			
S.E.M: Diet X Temp = 0.797*		S.E.M: Diet X Sex = 0.651^{NS}						
S.E.M: Temp X Sex = 0.460^{NS}		S.E.M: Temp X Sex X Diet = 1.127^{NS}						
GE gain	1	323	355	333	335	341	309	333
	2	335	456	317	299	472	257	356
	3	397	488	491	389	466	392	437
	4	439	577	552	363	599	482	502
	5	460	578	524	445	508	372	481
	6	675	688	372	496	492	474	533
Mean Temp vs. Sex		438	524	432	388	480	381	440
S.E.M: Diet = 22.22***		S.E.M: TEMP = 15.71***			S.E.M: Sex = 12.83 **			
S.E.M: Diet X Temp = 38.49*		S.E.M: Diet X Sex = 31.43^{NS}						
S.E.M: Temp X Sex = 22.22^{NS}		S.E.M: Temp X Sex X Diet = 54.43^{NS}						

***P < 0.001 **P < 0.01 *P < 0.05 NS = non significant

3.3.3. Heat loss

To investigate the effect of dietary lysine, sex and environmental temperature on heat loss, ANOVA were conducted using GenStat release 6.1 on the data from each trial separately, and then combined. To avoid confusion, the result from the combined analysis is termed "combined data"

3.3.3.1. Pilot trial

The mean heat loss of broilers between one and three weeks is shown in Table 3.13. Temperature and dietary lysine were found to have a significant effect (temperature at $P < 0.001$, dietary lysine at $P < 0.05$) on heat loss. Neither sex alone nor its interaction with dietary lysine or temperature was found to have significant effect on heat loss. Maximum heat loss occurred on dietary lysine treatment 6 and treatment 5 at 24^0C, treatment 5 and treatment 4 at 28^0C, treatment 3 and treatment 2 at 32^0C, for female and male growing broilers, respectively.

3.3.3.2. Trial 1

Table 3.14 shows the mean heat loss of growing broilers between one and three weeks of age. All the main effects, temperature, sex, and dietary lysine were found to have significant effects (dietary lysine at $P < 0.001$, temperature at $P < 0.01$, and sex at $P < 0.05$) on heat loss. None of the interactions were significant. The interaction between temperature and dietary lysine was close to being significant ($P = 0.053$). Maximum heat loss for females, at 24, 28, and 32^0C was found to be on treatments 4, 3, and 5, and for males it was on treatments 4, 5, and 5, respectively.

3.3.3.3. Trial 2

Table 3.15 shows the mean heat loss of growing broilers between three and five weeks of age. All the main effects and the interactions between dietary lysine and sex were found to have a significant effect on heat loss (dietary lysine, temperature, and sex *vs.* lysine at $P < 0.001$, sex at $P < 0.05$). Maximum heat loss was observed at 18^0C. Treatments with high lysine content resulted in lower heat losses; with a maximum heat loss being on treatment 5 at all temperatures.

3.3.3.4. Combined data

Table 3.16 shows the mean heat loss of growing broilers between one and three, and three and five weeks of age. All the main effects, dietary lysine, sex and temperature were found to have a significant effect on the heat loss (dietary lysine and temperature at $P<0.001$, and sex at $P<0.01$). Maximum heat loss occurred at 18^0C.

Table 3.13. *Effect of dietary treatment and temperature on mean heat loss (kJ/bird d) of male and female broilers between 1 and 3 weeks of age (pilot trial)*

Diet treatment (g/bird d)	Sex						Mean
	Females			Males			
	Temperature (^0C)						
	24	28	32	24	28	32	
1	345	367	286	383	382	335	350
2	441	393	331	425	401	378	395
3	394	373	428	468	388	359	402
4	417	416	364	450	415	371	406
5	408	424	376	412	337	326	381
6	474	303	317	449	336	344	371
Mean	413	379	350	431	377	352	384
S.E.M: Diet = 13.88*			S.E.M: Tem = 9.82 ***			S.E.M: Sex = 8.02^{NS}	
S.E.M: Diet X Sex = 19.64^{NS}			S.E.M: Temp X SEX = 13.88^{NS}				
S.E.M: Diet X Temp = 24.05^{NS}			S.E.M: Temp X Sex X Diet = 34.01^{NS}				
***P < 0.001		*P < 0.05			NS = non significant		

Table 3.14. *Effect of dietary treatment and temperature on mean heat loss (kJ/bird d) of male and female broilers between 1 and 3 weeks of age (trial 1)*

Diet treatment (g/bird d)	Sex						Mean
	Females			Males			
	Temperature (^{0}C)						
	24	28	32	24	28	32	
1	326	314	259	315	355	260	305
2	338	313	281	342	310	312	316
3	298	357	264	359	330	264	312
4	377	298	335	394	389	315	351
5	311	340	406	389	459	353	376
6	423	345	384	475	368	366	394
Mean	346	328	322	379	369	312	342
S.E.M: Diet = 12.66***			S.E.M: Temp = 8.95**			S.E.M: Sex = 7.31*	
S.E.M: Diet X Temp = 21.93^{NS}			S.E.M: Diet X Sex = 17.90^{NS}				
S.E.M: Temp X Sex = 12.66^{NS}			S.E.M: Temp X Sex X Diet = 31.01^{NS}				

***P < 0.001 **P < 0.01 *P < 0.05 NS = non significant

Table 3.15. *Effect of dietary treatment and temperature on mean heat loss (kJ/bird d) of male and female broilers between 3 and 5 weeks of age (trial 2)*

Diet treatment (g/bird d)	Sex						Mean
	Females			Males			
	Temperature (^{0}C)						
	18	25	32	18	25	32	
1	1038	849	925	1104	741	931	931
2	1011	824	824	1317	1064	907	991
3	1074	876	775	1252	1027	1050	1009
4	1116	911	957	1289	884	918	1013
5	1414	1197	1073	1053	1117	1012	1144
6	1153	1070	1085	1398	1049	1187	1157
Mean	1134	955	940	1236	980	1001	1041
S.E.M: Diet = 32.2***			S.E.M: Temp = 22.8***			S.E.M: Sex = 18.6*	
S.E.M: Diet X Temp = 55.8^{NS}			S.E.M: Diet X Sex = 45.6***				
S.E.M: Temp X Sex = 32.2^{NS}			S.E.M: Temp X Sex X Diet = 78.9^{NS}				

***P < 0.001 *P < 0.05 NS = non significant

Table 3.16. *The effect of dietary treatment and temperature on mean heat loss (kJ/kg$^{0.67}$) of male and female broilers between one and three, and three and five weeks of age (combined data)*

Diet treatment	Sex										
	Females					Males					Mean
	Temperature treatment										
	18	24	25	28	32	18	24	25	28	32	
1	10.22	7.30	8.01	7.03	6.73	11.14	7.52	7.02	8.38	7.10	8.05
2	9.84	8.20	7.58	7.75	7.11	11.65	8.28	9.11	7.85	7.79	8.52
3	10.39	7.37	7. 98	7.75	7.22	11.67	8.95	9.35	7.68	7.51	8.65
4	10.38	8.56	8.65	7.27	8.00	12.29	9.82	8.41	8.56	7.74	8.97
5	13.44	7.97	11.54	8.59	8.88	10.59	9.45	10.13	9.06	8.21	9.79
6	10.66	9.85	9.73	6.61	8.56	12.43	10.23	9.97	7.69	8.69	9.44
Mean	10.82	8.21	9.10	7.50	7.75	11.63	9.04	9.00	8.20	7.84	8.91

3.3.4. Estimation of heat loss of male and female broilers

3.3.4.1. Pilot trial

Table 3.17 shows the estimates of the coefficients of the relationships between the amount of heat loss (kJ/bird d), environmental temperature (^{0}C), feed intake (g), degree of maturity (g/g), and feather weight (g) of broilers between one and three week of age. While the constant term and feed intake were found to have positive relationship with heat loss, feather weight, degree of maturity and environmental temperature were found to have negative relationship with heat loss. Feed intake, environmental temperature, degree of maturity and feather weight were found to have a significant relationship with heat loss ($P<0.001$). The regression relationship accounted for 69.3 percent of the variation in the data.

3.3.4.2. Trial 1

Table 3.18 shows the relationship between the amount of heat loss, environmental temperature, feed intake, degree of maturity and feather weight of growing broilers between one and three weeks of age. All the parameters considered for the estimation of heat loss were found to have a significant relationship ($P < 0.001$). While the constant term and feed intake were found to have positive relationship, degree of maturity, feather weight, and temperature were found to have negative relationship with heat loss. The regression accounted for 72.2% of the variation in the data.

3.3.4.3. Trial 2

Table 3.19 shows the estimates of the coefficients of regression of heat loss from its relationship with environmental temperature, feed intake, degree of maturity, and feather weight of growing broilers between three to five weeks of age. Feather weight, and feed intake were found to have significant relationship with heat loss ($P<0.001$). Degree of maturity and temperature were found to have no relationship with heat loss. Heat loss showed positive relationship with feed intake, and degree of maturity, and negative relationship with feather weight and temperature. In the regression analysis the constant term was non-significant and was therefore excluded. The regression relationship accounted for 80% of the variation in the data.

3.3.4.4 Combined data

Table 3.20 shows the estimates of the coefficients of the relationships between the amount of heat loss, environmental temperature, feed intake, degree of maturity, and feather weight of broilers between one and three, and three and five of week of age when all data was combined together. While feed intake and the constant term were found to have positive relationship with heat loss, feather weight, environmental temperature and degree of maturity were found to have negative relationship with heat loss. Feed intake, feed intake, degree of maturity and environmental temperature were found to have a significant relationship with heat loss ($P<0.001$). The regression relationship accounted for 67.8 percent of the variation in the data.

Table 3.17. *Estimates of the coefficients of the relationship between the amount of heat loss ($kJ/kg^{0.67}$), environmental temperature, feed intake, degree of maturity, body protein, and feather weight of broilers between one and three weeks of age (pilot trial)*

Predictor	Estimate	S.E	T – ratio	T – probability	R^2
Constant term	218.1	45.9	4.75	< 0.001	69.3
Degree of maturity (g/g)	-663	181	-3.66	< 0.001	
Feed intake (g/ b d)	8.448	0.570	14.82	< 0.001	
Feather weight (g)	-1.724	0.348	-4.96	< 0.001	
Temperature (^{0}C)	-6.01	1.08	-5.59	< 0.001	

Table 3.18. *Estimates of the coefficients of the relationship between the amount of heat loss ($kJ/kg^{0.67}$), environmental temperature, feed intake, degree of maturity, body protein, and feather weight of broilers between one and three weeks of age (trial 1)*

Predictor	Estimate	S.E	T – ratio	T – probability	R^2
Constant term	213.8	39.0	5.48	< 0.001	72.2
Degree of maturity	-1337.0	192	-6.95	< 0.001	
Feed intake	7.880	0.467	16.87	< 0.001	
Feather weight	-1.655	0.336	-4.92	< 0.001	
Temperature	-4.41	1.00	-4.39	<0.001	

Table 3.19. *Estimates of the coefficients of the relationship between the amount of heat loss ($kJ/kg^{0.67}$), environmental temperature, feed intake, degree of maturity, body protein, and feather weight of broilers between three and five weeks of age (trial 2)*

Predictor	Estimate	S.E	T – ratio	T – probability	R^2
Degree of maturity	1756	912	1.93	NS	80.0
Feed intake	9.968	0.0492	20.27	< 0.001	
Feather weight	-3.852	0.526	-7.32	< 0.001	
Temperature	-0.06	1.63	-0.04	NS	

Table 3.20. *Estimates of the coefficients of the relationship between the amount of heat loss ($kJ/kg^{0.67}$), environmental temperature, feed intake, degree of maturity, and feather weight of broilers between one and three, and three and five weeks of age (combined data)*

Predictor	Estimate	S.E	T – ratio	T – probability	R^2
Constant term	8.992	0.600	14.99	<0.001	67.8
Degree of maturity (g/g)	-16.83	3.20	-5.26	<0.001	
Feed intake (g/ b d)	0.06310	0.00340	18.57	<0.001	
Feather weight (g)	-0.06099	0.00415	-14.71	<0.001	
Temperature (^{0}C)	-0.0782	0.0145	-5.41	<0.001	

Table 3.21. *The relationship between the amount of heat lost ($kJ/kg^{0.67}$) and environmental temperature, degree of maturity, feed intake, and feather weight for treatments 1 and 2 and 3 – 4 in broilers between 1 and 3 (pilot trial and trial 1), and between 3 and 5 (trial 1) weeks of age, as well as when all data were combined*

Diet treatment	Variable	Estimate	S.E	T – ratio	Significance	R^2	S.E
T1 – T2	Constant term	7.00	2.25	3.11	<0.01	54.5	1.01
	Degree of maturity	-21.46	7.91	-2.71	0.01		
	Feed intake	0.1374	0.0237	5.79	<0.001		
	Feather weight	-0.0198	0.0132	-1.50	NS		
	Temperature	-0.1214	0.0470	-2.59	<0.05		
T3 – T5	Constant term	8.32	1.59	5.23	<0.001	61.3	1.20
	Degree of maturity	-31.72	6.70	-4.74	<0.001		
	Feed intake	0.1811	0.0217	8.33	<0.001		
	Feather weight	-0.0682	0.0134	-5.08	<0.001		
	Temperature	-0.1703	0.0381	-4.47	<0.001		
Trial one (1 to 3 weeks of age)							
T1 – T2	Constant term	6.37	1.68	3.79	<0.001	55.9	0.849
	Degree of maturity	-30.12	7.52	-4.01	<0.001		
	Feed intake	0.1274	0.0227	5.61	<0.001		
	Feather weight	-0.0342	0.0118	-2.91	<0.01		
	Temperature	-0.0741	0.0288	-1.91	NS		
T3 – T5	Constant term	8.38	1.41	5.93	<0.001	64.2	1.14
	Degree of maturity	-52.99	7.11	-7.46	<0.001		
	Feed intake	0.1603	0.0184	8.374	<0.001		
	Feather weight	-0.0387	0.0129	-2.99	<0.01		
	Temperature	-0.1336	0.0358	-3.74	<0.001		
Trial two (3 to 5 weeks of age)							
T1 – T2	Constant term	7.60	1.94	3.92	<0.001	71.3	0.888
	Degree of maturity	-5.70	15.5	-0.37	NS		
	Feed intake	0.06415	0.00924	6.94	<0.001		
	Feather weight	-0.05523	0.00931	-5.93	<0.001		
	Temperature	-0.0526	0.0272	-1.93	NS		
T3 – T5	Constant term	4.19	1.70	2.46	<0.05	63.1	1.25
	Degree of maturity	21.7	15.1	1.44	NS		
	Feed intake	0.07518	0.00786	9.57	<0.001		
	Feather weight	-0.05484	0.008858	-6.39	<0.001		
	Temperature	-0.0282	0.0261	-1.08	NS		
Combined data							
T1 – T2	Constant term	9.496	0.976	9.73	<0.001	61.9	1.07
	Degree of maturity	-10.02	5.24	-1.91	NS		
	Feed intake	0.05835	0.00574	10.17	<0.001		
	Feather weight	-0.05652	0.00658	-8.59	<0.001		
	Temperature	-0.0847	0.0228	-3.71	<0.001		
T3 – T5	Constant term	10.944	0.842	12.99	<0.001	58.2	1.41
	Degree of maturity	-16.51	4.47	-.369	<0.001		
	Feed intake	0.05976	0.00491	12.18	<0.001		
	Feather weight	-0.07229	0.00622	-11.61	<0.001		
	Temperature	0.0886	0.0206	-4.30	<0.001		

NS = Non significant

Discussion

There were two objectives to the three experiments conducted in this research project. The first was to ascertain whether the manipulation of the protein content of the feed could assist in overcoming the constraining effect of high temperatures on food intake and growth of young broilers, and the other was to calculate the maximum amount of heat that a broiler could lose to the environment.

Other than in the pilot trial, the data provided some evidence of an interaction between temperature and lysine concentration, showing a strong effect on FCE. The main effect, sex, had no significant effect on FCE, ADG or FI in the pilot trial, or in FI and FCE in trials 1 and 2, respectively.

The current experiment showed a decrease in food intake with an increase in environmental temperature. At all the temperatures studied, food intake on feeds with adequate lysine (dietary treatment 1 and 2) was lower as compared to the most limiting treatment (dietary treatment 5) and was numerically higher at the lowest temperature as compared to the moderate or high temperature considered in each trial (Tables 3.7, 3.8 and 3.9). The decrease in food intake with increasing temperature suggests that this intake was constrained by the increased environmental temperature. As the dietary lysine supply was reduced (dietary treatment 5) food intake at low temperature increased beyond those at high or moderate temperatures to compensate for the deficiency in the first limiting nutrient. This implies that broilers attempt to consume sufficient of the deficient feed to grow at their potential, but that a high environmental temperature constrains them from consuming what they desire.

As indicated in Table 3.7, broilers increased their intake of food at least until the lysine concentration was reduced to 10.2 g/kg (treatment 4). Birds failed to increase food intake on treatment 5, which is likely to be a combination of the feed being highly limiting in lysine, hence growth rate was severely hampered, and that the environmental temperature was not sufficiently low (even at 18^0C) for them to be able to lose the necessary heat to the environment.

Though the response in ADG in the pilot trial was dependent only on temperature and lysine concentration in the diet, the sex of broilers had a significant effect on the response in trials 1

and 2. As the environmental temperature increased, ADG decreased at higher lysine contents, more especially when the lysine concentration was above 10.2 g/kg, with the maximum response being achieved on treatment 6 (Tables 3.12, 3.13 and 3.14). In all trials, the lowest ADG was on treatment 5. From Table 3.12, 3.13, and 3.14 the improvement in ADG with decreasing environmental temperature could clearly be seen if observation is made starting from treatment 5 in the series of the diets. Unlike the responses the in pilot trial, the response in trial 1 implies that performance of growing broilers on an imbalanced food may be improved by decreasing the environmental temperature. The corollary is that at low temperatures it might be possible to supply growing broilers aged between 7 and 35 days diets with lower lysine content without influencing growth performance. Similar responses were noticed in an experiment conducted by Ojano-Dirain and Waldroup (2002), where broilers between 3 to 6 weeks of age fed with high lysine (1.16%) had numerically higher body weight gain but were not significantly different from the weights of birds fed the lowest lysine level at moderate temperature, and hence, these authors suggested that the low lysine level supports maximum weight gain and FCE of 3 to 6 weeks old broilers only at low temperatures.

As the lysine concentration in the diet was increasing (dietary treatment 1 – 5), birds required less food to achieve maximum daily gain, resulting in an increase in FCE. At a low temperature (24^0C for pilot trial and trial 1, and 18^0C for trial 2) growing broilers consumed more food in order to maintain their body temperature. Maximum FCE was achieved at 32^0C in each trial. As maximum FCE is most likely to occur at optimum environmental temperature, usually in the thermonuetral zone, the result of the present study showed that this zone could be somewhere in the range of 28 to 32^0C for chickens up to 21 days of age. This range is similar to the upper and lower critical temperatures, and thus the thermoneutral zone, of broilers at different ages, calculated by Meltzer (1983) in order to determine environmental temperatures that would ensure optimum growth rate, as is indicated in Table 2.2.

In growing broilers, such as those used in the current trials, the consumption of food is associated with body protein, body lipid and gross energy gains, with their rates of gain being highly dependent on the prevailing environmental temperature and nutrient concentration. Analysis of variance on protein, lipid and gross energy gain indicated that dietary lysine had a significant effect on these variables in all the experiments. At low environmental temperatures, birds attempted to satisfy their lysine requirement, resulting in over-consumption of protein and energy, which increased the deposition of lipid (Table 3.10, 3.11,

and 3.12). Had the same amount of food been consumed at the higher temperatures, birds would have retained more lipid and protein, and hence, become fatter as less energy would have been needed for maintaining body temperature. This difference in response, depending on the temperature, was the cause of the significant interaction between lysine content and environmental temperature.

The main objective can be addressed as: can the constraint of high temperatures on food intake and growth be overcome by feeding high protein feeds. The answer is no. Protein gain was lower on the high protein feeds than on lower protein, indicating that although the broilers didn't need to consume as much of this feed as of a lower protein feed, nevertheless they presumably were too hot to be able to grow at their potential. Therefore, unlike with laying hens, one cannot feed higher protein feeds at high temperatures as a means of overcoming heat stress.

One of the main objectives of this research was to determine the extent to which broilers are able to lose heat to the environment when forced to do so by conditions that would require them to lose more heat to the environment than would be possible for them to grow at their potential. The aim was based on the theory that the maximum amount of heat that the bird would lose to the environment is a function of its feather cover, its degree of maturity, food intake and the prevailing environmental temperature.

Should this hypothesis be true, the heat lost by broilesr in the current experiments must increase up to a maximum as the lysine content decreases. The results of these experiments indicated that the response in heat loss to the effect of dietary lysine followed a similar trend to the food consumption of the bird (Table 3.13, 3.14, 3.15 and 3.16), in such a way that heat loss increased as the lysine content of the diet decreased, with a maximum value occurring on treatments with the least amount of lysine. The similarities in the response to dietary lysine, sex, and environmental temperature in heat loss and food intake indicate that food intake is highly influenced by the amount of heat that the bird would lose to its environment. The result reinforces the theory of Emmans and Oldham (1988) who stated that the desired food intake could be constrained by the amount of heat that the bird could lose. From the results in Table 3.13, 3.14, 3.15 and 3.16 it may be suggested that growing broilers on treatments 3 - 5 were constrained at all temperatures by their maximum ability to lose heat to the environment when given a feed that is imbalanced in nutrient concentration, and hence, were not able to meet the

requirement for the first limiting amino acid. It is clearly indicated in Table 3.13 and 3.14, and especially, in Table 3.15, that high environmental temperatures imposed an upper limit on the amount of heat that broilers could lose to the environment. As is shown in Table 3.15, the responses in heat loss at 18^0C were numerically higher as compared to those at 25 or 32^0C, indicating that birds were able to lose more heat at 18 than at 25 or 32^0C. A similar trend is indicated in Table 3.16. This suggests that by reducing the prevailing environmental temperature, and hence, by creating an optimum environment, the amount of heat that a bird would lose may be increased, providing a greater chance for the bird to eat enough of an imbalanced food to meet its first limiting nutrient, resulting in a faster growth rate.

Emmans and Fisher (1986) suggested that the heat loss of the bird varies in some way with the prevailing environmental temperature, and as the ability to store heat is effectively zero, the rate of heat loss must equal its rate of heat production. They also suggested that the rate of intake of a given feed by a given type of bird in a given state will depend on the temperature of the environment in which the growing broiler is kept. In the pilot trial, trial 1, and in the combined data degree of maturity, feed intake, feather cover weight and temperature showed a strong relationship with heat loss and were found to have the greatest influence on the amount of heat that growing broilers produce. In trial 2, maximum heat loss was found to be dependent on feather cover weight and feed intake, but independent of degree of maturity and environmental temperature. As is indicated in Table 3.19, the constant term was not significant and therefore was not included. The coefficients in Tables 3.17, 418 and 3.20 indicate that as the amount of heat that the bird could lose to the environment increased, so the food intake was able to increase, but that an increase in degree of maturity, feather cover weight and environmental temperature caused a decrease in the maximum amount of heat that growing broilers between 7 and 21 days would lose to the environment. This suggests that feed intake is inversely related to degree of maturity, feather cover weight, and environmental temperature in such way that if any of the latter three increase, feed intake must decrease in order for the bird to remain in thermal equilibrium.

The degree of maturity did not play a role in controlling heat loss in trial 2 either with the limiting or the non-limiting feeds, nor was this important with the non-limiting feeds when the data were combined (Table 3.21). It was, however, significant in combined data in feeds that were considered to be most limiting, indicating that its inclusion had a significant effect in regulating body temperature in broilers (Table 3.21). The results in Table 3.21 also indicate

that while temperature, feed intake and degree of maturity were significant factors in determining heat loss in the pilot trial, only feed intake, degree of maturity and feather weight were significant determining factors in trial 2 in feeds that were considered to be non-limiting. In feeds that were considered to be most limiting, feed intake, feather cover weight, degree of maturity and environmental temperature played a significant role in determining the heat loss of birds in all cases. This suggests that while heat loss regulation on low lysine diets was dependent on feed intake, feather weight, degree of maturity and environmental temperature for all birds in pilot trial, trial 1 and in the combined analysis, feed intake and feather cover weight were the only factors influencing heat loss regulation of birds between 3 and 5 weeks of age (Trial 2). In all the regressions that were conducted, R^2 ranged between 53 and 72. This suggests that some factors other than those considered in this experiment may have had an effect on heat loss, thereby accounting for the unexplained variation.

It is not possible to overcome the effects of high temperatures in broiler production by feeding high protein feeds, and the amount of heat that a broiler can lose to the environment, and hence its food intake, may be quantified from the knowledge of its degree of maturity, its feather cover, and the prevailing environmental temperature.

GENERAL DISCUSSION

Nutritional challenges would, perhaps, be simple if variations in the genetic make-up of animals and their complex interaction with the existing environment and feeding systems did not exist. The challenges of a nutritionist would also be simplified if it were not for the demand of high production (meat and egg) at low input cost and the desire to accommodate aspects of variation in individuals, populations, feeding, environment and production into the area of nutrition to maximize profit.

There has been an increasing awareness of the extent to which environmental factors, especially, high temperature, has an adverse effect on poultry production. The response in the physiological, metabolic, behavioural and productive changes that occurred when broilers and laying hens were exposed to high environmental temperature, and the overall energy and nutrient requirement gave an understanding for the reasons why potential growth rates and egg productions are impaired, and hence enabled for suggesting a means of alleviating the problem. The methods applied for estimating the effective energy of the diet by linear coefficients to five measurable components of interaction that exist between broilers and laying hens and the diets used in adjusting ME for heat increment of feeding were helpful in understanding the relationship between the two energy scales, and thus in creating a means of combining them to see if the adverse effect of the environment could be relieved by using different energy ratios. Two trials were conducted in this research protocol. The objective of the first trial was to overcome heat stress in laying hens by designing feeds that had a lower heat than conventional diets, and to determine whether these were more beneficial at high than at low nutrient densities. Accordingly feeds were designed (Table 2.1), feed to 360, 46 weeks of age Hy-Line brown laying hens in cages in an open sided house during hot weather.

It is well understood that feed intake decreases as the energy content of the feed is increasing, but feeding cost increases with the increase of both EE and ME. Results from this trial indicated that feed intake remained higher on diets with high EE:ME ratio, but generally showed a linear decline as energy content of the diet increased resulting in a numerically higher egg output. However, the general trend reduction in feed intake with increasing energy content was not enough to prevent an increase in feeding cost. Thus, feeding cost increased linearly as the dietary energy content increased. Despite this, however, the lowest feeding cost

was realized at high EE:ME ratio than at low EE:ME ratio and vice versa. The feeding cost associated with high EE:ME ratio was observed to be R 0.0645, 0.0458, and 0.0457 lower that low EE:ME ratio feeds at 11.3, 12.15, and 13.00 MJ/kg dietary energy levels. For all the various pricing structures analysed, laying hens under high EE:ME ratio diets were being able to produce heavier eggs, and thus, an increased outcome from the sale of eggs. They also showed an increasing trend in gaining weight gain, which indicates that birds were being able to concur the adverse effect of environmental temperature, and thus an increase in the out come from the sale of the chicken if that sale was going to be considered. As profit is the difference between the cost of input and output of producing a dozen of eggs, it was obtained to be the highest in treatments with low dietary energy but high EE:ME ratio. Also, the profit generated showed a decreasing pattern when the energy content of the diet was increasing, but still highest at high EE:ME ration diets. Therefore, economic wise the high EE:ME ratio diets seems to be cost effective as compared to the low EE:ME diets. Also, the combination of low dietary energy with high effective energy seems to be more beneficial as compared to treatments with high dietary energy diets.

The optimum economic combinations of the EE:ME ratios and dietary energy content that maximize profit seems to be dependent on feeding costs and the rate of lay (rather than egg weight), as the variation in the proportion of eggs for the different EE:ME ratios and dietary energy combinations falling under the same egg grade is small, and hence, the combination with the lowest feeding cost and highest rate of lay resulted in the highest profit.

It is of importance to define the broiler as thermally active creature. An interaction with the environment defines the zone of comfort in which the broiler will be able to grow at its potential. Heat losses and heat productions are correlated to the comfort zone. Functions within the broiler contributes to heat loss and heat production, and it is necessary to find out how the feeding system should alleviate or aggravate the production and losses of heat. The objective of the second experiment was to determine the extent to which broilers are able to lose heat to the environment when forced with conditions that require them to lose heat to the environment than would be possible for them to grow at their potential. This objective was based on the theory that the maximum amount of heat that growing broilers are able to lose to the environment is a function of the feather cover weight, its degree of maturity, feed intake and of the prevailing environmental temperature. Three lysine-limiting trials were conducted, with their responses measured at three temperatures, six diets and two sexes, and over two

growth periods. The first trial was a pilot trial of the second trial, both of which were conducted on broilers between 1 and 3 weeks of age. The third trail was a finisher trial and was conducted using broilers from 3 to 5 weeks of age. These experiments were conducted in six environmentally controlled chambers, and the different temperature schedules were randomly assigned to each of the six environmentally controlled chambers making two replications per temperature treatment. Birds were randomly assigned to the two chambers, and within the chambers birds were randomly assigned to each cage. Six feeds were designed using the summit and dilution technique in such a way that the sixth diet had the same proportions with the summit plus dilution diets as the fifth diet, but was supplemented with the synthetic lysine to give the same content as diet four.

In all the three trials conducted, result in FCE, FI, and ADG showed a positive response to the addition of synthetic lysine to dietary treatment five, indicating that lysine was the fist-limiting nutrient in the series of diets. Comparison was made between treatments with adequate lysine content (treatment 1 and 2) and treatments with poor lysine contents (treatments 3, 4, and 5). At all temperatures studied, treatments with adequate lysine contents showed lower food intake as compared to treatments with poor lysine contents. The numerical values indicated were higher at the lowest temperatures as compared to the moderate or high temperature schedules used in all trials, indicating that the lowest temperature schedule used in each experiment was really cold and birds were forced to increase their food intake to maintain normal body temperature. The most interesting point about this was birds were forced to eat much food, and yet were being able to convert it efficiently to increase their body weight without producing superfluous heat.

As predicted the results in pilot trial, trial one and combined data showed that heat loss was strongly dependent on the main effects, including, feed intake, feather cover weight, degree of maturity, and environmental temperature. In trial two, heat loss was found to be independent of environmental temperature and degree of maturity, but dependent on feed intake and feather cover. Also in pilot trial, trial one and combined data the relationship was positive with feed intake but negative with degree of maturity, feather cover weight and environmental temperature. In trial two the relationship was positive with feed intake and negative with feather cover weight. The results in pilot trial, trial one, and especially in the combined data, confirmed the proposed hypothesis showing that the heat lost by growing broiler to the environment is a function of feather cover, feed intake, environmental temperature and degree

of maturity. The regression coefficient of food intake in these relationships was inversely related to degree of maturity, feather cover weight, and environmental temperature in such a way that if any of the latter three increase, feed intake must decrease in order for the bird to remain in thermal equilibrium. Excluding the regression coefficient of degree of maturity and temperature, the regression coefficient in trial two also suggest that for the bird to remain in a state of thermal equilibrium, the rate of feather cover weight must decrease as the rate of feed intake is increasing. On the other hand, at high temperature, higher lysine levels will be required to achieve maximum growth performance.

In conclusion the results from the last three experiments suggested that as the environmental temperature decreased broilers were able to increase their food intake, and hence increase in the lysine intake on feed treatments with the most lysine limiting because of the increased heat loss capability. Therefore, at low environmental temperatures broilers will better make use of poor quality foods, and thus compensate better for the deficiency in lysine in the series of diets. On the other hand, at high temperature, higher lysine levels will be required to achieve maximum growth performance. Also from the combined regression analysis, it can universally be concluded that heat loss is greatly influenced by degree of maturity, feather cover weight, the existing environmental temperature and feed intake in such a way that as the rate of the first three increases the rate of food must decrease for the bird to remain in a state of thermal equilibrium. But the low fit in the models suggest that care must be taken in interpreting the data, as other factors than those included here may have an effect in heat loss.

The result of the present study gave some indications on the profit trends that can be achieved when working with laying hens of 46 to 56 ages. These birds were quite old and were not at their peak rate of lay. Therefore, the research suggest that there is a need to investigate the economic benefits that can be conferred from the use of high EE:ME ratio diets by using young laying hens, and if possible, should comparison be to done between young and old laying hens, a more meaningful and conclusive results will be achieved. Further, the high-energy diets may have an influence in egg size. Therefore, the research recommends the need for an investigation regarding the effect of high EE:ME ratio diets on egg size, especially, at high temperature at constant and variable humidity levels. Although the results of the last three trials with broilers responded as expected at constant temperature and humidity levels, it might not be the case with variable temperature and humidity levels. Therefore, the research recommends the need for further investigation regarding the response that might be achieved

to lysine limiting diets at constant temperature *vs.* variable humidity levels, and at variable temperature *vs.* humidity levels.

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APPENDIX 1

Egg weight	Egg proportions					Egg price (C/egg)			
	Small	Medium	Large	X-large	Jumbo	Normal	15% Decrease	15% Increase	Jumbo
45	28.9	62.8	8.0	0.3		27.36	23.25	31.46	0.18
46	20.8	65.4	13.1	0.7		28.12	23.90	32.34	0.43
47	14.4	64.4	19.6	1.6		28.99	24.64	33.34	0.98
48	9.6	60.2	26.8	3.3	0.1	30.06	25.55	34.57	2.11
49	6.3	53.8	33.6	6.0	0.3	31.33	26.62	36.03	3.93
50	4.0	46.0	39.4	10.0	0.6	32.87	27.93	37.80	6.64
51	2.5	37.8	43.3	15.0	1.4	34.75	29.53	39.96	10.38
52	1.5	30.0	45.0	20.8	2.7	36.92	31.37	42.46	15.03
53	0.9	23.0	44.3	26.9	4.9	39.43	33.51	45.35	20.63
54	0.5	17.2	41.4	32.6	8.3	42.26	35.91	48.60	26.99
55	0.3	12.5	37.2	37.2	12.8	45.24	38.44	52.02	33.61
56	0.2	8.8	32.2	40.2	18.6	48.35	41.09	55.60	40.34
57	0.1	6.1	26.8	41.5	25.5	51.52	43.78	59.24	46.96
58	0.1	4.2	21.6	40.8	33.3	54.59	46.40	62.78	53.10
59	0.0	2.8	17.0	38.6	41.6	57.55	48.91	66.18	58.75
60		1.8	13.0	35.2	50.0	60.28	51.23	69.32	63.75
61		1.2	9.7	30.9	58.2	62.73	53.32	72.14	68.03
62		0.8	7.1	26.4	65.7	64.85	55.12	74.58	71.60
63		0.5	5.1	22.0	72.4	66.67	56.66	76.67	74.55
64		0.3	3.6	17.8	78.3	68.20	57.96	78.42	76.95
65		0.2	2.5	14.1	83.2	69.42	59.01	79.83	78.81
66		0.1	1.7	10.9	87.3	70.43	59.86	80.99	80.31
67		0.1	1.2	8.3	90.4	71.15	60.47	81.82	81.32
68			0.8	6.3	92.9	71.75	60.99	82.51	82.21
69			0.5	4.6	94.9	72.21	61.38	83.04	82.85
70			0.4	3.3	96.3	72.51	61.63	83.39	83.23
71			0.2	2.4	97.4	72.77	61.85	83.69	83.61
72			0.2	1.7	98.1	72.91	61.97	83.85	83.77
73			0.1	1.2	98.7	73.05	62.09	84.01	83.97
74			0.1	0.8	99.1	73.13	62.16	84.10	84.06
75				0.6	99.4	73.21	62.23	84.19	84.19