# Effects of dietary changes on heat stress in broiler and Kampung chickens

S y a f w a n

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## Abstract

Poultry meat production has increased drastically over the last 35 years. Most developing countries are in the tropics and often have high ambient temperatures. At high ambient temperatures, chickens exhibit a slower growth rate due to a reduced feed intake. In order to limit the reduction in feed intake, a feeding strategy should be applied which decreases the level of heat production and/or increases the possibilities for heat dissipation. Such a feeding strategy can be based on feed that gives less heat because of lower energy costs of digestion or provides fewer nutrients that lead to a high heat production. This thesis studied how birds subjected to chronic heat stress change feed intake of especially crude protein and total energy. In addition, a wet feeding strategy that might alleviate the adverse effects of heat stress on performance was applied. Chickens exposed to choice feeding responded similarly at normal and at high ambient temperatures. They composed from a choice of a control diet, an energy rich diet and a protein rich diet a diet with a higher energy (HE) content and a lower protein content compared to the standard control diet. At high temperatures, chickens will reduce feed intake because they want to avoid or reduce a high body temperature. When the chickens were fed a wet-HE diet, feed intake and BW gain were higher in broiler chickens compared with feeding a dry-HE diet. The most beneficial effects of a wet diet occurred with a high energy diet. The indigenous Kampung chickens have been acclimatized to a high ambient temperature and did not benefit from a wet diet.

In the present study, temperature had a major effect on relative lengths and empty weights of gastrointestinal tract segments in broiler chickens on day 42. Relative lengths of most gastrointestinal tract segments were affected by diet formulation at day 21 and 42, but not relative empty weight. Control-fed birds had shorter relative lengths than HE-fed birds, suggesting that the higher BW gain of control-fed birds were not accompanied by a similar increase in length of the gastrointestinal tract. Wetting the diet did not increase empty weights of intestines both at days 21 and 42. In Kampung chickens, effects of diet formulation on gastrointestinal tract development disappeared when the birds grew older, suggesting that this type of bird adapts easily to changes in dietary nutrient content. A positive effect of the relative weight of the intestine of birds fed a wet diet was observed in Kampung chickens. In general, it seems that the Kampung chickens grow proportionally but broiler carcass is growing faster than its gastrointestinal tract in control- and wet-fed birds.
**Key words**: Self-selection, temperature, wet diet, broiler, indigenous chicken

## Foreword

Grown up in a village with large families, I was not supplied with an adequate and nutritious food. We had animal protein in our daily food only on special occasions during a year. Our daily animal protein was mainly from a river fish and it was really hard to get an egg and poultry meat even though our indigenous chickens were scavenging around close to our feet every day. This situation is still common for many families in small villages in Indonesia.

Indigenous ‘Kampung chickens’ have been raised for many centuries by local farmers, but surprisingly, there are only three books and a few articles available regarding our indigenous chickens today and therefore it is far from just sufficient how to raise these chickens to get a sufficient production for daily consumption. On the other hand, broiler chickens have been investigating for decades mainly under temperate zone and specific nutritional information for hot regions is still far from complete.

This thesis consists of seven chapters including introduction and general discussion covering aspects of responses in intake and performance to high ambient temperatures. The five chapters contain papers that are accepted by or submitted to an international journal. These chapters describe the literature on heat stress, and strategies to find out the basic nutrient requirements, namely protein and energy, not only for broilers but also for indigenous ‘Kampung’ chickens that match with their physiological requirements under high ambient tropical conditions. Furthermore, the strategy to increase feed intake and alleviate the adverse effects of high temperature were also investigated.

This thesis does not cover everything that guarantees an increased poultry production under hot temperature conditions and further research is suggested. I would like to remind that researchers should not forget indigenous chickens because this food feeds all local people in developing countries.

With love to my wife,

*Muspirah*

My sons,

*Ahmad Dairobi Saputra*

*Ahmad Zohiri Saputra*

And my daughter,

*Nafisa Az-Zahra*

Table of Contents

# CHAPTER 1

## General Introduction

## Background

Worldwide poultry meat production increased fast between 1970 and 2005 (Windhorst, 2006). However, poultry meat production systems and market shares vary considerably between continents. North and Central America as well as Europe have lost some share of the world market in the time period 1970 to 2005. Asia and South America have increased their contribution to global production considerably. In 2005, the latter contributed by almost 50% to global poultry meat production, whereas their contribution was less than 24% in 1970. Chicken meat reached the highest share with 88.3% relative to other poultry meat type such as Turkey, Duck and Goose in the mid 1980s, after that it decreased to about 86% and has remained fairly stable since then. In 2005, five of the ten leading countries were developing countries and four were located in Asia. The United States were still in the top position with a share of 22.9%, but China, Brazil and Indonesia are now ranked number two, three and nine (Windhorst, 2006).

Within continents, states or countries differ in climatic conditions. In areas with a high ambient temperature (HT) above the thermoneutral zone for chickens (21-22$^{o}$C) throughout the year, broilers experience a difficulty in dissipating the body heat because of a small difference in temperature between the animal's body and the environment (Etches et al., 2008). So, as a result they try to reduce heat load. This leads to less feed consumption (NRC, 1981; Whittow, 1986; Scheele et al., 1987; Otten et al., 1989; North and Bell, 1990; May and Lott, 1992; Yahav, 2000) and more water intake (NRC, 1981; North and Bell, 1990) and slower rate of body weight gain (Cahaner and Leenstra, 1992; Yalçin et al., 1997) compared to birds in moderate climatic conditions. Other researchers have noticed changes in the development of the gastrointestinal tract (Mitchell and Carlisle, 1992). Some behavioral and physiological adjustments of birds during HT exposure are shown in Figure 1. High ambient temperature can be a major limiting factor in productivity of poultry in hot climate conditions, especially in developing countries where farmers cannot afford costly artificial control of ambient temperature in broiler houses (Deeb and Cahaner, 2002).

High ambient temperatures, as well as other constraints like health, housing and the lack of balanced diets have led to the development of a type of bird which is generally kept in developing countries. In Indonesia, the indigenous bird 'the Kampung chicken' does not have a high production. So with an increasing human population, it cannot provide sufficient animal protein for human consumption on a daily basis (Diwyanto and Iskandar, 1999). On the other hand, this type of bird has been developed by the rural population under tropical climatic conditions in Indonesia for a long time, and is adapted to conditions there. This Kampung chicken plays an important role in the cash flow for rural people. These chickens produce a considerable amount of poultry meat (32% of total poultry meat production in 1997 in Indonesia) and eggs (17% of total egg production in 1997 in Indonesia). In addition, the price of meat and eggs from Kampung chickens is relatively stable (Diwyanto and Iskandar, 1999; Riethmuller, 1999).

A constraint to an optimal productivity of Kampung chickens is our lack of knowledge concerning their actual potential and what nutrient requirements they would need for reaching that potential. In general, most diets for chickens are nowadays formulated with feeding table values that were based on trials that were carried out under temperate conditions. Considering that Indonesia has a tropical climate (most hot and humid) (WorldFactBook, 2007), this could mean that diets formulated for birds under temperate conditions may not be optimal for indigenous and broiler chickens under HT. Therefore, some researchers have adjusted dietary compositions for different intensities of heat stress as experienced at different geographical locations and for different management strategies (Balnave, 2004).

Nowadays, not much is published on nutrient requirements for protein and about nothing for Kampung chickens kept under HT. There are some adjustments to hot conditions made by NRC (1994). Body weight (BW) gains of (exotic) broilers, such as the Ross 308 bird, and Kampung chickens differ considerably. Broilers multiply their hatching weight (40 g) 73 times at 6 weeks of age (2.9 kg) (Havenstein et al., 2003), whereas Kampung chickens multiply their hatching weight (27 g) 29 times at 10 weeks of age (0.77 kg) (Kompiang et al., 2001). Such differences in BW gain and thermotolerance under HT may influence the thermal comfort temperature profile of both types of birds. In addition, there is no information available about the optimal temperature range for Kampung chickens to have an optimal production which should represent their potentials. When ambient temperature is above a certain threshold, evaporation by panting may be the only means by which birds can release heat. Therefore, evaporative heat loss becomes the major route in the heat balance in birds. The major part of evaporative heat loss is from the respiratory-evaporative mechanism through increased respiration, known as panting behavior (Marder and Arad, 1989). Heat loss through panting can account for 60% of the total heat loss (Etches et al., 2008) and even up to 80% at very high temperatures (32$^{o}$C) (Ahmand and Sarwar,

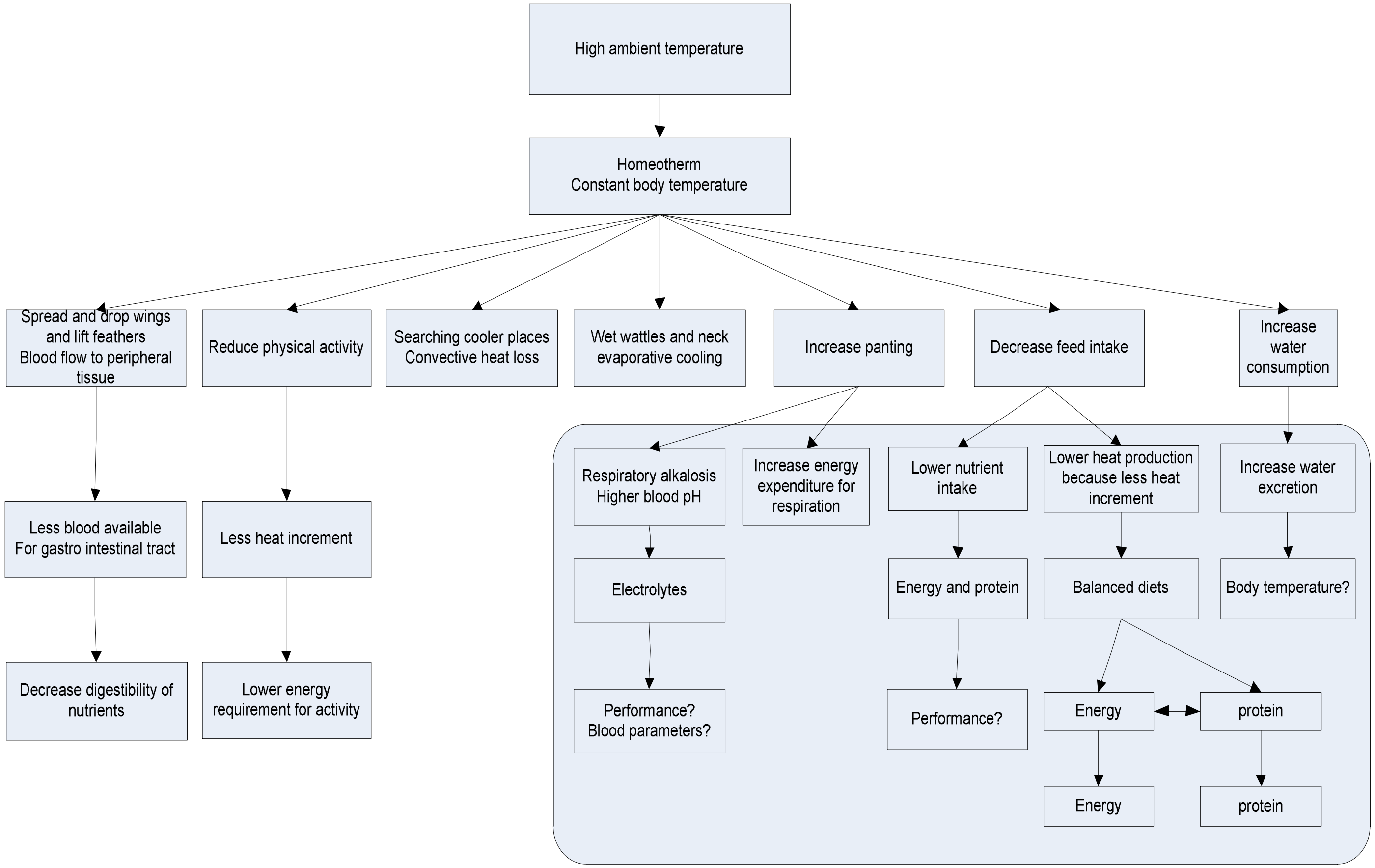


**Figure 1**. Behavioral and physiological adjustments of chickens at high ambient temperatures and its effects on nutrition. Modified after Veldkamp (2002).

2006). Also in confined housing, with no panting, the birds can give off their heat load by evaporation in addition to sensible heat loss. Evaporative heat loss is associated with loss of water and therefore dehydration can occur. Sufficient water intake will facilitate heat loss and contribute to thermotolerance at HT (Yahav et al., 2005).

Ideally, we would like to know the appropriate diet formulation for birds under HT that guarantees a good performance and does not compromise thermal comfort. In order to reach this situation for birds in a hot environment various strategies can be applied. One of them may be to allow the chickens to determine their own intake of protein and energy. Each nutrient will undergo changes after digestion and because of its use in synthesizing body protein and lipid those changes are associated with energy losses (Black, 1995). The bird is then able to influence the heat load associated with metabolism by enabling them to choose from a few feeds with different nutrient contents. This approach is one of the aspects studied in the present thesis.

Another approach to increase feed intake and thereby BW of chickens under HT is to provide feed in a form that may cost less energy in the animal body to process. For that purpose, wet diets were provided (water added 1:1 to a dry diet). Because this diet is soaked it may facilitate digestive enzymes to digest and may cause a more stable digestion with less heat peaks. This may also change the heat production pattern and thus may reduce heat load and at the same time will not be harmful for the bird. The ultimate goal is to design a feeding strategy that alleviates the adverse effects of heat stress. The study focuses on a comparison between the modern broiler and the Indonesian indigenous chicken (Kampung chicken). Broilers have been selected under conditions of abundance of energy and nutrients and have developed into very fast-growing animals. Kampung chickens have been developed under subsistence farming and it is thought that Kampung chickens may grow faster if provided a better diet. Apart from production performance, also the development in size and length of the gastrointestinal tract (GIT) will be investigated.

## Scope of the study

This thesis will focus on how chickens (both broilers and Kampung chickens) cope with HT when they can choose between energy and protein rich diets. In addition, we will investigate whether the physical form of a diet (by comparing wet with dry feeding) may also influence the response at HT. Traits to measure will be:

performance, gut development and heat tolerance. It is aimed to find out the optimal energy-to-protein ratio for every phase of growing for both types of chickens at HT.

Voluntary feed intake will decrease as ambient temperature increases. A reduced energy intake at a HT leads simultaneously to a reduced intake of other essential nutrients. This is a direct cause for the reduction of growth. A lower feed intake is a response of chickens to HT in order to reduce heat load. At HT, the animal has a reduced capacity to dissipate the heat load derived from the digestion of the feed and from further metabolism of the nutrients. So it will try to reduce this heat load.

An increased heat production with processing of extra feed has been referred to as heat increment. The heat increment produced after feed consumption and during digestion depends on the chemical composition of the diet. In literature it is stated that dietary protein has a higher heat increment when compared with fats and carbohydrates in birds under thermo neutral conditions (Black, 1995; Musharaf and Latshaw, 1999). Considering that protein has a high heat increment, a reduction of the dietary crude protein level has been recommended for broilers under heat stress. However, reports have shown that low-protein diets have negative effects on broiler performance when environmental temperature is high. During heat stress, a low feed intake is also seen with low protein diets. Such practice can result in amino acid deficiencies, resulting in poor feed efficiency and in a reduced BW gain (Buyse et al., 1992; Alleman and Leclercq, 1997; Furlan et al., 2004). Therefore, on the other hand, high energy density was suggested to be necessary under HT because high density diets mostly have high digestibility and thus less energy loss associated with processing. So the animals have less heat increment which does not need to be given off by panting (Balnave and Brake, 2005).

Dietary manipulations by adding water to the feed are known to improve feed intake, weight gain and/or feed conversion ratio under temperate conditions (Yasar and Forbes, 1999; Khoa, 2007). Water can also alter some aspects of the digestive tract of the birds like weight and length. It can increase passage rate and birds may consume more water under high temperature to reduce the increase in body temperature. Increasing total water intake under HT may benefit the bird because it facilitates heat loss. This may induce a lower rise in body temperature which in turn could result in maintaining a high level of feed intake and, as a consequence, a high growth. A general consequence of high intake is the increase in size of the gastro intestinal tract.

The main objectives of this study are to evaluate if chickens will adjust their diet under heat stress and to evaluate the impact of choice feeding, diet conformation (dry and wet) and composition on feed intake behavior, performance and GIT development, in broilers and Kampung chickens, when these birds are subjected to heat stress conditions. Also, the performance in various parts of the growing periods for Kampung chickens will be studied.

The aim of this thesis was to find out a preferred nutrient density in the diet and from that a desired feeding strategy to minimize the possible adverse effects of HT on performance and gut development of both types of chickens may be developed.

## Thesis outline

Chapter 2 is a literature review about feeding strategies that may reduce heat stress in growing meat chickens. The temperature effects for the animal and pathways for heat dissipation from the chicken are described. General effects of heat stress on bird physiology, feed, growth and water intake are included. Ambient temperature is one of the most important factors influencing energy requirement. Protein and amino acid are explored because protein and amino acid catabolism is associated with high heat production when compared with catabolism of fats and carbohydrates in birds under thermoneutral conditions. At the end of the review, management strategies to combat heat stress are presented. Some feeding strategies are discussed in more detail. Based on the conclusions of the review, experiments are proposed to get more detailed information on the responses of male broilers and indigenous chickens when given the opportunity to make a choice diet with regard to protein and energy content from various feeds under the constraint of HT.

In Chapter 3, the chronic exposure of fast growing male broilers to a normal or high ambient temperature is studied by applying a self-selection strategy. This is done to derive the preference for energy and protein density. The HT cycling or relative humidity are chosen to cover the same values as in a tropical climate. Performance, energy and protein intake are examined as well as feed intake behavior.

In Chapter 4, we conducted an experiment to determine the protein and energy intake in Indonesian’s indigenous chicken under hot tropical climatic conditions in Muaro Jambi District, Jambi Province, Sumatera, Indonesia when given the opportunity of choice feeding from an energy-rich and from a protein-rich feed. The results are compared with a control diet. At the moment, no standard nutrient requirements for this type of chickens are available. Self-selection may allow the chicken to choose a diet which optimizes the heat load associated with metabolism of nutrients ingested. Performance, energy and protein intake and feed intake behavior are examined. From this experiment, conclusions can be drawn to adjust dietary energy and protein density in diet.

Based on the results of Chapter 3, we designed an experiment to crosscheck the best self-selected feed formulation under HT for broilers and we additionally applied a wet or dry feeding strategy with the previously chosen feed composition at HT. We expect that wet feeding can undergo digestion with less work for the GIT compared to dry feeding. If less energy is needed for GIT to work then also less heat is produced and need to be dissipated. It was hypothesized that wet feed can be expected to alter some aspects of the digestive tract and the chicken may increase its feed intake and BW gain under HT. The aims are to increase feed intake and BW gain under heat stress by offering the possibilities to a lower rise in heat production. Feed intake behavior, performance and gut development are evaluated. The results are described in Chapter 5.

In Chapter 6, a wet feeding strategy is used to test the capacity of both Kampung chickens and broilers kept at tropical conditions to ingest a diet resulting from the self-selection studies as described in Chapters 3 and 4 and compared with a dry diet. There is no information available about wet feeding dealing with indigenous chickens and a few studies in broilers under tropical temperature. It can be expected that wet feeding may increase BW and birds will reach a higher BW in the same period of time. In addition, increasing water intake may benefit the bird by acting as a source of evaporation.

In the General Discussion (Chapter 7), the results reported in the Chapters 3 to 6 are reviewed and evaluated. The discussion will be based on how ambient temperature affects self-selection and how wet feeding strategies can reduce heat stress.

# CHAPTER 2

## Heat Stress and Feeding Strategies in Meat-Type Chickens

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## Abstract

Heat stress can induce hyperthermia in poultry. A reduction in heat load can be achieved by increasing the possibilities for heat dissipation, decreasing the level of heat production or by changing the heat production pattern within a day. Strategies to reduce the negative effects of heat stress can be based on a specific feeding strategy such as restricted feeding. Feed offered long enough before a hot period can avoid the harmful effects of a high temperature. Another strategy may be choice feeding from different feed ingredients, rich in protein or in energy. With such self-selection, the chicken can adjust its intake of individual components and this allows the bird to optimize the heat load associated with the metabolism of the ingested nutrients. Additional promising strategies involve offering a choice between feeds with a different feed particle size or structure. A large particle size contributes to the development of the gastro-intestinal tract (GIT), especially the gizzard and the caeca. A large gizzard will maximize the grinding process and potentially ease digestion down the GIT, thereby reducing heat production associated with digestive processing. Also wet feeding may be profitable under heat stress conditions as well. A wet diet may facilitate an increased water intake and larger particle sizes can limit water excretion with droppings resulting in more water available for evaporation during panting and cooling of the bird. In conclusion, these feeding strategies may help to reduce heat production peaks, facilitate evaporative activity and/or decreases the heat load, resulting in beneficial effects on performance and health of the bird kept in the tropical areas worldwide.

**Key words:** high temperature; self-selection; wet feed; broiler; particle size, gastrointestinal tract

## Introduction

In Western European countries, meat-type birds are mostly kept in confined systems in temperate zones. These broiler chickens have been selected for a high growth rate for decades (Havenstein et al., 2003). They show high feed intakes and thus high metabolic rates. In addition, they are able to regulate their heat balance relatively well and do not spend much energy on activity. In many other parts of the world, however, particularly in warm tropical and subtropical regions as well as in the southern part of Europe, poultry are kept in (semi)-intensive-outdoor-systems. In these systems, high ambient temperatures (HT) can have detrimental effects on production efficiency. A HT is known to depress growth rate and reduce meat yield of commercial broilers (Cahaner and Leenstra, 1992; Yalçin et al., 1997). Apart from inducing a high mortality rate, a decreased feed intake and a decreased body weight gain, HT also seems to negatively affect intestinal development (Mitchell and Carlisle, 1992; Garriga et al., 2006). At HT, broilers showed disturbance in the acid-base balance and increasing respiratory rate can lead to a respiratory alkalosis (Borges et al., 2007).

In nature, when chickens are exposed to HT, they may use water to splash their combs and wattles in order to increase evaporative cooling from these surfaces (Dawson and Whittow, 2000). They also use sand baths to dissipate the heat from the body, move to a shaded area or seek a micro-environment that avoids extremely highly environmental temperatures. They can also express their normal behavior like foraging, thereby ingesting those ingredients that avoid excessive heat loads while being ingested and metabolized. On the other hand, heat-stressed chickens tend to distance themselves from each other, pant, and often stand with their wings drooped and lifted slightly from the body to maximize heat loss under those conditions (Etches et al., 2008).

The bird in this condition increases the flux of heat from the tissues to the environment by behavioral changes. Under hot temperature conditions, the animal will apply physiological, anatomical and behavioral mechanisms aimed at facilitating heat loss to, or minimizing heat gain from the environment (Etches et al., 2008). Therefore, poultry farmers in regions with high temperatures must find ways to apply management and feeding strategies to facilitate the birds to cope with heat stress. Unfortunately, there are only few scientific studies that report on birds under heat stress in extensively managed systems, such as in tropical countries.

A number of approaches to reduce the impact of heat stress have been reported in literature. A solution for the prevention of heat stress deserves a multifactorial approach and may include disciplines such as genetics (Gowe and Fairfull, 2008), housing (Yahav et al., 2004), thermal conditioning (Yahav and McMurtry, 2001), and feeding and nutrition (Balnave and Mutisari Abdoellah, 1990; Moritz et al., 2001; Uni et al., 2001; De Basilio et al., 2003; Zarate et al., 2003a, b; Balnave and Brake, 2005; Ahmad and Sarwar, 2006; Daghir, 2008a). Recently, Lin et al. (2006) reviewed potential strategies to combat heat stress, including the use of Naked neck genes, thermal conditioning and the provision of certain micronutrients (vitamins and minerals).

The present review will focus on the within day heat production patterns, as a result of a change in feeding management (diurnal feeding patterns, self-selection, coarse particle and wet feeding) in meat-type chickens to alleviate heat stress.

## Effect of HT on heat production and heat loss

Development in the genetic selection of meat-type birds has led to rapid growth and a high metabolic rate, which is accompanied by a high heat production level due to increased feed intake Birds are homeothermic animals and able to maintain body temperature (**T**) within a narrow range (Yahav et al., 2005). When the ambient temperature is high (Yahav, 2009), the bird has less ability to dissipate the heat.

Body T of domestic chickens is within a narrow range that is reflected by an upper and lower limit of a circadian rhythm in deep body T (Etches et al., 2008). When exposed to a hot environment or by performing vigorous physical activity or both, body T can rise. This occurs when the additional heat cannot be dissipated within a short time. The increased body T will not last for a long period of time because the animal thrives to its normal body T. Conversely, when birds are exposed to a cold environment, heat is lost from the body and, unless the heat is compensated by extra metabolism, body T will decline until the bird is unable to survive and dies. These effects have been synthesized into the concept of the thermoneutral zone (TNZ), with lower and upper critical temperatures. For more details about the TNZ concept, see Mount (1979).

There is a large variation in the ideal temperature range for different classes and age-groups of poultry. This is due to variation in type of birds and variation in some aspects of the environment. As for the optimum temperature range, what is ideal for heat exchange may not be optimal for production such as for growth, for egg mass or for feed efficiency. The overall optimum range is mainly dependent on the relative market value of the product produced, in proportion to feed costs. As the ratio of price of feed to gain increases, the best temperature is the one which provides the lowest ratio (Daghir, 2008b).

### HEAT PRODUCTION AS A RESULT OF FEED INTAKE

Thermo neutral heat production (HP) at a given intake and ambient temperature determines the range of the comfort zone for an animal. In growing birds, maintenance metabolism is a large part of HP. HP from maintenance will be higher if an animal exerts physical activity in order to gather food and water (Gous and Morris, 2005). Foraging related activities need more energy (Andersson et al., 2001) and thus increase total heat production. The form of feed which is offered can also influence the energy expenditure related to feed consumption activity. The time spent for eating a meal in pelleted form was reduced to one third compared to meal in a mash form. Eating a pellet diet instead of a mash diet saves about 6% energy (Gous and Morris, 2005), which could be beneficial at HT.

HP is the resultant of the heat produced due to energy use associated with digestion processes like transport of digesta in the gastro-intestinal tract, release of enzyme, absorption and utilization of nutrients. Together these processes are part of the heat increment caused by feed consumption. It has been shown that heat stress could decrease the digestibility of dry matter, protein, and carbohydrates, whereas fat digestibility was relatively unaffected (Puvadolpirod and Thaxton, 2000b).

Broiler HP is particularly high because of a very high growth rate, mediated by a high feed consumption. The inefficiency of conversion of feed above maintenance into protein and lipid is about 20 to 25%. All feed energy needed for maintenance is heat. Wiernusz (1998) estimated that about 60% of total ME intake is lost as heat (maintenance plus 25 to 30% of the ME above maintenance). If feed intake diminishes at HT this means that HP from feed above maintenance decreases and thus also total HP. In addition, HP normally increases with an increase in total protein accretion (MacLeod, 1997).

HP in broiler is also dependent on the genetic line (Buys et al., 1999). Broiler lines selected for fast growth accompanied with a low FCR had a lower HP as compared to lines selected either for slow growth with a low FCR or slow growth with a high FCR. A bird with a very fast rate of growth has a problem with its respiratory and/or cardio-vascular system that is unable to cope with the increased metabolic demands. This is shown by a higher $pCO_2$ and lower $pO_2$ in their venous blood as compared to other genetic slow growing lines, indicating a lower total $O_2$ and $CO_2$ carrying capacity of these birds. This will lead to a low HP.

HP over a 24 h period is not constant and depends on the activity pattern of the animal over the day. Extra HP due to feed intake should preferably not be generated during the hottest period of the day. Broiler chickens kept in intensive, temperature controlled, dark houses experience no particular hot period. However, with a natural day light scheme (such as in extensive tropical poultry systems), this may not be achievable because the poultry houses often have a natural light and dark scheme. So it is dark during the coolest period of the day and feed intake will not occur. Therefore, feed intake mainly occurs during the hot day time and the birds can suffer with a high HP.

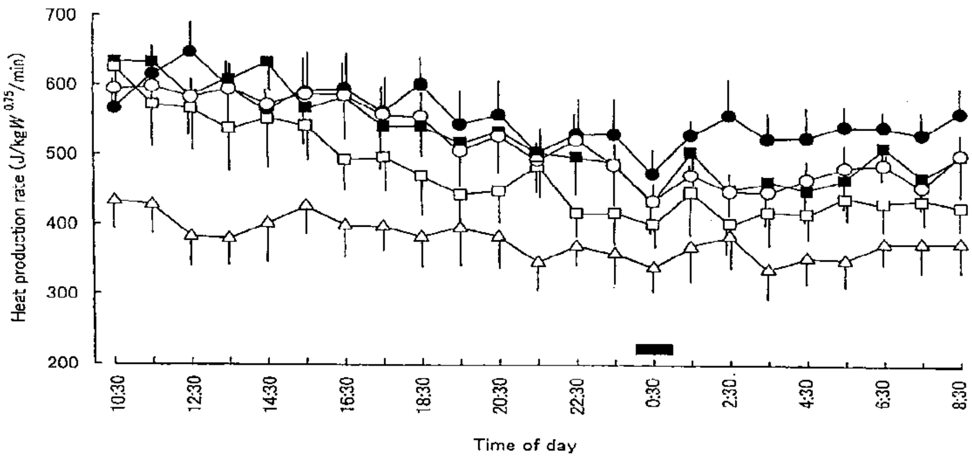


**Figure 1.** Pattern of circadian variation in HP rates of growing broilers given free access to feed (●), 75% of ad lib intake (○), 50% of ad lib intake (■), 25% of ad lib intake (□) and no feed at all 0% (Δ). The black horizontal bar represents the dark period and vertical bars are SEM of 5 birds (Koh and MacLeod, 1999a).

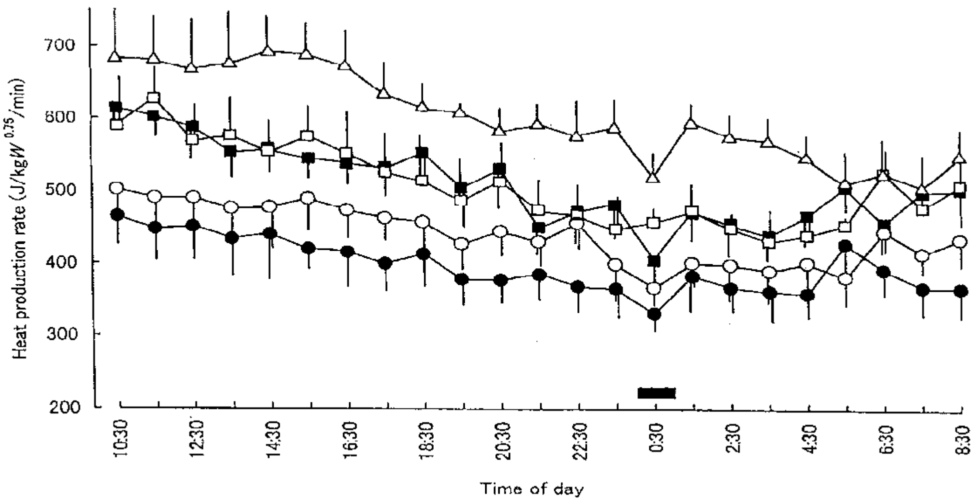


**Figure 2**. Pattern of circadian variation in HP rates of growing broilers maintained at 14°C (Δ), 17°C (□), 22° (■), 27°C (○) and 32°C (●). The black horizontal bar represent the dark period and vertical bars are SEM of 5 birds (Koh and MacLeod, 1999a).

A circadian variation in HP of growing broilers, maintained at different feed intake levels and ambient temperatures with a 23L:1D lighting pattern, is shown in Figures 1 and 2. Figure 1 shows that HP tends to decrease until the 1-h dark period (00:30 h). HP rates in the four fed groups were similar at 10:30 h and then decreased linearly until 00:30 h. HP decreased significantly with decreasing feed intake. This shows that regulated feeding may change the pattern of circadian variation in HP rate. In Figure 2, a clear negative relationship between ambient temperature and HP rate is demonstrated. The primary consequence of heat stress is that animals will reduce feed intake progressively while ambient temperature increases (May and Lott, 1992). This lower feed intake (energy intake) will reduce HP of the chicken.

## HEAT LOSS BY SENSIBLE AND EVAPORATIVE HEAT DISSIPATION

At relatively low temperatures, heat is dissipated through sensible heat loss (SHL) and is emitted by radiation, conduction and convection (Yahav et al., 2005). SHL can be dissipated when ambient and/or wall and floor temperatures are below the fowl's surface temperature (Hilman et al., 1985; Etches et al., 2008). Radiative heat transfer can occur between the bird and its environment if radiant surface temperature of the bird is different from that of the surrounding surface or in open air. Convection occurs by given off heat to the surrounding air by comb, wattles, face, legs, toes, neck, body and wings (Yahav et al., 2005). SHL from the comb and wattles represents 34% of the total SHL at 35$^{o}$C (Hilman et al., 1985). In addition, heat loss by convection plus radiation (Qt) can increase considerably with increasing air velocity. Air velocity also exposes the skin more and thus may increase radiant losses also. The SHL by Qt, expressed as a percentage of energy expended for maintenance, reach a level of about 45% in broilers subjected to 3.0 m/s air velocity (Yahav et al., 2004). Therefore, SHL can play a major role in heat loss to the environment. Exposure to high ambient temperatures enhances blood flow in the chicken foot due to the opening of arteriovenous anastomoses (Hilman et al., 1985). This warming of the foot can facilitate conductive heat loss.

An increase in body T above the regulated range may lead to a cascade of thermoregulatory events that may be lethal if body T cannot be maintained within certain limits (Yahav et al., 2005). If ambient temperature rises, heat dissipation is shifted from non-evaporation towards more evaporation (of moisture) at higher temperatures (Etches et al., 2008). In the bird, heat is mainly dissipated through an increased respiration (called *'Panting'*) (Marder and Arad, 1989) but also via cutaneous evaporative mechanisms (Ophir et al., 2002).

At the high end of the TNZ, heat loss through panting can account for 60% of the total heat loss (Etches et al., 2008) and even up to 80% at very high temperatures (32$^{o}$C) (Ahmad and Sarwar, 2006). Evaporative heat loss is associated with loss of water and therefore dehydration can occur. Sufficient water intake will facilitate this type of heat loss and contribute to thermo-tolerance at higher ambient temperatures (Yahav et al., 2005).

## Effect of HT on feed intake, water intake, body weight, physiology and GIT development

A high ambient temperature is a relative term especially since each animal has its own upper threshold of what is experienced as the comfort zone. When the ambient temperature exceeds the animal's thermoneutral zone, the animal is in heat stress. The ambient temperature at which this happens also depends upon the RH and air velocity (Veldkamp et al., 2002). A high ambient temperature affects feed and water intake, respiration rate, body T, heterophil/lymphocyte (H/L) ratio and GIT development. These aspects will be discussed in the next paragraphs.

### FEED INTAKE

Poultry production efficiency is affected by ambient temperatures and humidity (Wiernusz, 1998). Feed intake by broiler-type chickens is reduced at HT (NRC, 1994; Cheng et al., 1997). An increase in the ambient housing temperature from 21.1 to 32.2$^{o}$C caused a drop in feed intake of about 9.5% per bird/day from the first week to the sixth week of age. When ambient temperature rose from 32.2 to 37.8$^{o}$C, it caused a further drop in feed intake of about 9.9% per bird/day, as compared to 21.1$^{o}$C (North and Bell, 1990).

### WATER INTAKE

High ambient temperatures will increase water intake. Chickens drink 4 times more at 38$^{o}$C (North and Bell, 1990), as compared to 21$^{o}$C. The newest data showed that water consumption increases about 7% for each 1$^{o}$C above 21$^{o}$C (NRC, 1994). Stimulation of water intake may benefit the bird by facilitating the evaporation mechanism (Belay and Teeter, 1993). This will help cooling of the bird (Ahmad et al., 2005). Thus water is involved in many aspects of poultry metabolism including body T control, digestion processes, and absorption of feed and transport of nutrients. Water consumption during heat stress depends also on the amount of feed consumed. Birds that received feed 1 h before heat exposure had a larger increase in water consumption during the heat exposure period compared to birds that did not receive feed prior to heat exposure (Lott, 1991).

Furthermore, water consumption in low-high and high-low temperature cycles was affected by drinker types (bell versus nipple; Table 1) and the height of the nipple above the bird (Table 2). Data were summarized for 24 h periods that began and ended at a low temperature. At each age, daily water consumption was significantly lower for birds with nipple drinkers than for birds with bell drinkers. In particular, this difference was most pronounced during the quarters of the day that temperature was highest (29.4-35-29.4$^{o}$C) (Table 1). Daily water consumption was largest for birds with bell drinkers, intermediate for birds with low nipples and lowest for birds with high nipples (Table 2). Average water consumption over 3 d (54 to 56 d of age) in birds with a bell drinker increased with increasing temperature, but consumption from a nipple drinker decreased, surprisingly with increasing temperature (May et al., 1997).

In the study of May and Lott (1992), they reported that an increased water consumption at 24-35-24$^{o}$C cyclic temperature was accompanied by a decreased feed consumption. It is clear that a depressed feed consumption result in a lower body weight gain.

## BODY WEIGHT

Broilers that were subjected to high temperature (HT) gained less than those subjected to normal temperature (NT). Body weight of broilers at 6 weeks of age was decreased by about 14.3% and 21.2% at 32.2$^{o}$C and 37.8$^{o}$C, respectively (North and Bell, 1990) and even up to 34% at 32.2$^{o}$C as compared to 21.1$^{o}$C (Cheng et al., 1997). There have been few studies on the combined effects of temperature and RH in relation to BW. It is generally accepted that high humidity aggravates the detrimental effects of a high temperature. A bell-shaped response function of BW gain to RH was observed in 5 to 8-week-old broiler chickens in response to various RH levels with a maximum BW at 60 to 65% RH, both at 30 and 28$^{o}$C (Yahav, 2000).

**Table 1.** Effect of drinker type and high cyclic temperature on water consumption by broilers (May et al., 1997)

| Age (d) | Drinker type | Daily consumption | Quarterly water consumption during cyclic temperature interval[1] | | | |
|---|---|---|---|---|---|---|
| | | | 23.9 to 29.4°C | 29.4 to 35°C | 35 to 29.4°C | 29.4 to 23.9°C |
| 21 | Bell | 27.2[a] | 23.6[a] | 32.4[a] | 27.8[a] | 25.0[a] |
| | Nipple | 24.8[b] | 23.3[a] | 25.7[b] | 24.7[b] | 25.5[a] |
| 28 | Bell | 26.0[a] | 25.8[a] | 31.1[a] | 28.3[a] | 18.9[a] |
| | Nipple | 21.2[b] | 20.1[b] | 21.3[b] | 23.5[b] | 20.1[a] |
| 35 | Bell | 22.5[a] | 22.1[a] | 28.4[a] | 24.5[a] | 15.1[a] |
| | Nipple | 17.1[b] | 17.5[b] | 14.3[b] | 20.9[b] | 15.7[a] |
| 42 | Bell | 23.0[a] | 21.8[a] | 30.6[a] | 26.1[a] | 13.4[a] |
| | Nipple | 14.2[b] | 17.5[a] | 12.3[b] | 12.7[b] | 14.3[a] |
| 49 | Bell | 19.0[a] | 17.2[a] | 24.6[a] | 23.1[a] | 11.0[a] |
| | Nipple | 13.5[b] | 15.4[a] | 9.4[b] | 16.0[b] | 13.3[a] |

Means within an age and within daily consumption or quarterly consumption with no common subscript letters (a-b) differ significantly ($P < 0.05$).
[1]Consumption is given as percentage of body weight per day. Quarterly consumption is presented as the consumption per quarter times four.

**Table 2.** Effect of nipple height and high cyclic temperature on water consumption by broilers (May et al., 1997)

| Drinker type | Daily consumption | Quarterly water consumption during cyclic temperature interval[1] | | | |
|---|---|---|---|---|---|
| | | 23.9 to 29.4°C | 29.4 to 35°C | 35 to 29.4°C | 29.4 to 23.9°C |
| Bell | 16.9[a] | 17.6[a] | 20.5[a] | 18.1[a] | 11.5[a] |
| Nipple-high[2] | 11.2[c] | 13.1[b] | 8.0[c] | 10.0[c] | 13.7[a] |
| Nipple-low[3] | 14.7[b] | 17.2[a] | 12.7[b] | 15.1[a] | 13.8[a] |

Means within daily consumption or quarterly consumption with no common subscript letters (a-c) differ significantly ($P < 0.05$).
[1]Consumption is given as percentage of body weight per day. Quarterly consumption is presented as the consumption per quarter times four.
[2]High nipples were at a height that forced the broilers to extend their necks to reach the nipple.
[3]Low nipples were at approximately the height of the back of the broilers.

## BODY TEMPERATURE AND RESPIRATION RATE

Male turkeys (Waibel and MacLeod, 1995) and broilers (Yahav, 2000; De Basilio et al., 2003) raised under high ambient temperatures show an increased body T and respiration rate. Broilers maintained at 10$^o$C above TNZ had the highest core body T (CBT) compared to the other temperature treatments (40.1 vs. an average of 39.9$^o$C, respectively; $P < 0.001$). CBT was also influenced by gender and crude protein level in the diet. Protein level did not affect CBT of males but CBT was significantly elevated (0.07°C) in females on the higher protein level (Zuidhof et al., 2010). The higher CBT can be derived from three reasons. Firstly is the lower surface per BW. Male has a lower surface per BW because male is normally bigger than female. So, the heavy birds may suffer more at HT if they have the same HP per BW. Secondly is the efficiency in protein deposition. Males deposit protein more efficient than females. This means that males use less CP for lipid deposition than females, therefore male produce less heat. Thirdly is the ratio feed intake into BW (FCR). If FCR is higher, CBT is higher because more HP is produced.

Respiration rate is also dependent on the age of the bird, ambient temperature and RH. At 20 weeks of age, respiration rate of turkeys was increased from 195 breaths per minute at 25$^o$C to 230 breaths per minute at 32$^o$C. The increase in respiration rate was more pronounced when RH is high (Brown-Brandl et al., 1997). This finding is also in line with reports by Yahav (2000) who found that the rate of panting estimated from blood pH and $pCO_2$ was higher in chickens exposed to 30°C than to 28°C.

## HETEROPHIL/LYMPHOCYTE RATIO

The H/L ratio is affected by a number of stressors such as high ambient temperature and can be used as an indicator of heat stress (Gross and Siegel, 1983; Puvadolpirod and Thaxton, 2000a). A high H/L ratio is negatively correlated with BW and positively correlated with mortality rate (Puvadolpirod and Thaxton, 2000a; Al-Murrani et al., 2006).

## GASTROINTESTINAL TRACT DEVELOPMENT

Gastrointestinal tract (GIT) development can be influenced by heat stress also. GIT development, as indicated by total wet and dry weights of the whole small intestine were reduced by about 22 and 23%, respectively, in birds kept at 35°C compared to those kept at 22$^{o}$C. Moreover, the size of the absorptive was also reduced as indicated by villus height ($\mu$m) of about 19% ($P$<0.001) in birds maintained at 35°C (762 $\mu$m) compared to those kept at 22$^{o}$C (938 $\mu$m) per unit length of jejunum for 14 d (Mitchell and Carlisle, 1992). In terms of intestinal development, birds exposed to HT and RH (30$^{o}$C and 70%) showed a 27.2% reduction in fresh weight of jejunum and a 3.8% reduction in jejunum length compared to birds exposed to a low temperature and RH (20$^{o}$C and 50%) (Garriga et al., 2006). The decrease in jejunum weight was mainly attributed to the effect of the reduced intake with high ambient temperature because restrictedly pair-fed birds showed similar values as the control birds. This indicates that high temperature reduces intestinal weight also in connection to the lower feed intake. Furthermore, the decrease in intestinal weight and villus height may be influenced by the reduction in $T_3$ production (Mitchell and Carlisle, 1992; Garriga et al., 2006) under heat stress, because thyroid hormones stimulate the growth of the intestines (Levin, 1994; McNabb, 2007). Therefore, functional hypothyroidism mediates the reduction in jejunal mass and villus height in heat stressed birds (Garriga et al., 2006).

## Effect of HT on energy and protein requirements

It appears that birds under HT conditions have a preference for those nutrients that will result in less HP at a given physiological status of the birds. Accordingly, they will adjust their production level also.

### ENERGY REQUIREMENTS

The advantages of using high-energy ratio for broilers by adding fat in feeding program at high ambient temperature areas are well documented (Daghir, 2008b). Added fat (5%) at 31$^{o}$C improved feed intake in laying hens by about 17%, whereas added fat at 10-18$^{o}$C improved feed intake by only 4.5% (Daghir, 2008a). Compared to either protein or carbohydrates, fat gives less heat production because dietary fat used for deposition has a lower heat increment than protein or carbohydrates (Musharaf and Latshaw, 1999). However, a significant additional weight response to additional energy (like extra fat) occurred only at an adequate amino acid (lysine) level in the diet (McNaughton and Reece, 1984). On the other hand, digestibility of energy, protein and fat in diets with a high energy to protein ratio was reduced during a 2 weeks exposure to 32°C in 6 week old male broilers. So, high quality oil and protein sources with a high digestibility should be used (Bonnet et al., 1997).

Male broilers housed at ambient temperatures ranging from 21.1°C to 35°C and fed grower diets containing either 12.7 or 13.4 MJ ME/kg with five different levels of CP and amino acids showed that high energy diets across all temperatures had significantly improved feed conversions and a better protein utilization (Cheng et al., 1997). This means that high level energy densities may be required under hot temperature conditions to reduce the heat load (Balnave and Brake, 2005). So also less heat will need to be given off by panting.

The energy requirement for maintenance (MEm) at different temperatures can be derived from linear regression of energy retention on ME intake at each temperature. The MEm was estimated from the regression equation of energy retention on ME intake. The MEm requirements were 157.8, 112.1, and 127.2 kcal of ME/kg of $W^{0.75}$/d for chickens reared at 13, 23, and 32°C, respectively. Temperature had a quadratic effect on MEm with a minimum MEm near 26°C: MEm = $W^{0.75}$ (307.87 + 15.63 T + 0.31 T2) (Sakomura et al., 2005). The quadratic effect of temperature on MEm implies that the MEm requirement is increased at HT. This estimation suggests that metabolism of the birds change when they are reared above or below their TNZ to dissipate heat or increase heat production.

Adaptive changes in feed intake and energy expenditure over the long-term contribute to homeostatic control of body energy stores and maintaining a constant BW. In addition to meeting immediate energy demands, feed intake can be adjusted to ensure that energy and nutrients are stored in anticipation of periods of high demand or periods of feed shortage (Richards and Proszkowiec-Weglarz, 2007). Therefore, it is a challenge to estimate the energy requirement at HT.

The estimate of average efficiency of total energy use of different nutrients for different body functions is given in Table 3. Each nutrient has its own ATP potential. So when starch and lipids are used for maintenance about the same amount of ME can be used as ATP and this means also the same amount of heat is produced per calorific value of the nutrient when used for maintenance. if fatty acids, for example, are for energy supply for activity than about 66% of the calorific value of fatty acids can be converted into ATP and the rest (34%) is lost as waste heat. If the body produces fat, it does not need to make many changes with fatty acids and it will deposit about 90% of the calorific value of these fatty acids into fat and only 10% finally results in “waste” heat. When protein is used for ATP, therefore, more heat is produced per calorific value (42%). It is clear that lipid is deposited with a high efficiency. So in that case only a small part of the calorific value of lipid is produced as heat. Therefore, a high energy diet with relatively high fat content mostly gives less heat load per energy unit after digestion. Animals will deposit a part of the dietary fat directly as body fat. So in that case not many changes are needed to convert fatty into body lipids. Dietary protein has to be hydrolyzed first to amino acids (AAs) and peptides. From these AAs body protein can be made if AA pattern is balanced. So if the dietary amino acid pattern resembles the protein needed for accretion well, not many changes are needed and this will cost energy. Synthesis of body fat from fatty acids does not require much additional changes and energy compared synthesizing body fat from e.g. carbohydrates. In addition, the body does not store much carbohydrate. So carbohydrate molecules have to change before they can be used for fat synthesis or for ATP. So with carbohydrates several metabolic changes occur when it is used for lipid synthesis.

PROTEIN AND AMINO ACIDS REQUIREMENTS

Dietary protein has received considerable attention in relation to heat stress, because its catabolism is associated with higher heat production when compared to catabolism of fats and carbohydrates in birds under TNZ conditions (see also Table 3).

**Table 3**. Biochemical efficiency of absorbed nutrients for ATP and for lipid synthesis (Black, 1995).

| Nutrients | Calorific value (kJ/g) | ATP production (%) | Lipid synthesis (%) |
|---|---|---|---|
| Fatty acids | 39.8 | 66 | 90 |
| Starch | 17.7 | 68 | 74 |
| Protein | 23.8 | 58 | 53 |

Raising the protein level of a diet above NRC (1994) recommendations did not improve performance at 33$^{o}$C (Cheng et al., 1997). Low protein diets, on the other hand, had negative effects on broiler performance when ambient temperature was high. This is clear because lower feed intake results in reducing intake of amino acids and further results in a poor feed efficiency and poor BW gain (Alleman and Leclercq, 1997). Broiler chickens exposed to acute heat stress (36.4 and 40.0$^{o}$C) had reduced plasma amino acid concentrations. The most significant change was at 40°C compared to lower temperatures (Tabiri et al., 2000). These authors speculated that changes in plasma Trp/LNAA (large neutral amino acid, sum of isoleucine, leucine, valine, tyrosine and phenylalanine) ratio and in Tyr may be related to a reduced feed intake and also to an altered thyroid function. Both phenomena are usually measured in heat stressed birds.

Under heat stress conditions, broilers ageing 21 to 49 days should be fed diets that contain between 90 to 100% of the NRC (1994) recommended levels of amino acids and protein in diets containing 13.4 MJ ME/kg (Cheng et al., 1999). According to Cheng *et al.* (1999), nutritionists should not compensate for a decreased intake in hot temperatures by increasing the concentration of protein and amino acids. So the final effect on performance then depends on the 'amounts of ideal protein'. The ideal amino acids pattern is not similar among species as shown in Table 4. Relative to lysine, the chicken requires more methionine+cystine, threonine and less leucine than the turkey and the pig. The ideal amino acid balance for broilers may vary somewhat with ambient temperature. This is logic as the amino acid part of the diet that is used for maintenance or growth changes due to metabolic stress (Moughan, 1999; Moughan and Fuller, 2003). They concluded that if intake is reduced the overall pattern needed will resemble more the maintenance pattern.

**Table 4**. Estimated ideal protein ratio for a starting hen turkey, broiler chicken and pig, expressed as a percentage of the lysine requirement (Firman and Boling, 1998).

| Amino Acid | Turkeys | Broiler Chickens | Pigs |
|---|---|---|---|
| Lysine | 100 | 100 | 100 |
| Methionine+Cystine | 59 | 72 | 60 |
| Threonine | 55 | 67 | 65 |
| Valine | 76 | 77 | 68 |
| Arginine | 105 | 105 | NA[1] |
| Histidine | 36 | 31 | 32 |
| Isoleucine | 69 | 67 | 60 |
| Leucine | 124 | 100 | 111 |
| Phenylalanine+Tyrosine | 105 | 105 | 95 |
| Tryptophan | 16 | 16 | 18 |

[1]NA = not available

At 31°C, broilers on low sodium chloride diets had an improved FCR at similar feed intake and numerical larger BW on a 1.36 Arg:Lys ratio diet compared to a 1.10

Arg:Lys ratio (Brake et al., 1998). These authors showed a beneficial effect of an increased Arg:Lys ratio at HT. However, others reported that an increased level of lysine in broilers (Mendes et al., 1997) or Arg:Lys ratio in turkeys (Veldkamp et al., 2000) was unable to improve weight gain and breast meat yield, or attenuate the adverse effects of heat stress.

Raising the protein level at HT without raising the level of essential amino acids such as lysine, will not help very much in overcoming the reduction in BW gain and in feed conversion (Ait-Tahar and Picard, 1987). However, an increased dietary lysine concentration appears necessary to compensate at least partly for the reduced feed intake (Corzo et al., 2003). The improvements were found in intake and growth by feeding broilers with 2-hydroxy–4-(methylthio) butanoic acid (HMB), but not with DL-Methionine (Chen et al., 2003). Amino acid supplementation had a minimum effect on heat production (Zarate et al., 2003a). Moughan (1999) stated that the ideal amino acid pattern for maintenance is not the same as the ideal amino acid pattern for growth.

## Feeding strategies to combat heat stress

Heat stress can only be reduced by feeding strategies if the animal produces less heat and/or losses more heat. A lower HP can be realized by e.g. a reduced heat increment, catabolism of fewer nutrients above requirements or a more efficient nutrient digestion. More heat loss can be realized through more water evaporation from the body. The possible ways to combat heat stress by feeding strategies are applying diurnal feeding patterns, self-selection strategies, feeding coarser diets and wet feeding.

### DIURNAL FEEDING PATTERNS

As the production cycle shortens due to improvements in genetic selection and nutrition, the implementation of restricted feed at HT may not produce a better performance on one hand, but could reduce the adverse effect of HT on the other.

Early growth restriction induced by feed restriction cannot completely compensate the adverse effect of high ambient temperature on performance, nor can high ambient temperatures recover the beneficial effect of an early growth restriction in improving feed efficiency and reducing fattening (Plavnik and Yahav, 1998). Others reported that feed withdrawal two hours before the hot period of the day improved feed conversion and lowered mortality without affecting BW (Yalçin et al., 2001). This suggests that feed allowance could also be restricted under normal conditions. Furthermore, chickens fed restrictedly for 2 h prior to a hot period of the day gained 2.8% more and showed a lower H/L ratio than heat stressed bird fed ad libitum (Yalçin et al., 2003). This means that feed restriction during the heat stress period can reduce the harmful effects of HT.

Feed withdrawal during the warmest part of the day (09:00 to 16:00 h) was compared with the distribution of ground corn during 09:00 to 16:00 h and commercial feed consumed ad libitum. The broilers were exposed to tropical climatic conditions from 28 to 42 d (averaged Ta = 25°C; RH = 72%). From 16:00 to 09:00 h, continuous light was provided in the poultry house and all chickens received a commercial diet. Results showed that both feed withdrawal and corn distribution during the day period reduced growth and deteriorated feed conversion. However, the body T in birds on the control diet was higher than of those fed both feed withdrawal and corn distribution (Lozano et al., 2006). Separate feeding of a high protein fraction (16:00 to 09:00 h) and an energy-rich fraction (09:00 to 16:00 h) at diurnally cyclic temperature: 26$^{o}$C (16:00 to 09:00 h) and 30$^{o}$C (09:00 to 16:00 h) compared with a control diet show that growth and feed efficiency were slightly reduced (-4%) by separate feeding. However, during the thermal challenge period at the age of 34 d to 36 ±2$^{o}$C and 40 to 58% RH for 7 h, body T was reduced by separate feeding (De Basilio et al., 2001). Certainly the gap between two feeds is important with regard to its influence on performance at HT. It can certainly help to reduce heat stress as shown by the lower body T.

Feed withdrawal between 10:00 to 16:00 h during the day from weeks 5 to 6 or in week 6 was compared with control feeding with the same feed on broilers reared under natural summer conditions. BW and daily weight gain were higher in the feed withdrawal groups during week six. Feed withdrawal during weeks five to six produced the lowest body weight and daily weight gain. However, Tb was also lower in the feed withdrawal treatment (Özkan et al., 2003). Because total feed intake and feed conversion ratio by 6 h feed withdrawal during 7 day before market age did not affect slaughter weight, it can be suggested that feed withdrawal during the hot period of the day can be used to alleviate heat stress.

Broiler breeder fowl responded to a restricted feed intake by a reduction in HP resulting in a reduced BW gain. The reduced metabolic rate per unit of weight is associated with a thermoregulatory advantage at high ambient temperature (MacLeod et al., 1993). Reducing weight gain by restricted feeding results in a longer growing period and delay in marketing age. Therefore during a heat stress period the producer has to balance the benefits of a faster growth rate and a greater risk of mortality.

Feed intake at different levels of feed restriction under various environmental temperatures is shown in Figure 3 (Koh and MacLeod, 1999b). The HP in relation to feed intake and ambient temperature is shown in Figure 4 (Koh and MacLeod, 1999a). HP data were calculated for each combination of temperature and feed intake. When HP (kJ/kg $W^{0.75}$/14 h) was calculated at each combinations, HP significantly decreased with a decreasing feed intake and increasing ambient temperature.

SELF-SELECTION

A direct measure of a separate regulation of protein and energy intake can be made with a self-selection method of feeding. There is evidence that both wild and domesticated fowl are able to adjust their nutrient intake by selecting from various feed ingredients that matches their physiological requirements (Hughes, 1984; Yo et al., 1998). Self-selection may allow the bird to meet their daily cyclic requirements for nutrients more effectively compared to offering a complete diet. For example, during hot cyclic periods, the birds may prefer to change energy intake from the hot (afternoon) to the cooler periods (morning).

They may do this in order to reduce heat production during the hot period. In this way they can fulfill their energy demand throughout the entire 24h-period of the day. The information concerning the ability of chickens to separately regulate their consumption of protein and energy under heat stress is still limited. This is also the case for village chickens kept under tropical climate condition. Factors that influence dietary choice by chickens include genotype, chronological age, physiological state, ambient temperature, health status, stress and prior experiences (Forbes and Shariatmadari, 1994).

Some researchers have reported an improved (Gabriel et al., 2003), similar (Rose et al., 1995) or decreased (Amerah and Ravindran, 2008) growth performance of chickens under NT conditions after subjecting them to choice feeding (with wheat). Also an additional pea meal did not give differences in performance with control feed. So the response depends on feed ingredients (McNeill et al., 2004).

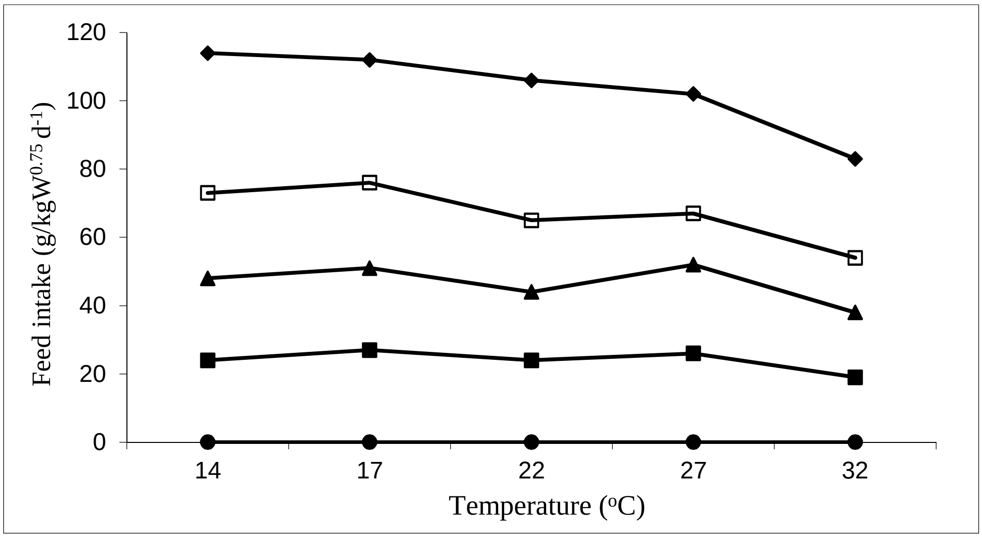


**Figure 3**. Feed intake at different ambient temperatures of growing broilers provided feed ad lib (♦), 75% of ad lib (□), 50% of ad lib (▲), 25% of ad lib (■) and no feed 0% (●) (Koh and MacLeod, 1999b)

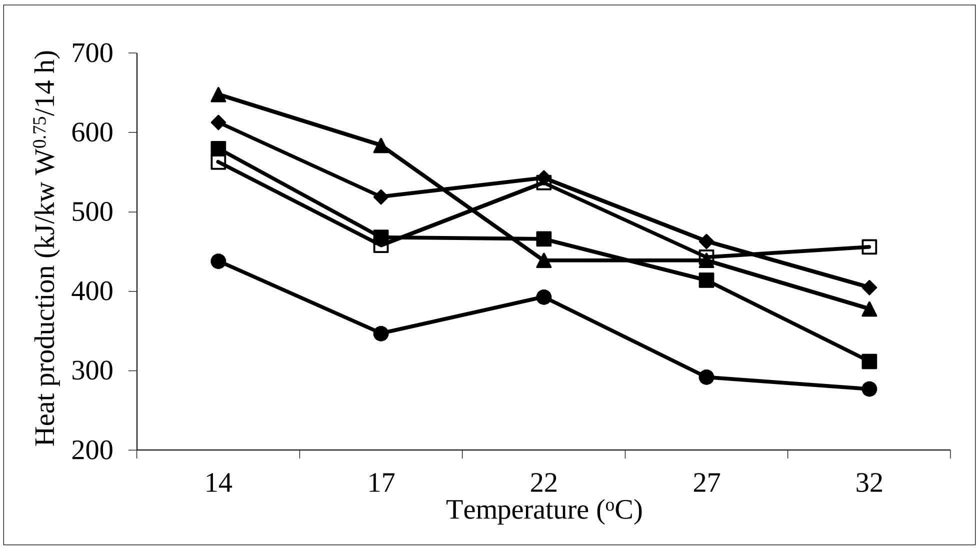


**Figure 4**. Heat production in relation to ambient temperature and feed intake level of growing broilers provided feed ad lib (♦), 75% of ad lib (□), 50% of ad lib (▲), 25% of ad lib (■) and no feed 0% (●) (Koh and MacLeod, 1999a)

From choice feeding studies, a broiler at HT (cycling diurnally between 25 and 35$^{o}$C) prefers to consume less protein and more energy compared to a complete diet (Sinurat and Balnave, 1986). Also Japanese quail preferred to eat more energy and less protein if they were offered a choice as compared to a single complete diet between 20$^{o}$C and 35$^{o}$C (MacLeod and Dabutha, 1997). This response of birds at HT is probably caused by birds trying to avoid the increased risk of heat increment from protein conversion associated with HT. Indeed, high fat diets (5%) reduced the detrimental effect of heat stress in broilers raised at 29 to 36$^{o}$C (Ghazalah et al., 2008). These results agree with Veldkamp *et al.* (2002), who concluded that turkeys modulate their feed intake when exposed to HT in relation to caloric diet density.

In a hot environment, self-selection would mean that animals can select a diet and optimize the heat load associated with metabolism. It may enable the bird to more precisely balance nutrient intake to its nutrient requirements. This has been particularly evident in laying hens with increased egg mass output when a high energy, complete diet was fed with an additional protein concentrate at a hot ambient temperature (Balnave and Mutisari Abdoellah, 1990).

The effect of choice feeding on performance seems to be determined also by the age of birds when the choice is offered for the first time. BW of choice-fed chickens under tropical condition from 1 to 42 d of age and from 8 to 42 d of age was lower at 35 and 42 d. When chickens changed from a complete diet to choice feeding from 22 to 42 d and from 36 to 42 d, their BW at 42 d did not differ from those that had received the control diet (Yo et al., 1998). The lower BW of choice-fed birds was presumably due to a dramatic decline in the level of protein concentrate consumed during 1 to 42 d period (control-fed birds: 16.3 g/kg, choice-fed birds: 13.8 g/kg).

The length of exposure to heat stress can also affect the response of birds to a choice diet with dietary protein. Gonzalez-Esquerra and Leeson (2005) fed broilers two levels of dietary CP (10% and 30%) in a choice feeding experiment and compared them with a single diet with 26% CP with the same ME at a) NT (23$^{o}$C at 21 d), b) acute heat stress (AHS; sudden temperature increase to 29.4$^{o}$C at 21 d) and c) chronic heat stress (CHS; gradually temperature increase to 29.4$^{o}$C at 7 d). Result from 28 to 42 d of age showed that feed intake was 25% and 27% lower and BW gain was 19% and 23% lower at AHS and CHS respectively as compared to NT. Within temperature, the choice-fed birds consumed 25.4, 24.9 and 26.6% at NT, AHS and CHS, respectively. However, BW gain of choice-fed birds was 14%, 2% and 7% lower than single diet fed birds at NT, AHS and CHS, respectively. The small differences in BW gain between choice and control-fed birds under heat stress reflects adaptation mechanisms to balance energy to CP intake ratios, as a means to avoid excessive heat load. The effect of choice feeding may also depend on ingredient quality, since some ingredients may contribute less to a balanced intake of nutrients, due to differences in palatability or differences in particle sizes (Cruz et al., 2005).

Yo et al. (1997) concluded that physical form of feed offered is an important factor to be considered in a choice feeding system. They fed whole, cracked or ground corn associated with a protein concentrate (pellet or mash) to broilers at tropical climate conditions with temperatures varying between 23.6±1.3 and 29.2±1.8$^{o}$C. Results showed that birds fed on whole corn had a lower corn intake (73.3 g/d) than those fed cracked (87.1 g/d) or ground corn (84.1 g/d). Presenting the protein concentrate as a pellet resulted in a higher intake (40.1 g/d) and a higher proportion of concentrate in the selected diet (33.0%) than presenting it as a mash (33.4 g/d and 29.6%), respectively. So, total feed intake was highest on cracked corn and a pellet protein concentrate. When corn was fed as whole grain, the intake of the protein concentrate in the self-selected diet was higher (35.1%) than if corn was fed as cracked (29.3%) or ground (29.1%). Although none of the diets affected BW. Feed conversion ratio was lower for the diets with the large particle sizes.

In a choice feeding situation, physical appearance can have an effect on development of GIT. The birds that chose whole wheat were accompanied by an increased development of the upper part of the GIT (Gabriel et al., 2003). Literature data show that the inclusion of whole wheat or the use of a coarse mash (larger particle sizes) is associated with heavier gizzards. This resulted in an increase in total transit time in the GIT due to a longer retention time in the gizzard (Banfield et al., 2002; Plavnik et al., 2002; Hetland et al., 2004; Amerah et al., 2007). There is no relationship between the volume of ingesta in the gizzard and the mass of the empty gizzard nor between maximal gizzard length and width with dietary treatment (Amerah et al., 2007). A large muscular gizzard will maximize the grinding capacities of the gastrointestinal tract (Kwakkel et al., 1997) which may increase the digestive capacity and in this way contribute to a better performance of broilers. The coarse diet increases the chemical (pepsin in the proventriculus) and physical (gizzard muscle) functionality of the upper part of the digestive tract and, consequently, feed intake and BW gain will increase (Khoa, 2007).

The advantage of choice feeding is that birds are able to adjust their nutrient intake over the day by selecting from various feed ingredients to match their physiological requirements. This may be of particular interest for birds kept under tropical condition. However, the results of a choice feeding strategy could be potentially affected by feed properties such as particle size, the initial age from which moment the choice was offered, the quality of protein and energy sources and the level of CP. The economic advantages of choice feeding could be a reduction of feed costs because mixing ingredients is no longer necessary and formulations of feeds become less crucial. Summarizing, a self-selection feeding strategy is relevant for a poultry farmer, perhaps even a large poultry producer, at high temperature zones in both developing and developed countries.

COARSE PARTICLE

It is unknown whether a change from a fine to a coarsely ground diet for broilers under HT may assist digestive function and therefore reduce heat generated by such processes. However, water excretion in droppings is negatively related to the proportion of coarse particles as shown by the negative correlation with the residue from the regression relating water excretion to food intake by coarse particles. This relationship is due to a direct effect of an increased retention time of coarse particles in the GIT (Carré et al., 2002). Therefore, coarse diets may allow more water to be reabsorbed from the GIT compared to fine diets. If more water is available in the metabolism, heat loss trough evaporation of moisture during panting may be facilitated. On the other hand, more heat loss via evaporative cooling emphasized the importance of increasing water consumption in heat stressed broilers. Therefore, coarsely ground diet may in this way help to dissipate the heat load under HT conditions.

WET FEEDING

Changes in feed management such as adding water to the feed are well known for their positive effects on performance of birds. This strategy has been particularly evident in broilers at NT because it improved feed intake, weight gain and/or feed conversion ratio and weight of the GIT compared to birds on dry feed (Yasar and Forbes, 2000; Moritz et al., 2001; Shariatmadari and Forbes, 2005; Khoa, 2007). In addition, a higher rate of passage through the GIT with wet feeding has been suggested because the weight of digesta in the whole digestive tract of birds fed wet diets was less while feed intake was higher (Yasar and Forbes, 2000).

The improvements in digestive efficiency with wet feeding are thought to come from a heavier empty weight, a higher full gut length and more wall thickness in some parts of the digestive tract (Yasar and Forbes, 2000). Viscosity of digesta was significantly reduced by wet feeding and this may indicate a faster passage rate of digesta. Moreover, a thicker gut wall layer with wet feeding may also improve digestive function (Yasar and Forbes, 2000). Adding water to the feed reduces digesta viscosity to a similar extent and stimulates pre-digestion and absorption possibly due to a faster penetration of digestive enzymes into feed particles than with the ingestion of dry food. As a result, nutrient digestibility may increase.

In addition to the dilution of dry feed by water, the inclusion of exogenous enzymes to the wet feed may have an extra potential effect for absorption. It may increase substrate accessibility by enzymes thereby also enhancing the absorption of nutrients. Acidified feed with Calprona AL® (Khoa, 2007) or fungal origin (Yasar and Forbes, 2000) had some additional effect to watering the diet in terms of feed intake, BW gain and FCR. However, using yeast (*Saccaharomyces cerevisiae*) to a wet diet resulted in a heavier BW and similar FCR than those fed on a dry diet with yeast or feed without yeast in dry and wet form (Afsharmanesh et al., 2010). The different results of these studies are probably due to the differences in enzyme activity. Yeast has been traditionally used as a growth promoter in poultry and other animal diets and to stimulate micro-organisms capable of modifying the gastrointestinal environment to improve health status and performance. The improvement of performance is thought to be mediated by alterations of the intestinal flora e.g. the increase of the growth of non-pathogenic facultative anaerobic and gram positive bacteria. These can forming lactic acid and hydrogen peroxide and may suppress the growth of intestinal pathogens. It may well that the enhancement of digestion and the utilization of nutrients works in this way (Afsharmanesh et al., 2010).

Three studies of wet feeding and the effect on laying performance at HT have been reviewed by Lin et al. (2006). These authors reported that the increased performance by wet feeding can be the result of an elevated dry matter (DM) intake at HT. In this way, egg production and egg weight can be alleviated at HT conditions (Lin et al., 2006). In broilers, only a few studies are available in literature. Water addition to diets fed to broilers housed at HT contributed to an increase in feed intake, live weight, feed efficiency, weights of heart, crop, and abdominal fat (Awojobi and Meshioye, 2001; Kutlu, 2001; Awojobi et al., 2009). Empty gizzard weights were higher in broilers fed a wet diet (2 parts of water and 1 part of feed) without drinking water (Awojobi et al., 2009). This study gave results contrary to the higher gizzard weights in broilers fed dry diet in the first reported study by Awajobi and Meshioye (2001). The most optimal water-to-feed ratio was a 1:1 ratio for feed conversion efficiency and carcass weight in finishing broiler (Awojobi et al., 2009). Wet feeding is desirable not only during hot weather conditions, but also during the rainy season in a tropical climate (Awojobi and Meshioye, 2001). We speculate that a high feed intake of birds housed at HT conditions as facilitated by wet feeding is enabled by the cooling of fresh water or wetted feed. In addition, extra water in the body in association with a high DM intake can help to reduce the increase in Tb. Extra water in the metabolism will facilitate heat loss by means of evaporation. With wet feeding the feed is already soaked to stimulate pre-digestion and absorption, ready for gastric and intestinal digestion (Khoa, 2007). The contribution to the digestive process before the feed arrives in the crop could be more important than digestion occurring in the crop its self.

## Conclusions

Heat stress induces hyperthermia and reducing heat load can be achieved by increasing heat dissipation and/or decreasing heat production. In a hot environment, the bird reduces its feed intake and increases its water intake. Reduction in feed intake can result in a shortage of some nutrients such as protein, amino acids and also energy. Overall, heat stress will affect the performance of the chicken.

The use of high fat diet for optimal broiler performance is suggested in warm regions because high fat diets generate less heat increment per unit of energy than high carbohydrate diets. However, this only functions if also an adequate level of essential amino acids (lysine) is provided. However, increased lysine or Arg:Lys ratio at HT was not able to improve growth rate and to reduce the adverse effects of heat stress.

Predicting the optimal energy and protein content in a diet for meat-type chickens (both native and exotic birds) under hot environmental temperature has not been studied in great detail yet. Therefore there is a challenge of accurately defining the optimal nutrient contents in a diet of various birds under hot ambient temperature. This may be done by self-selection strategies of the bird.

Another promising strategy to increase performance of birds under potential heat stress conditions is water addition to the feed in combination with adjusting the particle size to promote GIT development. A well-developed gizzard and longer small intestine will enhance the grinding capacity, potentially improve digestion down the GIT and provide a large area for absorption. Larger particle sizes will allow more water available in the metabolism and dissipate the heat load through panting activity. Wet feeding increases voluntary feed intake, increases development of some parts of the GIT, and reduces digesta viscosity. Therefore, together with wet feeding, a large particle size will keep the litter quality more adequate and may cool the chickens and improve the bird's welfare. Further research should include the native chicken kept by many farmers in rural areas under hot tropical temperatures and RH cycling.

# CHAPTER 3

# Dietary Self Selection by Broilers at Normal and High Temperature Change Feed Intake Behavior, Nutrient Intake and Performance

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## Abstract

Self-selection assumes that at a high ambient temperature, birds are able to select a diet from different sources in order to minimize the heat load associated with the ingested nutrients metabolism. The objective was to test the hypothesis that young chickens are able to compose an adequate ration by adjusting dietary nutrient intake from three different diets that vary in energy and in protein contents from a cafeteria system at high temperature (HT; 31-32$^{o}$C) and at normal temperature (NT; 31-21$^{o}$C). Night temperature was set at 25$^{o}$C at HT and at 18$^{o}$C at NT and 12 h dark:12 h light. Control birds were fed a standard control diet (CP: 215 g/kg; ME: 2,895 kcal/kg) for broiler chickens. The choice-fed birds could choose between the control diet, a high protein diet (CP: 299 g/kg; ME: 2,780 kcal/kg) and a high energy diet (CP: 150.7 g/kg; ME: 3,241 kcal/kg). The diets had similar pellet size and colour. Birds had access to each diet in a separate feeding trough from 1 to 42 d of age. Results showed that broilers spent 3.3% more time eating at NT than at HT and showed 42% more panting behavior at HT than at NT. HT decreased feed intake, protein intake, energy intake and BW gain. Choice-fed birds had similar feed intake and BW gain, 14% lower protein intake and 6.4% higher energy intake than control-fed birds. Body T and heterophil/lymphocyte ratio were higher at HT than at NT. Water intake was 8% higher in control-fed birds than in choice-fed birds but similar at both temperatures regimens. It can be concluded that broilers can compose a diet by selecting less protein but higher energy density from different diets compared with the control. Choice-fed birds had similar feed efficiency as control-fed birds at HT, indicating similar body composition for both groups. Extra energy intake of choice-fed birds at HT was used for panting activity.

**Key words:** self-selection, protein, energy, heat stress, broiler

## Introduction

There is evidence that both wild and domesticated fowl can reach their desired nutrient intake by selecting from a range of feedstuffs. They can compose a diet that matches their physiological requirements (Hughes, 1984; Yo et al., 1998). When given the opportunity to select from a set of different dietary ingredients they may do so to optimise performance (Gous and Swatson, 2000). This phenomenon can be used as a basis to design strategies for nutrient self-selection and it also can give insight in the nutrient requirements throughout aging and at different ambient temperature (**T**) conditions. Moreover, nutrient composition in a complete single diet may not exactly match the composition which would have been chosen by the bird itself. In addition, the selected mixture of diet components may differ at normal (**NT**) and high temperature (**HT**). We assume that with self-selection at HT, birds will select an optimum diet (from different feed sources) to adjust (minimize) heat load associated with the metabolism of the ingested nutrients.

Most diets for chickens are nowadays formulated on the basis of trials that were carried out under constant temperate thermal conditions. These diets may not deliver the amounts of various nutrients which broilers would like to eat at HT conditions. At HT, when feed intake is generally low, birds may choose a different composition compared to NT. The choice feeding may enable them to make such a choice and in this way add to the possibilities for managing heat production (Etches et al., 2008). At HT, the bird will attempt to adjust its heat production by reducing feed intake (North and Bell, 1990) or change its intake of especially those nutrients that are most needed and at the same time reducing the intake of those nutrients that yield a lot of heat during processing (and in this way reducing the heat load). The evidence from literature that birds are able to select a proper mix of dietary ingredients to reduce heat load at HT is scarce and conflicting. Thus it is hardly possible to predict the outcome in term of feed intake and BW gain while offering high energy and high protein in a choice feed setting (Forbes and Shariatmadari, 1994). Based on the theory of certain nutrients inducing a high heat load, several experiments have been conducted with different levels of protein and energy at HT. A surplus of amino acids or imbalanced amino acid supply in a diet gives a high heat increment (Musharaf and Latshaw, 1999). Thus, a reduction of the dietary CP level has been recommended for broilers under heat stress conditions (Furlan et al., 2004). Most studies, however, have shown that low-protein diets have a negative effect on broiler performance also when ambient temperature is high. Reduced feed intake of a diet with a low protein level at HT will induce amino acid deficiency (Buyse et al., 1992; Alleman and Leclercq, 1997; Furlan et al., 2004).

In an ideal situation, protein is used for net gain (including turnover), thus preferably for protein deposition. By adjusting dietary protein levels and giving ideal protein one can avoid protein breakdown and extra heat production (Furlan et al., 2004). Also extra fat in a diet as compared to starch means less heat increment at the same intake level of metabolizable energy. This agrees with findings that broilers that are fed energy dense diets with a good amino acid composition across all T (21-35$^{o}$C) had a significantly improved feed efficiency and protein utilization (Cheng et al., 1997). In laying hens, adding fat at the expense of carbohydrates to a diet at 31$^{o}$C improved 17.2% feed consumption and only improved 4.5% feed consumption at 10 to 18$^{o}$C as compared without adding fat (Daghir, 2008b). When this dietary fat is used for fat deposition, this result in a less heat per unit of energy intake compared with starch or protein, for example. So less heat is dissipated from a diet high in fat compared with the same metabolizable energy from starch and protein (Balnave and Brake, 2005). Therefore, under high ambient T, both high densities (by dietary fat) and an ideal protein composition in terms of amino acid content are recommended (Bonnet et al., 1997). It is of interest to know how birds exposed to HT will select from high-energy and high-protein diets and compose an adequate feed intake. This selection may result in an optimal growth compared with single control diets.

The objectives of the present investigations were (1) to test the hypothesis that young chickens are able to compose an adequate ration by adjusting dietary nutrient intake from three different diets that vary in energy and protein contents from a cafeteria system; (2) to investigate if they compose ration with similar content of protein and energy under HT and NT conditions, and (3) to study if the selection of a certain ration differs with age of the birds.

## Materials and Methods

### *Animal Ethics*

All of the procedures involving animals in this experiment were approved by the Animal Experimental Committee of Wageningen University, the Netherlands.

***Birds, Housing and Care***

A total of 144 one-day-old male broiler chickens (Ross 308) were purchased from a commercial hatchery (Morren Breeders B.V., Lunteren, The Netherlands). After arrival, each bird was weighed and wing-tagged for identification. After that, each bird was randomly allotted to one of 24 floor pens with 6 birds each. Unique colouring of each bird within a pen was done to allow observation of the individual feed intake and panting behavior.

Twelve identical pens were made in each of two identical T-controlled rooms. Each pen had three drinking nipples with a cup underneath connected to a water tank of 10 litres capacity. To enable the birds an easy and equal access to feed, three feed troughs were placed in each pen. Wood shavings were used as litter and were regularly added to each pen to maintain good litter conditions. Pen dimensions were 1.75 m x 1.15 m and 0.80 m (W x L x H). All birds were exposed to a 23-h light (**L**) and 1-h dark (**D**) cycle for the first three days. Thereafter, a schedule of 12-h L (between 07:00 and 19:00 h) and 12-h D (19:00 to 07:00 h) per day was used. This light scheme resembles the natural situation in countries near the equator. Light intensity was maintained at 20 lux during the light periods throughout the experiment.

The T inside the room was initially kept at 32±2$^0$C with relative humidity (**RH**) of 70 to 80% from day 0 to 7. This was done to allow the birds to develop thermotolerance and to prevent mortality (May and Lott, 2000). The T and RH cycles were set up for day and night rhythm. After 7 d of age, the T in each room was set according to the experimental design.

***Experimental Design and Treatments***

This study was conducted as a split plot in a completely randomized design with repeated measures. The main plot was T (NT and HT) and sub plots were dietary treatments (control, single diet-fed birds versus choice-fed birds). Each dietary treatment was assigned randomly to six replicated pens in each T room with six birds each. Thus, the pen was the experimental unit.

One room was maintained at HT and the other room was maintained at NT. The HT regimen was maintained at 32±2$^o$C during the day (from 07:00 to 19:00 h) and at 25±2$^o$C during the night (from 19:00 to until 07:00 h) with RH 70 to 80% from 8 to 42 d of age. The NT regimen was set at 20$^o$C during the day and at 18$^o$C during the night with RH of 40 to 50% from 21 to 42 d of age. From d 8 to 20 onwards, a step-down decrease to a T of 20$^{o}$C was applied by 0.5$^{o}$C per day (d 8 to13) and 1$^{o}$C per day (d 14 to 20). Relative humidity was maintained about constant with no more than 5% variation at the level of the birds. Ventilation rates were similar in both rooms and only artificial light with 20 lux was provided.

The control animals were fed a diet that contained the recommended nutrient levels with regard to CP, essential amino acids and metabolizable energy (ME) level (NRC, 1994) for broiler chickens for the entire growing period (CP: 215 g/kg; ME: 2,895 kcal/kg). The choice-fed birds could choose from (1) the control diet (2) a high-protein diet (CP: 299 g/kg; ME: 2,780 kcal/kg), and (3) a high-energy diet (CP: 150.7 g/kg; ME: 3,241 kcal/kg). Ratios between all amino acids relative to lysine were similar on total and digestible bases among the diets. The choice-fed animals had free access to each diet in a separate feed trough. This gave the birds the opportunity to eat from the control, high-protein and high-energy diets and, thus, compose their own diet. In order to avoid any confounding in place and choice of feed from feeding troughs, the site of each feeding trough in a pen was changed every day according to a predetermined random schedule.

Each diet was supplied as pellets with a similar size (2 mm) and colour. Protein sources were soybean meal and heat-treated soybeans. Energy sources were maize, wheat, and vegetable oil. All ingredients were ground by using a hammer mill. Dietary compositions of the diets are presented in Table 1. Within each of the two ambient T rooms (NT and HT), both dietary treatments were offered. Both rooms were identical as this was shown in a previous experiment (Khoa, 2007).

### *Traits Measured*

Feed intake and panting behavior was recorded by direct observation during a 5 to 7 min walk through each room. Each bird in each of the 12 pens in each room was observed instantaneously for a moment during this period (a scan). So, after 5 to 7 min, the behavior of each bird in the room was scanned at a single moment. This procedure was repeated with 15-min pauses several times between 09:00 to 1:00 h and between 14:00 to 17:00 h. In total, one-day scan sampling lasted 3 h in the morning and 3 h in the afternoon and represented a 12-h daylight period. Scan sampling was done two days per week. Observations were conducted from wk 1 to 6 by an experienced assessor standing in front of the pen. Based on these observations (instantaneous scan sampling), we calculated the percentage of time the chickens in a certain pen spent on eating and

**Table 1.** The ingredients (g/kg) and calculated nutrient composition of the broiler dietary treatments.

| Ingredients | Dietary Treatments | | |
|---|---|---|---|
| | Control diet | High-Protein diet | High-Energy diet |
| Maize | 368.8 | 204.6 | 441.0 |
| Wheat | 200.0 | 150.0 | 250.0 |
| Soybean meal, Crude fiber<50 | 260.0 | 365.0 | 20.0 |
| Soybean, heat treated | 100.0 | 150.0 | 200.0 |
| Maize gluten meal | 0.0 | 60.0 | 0.0 |
| Fat/Oil plan origin (highly digestible vegetable oil, hg VC) | 35.0 | 35.0 | 50.0 |
| Limestone | 14.0 | 14.0 | 15.0 |
| Monocalcium phosphate | 9.0 | 7.0 | 10.0 |
| Premix[1] | 5.0 | 5.0 | 5.0 |
| Salt (NaCl) | 2.3 | 2.1 | 1.9 |
| $NaHCO_3$ | 2.0 | 2.2 | 2.7 |
| DL-Methionine | 2.1 | 2.8 | 1.3 |
| L-lysine HCl | 1.3 | 1.9 | 2.4 |
| L-Threonine | 0.4 | 0.3 | 0.6 |
| Natuphos 5000G[3] | 0.1 | 0.1 | 0.1 |
| Total | 1,000.0 | 1,000.0 | 1,000.0 |
| Calculated composition (g/kg) | | | |
| ME (kcal/kg) | 2,895.0 | 2,780.0 | 3,241.0 |
| CP | 215.0 (210)[2] | 299.9 (308.0) | 150.7 (154.0) |
| Moisture | 119.5 | 116.6 | 116.6 |
| Crude fat | 76.0 (78.3) | 84.2 (84.1) | 108.7 (113.1) |
| Crude fibre | 28.2 (26.9) | 30.7 (28.3) | 27.8 (24.4) |
| Total lysine | 12.2 | 16.9 | 8.6 |
| Digestible lysine | 10.5 | 14.7 | 7.4 |
| Total methionine | 5.3 | 7.4 | 3.6 |
| Digestible methionine | 4.9 | 6.9 | 3.3 |
| Total methionine + cysteine | 8.8 | 12.1 | 6.2 |
| Digestible methionine + cysteine | 7.7 | 10.7 | 5.4 |
| Total threonine | 8.2 | 11.3 | 5.8 |
| Digestible threonine | 6.9 | 9.5 | 4.8 |
| Total tryptophan | 2.5 | 3.4 | 1.6 |
| Digestible tryptophan | 2.2 | 3.0 | 1.4 |
| Calcium | 8.8 | 8.8 | 8.9 |
| Phosphorus | 5.5 | 5.6 | 5.1 |
| Available phosphorus | 3.0 | 2.7 | 3.0 |

*Continued...*

[1]Composition of 1 kg premix:vitamin A (retinyl acetate), 12,000 IU; vitamin $D_3$ (cholecalciferol), 2,400 IU; vitamin E (dl-α-tocopherol), 30 mg; vitamin $K_3$ (menadion), 1.5 mg; vitamin $B_1$ (thiamin), 2.0 mg; vitamin $B_2$ (riboflavin), 7.5 mg; vitamin $B_6$ (pyridoxine-HCl), 3.5 mg, vitamin $B_{12}$ (cyanocobalamin), 20 µg; niacin, 35 mg; vitamin $B_5$ (d-pantothenic acid), 10 mg; choline chloride, 460 mg; folic acid, 1.0 mg, vitamin B-complex, 0.2 mg; iron, 80 mg; copper, 12 mg; manganese, 85 mg; zinc, 60 mg; cobalt, 0.4 mg; iodine, 0.8; selenium, 0.1 mg and antioxidant 125 mg.
[2]Values in parenthesis were analyzed values.
[3]BASF, Ludwigshafen, Germany

panting behaviors relative to other behaviors. Eating was defined as a chicken with the head in or above the feeder, and panting was defined as breathing rapidly with short gasps with open beak and split wing feather alignment. All other behaviors were described by Bokkers and Koene (2003). Because previous experience showed that making more than one scan per observation of a pen increased behavioral activity of the birds as a reaction to the presence of the observer (Bokkers and Koene, 2003), only one scan per observation was made. All scan data per pen per week were pooled.

Feed intakes per pen in each room were recorded daily by weighing the feed troughs and averaged per bird per week. Water intake was measured by subtracting the given amount of water with left over (mL) each time adding water to the tank and averaged per bird per week. Protein and energy intake were calculated from the intake of each of the three diets and their concentrations. Birds were weighed weekly and BW gain was calculated accordingly. Weight of birds that died or had to be culled was determined and their BW gain was included in the calculation of the feed conversion ratio (**FCR**) per pen.

Blood samples, collected from the wing veins in birds from each pen, were taken four times at 7, 21, 35 and 42 d of age. Two birds were randomly taken out of each pen and blood was collected within 2 min after the chick was caught. Birds were marked and the same birds were sampled again at the next sampling. Blood samples of approximately one mL per bird were collected into syringe-needle assemblies that had been flushed with a solution of EDTA. One blood smears for each broiler was prepared and fixed with methanol. Then, the smears were stained immediately with Wright's stain 100% and rinsed with distilled water and ran air dry. Heterophil/Lymphocyte (**H/L**) ratio was counted from 100 cells per slide and classified using oil immersion microscopy at 100X (Dumonceaux and Harrison, 1994).

Just before blood sampling, body T was measured by a digital thermometer on same birds which were used for H/L ratio determination. Data of the two birds per pen were averaged.

***Statistical Analysis***

In this experiment, birds were randomly allocated to pens representing different diet treatments. All performance data were taken on the same experimental units, repeated in time: 6 weekly observations were available for each individual experimental unit. Repeated measurements on the same animal cannot be regarded as independent units of observation and mixed models can be used to account for the covariance structure among repeated observations (Littell et al., 1998). In the analysis, the repeated statement PROC MIXED in SAS (version 9.1; SAS Inst. Inc., Cary, NC) was used adding the factor time ("wk" as the time factor) and pen was considered as an additional random effect. The following statistical model was used:

$$Y_{ijkl} = \mu + T_i + F_j + T_i \times F_j + W_k + T_i \times W_k + F_j \times W_k + T_i \times F_j \times W_{k+} e_{ijkl}$$

where $Y_{ijkl}$ = measurement of response of the *l*th bird kept on *i*th temperature having the *j*th feed at *k*th time, μ is overall mean effect, $T_i$ is the *i*th fixed temperature effect (*i*=1 is NT and i=2 is HT), $F_j$ is the *j*th fixed feed effect (*j*=control or choice feed), $T_i$ x $F_j$ is interaction between T and feed, $W_k$ is the *k*th random week of measurement (*k*=1..6). $T_i$ x $W_k$ is the random interaction effect between temperature and week of measurement, $F_j$ x $W_k$ is the random interaction between feed and week of measurement, $T_i$ x $F_j$ x $W_k$ is the random interaction between temperature, feed and week of measurement and $e_{ijkl}$ is random error associated with *j*th diet assigned to the *i*th temperature at week measurement *k*, $e_{ijkl} \sim NID(0, \sigma^2_e)$.

Differences were considered significant at a probability level of $P < 0.05$. If significances of main effects or their interactions were detected, then means were compared using least squares means comparison. Means of significant effects were separated using the PDIFF option with the SAXTON macro in SAS at the $P < 0.05$ level (Saxton, 1998). The Kenward-Roger method was used for computing the denominator degrees of freedom for the tests of main effects.

The best covariance structure was based on the corrected Akaike Information Criteria (AICC). The first-order ante-dependence covariance structure [ANTE (1)] fitted the data best for feed intake, energy intake, BW gain, water intake and protein to gain ratio. The heterogenous autoregressive covariance structure [ARH(1)] fitted the data best for protein intake and energy-to-gain ratio. The Simple covariance structure fitted the data best for FCR and water-to-feed ratio.

Body T and heterophil/lymphocyte ratio were analyzed by PROC MIXED in SAS with the following linear model:

$Y_{ijk} = \mu + T_i + F_j + T_i \times F_j + e_{ijk}$

where $Y_{ijk}$ = measurement of response of the *k*th bird kept on *i*th temperature having the *j*th feed, $\mu$ is overall mean effect, $T_i$ is the *i*th fixed temperature effect (*i*=1 is NT and i=2 is HT), $F_j$ is the *j*th fixed feed effect (*j*= control or choice feed), $T_i \times F_j$ is interaction between T and Feed and $e_{ijk}$ is the residual error.

Behavior data were analysed with replicated observations per pen per week. Feed intake and panting behavior were analysed with the Kruskal-Wallis test and post hoc with the Wilcoxon two sample test by nonlinear procedure of SAS.

## Results

***Temperature and Relative Humidity.*** The T and RH are given as the average minimum and maximum ± SD for the respective interval. The T in the HT room during the first week was between 30.7±0.5°C and 31.9±0.4°C and the RH was between 58±6% and 74±6%,.whereas the T in the NT room was between 30.0±0.9°C and 31.9±0.6°C and the RH was between 64±8% and 80±7%. The T in the HT room from wk 2 to 6 was between 25.2±2.0°C and 31.5±0.5°C and the RH was between 61.0±7% and 79±10%. The T at the NT room from wk 4 to 6 was between 18.5±0.6°C and 21.0±0.4°C and the RH was between 40±5% and 49±5%.

***Eating and Panting Behavior.*** Results on the percentage time spent on eating and panting behavior per week are given in Figure 1. Eating time declined from wk 1 to 6 of age for both T conditions and for both dietary treatments. The time budget for eating was higher ($P < 0.01$; Figure 1 upper-left panel) for birds kept at NT than for those kept at HT in the first, fifth and sixth week of age. Birds on the choice-feeding regimen used a similar eating time as animals on the control-feeding regimen ($P > 0.05$; Figure 1 upper-right panel). Panting behavior was more at HT after wk 1 onwards ($P < 0.05$; Figure 1 down-left panel) and it was similar for both dietary treatments ($P > 0.05$; Figure 1 down-right panel).

***Bird Performance.*** Mortality in this study was very low (1.4%). All performance data in the tables are data corrected for mortality by week. Probability values for every parameter are presented in Table 2. Differences in performance of the broilers in each week at different T and dietary treatments are presented in Table 3.

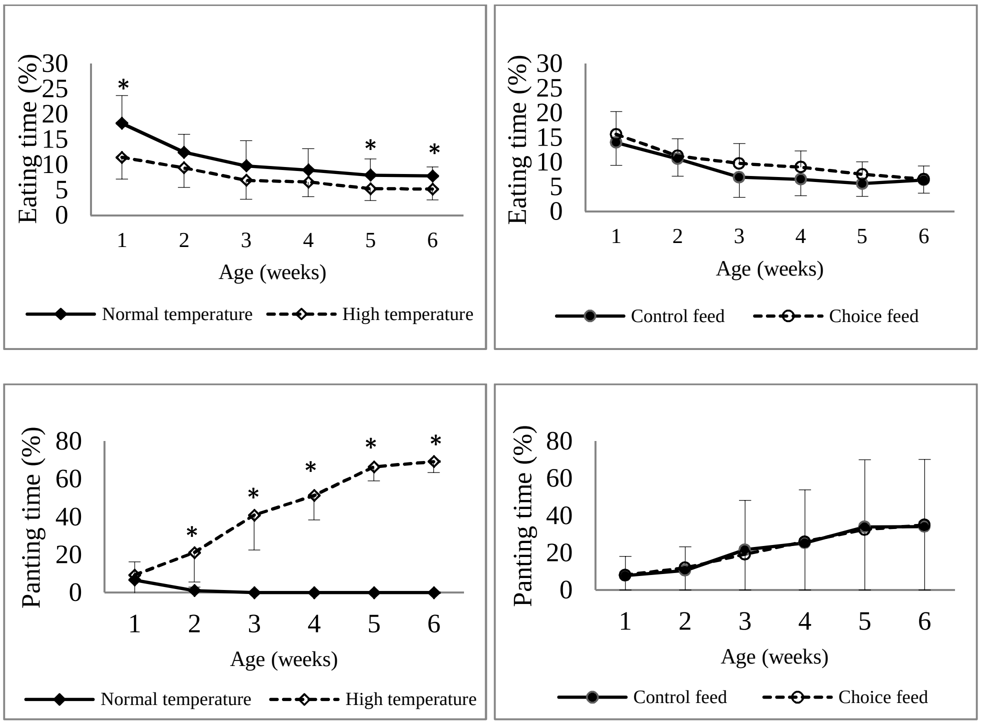


**Figure 1**. Eating time (expressed as a percentage within observation time) (upper panel) and time spend on panting (lower panel) at different temperatures (left panels) and at different diets (right panels). Vertical bars represent SD. The asterisk (*) means a significant difference of at least $P$<0.05 at a certain age.The T and relative humidity (RH) in the high-T (HT) room during the first week were 31.3±0.5°C and 63.7±6.8%, respectively, whereas the T and RH at the normal-T (NT) room were 31.0±0.7°C and 63.7±6.8%, respectively. For NT, the T ranged from 18.5±0.7°C to 21.0±0.5°C and the RH ranged from 40.5±5.2% to 49.0±5.3% from wk 4 to 6); for HT, the T ranged from 25.2±2.0°C to 31.5±0.5°C and the RH ranged from 61.0±7.8% to 79.2±10.2% from wk 2 to 6. Control diet= 215 g of CP/kg and 2,895 kcal of ME/kg; Choice diet= (1) the control diet, (2) a high-protein diet (299 g of CP/kg and 2,780 kcal of ME/kg), and (3) a high-energy diet (150.7 g of CP/kg and 3,241 kcal of ME/kg).

*Feed Intake, Protein Intake and Energy Intake*. Temperature had a major effect on feed, protein and energy intake after the change in ambient temperature at d 7 (Tables 2 and 3). Birds that were choice-fed consumed most feed from the energy-rich diet (59% at NT and 52% at HT). The amount chosen as second choice was the control diet (25% at NT and 27% at HT) and the third choice was the high-protein diet (16% at NT and

20% at HT) (Figure 2). The increase in intake of the high energy diet at HT occurred at 10 d of age and at NT occurred at 14 d of age. Overall, feed, protein and energy intakes were higher at NT ($P < 0.001$) compared with HT (Table 2). An interaction between T and week showed that the difference in feed, protein and energy intake between temperatures regimens (HT and NT) increased over time. The difference was largest after 3 wk of age (Figure 3). Total feed intake in weight was similar between control- and choice-fed birds (Table 2). Protein intake was higher ($P < 0.001$) and energy intake was lower ($P < 0.030$) for control-fed birds compared with choice-fed birds (Table 2 and Figure 3). Protein intake during the last week was higher than all other week of trial for all dietary treatment groups (Figure 4). There was also a T x dietary treatment x week interaction effect on protein intake (Table 2). The protein intake was similar for choice-fed birds at NT and control-fed birds at HT in wk 3 to 6 (Table 4). Energy intake in choice-fed birds was higher than in control-fed birds at NT in wk 5 and 6. However, the intake with choice feeding was similar compared to energy intake of the control diet at HT at all ages (Table 3).

*Body Weight Gain and Feed Conversion Ratio.* Temperature did significantly affect BW gain but BW gain was not affected by dietary treatment (Tables 2 and 3). An interaction between T and week showed that broilers at NT continued to increase in BW until wk 6, whereas broilers at HT continued to increase in BW until wk 4 after which the BW gain of birds until the end of the experiment increased much less (Figure 3). Overall, FCR was not affected by either T or dietary treatment, but increased over time (week; Table 2). However, FCR was lower for birds at HT in wk 2 and higher for birds at HT in wk 6 as compared with birds at NT (Table 3).

*Ratios of Protein and Energy-to-Gain.* Temperature did not significantly influence the protein-to-gain ratio, but it affected the dietary energy-to-gain ratio (Table 2). Energy-to-gain ratio was higher at NT than at HT (Table 3). Protein- and energy-to-gain ratios were affected by dietary treatment (Table 2). The ratio of dietary protein to gain was higher ($P < 0.001$) and the ratio of dietary energy-to-gain was lower ($P < 0.001$) for control-fed birds compared to choice-fed birds (Table 3). An interaction between T and week (Table 2) showed that the difference in protein-to-gain ratio between HT and NT was most pronounced at wk 2 and 3 (Figure 3). The interaction between dietary treatment and week showed that the difference in protein- and energy-to-gain ratio between control- and choice-fed birds were most clear from wk 3 onwards (Figure 4).

**Table 2**. Probability values of main effects and interaction between ambient temperatures (T), dietary treatment (F) and week (W) for different traits[1].

| Main effects[2] | Feed intake (g/bird per wk) | Protein intake (g CP/ bird per wk) | Energy intake (kcal ME /kg per bird/wk) | BW gain (g/bird per wk) | Water intake (g/bird per wk) | Protein-to-gain ratio (g CP intake/100 g BW gain) | Energy-to-gain ratio (kcal of ME / kg per 100 g BW gain) | FCR (g/g) | Water-to-feed ratio (g/g) |
|---|---|---|---|---|---|---|---|---|---|
| T | **<0.001** | **<0.001** | **<0.001** | **<0.001** | 0.904 | 0.838 | **0.032** | 0.062 | **<0.001** |
| F | 0.826 | **<0.001** | **0.030** | 0.159 | **0.004** | **<0.001** | **<0.001** | 0.179 | **0.001** |
| W | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **0.080** |
| T x F | 0.306 | 0.336 | 0.133 | 0.650 | 0.798 | 0.458 | 0.089 | 0.179 | 0.864 |
| T x W | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **0.039** | **0.006** | 0.415 | 0.171 | **0.001** |
| F x W | 0.960 | **<0.001** | 0.276 | 0.356 | 0.096 | **0.016** | **0.031** | 0.461 | 0.853 |
| T x F x W | 0.333 | **0.023** | 0.179 | 0.498 | 0.272 | 0.393 | 0.397 | 0.565 | 0.187 |

[1]The T and relative humidity (RH) in the high-T (HT) room during the first week were 31.3±0.5°C and 63.7±6.8%, respectively, whereas the T and RH at the normal-T (NT) room were 31.0±0.7°C and 63.7±6.8%, respectively. For NT, the T ranged from 18.5±0.7°C to 21.0±0.5°C and the RH ranged from 40.5±5.2% to 49.0±5.3% from wk 4 to 6); for HT, the T ranged from 25.2±2.0°C to 31.5±0.5°C and the RH ranged from 61.0±7.8% to 79.2±10.2% from wk 2 to 6.
Control diet= 215 g of CP/kg and 2,895 kcal of ME/kg; Choice diet= (1) the control diet, (2) a high-protein diet (299 g of CP/kg and 2,780 kcal of ME/kg), and (3) a high-energy diet (150.7 g of CP/kg and 3,241 kcal of ME/kg).
[2]T x F=interaction between T and F;.
T x W=interaction between T and W.
F x W=interaction between feed and W.
T x F x W=interaction between T, F and W.

**Table 3**. Least squares means of performance parameters in broiler chicken from 0 to 6 wk of age as affected by temperature and dietary treatment[1].

| Parameters | Week | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| Feed intake (g/bird per week) | | | | | | |
| NT Control diet | 119.9 | 311.5[a] | 497.9 | 780.8[a] | 988.9[a] | 1186.3[a] |
| Choice diet | 119.9 | 317.5[a] | 493.0 | 788.9[a] | 1024.9[a] | 1215.4[a] |
| NT Control diet | 119.9 | 274.3[b] | 465.5 | 691.0[b] | 806.2[b] | 935.4[b] |
| Choice diet | 114.3 | 259.9[b] | 454.6 | 657.8[b] | 779.1[b] | 912.5[b] |
| SEM | 4.0 | 6.4 | 14.8 | 22.0 | 30.5 | 30.1 |
| Protein intake (g of CP/bird per week) | | | | | | |
| NT Control diet | 25.8 | 67.0[a] | 107.0[a] | 167.9[a] | 212.6[a] | 255.1[a] |
| Choice diet | 25.6 | 66.7[a] | 93.3[bc] | 142.1[b] | 178.5[b] | 207.5[b] |
| NT Control diet | 25.8 | 59.0[b] | 100.1[ab] | 148.6[b] | 173.3[bc] | 201.1[b] |
| Choice diet | 24.9 | 51.2[c] | 85.3[c] | 128.4[c] | 157.0[c] | 171.7[c] |
| SEM | 0.8 | 1.5 | 3.4 | 4.1 | 6.2 | 5.6 |
| Energy (kcal of ME/ kg per bird per week) | | | | | | |
| NT Control diet | 347.0 | 901.8[a] | 1441.3[ab] | 2260.3[a] | 2862.9[b] | 3434.5[b] |
| Choice diet | 358.5 | 954.2[a] | 1521.4[a] | 2463.2[a] | 3222.2[a] | 3836.9[a] |
| NT Control diet | 347.3 | 794.0[b] | 1347.6[b] | 2000.3[b] | 2334.0[c] | 2708.1[c] |
| Choice diet | 340.1 | 794.3[b] | 1405.1[ab] | 2020.2[b] | 2374.0[c] | 2815.6[c] |
| SEM | 11.9 | 19.4 | 43.6 | 67.8 | 96.4 | 97.0 |
| BW gain (g/bird per week) | | | | | | |
| NT Control diet | 110.6 | 227.1[ab] | 364.2 | 536.1[a] | 598.8[a] | 667.3[a] |
| Choice diet | 104.8 | 245.8[a] | 354.6 | 515.2[ab] | 585.7[a] | 642.4[a] |
| NT Control diet | 110.5 | 218.3[b] | 358.1 | 481.8[bc] | 480.7[b] | 507.1[b] |
| Choice diet | 111.5 | 212.8[b] | 328.3 | 455.2[c] | 464.6[b] | 478.0[b] |
| SEM | 3.4 | 6.9 | 13.6 | 15.2 | 24.7 | 28.1 |
| Water (g/bird per week) | | | | | | |
| NT Control diet | 220.7 | 538.9[ab] | 919.4 | 1394.4[ab] | 1813.9 | 2175.0[a] |
| Choice diet | 228.6 | 555.6[a] | 852.8 | 1305.6[b] | 1661.1 | 1852.8[b] |
| NT Control diet | 245.2 | 536.1[ab] | 961.1 | 1480.6[a] | 1827.8 | 1944.4[b] |
| Choice diet | 216.7 | 475.0[b] | 858.3 | 1319.4[b] | 1719.4 | 1891.7[b] |
| SEM | 10.1 | 21.7 | 37.1 | 51.7 | 68.9 | 71.8 |
| FCR (g/g) | | | | | | |
| NT Control diet | 1.08 | 1.37[a] | 1.37 | 1.46 | 1.67 | 1.78[a] |
| Choice diet | 1.15 | 1.30[ab] | 1.40 | 1.53 | 1.76 | 1.89[ab] |
| NT Control diet | 1.09 | 1.26[b] | 1.30 | 1.43 | 1.68 | 1.91[b] |
| Choice diet | 1.03 | 1.23[b] | 1.39 | 1.45 | 1.68 | 1.91[b] |
| SEM | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |

*Continued...*

**Table 3**. (Continued). Least squares means of performance parameters in broiler chicken from 0 to 6 wk of age as affected by temperature and dietary treatment[1].

| Parameters | Week (W) | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| Protein to gain ratio(g of CP intake/100 g BW gain) | | | | | | |
| NT Control diet | 23.3 | 29.5[a] | 29.4[a] | 31.3[a] | 36.0[a] | 38.3[a] |
| Choice diet | 24.4 | 27.3[a] | 26.3[c] | 27.6[b] | 30.6[b] | 32.3[b] |
| HT Control diet | 23.4 | 27.1[a] | 27.9[b] | 30.8[a] | 36.0[a] | 41.1[a] |
| Choice diet | 22.4 | 24.2[b] | 26.3[c] | 28.2[b] | 33.8[a] | 36.0[ab] |
| SEM | 0.8 | 0.8 | 0.3 | 0.3 | 1.0 | 1.7 |
| Energy-to-gain ratio (kcal ME intake/kg per 100 g BW gain) | | | | | | |
| NT Control diet | 313.4 | 397.0 | 395.9[b] | 421.6[c] | 484.6[b] | 515.5[b] |
| Choice diet | 341.6 | 389.7 | 432.0[a] | 478.4[a] | 553.2[a] | 597.8[a] |
| HT Control diet | 314.9 | 365.2 | 376.4[b] | 415.3[c] | 485.4[b] | 553.8[ab] |
| Choice diet | 305.0 | 374.7 | 429.4[a] | 444.1[b] | 510.8[ab] | 589.5[a] |
| SEM | 11.7 | 10.8 | 8.9 | 5.5 | 16.6 | 23.8 |
| Water-to-feed ratio (g/g) | | | | | | |
| NT Control diet | 1.84 | 1.74 | 1.85[ab] | 1.79[b] | 1.83[b] | 1.83[b] |
| Choice diet | 1.92 | 1.75 | 1.73[b] | 1.65[b] | 1.62[b] | 1.52[c] |
| HT Control diet | 2.06 | 1.95 | 2.07[a] | 2.14[a] | 2.28[a] | 2.08[a] |
| Choice diet | 1.90 | 1.84 | 1.90[ab] | 2.01[ab] | 2.23[a] | 2.08[a] |
| SEM | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 | 0.08 |

[a-c] Means within a week between treatments without a common superscript differ significantly (*P*<0.05); no superscript letter means nonsignificant differences.

[1]The temperature (T) and relative humidity (RH) in the high-T (HT) room during the first week were 31.3±0.5°C and 63.7±6.8%, respectively, whereas the T and RH at the normal-T (NT) room were 31.0±0.7°C and 63.7±6.8%, respectively. For NT, the T ranged from 18.5±0.7°C to 21.0±0.5°C and the RH ranged from 40.5±5.2% to 49.0±5.3% from wk 4 to 6); for HT, the T ranged from 25.2±2.0°C to 31.5±0.5°C and the RH ranged from 61.0±7.8% to 79.2±10.2% from wk 2 to 6. Control diet= 215 g of CP/kg and 2,895 kcal of ME/kg; Choice diet= (1) the control diet, (2) a high-protein diet (299 g of CP/kg and 2,780 kcal of ME/kg), and (3) a high-energy diet (150.7 g of CP/kg and 3,241 kcal of ME/kg).

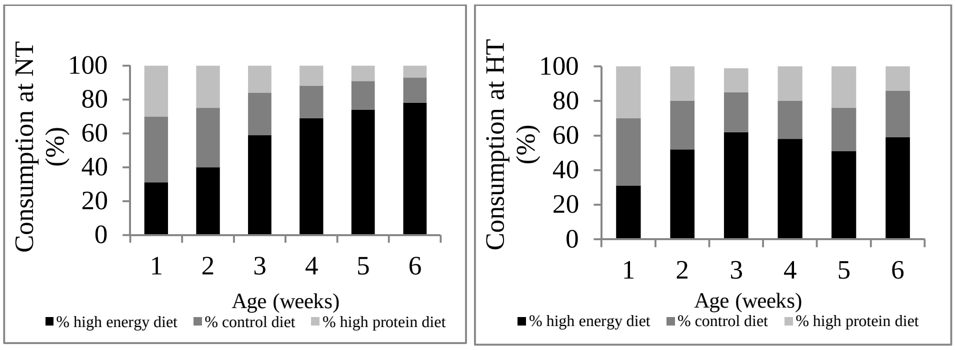


**Figure 2**. Consumption of a control diet, high-protein diet and high-energy diet as a proportion of total feed intake at normal temperature (NT; left panel) and high temperature (HT; right panel). The T and relative humidity (RH) in the high-T (HT) room during the first week were 31.3±0.5°C and 63.7±6.8%, respectively, whereas the T and RH at the normal-T (NT) room were 31.0±0.7°C and 63.7±6.8%, respectively. For NT, the T ranged from 18.5±0.7°C to 21.0±0.5°C and the RH ranged from 40.5±5.2% to 49.0±5.3% from wk 4 to 6); for HT, the T ranged from 25.2±2.0°C to 31.5±0.5°C and the RH ranged from 61.0±7.8% to 79.2±10.2% from wk 2 to 6. Control diet= 215 g of CP/kg and 2,895 kcal of ME/kg; Choice diet= (1) the control diet, (2) a high-protein diet (299 g of CP/kg and 2,780 kcal of ME/kg), and (3) a high-energy diet (150.7 g of CP/kg and 3,241 kcal of ME/kg).

**Table 4**. Effect of 3-way interaction between temperature, dietary treatment, and week on protein intake of broiler from 0 to 6 wk[1].

| Protein intake (g of CP/bird per week) | Week (W) | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| NT Control diet | 25.8$^{a}$ | 67.0$^{d}$ | 107.0$^{g}$ | 167.9$^{jk}$ | 212.6$^{l}$ | 255.1$^{m}$ |
| Choice diet | 25.6$^{a}$ | 66.7$^{d}$ | 93.3$^{ef}$ | 142.1$^{i}$ | 178.5$^{k}$ | 207.5$^{l}$ |
| HT Control diet | 25.8$^{a}$ | 59.0$^{c}$ | 100.0$^{fg}$ | 148.5$^{i}$ | 173.3$^{jk}$ | 201.1$^{l}$ |
| Choice diet | 24.9$^{a}$ | 51.2$^{b}$ | 85.3$^{e}$ | 128.4$^{h}$ | 157.0$^{ij}$ | 171.7$^{k}$ |

[a-m] Means without common superscript letters differ significantly ($P < 0.05$).

[1]The temperature (T) and relative humidity (RH) in the high-T (HT) room during the first week were 31.3±0.5°C and 63.7±6.8%, respectively, whereas the T and RH at the normal-T (NT) room were 31.0±0.7°C and 63.7±6.8%, respectively. For NT, the T ranged from 18.5±0.7°C to 21.0±0.5°C and the RH ranged from 40.5±5.2% to 49.0±5.3% from wk 4 to 6); for HT, the T ranged from 25.2±2.0°C to 31.5±0.5°C and the RH ranged from 61.0±7.8% to 79.2±10.2% from wk 2 to 6. Control diet= 215 g of CP/kg and 2,895 kcal of ME/kg; Choice diet= (1) the control diet, (2) a high-protein diet (299 g of CP/kg and 2,780 kcal of ME/kg), and (3) a high-energy diet (150.7 g of CP/kg and 3,241 kcal of ME/kg).

*Water Intake and Water-to-Feed Ratio*. Temperature did not significantly affect water intake, but it affected the water-to-feed ratio (Table 2). The water-to-feed intake ratio was higher at HT than at NT (Table 3). Water intake ($P<0.004$) and the water-to-feed ratio were higher ($P < 0.001$) for control-fed birds as compared with choice-fed

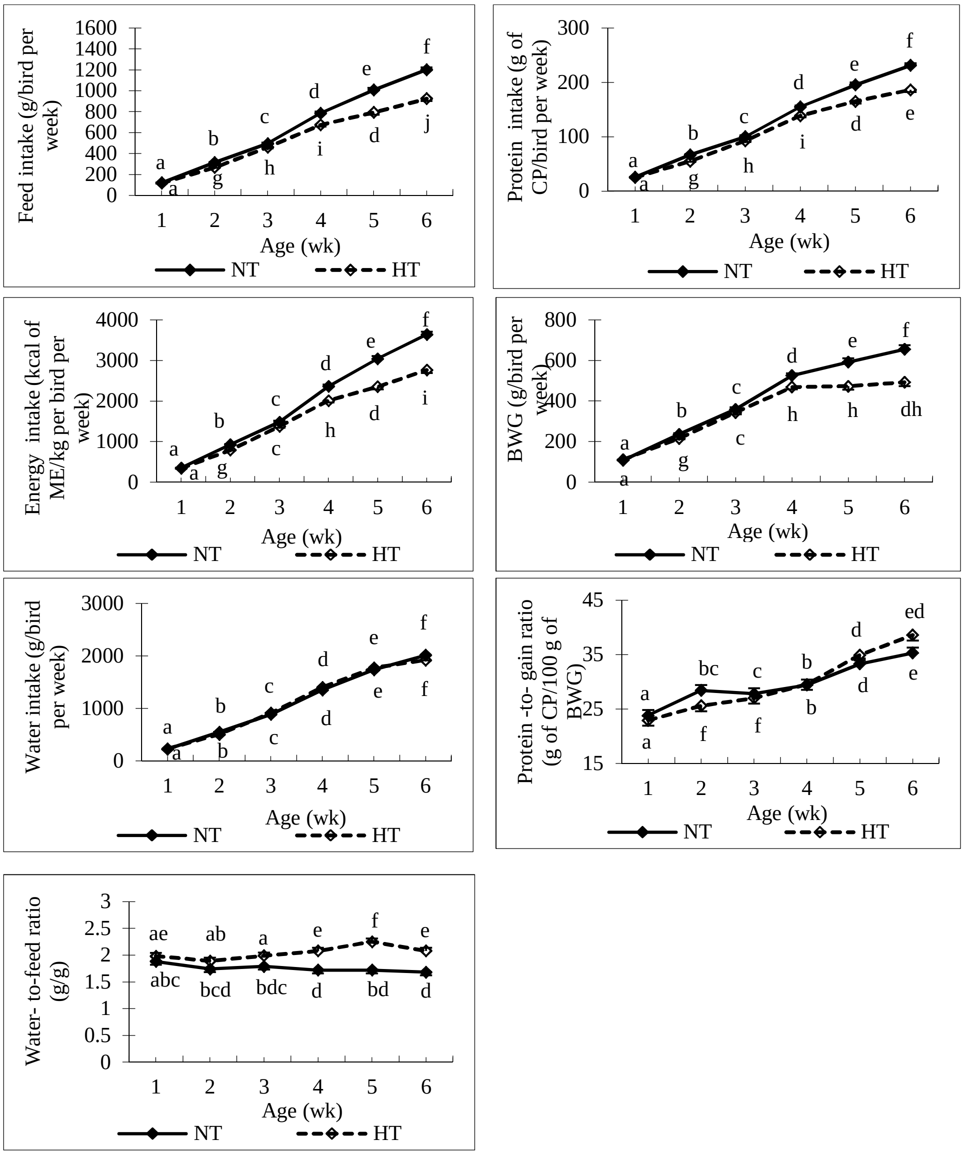


**Figure 3**. Least square means for traits that show a significant temperature (T) and week interaction.Means within and between lines without common letters (a-j) differ significantly ($P < 0.05$). The temperature (T) and relative humidity (RH) in the high-T (HT) room during the first week were 31.3±0.5°C and 63.7±6.8%, respectively, whereas the T and RH at the normal-T (NT) room were 31.0±0.7°C and 63.7±6.8%, respectively. For NT, the T ranged from 18.5±0.7°C to 21.0±0.5°C and the RH ranged from 40.5±5.2% to 49.0±5.3% from wk 4 to 6); for HT, the T ranged from 25.2±2.0°C to 31.5±0.5°C and the RH ranged from 61.0±7.8% to 79.2±10.2% from wk 2 to 6. BWG= BW gain.

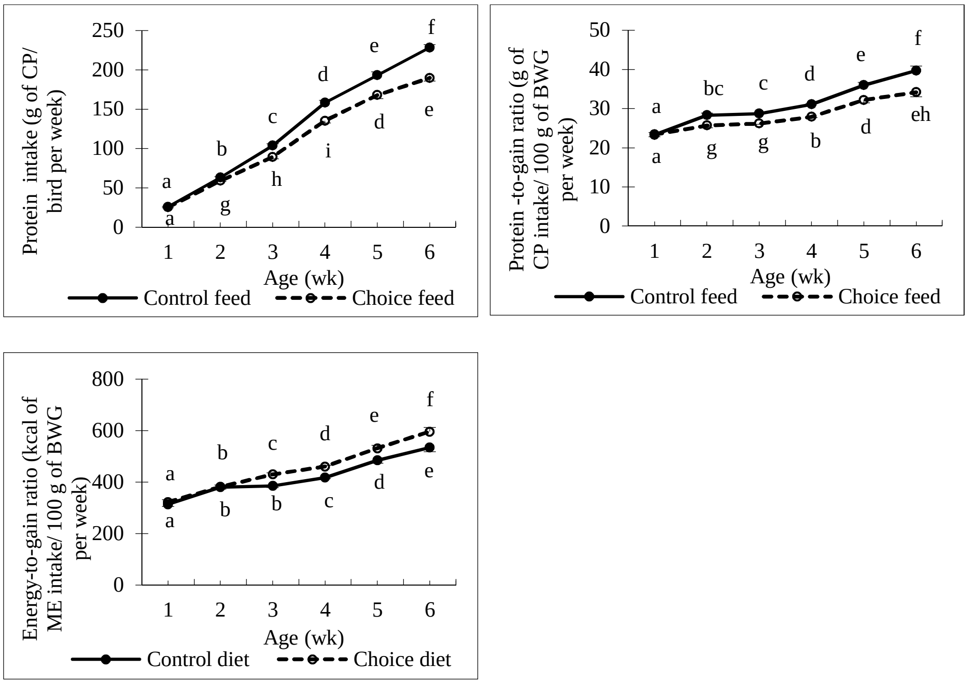


**Figure 4**. Least square means for traits that show a significant dietary treatmentand week interaction. Means within and between lines without common letter s (a-j) differ significantly ($P < 0.05$). Control diet= 215 g of CP/kg and 2,895 kcal of ME/kg; Choice diet= (1) the control diet, (2) a high-protein diet (299 g of CP/kg and 2,780 kcal of ME/kg), and (3) a high-energy diet (150.7 g of CP/kg and 3,241 kcal of ME/kg).

birds (Table 2 and 3). The interaction between T and week for water intake could be fully explained by the significant week effect. The difference between HT and NT in water-to-feed ratio increased over time, most clearly after 3 wk of age (Figure 3).

***Body Temperature and Heterophil/Lympocyte Ratio*.** Results of T and dietary treatment on body T and H/L ratio are given in Table 5. Temperature had a major effect on body T and H/L ratio at d 21, 35 and 42. Body T and H/L ratio were higher at HT than at NT at these ages. No effect of dietary treatment on body T and H/L ratio could be detected.

**Table 5**. Effect of ambient temperatures (T) and dietary treatment (F) on body temperature and H/L ratio[1]

| Variables | NT | | HT | | | Source of variation[2] | | |
|---|---|---|---|---|---|---|---|---|
| | Control diet | Choice diet | Control diet | Choice diet | SEM | T | F | T x F[3] |
| | | | | | | ------------*P*------------- | | |
| Body temperature (°C) | | | | | | | | |
| Day 7 | 41.03 | 41.02 | 40.95 | 40.93 | 0.106 | 0.559 | 0.890 | 0.890 |
| Day 21 | 40.82 | 40.98 | 41.13 | 41.19 | 0.078 | **0.003** | 0.182 | 0.531 |
| Day 35 | 41.14 | 41.22 | 42.16 | 41.98 | 0.125 | **<0.001** | 0.573 | 0.176 |
| Day 42 | 41.30 | 41.29 | 42.12 | 41.88 | 0.980 | **<0.001** | 0.231 | 0.264 |
| H/L ratio | | | | | | | | |
| Day 7 | 0.10 | 0.10 | 0.07 | 0.12 | 0.018 | 0.850 | 0.215 | 0.206 |
| Day 21 | 0.10 | 0.14 | 0.19 | 0.21 | 0.020 | **0.007** | 0.138 | 0.491 |
| Day 35 | 0.14 | 0.13 | 0.37 | 0.43 | 0.081 | **0.009** | 0.752 | 0.648 |
| Day 42 | 0.23 | 0.17 | 0.37 | 0.49 | 0.083 | **0.011** | 0.687 | 0.303 |

[1]Mean values are expressed as an average of 6 replicate pens of 2 birds each. The temperature (T) and relative humidity (RH) in the high-T (HT) room during the first week were 31.3±0.5°C and 63.7±6.8%, respectively, whereas the T and RH at the normal-T (NT) room were 31.0±0.7°C and 63.7±6.8%, respectively. For NT, the T ranged from 18.5±0.7°C to 21.0±0.5°C and the RH ranged from 40.5±5.2% to 49.0±5.3% from wk 4 to 6); for HT, the T ranged from 25.2±2.0°C to 31.5±0.5°C and the RH ranged from 61.0±7.8% to 79.2±10.2% from wk 2 to 6.
[2]Significant effects ($P$<0.05) are printed in bold.
[3]T x F=interaction between T and F.

## Discussion

***Eating and Panting Behavior***. Birds at NT needed about 3.3% more time to consume their feed than the birds at HT did, although the feed intake was 17.5% higher at NT. It seems that birds at NT did eat a larger meal size per unit of time than the birds at HT. The latter birds showed much more panting behavior (42%) than the birds at NT (Figure 1). Therefore panting behavior may have reduced meal size by frequently interrupting eating behavior.

***Feed Intake, Protein Intake and Energy Intake*.** The observed higher feed intake of broilers at NT than that of broilers at HT (2% in wk 1 to 23% in wk 6) in this study is in agreement with observations reported by others in broilers (Cheng et al., 1997; Yo et al., 1998; Temim et al., 1999) and in turkeys (Veldkamp et al., 2000; Veldkamp et al., 2003). These studies showed that at high ambient T, the decrease in feed intake due to heat stress ranged from 25 to 30%. Despite a similar total feed intake between both dietary treatments (control and choice-fed), choice-fed birds within both T regimens had a higher intake of the high-energy diet at the expense of the intake of the high-protein diet (Figure 2). Siegel et al. (1997) similarly reported that broilers preferred a higher proportion of a low protein-high energy diet than low energy-high protein diet when given the choice. This suggests that formulated diet for maximizing growth may not reflect dietary preferences of birds. Our data confirmed this observation. Overall, broilers exposed to HT consumed 14.4% less protein and 18.3% less energy as compared with broilers exposed to NT, which is in agreement with results of Cheng et al. (1997) and Sakomura et al. (2005). Within NT, choice-fed broilers consumed 14.6% less protein and 9.9% more energy compared to controls. Choice-fed broilers exposed to HT consumed 12.6% less protein and 2.3% more energy compared to control-fed broilers.

Changes of these preferences for certain diets are illustrated in Figure 2. This is particularly evident up to 21 d of age where intake of the high-energy diet increases with age. Surprisingly, the increased consumption of the high-energy diet at HT reaches a plateau at 3 wk of age. At NT, however, the intake of this diet still increased until 6 wk of age. It is suggested that at older ages, birds at HT may have a lower demand for energy needed for protein synthesis that is only partially counterbalanced by a higher demand for energy needed for activity (locomotion and panting). If the energy part of the diet is used for energy function, it can convert 66% of the calorific value into adenosine triphosphate (**ATP**) and the rest (34%) results as waste heat. When protein is used for ATP, it can convert about 58% of calorific value into ATP; therefore, more heat is produced per unit of calorific value (42%) (Black, 1995). The use of high-energy rations for broilers in warm regions by including more fat may thus be beneficial for the animal, since fat gives less heat load per unit of kilojoules than do carbohydrate and protein (Musharaf and Latshaw, 1999). So, when the birds get access to more energy in the choice-feed system, they avoided a higher heat load by ingesting more of the energy diet for energy purposes. In the control-fed birds, the energy demand was probably met by converting protein into energy, which increased the heat load. Adaptive changes in feed intake and energy expenditure over the long-term contribute to homeostatic control of body energy stores and maintenance of a constant BW (Richards and Proszkowiec-Weglarz, 2007). Therefore, the birds change their metabolism when they are reared above or below their thermoneutral temperatures to dissipate heat or increase heat production (Sakomura et al., 2005).

***Body Weight Gain and Feed Conversion Rate.*** Broilers that were subjected to HT gained less BW than those subjected to NT. This negative effect of HT on BW gain increased with increasing age. This result was confirmed by Alleman and Leclercq (1997), Cheng et al. (1997) and May et al. (1998). Overall, BW gain after 6 wk of age was about 15% lower at HT than at NT.

Within each T regimen, BW gain was slightly lower in choice-fed than in control-fed birds. This was probably because protein intake was significantly lower for the choice-fed birds as compared with the control-fed birds. For energy intake, differences were not significantly different, although choice-fed bird had, on average, a numerically higher energy intake than the control-fed birds. As expected in young birds, BW gain is most determined by protein intake, whereas energy intake only sustains BW gain. In a similar choice-feeding experiment, Yo et al. (1998) found a similar relationship between BW gain and protein intake.

FCR did not differ significantly between T regimens and between dietary treatments, except for the T in wk 2 and 6. It is shown by the data that the high energy intake (and thus, low protein intake) of the choice-fed birds at NT had a negative effect on FCR. For a better performance, these birds should have consumed more protein than they did. So, protein intake of choice-fed birds at NT was limiting, which seemed not to be the case at HT, probably due to less deficient protein levels at the lower feed intake level.

***Ratios of Protein and Energy-to-Gain.*** The protein-to-gain ratio was lower and the energy-to-gain ratio was higher in choice-fed birds than in control-fed birds at both T. These ratios were confirmed in choice-feeding study by Yo et al. (1998) and Siegel et al. (1997). The larger energy intake of choice-fed birds at both T and, as a consequence the lower protein intake, had only a slightly negative effect on BW gain. This suggests that energy was limiting in the control-fed birds and it justifies a somewhat higher energy-to-protein ratio in the diets at both T. An adequate protein and energy concentration in the diet by week at NT and HT is illustrated in Figure 5. The protein and energy concentration varies between 171 to 214 g CP/kg and 2,990 to 3,157 kcal of ME/kg at NT and between 188 to 218 g CP/kg and 2,997 to 3,086 kcal of ME/kg at HT as the birds become older.

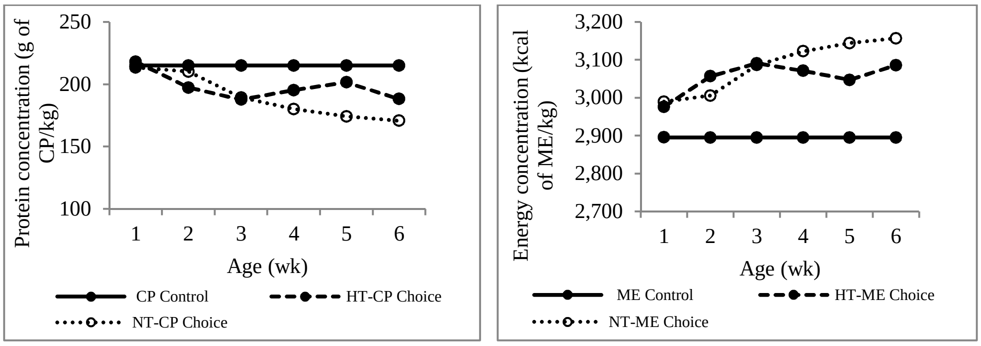


**Figure 5**. Crude protein and energy concentration of feed intake under choice conditions at normal temperature (NT) and high temperature (HT). CP control= CP concentration in control diet, HT-CP choice= CP concentration eaten at HT, NT-CP choice=CP concentration eaten at NT. ME control=ME concentration in control diet, HT-ME choice= ME concentration eaten at HT, NT-ME choice=ME concentration eaten at NT. The temperature (T) and relative humidity (RH) in the high-T (HT) room during the first week were 31.3±0.5°C and 63.7±6.8%, respectively, whereas the T and RH at the normal-T (NT) room were 31.0±0.7°C and 63.7±6.8%, respectively. For NT, the T ranged from 18.5±0.7°C to 21.0±0.5°C and the RH ranged from 40.5±5.2% to 49.0±5.3% from wk 4 to 6); for HT, the T ranged from 25.2±2.0°C to 31.5±0.5°C and the RH ranged from 61.0±7.8% to 79.2±10.2% from wk 2 to 6.

***Water Intake and Water-to-Feed Ratio.*** Ambient T is known to influence water intake. Chickens consume water about 2-fold at 32°C and 2.5-fold at 37°C (NRC, 1981) and 3.6-fold at 38°C (North and Bell, 1990) compared with 21°C. It has been reported that birds seek to manage heat stress by increasing water consumption and then increase urinary production (Borges et al., 2004). However, we did not find a significant difference in water intake between T. It should be pointed out here that the use of nipples by panting birds at HT may inhibit somewhat the natural way of drinking from a bell drinker. Previous research demonstrated that water consumption from nipples as compared with bell drinkers at cyclic HT by panting broilers was 21% lower (May et al., 1997). This suggests that the chickens may not be able to coordinate the water intake while panting. The inability of broilers to drink sufficient amounts of water at HT may lead to an unbalanced total body water content due to a high degree of water loss from the body and this could result in poor performance of at HT (Yahav et al., 2004).

Water consumption in this study was 8% higher in control-fed birds as compared to choice-fed birds. A higher water intake was not related to a higher feed intake (only 0.5% higher feed intake for control-fed birds compared with choice-fed birds), but it was positively correlated to a 14% higher protein intake of control-fed birds as compared to choice-fed birds. Increasing the protein level in the diet increases water intake and also the water-to-feed ratio (Marks and Pesti, 1984). Thus, a high protein consumption by broilers need to be catabolized and excreted via the kidneys in the form of uric acid, which implies extra water (Francesch and Brufau, 2004).

The higher water-to-feed ratio at HT indicates that the bird uses the extra water to enable evaporation during panting (Figure 1). Increasing water intake may benefit the birds by facilitating evaporation (Belay and Teeter, 1993) and this may prevent the increase in body T (Furlan et al., 2004; Ahmad et al., 2005). Heat loss through evaporation can account for 60% of the total heat loss (Etches et al., 2008) and even more than 80% at high T (Ahmad and Sarwar, 2006). Evaporative heat loss is associated with loss of water and therefore dehydration can occur. Sufficient water intake will facilitate this type of heat loss and contribute to thermotolerance at high ambient T (Yahav et al., 2005).

***Body Temperature and Heterophil/Lymphocyte Ratio.*** The somewhat higher body T at HT agrees with other studies (Yahav, 2000). The higher body T at HT was associated with a higher H/L ratio. This H/L ratio is often used as an indicator for long-term stress. The results of H/L ratio agrees with other studies in which the H/L ratio is negatively correlated with BW (Puvadolpirod and Thaxton, 2000a). We expected that body T in the choice-fed group would be lower than in the control group because choice-fed birds were able to consume a better balanced energy-to-protein diet. However, the choice-fed birds did not have a decreased body T or a lower H/L ratio at HT possibly due to similar BW in both groups (Puvadolpirod and Thaxton, 2000a).

## Conclusion

It can be concluded that broilers spent more time eating at NT than at HT and showed more panting behavior at HT. Broilers can compose a diet by selecting less of a protein-rich diet and more of an energy-rich diet than eating the control diet. At NT, choice-fed birds had a similar feed intake and BW gain, but a lower protein and higher energy intake than control-fed birds. At HT, choice-fed birds had a similar feed intake, BW gain, energy intake and a lower protein intake than control-fed birds. The intake of protein and energy differed between the two T regimens and differed between ages. High T decreased feed intake, protein intake, energy intake and BW gain. Choice-fed birds had a similar feed efficiency as control-fed bird at HT, indicating similar body composition for both groups. Extra energy intake of choice-fed birds at HT was used for panting activity. Body T and H/L ratio were higher at HT than at NT. Water intake was similar between both temperatures, but higher in control-fed birds than in choice-fed birds.

## Acknowledgments

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# CHAPTER 4

# Feed Intake Behavior, Nutrient Intake and Performance of Indigenous Chickens Fed a Choice Diet under Tropical Climatic Conditions in Jambi Province, Indonesia

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## Abstract

Birds are able to make adaptation in feed intake and energy intake and change their metabolism to maintain the growth rate at various environments. The objectives of this study were to investigate the intake of protein and energy with self-selection by indigenous chicken (Kampung chickens) under tropical climatic conditions in Jambi Province of Indonesia in an intensive cafeteria system. The temperature ranged from 25.6±1.0$^o$C to 31.2±1.4$^o$C and RH ranged from 63±8% to 89±5%. The control animals were fed with a standard control diet for broiler chickens. The choice-fed birds could choose from 1: control diet, 2: a high-protein diet and 3: a high-energy diet. Birds had free access to each diet in separate feeding troughs. Diets were offered as mash from 1 to 21 d of age with control diet (CP: 233 g /kg; ME: 2,999 kcal/kg), a high-protein diet (CP: 290.6 g/kg; ME: 2,895 kcal/kg), a high-energy diet (CP: 138 g/kg; ME: 3,258 kcal /kg) and as crumbles from 22 to 84 d of age with control diet (CP 216 g/kg; ME: 3,104 kcal/kg), a high protein diet (CP: 303.6 g/kg; ME: 3,072 kcal/kg), a high energy diet (CP: 142.4 g/kg; ME: 3,293 kcal/kg). Results showed that the choice-fed birds spent 28% more time eating than the control-fed birds. Feed intake and ME intake were similar between the two dietary treatments. Protein intake was 22% higher in control-fed birds than in choice-fed birds. BW gain was 20% higher in control-birds and FCR was 18% lower in control-birds. Water intake was 24% higher in control-fed birds. Body T was similar between dietary treatments. It can be concluded that Kampung chickens can compose a diet by selecting less of a protein-rich diet and more of an energy-rich diet than eating the control diet. The choice-fed birds had a lower BW gain than the control-fed birds.

**Key words**: self-selection, protein, energy, tropical climate, Kampung chickens

## Introduction

Ambient temperature can be considered to be the most important factor which affects heat exchange between birds and environment (Yahav et al., 2005). As a consequence factors which effect heat production like feed intake can be affected in a hot environment (Whittow, 1986; May and Lott, 1992; Daghir, 2008b). This response is one of the ways for birds to reduce part of the heat load which originates from digestion of feed and processing of nutrients in the body. However, such a feed intake reduction can result in a shortage for essential nutrients (amino acids and energy) and thus BW gain may be less.

Another way to reduce the heat load in a hot environment is to adjust dietary nutrient concentration or to allow the birds to select from a set of nutrients in order to reduce heat production and thus heat load. Also in Indonesia, protein and energy requirement for slow growing meat-type chickens are based on international feeding tables such as the NRC (1994). These tables do not account for nutrient requirements and intake levels as occur with indigenous chickens under tropical conditions. So, there are no standards for adequate nutrient contents under different heat stress conditions. In order to derive the protein and energy requirement for the indigenous breed in Indonesia, the so-called Ayam (chicken) Kampung (village) in a hot environment, various strategies can be followed. The classical way is to test feeds with various protein and energy contents and measure response. One can also measure directly intakes of protein and energy by the birds in a choice feeding setting (Balnave and Mutisari Abdoellah, 1990). This method allows the chickens to determine their own dietary composition and enables them to reduce heat production associated with metabolism of these nutrients.

A choice between one energy rich ingredient and only one protein rich ingredient may be only partially effective in composing optimal diets. Cruz et al. (2005) showed that broilers could compose a balanced diet that alleviated the negative effect of heat stress on BW gain by offering various choices of ingredients with different contents of protein and energy. Therefore, it seems that a combination of more than one protein and more than one energy source need to be offered in an ideal situation. On the other hand, choice feeding in a hot climate does not always avoid a decreased BW gain. Yo et al. (1998) reported that under tropical climatic conditions total feed intake of a choice-fed composition until 6 weeks of age did not differ from total feed intake of a single (control) diet. Broilers on the choice feeding regimen did select a diet with a lower CP content than the CP content present in the control diet and showed a lower BW at 6 week of age than the birds fed the control diet. So, results of various studies do not give the same effects but this may be related to type of chickens and how feed is offered and it also may depend on the climatic treatment applied (Shariatmadari and Forbes, 1993; Forbes and Shariatmadari, 1994).

The knowledge on the ability of Kampung chickens to regulate the separate consumption of protein and energy sources under tropical climatic conditions is limited (Kompiang et al., 2001). From literature it is clear that birds can compose a diet from different diets. It is however not known what kind of diet Kampung chickens will compose from choice diets differing in energy and protein when exposed to tropical climatic conditions.

The objectives of this study were to investigate the intake of protein and energy by self-selection in indigenous chickens (Kampung chickens) under tropical climatic conditions in Muaro Jambi District, Jambi Province of Indonesia. The hypothesis is that the birds will be able to select different ingredients to minimize heat load, therefore optimising feed intake and performance as compared to a single control diet.

## Materials and Methods

### *Bird, Housing and Care*

120 day-old chicks of Kampung chickens were obtained from the hatchery of Jambi University. After hatch, each bird was weighed and wing-tagged for identification. After that, all chickens were randomly allotted to 12 floor pens with 10 birds each. In addition, unique colouring of each bird was done to identify the feed intake and panting behavior of each bird during the trial. The birds were kept in an open sided house under tropical temperature conditions. The floor pen was about 1 meter above the ground. Each pen had one bell shaped drinker with a 3.3 litre capacity. To enable the birds an easy and equal access to feed, three feed troughs were placed in each pen. Birds had access to feed and water ad libitum. Wood shavings were used as litter and it was regularly added to each pen to maintain a good litter conditions. The pen dimension was 1.75 m (l) x 1.2 m (w) and 2 m (h) and made of wire.

For the first two weeks of age, the house was covered by black plastic suspended at half-height of the wall during the whole day. The weeks thereafter, it was covered during the night only (from 07:00 to 19:00 h). The temperature and relative humidity cycle were recorded daily by using a minimum-maximum thermo-hygrometer. During days 0 to 14, temperature inside each pen was warmed by a 40 Watt bulb. The bulbs were hanged on under a bluish white metal in the form of Chinese-head shaped. Artificial light was at a schedule of 23-h light (L) and 1-h dark (D) for the first three days only. The dark hour was created by covering the bulb with a thin attached closely to the Chines-head shaped. Thereafter, the temperature (T), relative humidity (RH) and light followed the natural rhythm with about 12L:12D.

***Experimental Design and Treatments***

The experiment was conducted as a completely randomized design with repeated measures. The factor was a dietary treatment (control, single diet fed birds versus choice-fed birds). Each dietary treatment was assigned to six replicated pen with ten birds each. Thus, the pen was the experimental unit.

Due to a limited availability of raw materials at one time, the diets were made twice. The first batch was made three days before the start and the second batch was made two weeks later. Dietary treatments were analysed by proximate analysis at Andalas University, West Sumatera. The first rations were made as a mash from 1 to 21 d of age (control diet = 233 g of CP/kg and 2,999 kcal of ME/kg, a high-protein diet = 290.6 g of CP/kg and 2,895 kcal of ME/kg, a high-energy diet= 138 g of CP/kg and 3,258 kcal of ME/kg) and as crumbles from 22 to 84 d of age (control diet = 216 g of CP /kg and 3,104 kcal of ME/kg, a high-protein diet = 303.6 g of CP/kg and 3,072 kcal of ME/kg, a high-energy diet = 142.4 g of CP/kg and 3,293 kcal of ME/kg). All diets had similar colour. The control birds were fed with a single control diet and the choice-fed birds could choose from (a) the control diet, (b) the high protein diet and (c) the high energy diet, each provided in a separate feed troughs. This gave the birds the opportunity to eat from the control, a high-protein or a high-energy diet and thus compose their own diet. In order to avoid a habituation, the site of each feed trough in a pen was changed every day according to a predetermined random schedule.

The feed was offered at 12:00 h every day and left over was weighed next day at 12:00 h. Before meal time, all feeders were taken out of the pens and weighed, and new feeders were placed back. Water was recorded before and after refilled. The sources for protein were soybean meal and local fish meal. The energy sources were corn, palm kernel meal, polished rice and vegetable oil. Dietary compositions of the experimental diets are presented in Table 1.

**Table 1**. The ingredient (g/kg) and analysed nutrient composition of Kampung chickens dietary treatment.

| Ingredient | Dietary treatment | | |
|---|---|---|---|
| | Control diet | High-Protein diet | High-Energy diet |
| Soybean meal | 247.6 | 446.5 | 40.0 |
| Polished | 330.0 | 387.8 | 250.0 |
| Maize | 340.8 | 50.0 | 520.0 |
| Fish meal | 50.0 | 80.0 | 30.0 |
| Palm kernel meal (expeller extracted) | 10.0 | 10.0 | 100.0 |
| Vegetable oil | 0.0 | 0.0 | 39.8 |
| Limestone | 10.0 | 13.0 | 10.0 |
| Salt | 4.0 | 4.0 | 4.0 |
| Top Mix[1] | 5.0 | 5.0 | 5.0 |
| Dicalcium phosphate | 0.8 | 0.8 | 0.8 |
| DL-Methionine | 1.7 | 2.7 | 0.2 |
| L-lysine HCl | 0.1 | 0.2 | 0.2 |
| Total | 1000.0 | 1000.0 | 1000.0 |
| Analysed nutrient content (g/kg) | | | |
| ME (kcal/kg)[2] from 0-21 d of age | 2999.1 | 2895.1 | 3257.8[3] |
| ME (kcal/kg)[2] from 22-84 d of age | 3104.2 | 3072.0 | 3292.7 |
| Crude protein from 0-21 d of age | 233.0 | 290.6 | 138.1 |
| Crude protein from 22-84 d of age | 216.2 | 303.6 | 142.4 |

[1]Composition of 1 kg Top Mix: vitamin A (retinyl acetate), 12,000 IU; vitamin $D_3$ (cholecalciferol), 2,000 IU; vitamin E (dl-α-tocopherol), 8.0 mg; vitamin K, 2.0 mg; vitamin $B_1$ (thiamin), 2.0 mg; vitamin $B_2$ (riboflavin), 5.0 mg; vitamin $B_6$ (pyridoxine-HCl), 0.5 mg, vitamin $B_{12}$ (cyanocobalamin), 12 mg; vitamin C, 25 mg; niacin, 40 mg; vitamin $B_5$ (d-pantothenic acid), 6.0 mg; choline chloride, 10 mg; methionine, 30 mg; lysine, 30 mg; iron, 20 mg; copper, 4 mg; manganese, 120 mg; zinc, 100 mg; cobalt, 0.2 mg; iodine, 0.2; zinc bacitracin, 21 mg and santoquin (antioxidant), 10 mg.
[2]Metabolisable energy was calculated by determining (combustion) gross energy of the entire diet multiplied with a ME/GE-convection factor (0.70).
[3]Value was calculated.

### *Measurements*

Feed intake behavior and panting behavior were recorded by direct observation during a 5- to 7- min walk in the room. Each bird in each of the 12 pens was observed instantaneously for a moment during this period (a scan) similar to Bokkers and Koene

(2003). After 5 to 7 min, the behavior of each bird was scanned at a single moment. This procedure was repeated with 15 min pauses several times between 09:00 to 12:00 h and between 14:00 to 17:00 h to represent a 12 h daylight period. Scan sampling was done one day per wk. Observations were conducted from wk 1 to 12 by an experienced assessor standing in front of the pen. Based on these observations (instantaneous scan sampling), we calculated the percentage of time the chickens in a certain pen spent on eating and panting behaviors relative to other behaviors. Eating was defined as a chicken with the head in or above the feeder, and panting was defined as breathing rapidly with short gasps with open beak and split wing feather alignment. All other behaviors were described by Bokkers and Koene (2003). Since previous experience showed that making more than one scan in each bird per observation of a pen increased behavioral activity of the birds as a reaction to the presence of the observer (Bokkers and Koene, 2003), only one scan in each bird per observation was made. All scan data per pen per wk were pooled.

Feed intake per pen was recorded daily. Water intake per pen was measured by subtracting the given amount of water with left over (ml) each time adding water to the tank. Protein and energy intakes were calculated from the intake of each of the three diets and their concentrations. Birds were weighed weekly and BW gain was calculated accordingly. The weight of birds that died or had to be culled was determined and their BW gain was included in the calculation of the feed conversion ratio (**FCR**) per pen. All performance data were finally averaged per bird in each pen for each two wk period.

Body T was measured from two birds per pen by a digital thermometer in the cloaca each time before the weekly weighing. Data of the two birds per pen were averaged.

### *Statistical Analysis*

In this experiment, birds were randomly allocated to pens representing different diet treatments. All performance data were taken on the same experimental units (pens), repeated in time: 12 weekly observations were available for each individual experimental unit. One experimental unit of choice-fed birds in the last week of trial was omitted from the analysis due to a five times higher FCR in that pen as compared with the average of all other replicated pens. All performance data were analysed after pooling daily data into six 14-d periods. Repeated measurements on the same animal cannot be regarded as independent units of observation and mixed model can be used to account for the covariance structure among repeated observation (Littell et al., 1998). In the analysis, the repeated statement PROC MIXED in SAS (version 9.1; SAS Inst. Inc., Cary, NC) was used adding the factor time (“period” as the time factor) and pen was considered as an additional random effect. The following statistical model was used:

$$Y_{ijk} = \mu + F_i + P_j + F_i \text{ x } W_j + e_{ijk}$$

Where $Y_{ijk}$ is measurement of response of the *k*th birds having the *i*th diet at *j*th period, μ is overall mean effect, $F_i$ is the *i*th fixed diet effect (*i*= complete diet or choice diet), $P_j$ is the random *j*th period measurement (*j*=1..6), $F_i$ x $P_j$ is the random interaction effect between diet and period of measurement and $e_{ijk}$ is random error associated with *i*th diet at period measurement *j*th, $e_{ijk}$~ NID (0, $\sigma^2_e$).

Differences were considered significant at a probability level of $P < 0.05$. If significant of main effects or their interactions were detected, then means were compared using least squares means comparison. Means of significant effects were separated using the PDIFF option with the SAXTON macro in SAS at the $P < 0.05$ level (Saxton, 1998). The Kenward-Roger method was used for computing the denominator degrees of freedom for the tests of main effects.

The best covariance structure was based on the corrected Akaike Information Criteria (AICC). The first-order heterogenous autoregressive covariance structure [ARH (1)] fitted the data best for feed intake, protein intake, energy intake, BW gain, FCR, water intake, protein to gain ratio and energy-to-gain ratio. The unstructured covariance structure [UN] fitted the data best for water-to-feed ratio.

Body T was analyzed by PROC GLM in SAS with the following linear model:

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

Where $Y_{ij}$ = measurement of response of the *j*th bird having the *i*th diet, μ is overall mean effect, $F_i$ is the *i*th fixed diet effect (*i*=complete or choice), and $e_{ij}$ is the residual error.

Behavior data were analysed with replicated observations per pen per week. Feed intake and panting behavior traits were analysed with the Kruskal-Wallis test and post hoc with the Wilcoxon two sample test by nonlinear procedure of SAS.

## Results

***Temperature and Relative Humidity.*** The T and RH are given as the average minimum and maximum ± SD for the respective interval. The natural variation in T inside the house was between 25.6±1.0°C and 31.2±1.4°C and the RH was between 63±8% and 89±5%. During the period of this experiment (September to December, 2008), the season in Muaro Jambi District, Jambi Province of Indonesia was rainy season with normal rainfall (MGA, 2008).

***Eating and Panting Behavior.*** Results on the percentage of time spent on eating behavior are given in Figure 1. Eating time in wk 2 was not recorded for practical reasons. Eating time declined with increasing age of the birds at both dietary treatments. Birds on the choice feeding regimen spent more time on eating than birds in the control feeding regimen after 32 d of age ($P < 0.05$). Time spent on panting is presented in Figure 2. It was observed that Kampung chickens subjected to choice dietary treatment spent similar time for panting behavior as compared to chickens subjected to control dietary treatment ($P > 0.05$).

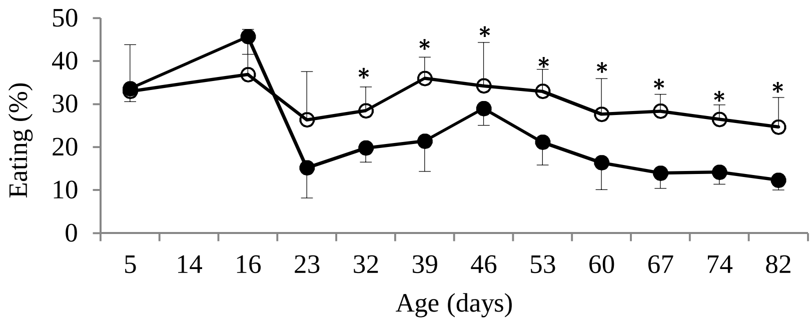


**Figure 1**. Eating time (expressed as percentage within observation time) for control- (●) and choice-feeding (○). Vertical bars represent SD. The asterisk (*) means a significant difference of at least $P < 0.05$ at a certain age.Diet: from 1 to 21 d of age; control diet (CP: 233 g /kg; ME: 2,999 kcal/kg); choice diet = (1) the control diet, (2) a high-protein diet (CP: 290.6 g/kg; ME: 2,895 kcal/kg), or (3) a high-energy diet (CP: 138 g/kg; ME: 3,258 kcal /kg) as a mash. From 22 to 84 d of age; control diet (CP 216 g/kg; ME: 3,104 kcal/kg); choice diet = (1) the control diet, (2) a high-protein diet (CP: 303.6 g/kg; ME: 3,072 kcal/kg), or (3) a high-energy diet (CP: 142.4 g/kg; ME: 3,293 kcal/kg).

***Bird Performance.*** Mortality in this experiment was 7%, consisting of 5% in control-fed birds and 2% in choice-fed birds.

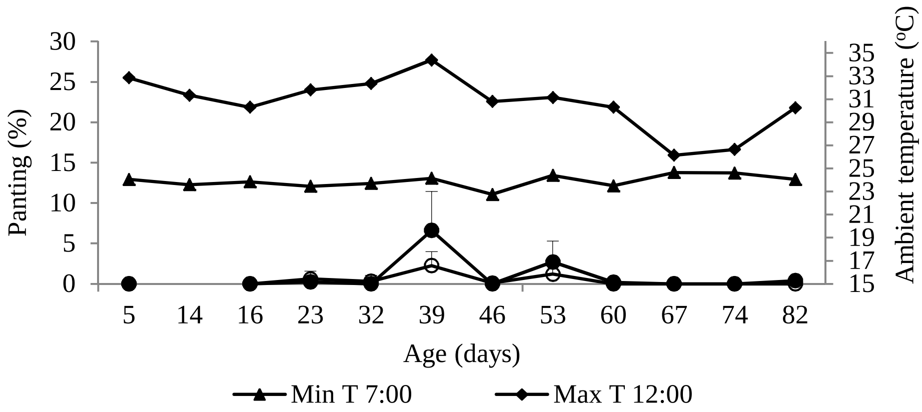


**Figure 2**. Time (expressed as percentage) spends for panting behavior for control- (●) and choice-feeding (○).Vertical bars represent SD. Diet: from 1 to 21 d of age; control diet (CP: 233 g /kg; ME: 2,999 kcal/kg); choice diet = (1) the control diet, (2) a high-protein diet (CP: 290.6 g/kg; ME: 2,895 kcal/kg), or (3) a high-energy diet (CP: 138 g/kg; ME: 3,258 kcal /kg) as a mash. From 22 to 84 d of age; control diet (CP 216 g/kg; ME: 3,104 kcal/kg); choice diet = (1) the control diet, (2) a high-protein diet (CP: 303.6 g/kg; ME: 3,072 kcal/kg), or (3) a high-energy diet (CP: 142.4 g/kg; ME: 3,293 kcal/kg).

**Table 2**. Probability values of main effects and interaction between dietary treatment1 (F) and period for different traits.

| Parameters | F | Period | F x Period[2] |
|---|---|---|---|
| Feed intake (g/bird per period) | 0.119 | **<0.001** | 0.273 |
| Protein intake (g of CP/bird per period) | **<0.001** | **<0.001** | **0.001** |
| Energy intake (kcal of ME/kg per bird/ period) | 0.456 | **<0.001** | 0.453 |
| BW gain (g/bird per period) | **<0.001** | **<0.001** | **0.003** |
| Water intake (g/bird per period) | **<0.001** | **<0.001** | **0.001** |
| FCR (g/g) | **<0.001** | **<0.001** | **0.001** |
| Protein to gain ratio (g of CP/100 g of BW gain per bird/period) | 0.410 | **<0.001** | 0.067 |
| Energy-to-gain ratio (kcal of ME/kg per 100 g of BW gain per bird/period) | **<0.001** | **<0.001** | **<0.001** |
| Water-to-feed ratio (g/g) | **<0.001** | **<0.001** | **<0.001** |

[1]Dietary treatment: from 1 to 21 d of age; control diet (CP: 233 g /kg; ME: 2,999 kcal/kg); choice diet = (1) the control diet, (2) a high-protein diet (CP: 290.6 g/kg; ME: 2,895 kcal/kg), or (3) a high-energy diet (CP: 138 g/kg; ME: 3,258 kcal /kg) as a mash. From 22 to 84 d of age; control diet (CP 216 g/kg; ME: 3,104 kcal/kg); choice diet = (1) the control diet, (2) a high-protein diet (CP: 303.6 g/kg; ME: 3,072 kcal/kg), or (3) a high-energy diet (CP: 142.4 g/kg; ME: 3,293 kcal/kg). Period: six 14-d periods.
[2]F x Period=interaction between dietary treatment and period.

**Table 3**. Least square means of performance parameter in Kampung chickens from period 1 to 6 (12 weeks) as affected by dietary treatments[1].

| Parameter | Period[2] | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| Feed intake (g/bird per period) | | | | | | |
| Control diet | 163.4 | 326.2 | 438.4 | 547.4 | 708.2[a] | 843.8 |
| Choice diet | 164.9 | 325.3 | 443.9 | 523.2 | 634.7[b] | 751.6 |
| SEM | 5.5 | 12.7 | 17.3 | 15.9 | 21.4 | 34.0; 36.1 |
| Protein intake (g of CP/bird per period) | | | | | | |
| Control diet | 38.5[a] | 72.8[a] | 94.8 | 118.3[a] | 153.1[a] | 182.4[a] |
| Choice diet | 33.8[b] | 60.1[b] | 83.4 | 93.5[b] | 112.8[b] | 131.8[b] |
| SEM | 1.1 | 2.6 | 3.8 | 3.7 | 4.7 | 6.9; 7.3 |
| Energy intake (kcal of ME/kg per bird per period) | | | | | | |
| Control diet | 699.9 | 1426.0 | 1944.2 | 2427.3 | 3140.4 | 3742.0 |
| Choice diet | 688.5 | 1433.8 | 2031.0 | 2398.9 | 2909.5 | 3453.0 |
| SEM | 23.7 | 56.0 | 77.2 | 70.4 | 95.6 | 154.3;163.7 |
| BW gain (g/bird per period) | | | | | | |
| Control diet | 73.9 | 133.1 | 172.4[a] | 200.0[a] | 201.6[a] | 236.7[a] |
| Choice diet | 76.1 | 116.9 | 144.4[b] | 164.0[b] | 143.2[b] | 167.7[b] |
| SEM | 1.9 | 8.7 | 6.5 | 8.0 | 12.8 | 12.7; 13.8 |
| Water intake (g/bird per period) | | | | | | |
| Control diet | 252.7 | 563.9[a] | 931.3[a] | 1265.8[a] | 1700.9[a] | 2166.8[a] |
| Choice diet | 244.5 | 479.0[b] | 736.7[b] | 964.0[b] | 1282.5[b] | 1545.8[b] |
| SEM | 11.7 | 19.7 | 28.2 | 45.2 | 70.7 | 110.5;115.1 |
| Protein to gain ratio(g of CP/100 g of BW Gain/bird per period) | | | | | | |
| Control diet | 52.1a | 54.7 | 55.5 | 59.3 | 78.0 | 77.4 |
| Choice diet | 44.4[b] | 53.0 | 57.7 | 57.2 | 78.7 | 78.7 |
| SEM | 1.0 | 2.6 | 2.4 | 1.3 | 4.4 | 2.4; 2.6 |
| Energy-to-gain ratio (kcal of ME /kg per 100 g of BW Gain/bird/ period) | | | | | | |
| Control diet | 663.0 | 749.8[b] | 796.6[b] | 851.4[b] | 1119.6[b] | 1110.9[b] |
| Choice diet | 670.7 | 920.9[a] | 985.4[a] | 1031.1[a] | 1425.4[a] | 1451.2[a] |
| SEM | 17.6 | 50.9 | 35.5 | 25.6 | 65.1 | 42.6;46.8 |

*Continued…*

**Table 3.** (Continued). Least square means of performance parameter in Kampung chickens from period 1 to 6 (12 weeks) as affected by dietary treatments1.

| Parameter | Period[2] | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| FCR (g/g) | | | | | | |
| Control diet | 2.21 | 2.45 | 2.57[b] | 2.74[b] | 3.61[b] | 3.58[b] |
| Choice diet | 2.17 | 2.89 | 3.08[a] | 3.21[a] | 4.44[a] | 4.51[a] |
| SEM | 0.16 | 0.16 | 0.11 | 0.09 | 0.21 | 0.13;0.14 |
| Water-to-feed ratio (g/g) | | | | | | |
| Control diet | 1.55 | 1.74[a] | 2.14[a] | 2.32[a] | 2.40[a] | 2.56[a] |
| Choice diet | 1.48 | 1.47[b] | 1.66[b] | 1.83[b] | 2.01[b] | 1.95[b] |
| SEM | 0.14 | 0.05 | 0.08 | 0.05 | 0.09 | 0.08; 0.09 |

Means within a period between treatments without a common superscript letters (a-b) differ significantly at ($P <$ 0.05). No superscript means non-significant differences.

[1]Dietary treatment: from 1 to 21 d of age; control diet (CP: 233 g /kg; ME: 2,999 kcal/kg); choice diet = (1) the control diet, (2) a high-protein diet (CP: 290.6 g/kg; ME: 2,895 kcal/kg), or (3) a high-energy diet (CP: 138 g/kg; ME: 3,258 kcal /kg) as a mash. From 22 to 84 d of age; control diet (CP 216 g/kg; ME: 3,104 kcal/kg); choice diet = (1) the control diet, (2) a high-protein diet (CP: 303.6 g/kg; ME: 3,072 kcal/kg), or (3) a high-energy diet (CP: 142.4 g/kg; ME: 3,293 kcal/kg).

[2]Period: 14-d periods

All performance data in the tables are data corrected for mortality by period. Probability values for every parameter are presented in Table 2. Differences in performance of the Kampung chickens in each period at different dietary treatments are presented in Table 3. The interaction between diet and period is presented in Figure 3.

*Feed Intake, Protein Intake and Energy Intake.* Total feed intake was similar between control- and choice-fed birds, but protein intake was higher ($P < 0.001$) in control animals. (Table 2). Feed intake and energy intake per period increased over time but it did not significantly differ between control- and choice-fed birds at all period interval except in period 5 for feed intake (Table 3). Protein intake increased over time and was significantly higher for the control-fed birds than the choice-fed birds except in period 3 (Table 3). There was an interaction effect between dietary treatment and period on protein intake (Table 2). The difference in protein intake between both treatments significantly increased after period 3 (Figure 3, panel A). Birds that were choice-fed consumed about 47% feed from the energy-rich diet, 33%

from the control diet and 20% from the protein-rich diet (Figure 4). The increase in intake of the energy-rich diet started at 4 d of age.

*Body Weight Gain and Feed Conversion Ratio.* BW gain and FCR were affected by dietary treatments (Table 2). BW gain and FCR increased over time. BW gain was higher ($P < 0.05$) and FCR was lower for control-fed birds compared with choice-fed birds after period 2 (Table 3). An interaction between dietary treatment and period showed that Kampung chickens fed the control diet continued to increase in rate of BW gain until period 6, whereas Kampung chickens fed the choice diet increased BW gain until period 4 after which they maintained the same growth rate (Figure3, panel B). FCR increased after period 4 in both dietary treatments (Figure 3, panel C).

*Ratios of Protein and Energy-to-Gain.* Dietary treatment did not affect ratios of protein to gain but it affected the ratios of energy intake to gain ratio (Table 2). The ratio of energy-to-gain was higher ($P < 0.05$) for choice-fed birds compared with control-fed birds after period 1 (Table 3). An interaction between dietary treatment and period on ratios of energy-to-gain showed that the ratio highly increased after period 4 and stabilized during period 5 to 6 in both dietary treatments (Figure 3, panel D).

*Water Intake and Water-to-Feed Ratio.* Water intake and water-to-feed ratios were affected by dietary treatment (Table 2). Water intake and water-to-feed ratio were clearly higher in the control-fed birds than in the choice-fed birds in each period after period 1 (Table 3). There was an interaction effect between dietary treatment and period for water intake and for water-to-feed ratio (Table 2). The differences in water intake and water-to-feed ratio between control- and choice-fed birds increased over time (Figure 3, panel E and F)

***Body Temperature.*** Results of dietary treatment on body T are presented in Table 4. Body T of control- and choice-fed birds was similar in each week except at 3 wk of age, where body T was higher ($P < 0.05$) for control-fed birds as compared with choice-fed birds.

## Discussion

***Eating and Panting Behavior.*** Choice-fed birds needed about 28% more time to consume their feed than control-fed birds although their feed intake was not significantly less (6%) than control-fed birds. The time spent on eating for choice-fed birds is most likely related to the extra time for the birds to sample each dietary treatment before making the choice (we changed the position of the feed through in the

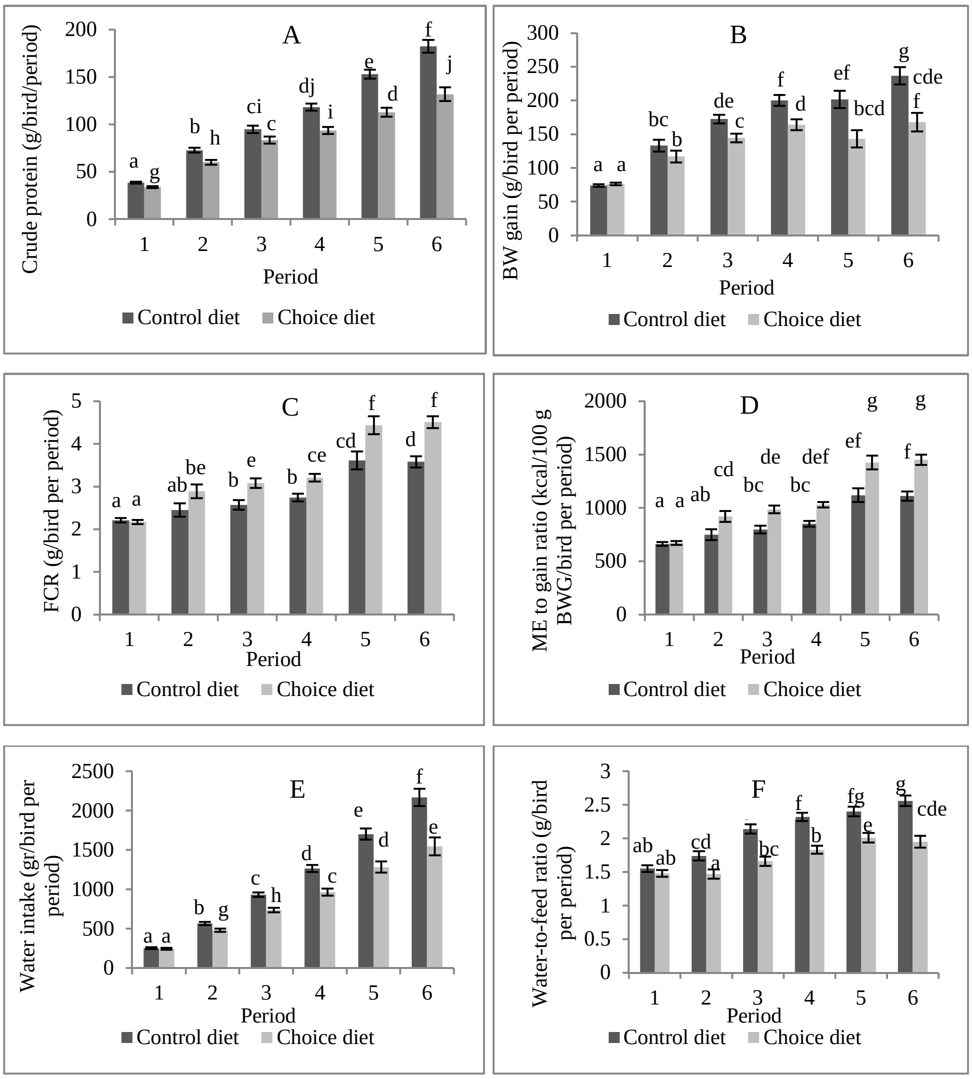


**Figure 3**. Least square means for traits that show feed x period interaction. Means within and between bars with common letters (a-j) differ significantly ($P < 0.05$). Dietary treatment: from 1 to 21 d of age; control diet (CP: 233 g /kg; ME: 2,999 kcal/kg); choice diet = (1) the control diet, (2) a high-protein diet (CP: 290.6 g/kg; ME: 2,895 kcal/kg), or (3) a high-energy diet (CP: 138 g/kg; ME: 3,258 kcal /kg) as a mash. From 22 to 84 d of age; control diet (CP 216 g/kg; ME: 3,104 kcal/kg); choice diet = (1) the control diet, (2) a high-protein diet (CP: 303.6 g/kg; ME: 3,072 kcal/kg), or (3) a high-energy diet (CP: 142.4 g/kg; ME: 3,293 kcal/kg).

pen daily). Panting behavior was similar. It seems that the control-fed birds had a larger meal size per unit of time than the choice-fed birds.

Panting behavior of Kampung chickens only showed more panting at day 39 (6.6% for control and 4.8% for choice-fed birds) than the other days. At this day, the minimum temperature was 24.2°C at 7:00 h and the maximum temperature was 34.4°C at 12:00 h. This was a difference of 10.2°C at that particular day. It should be noted that feed intake, protein intake and energy intake at this day were the same.

**Table 4**. Effect of feeding strategies on body temperature at various age[1].

| Week | Control diet | Choice diet | SEM[2] | Source of variation[3] |
|---|---|---|---|---|
| | | | | Feed |
| | ------ (in Celsius)------ | | | |
| 1 | 40.8 | 41.0 | 0.15 | 0.265 |
| 2 | 40.9 | 40.9 | 0.15 | 0.265 |
| 3 | 41.2 | 40.9 | 0.08 | **0.034** |
| 4 | 41.1 | 40.9 | 0.12 | 0.287 |
| 5 | 41.1 | 41.0 | 0.10 | 0.525 |
| 6 | 41.3 | 41.2 | 0.07 | 0.640 |
| 7 | 41.3 | 41.4 | 0.04 | 0.611 |
| 8 | 41.4 | 41.4 | 0.08 | 0.942 |
| 9 | 41.4 | 41.3 | 0.09 | 0.798 |
| 10 | 41.4 | 41.6 | 0.08 | 0.165 |
| 11 | 41.5 | 41.5 | 0.08 | 0.721 |
| 12 | 41.6 | 41.6 | 0.08 | 0.890 |

[1]Mean values are expressed as an average of 6 replicate pens. Dietary treatment: from 1 to 21 d of age; control diet (CP: 233 g /kg; ME: 2,999 kcal/kg); choice diet = (1) the control diet, (2) a high-protein diet (CP: 290.6 g/kg; ME: 2,895 kcal/kg), or (3) a high-energy diet (CP: 138 g/kg; ME: 3,258 kcal /kg) as a mash. From 22 to 84 d of age; control diet (CP 216 g/kg; ME: 3,104 kcal/kg); choice diet = (1) the control diet, (2) a high-protein diet (CP: 303.6 g/kg; ME: 3,072 kcal/kg), or (3) a high-energy diet (CP: 142.4 g/kg; ME: 3,293 kcal/kg). [2]1 df.
[3]Significant effects (*P*<0.05) are printed in bold.

***Feed Intake, Protein Intake and Energy Intake***. In the present study the choice-fed birds consumed the same amount of feed as the control-fed birds. This is not an agreement with the results of a study by Kompiang et al. (2001). They found that feed intake of Kampung chickens that were choice-fed was significantly lower (6.6%) than those of control-fed birds.

Despite a similar total feed intake as control-fed birds, the choice-fed birds preferred a higher intake of the high-energy at the expense of the intake of the high-protein diet (Figure 4). Our results confirmed our previous observation (Chapter 3) that broilers kept at HT chose a higher proportion of the high-energy diet than of the high-protein diet. This suggests that a formulated diet for maximizing growth may not reflect dietary preferences of birds (Siegel et al., 1997; Delezie et al., 2009). Overall, the birds subjected to the choice feeding treatment consumed 22% less protein and 3% less energy. The lower intake of protein and similar intake of energy are in agreement with the results of a choice feeding study in Kampung chickens (Kompiang et al., 2001) and also of a broiler study at high temperatures (Yo et al., 1998).

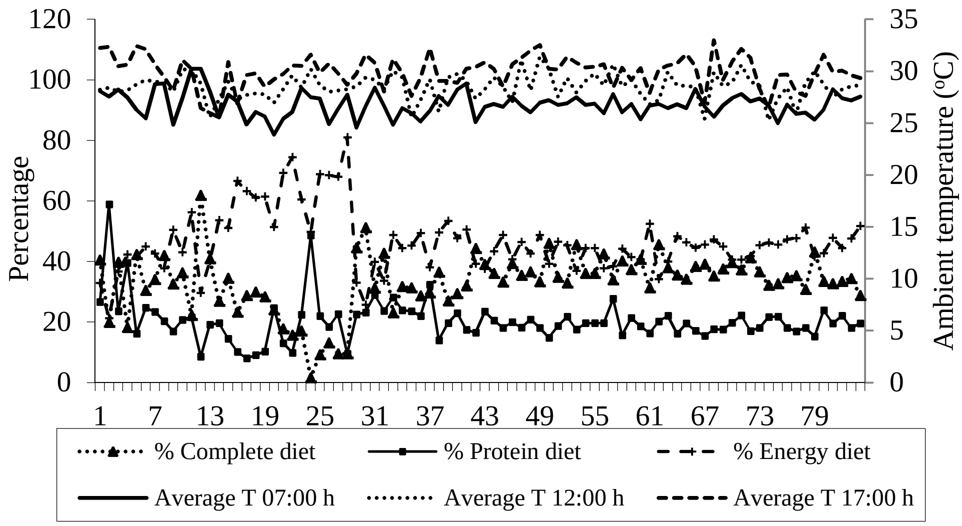


**Figure 4**. Consumption of control diet, high protein diet and high energy diet as a proportion of total feed intake. Dietary treatment: from 1 to 21 d of age; control diet (CP: 233 g /kg; ME: 2,999 kcal/kg); choice diet = (1) the control diet, (2) a high-protein diet (CP: 290.6 g/kg; ME: 2,895 kcal/kg), or (3) a high-energy diet (CP: 138 g/kg; ME: 3,258 kcal /kg) as a mash. From 22 to 84 d of age; control diet (CP 216 g/kg; ME: 3,104 kcal/kg); choice diet = (1) the control diet, (2) a high-protein diet (CP: 303.6 g/kg; ME: 3,072 kcal/kg), or (3) a high-energy diet (CP: 142.4 g/kg; ME: 3,293 kcal/kg).

Distinct preferences for the protein and energy diets in this experiment demonstrate the ability of Kampung chickens to discriminate among diets that differ in protein and energy content independently (Figure 4) because the colour of the diets was similar. It seems that after 28 d of age, choice-fed Kampung chickens reduced the intake from the high energy diet and shifted to the control diet. This shifted preference was also observed in broilers at high temperature (Chapter 3). Adaptive changes in feed intake and energy expenditure over the long-term contribute to a homeostatic control of body energy stores and maintenance of a constant BW (Richards and Proszkowiec-Weglarz, 2007).

***BW gain and FCR.*** BW gain of Kampung chickens was clearly highest when they were fed a control diet. This result is in line with a previous study which found that the growth rate of Kampung chickens fed a high-protein and high-energy diet (20% CP and 3,100 kcal ME/kg) was higher than when fed a low-protein and low-energy diet (14% CP and 2,833 kcal ME/kg) (Argono and Iskandar, 2005). Therefore, BW gain of choice-fed birds until 84 d of age, in our study, was most determined by protein intake because choice-fed birds consumed less protein (22%, $P < 0.01$) than control-fed birds.

Overall, BW gain after 84 d of age was about 20% lower in the choice-fed birds as compared with the control-fed birds. The higher BW gain of Kampung chickens given the control diet in our study showed that this bird tolerates in a high cyclic ambient T and they perform well with the high protein diet. It should be emphasised that rearing the birds in a cycling hot environment (as in our study) may be less stressful than when rearing them in an environment with a constant high T during the day. Birds can recover during the part of the day in which the temperature is lowest. Cheng at al. (1999) also found that BW gain was higher in broilers kept at a high cyclic T (26.6 $^{o}$C;16 h to 35$^{o}$C; 8h) compared with broilers continuously kept at a constant (32.2$^{o}$C) high T.

FCR increased with increasing age of the chickens in all dietary treatments. However, the increase was more pronounced on choice-fed birds from wk 10 onwards. Low protein intake of the choice-fed birds had a negative effect on FCR. Therefore, protein intake of the choice-fed birds was insufficient for high rate of gain due to a low feed intake level.

***Ratios of Protein- and Energy-to-Gain***. The ratio of protein-to-gain was similar between choice-fed birds and control-fed birds, emphasizing the fact that protein intake has probably determined BW gain as suggested above. This finding was in contrast with a similar study in Kampung chickens done by Kompiang et al. (2001). They found a lower ratio of protein-to-gain in choice-fed birds compared with control-fed birds. The higher ratio of energy-to-gain in choice-fed birds in our study was related to similar energy intake but BW gain was 20% lower than control-fed birds. When the ME/CP ratios in the diet are considered, it should be mentioned that the protein and energy concentration in the choice diet eaten by the birds varies from 212 to 145 g CP/kg and from 3,071 to 3,217 kcal of ME/kg as age increased (Figure 5).

These ratios also suggests that Kampung chickens preferred the same amount of protein per 100 g BW gain but require more energy per 100 g BW gain (Table 3). Control-fed birds had a higher growth rate. Protein- and energy-to-gain ratios after wk 10 were significantly increased both in control- and in choice-fed birds. This means that after this period, Kampung chickens requires more dietary protein and energy for each unit increase in body weight than before wk 10.

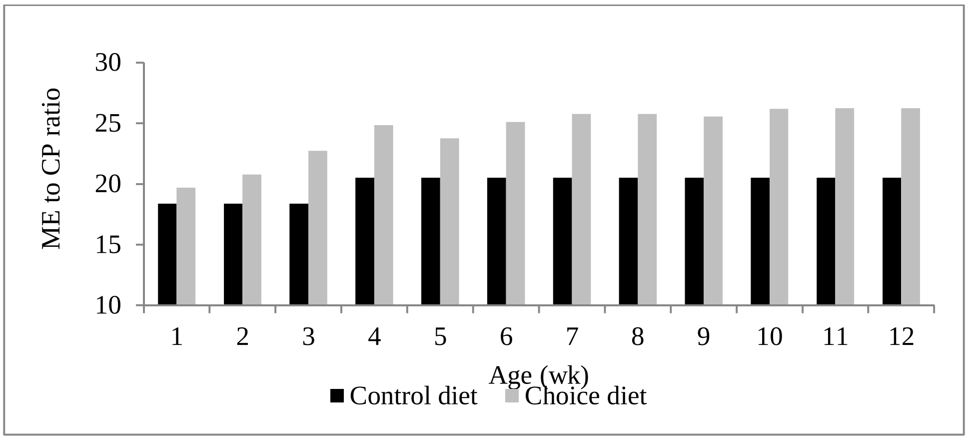


**Figure 5**. Energy to protein ratio of control feed and choice feed intake of Kampung chickens under tropical climate conditions. Dietary treatment: from 1 to 21 d of age; control diet (CP: 233 g /kg; ME: 2,999 kcal/kg); choice diet = (1) the control diet, (2) a high-protein diet (CP: 290.6 g/kg; ME: 2,895 kcal/kg), or (3) a high-energy diet (CP: 138 g/kg; ME: 3,258 kcal /kg) as a mash. From 22 to 84 d of age; control diet (CP 216 g/kg; ME: 3,104 kcal/kg); choice diet = (1) the control diet, (2) a high-protein diet (CP: 303.6 g/kg; ME: 3,072 kcal/kg), or (3) a high-energy diet (CP: 142.4 g/kg; ME: 3,293 kcal/kg).

***Water Intake and Water-to-Feed Ratio.*** Water consumption was 24% higher in control-fed birds than in choice-fed birds. As a consequence, overall water-to-feed ratio in the control-fed group was 18% higher than in the choice-fed group (Table 3). The progressive increase in water intake in control-fed birds was related to the 22% higher protein intake of control-fed birds. Alleman and Leclercq (1997) reported that increasing the level of crude protein in the diet increases both water intake and water-to-feed ratio.

***Body Temperature.*** Body T was similar between dietary treatments except at d 21. The higher body T of control-fed birds at this age was thought to be related to the higher intake of feed and especially protein (5.12 g/d in control-fed birds and 4.51 g/d in choice-fed birds; $P < 0.03$). It seems that control-fed birds maintained their body T by drinking more water as a consequence of higher protein intake. Higher water intake in control-fed birds will be very effective if it has been used to increase heat dissipation by means of evaporation, particularly when the ambient T is high. In this way heat increment caused by higher intake of crude protein is possible to release although these effects were not measured.

## Conclusion

It can be concluded that Kampung chickens spent more time eating in choice feeding than control feeding system. Birds compose a diet by selecting less of a protein-rich diet and more of an energy-rich diet than the control diet, but energy intake was similar with the energy in the control diet. It seemed that Kampung chickens did not eat to maximise growth rate when they have the opportunity to select a ration. A low rate of growth is probably more beneficial for survival under tropical conditions than a high rate of gain. Water intake was higher in the control-fed birds than in the choice-fed birds.

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# CHAPTER 5

## Effects of Wet Feeding and a High Dietary Energy to Protein Ratio on Feed Intake Behavior, Performance, and Gastrointestinal Tract Development of Broilers at Normal and High Ambient Temperatures

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## Abstract

Wetting a high energy-to-protein ratio (HE) diet has not been evaluated on performance and gastrointestinal (GIT) development in broilers at normal temperature (NT) and high temperatures (HT). The objectives of this experiment were to study the effects of wet feeding and a high energy-to-protein ratios on feed intake behavior, feed intake, GIT development and performance in broiler chickens at NT (31o to 21$^{o}$C) and HT (31o to 32$^{o}$C) conditions. Night temperature was set to 18$^{o}$C for NT and 25$^{o}$C for HT with an 12L:12D schedule. The control diet was a standard diet for broiler chickens with 215 g CP/kg and 2,895 kcal ME/kg. The HE diet contained 195 g CP/kg and 3,067 kcal ME/kg. Birds had ad libitum access to the diet in dry or wet (added water 1:1) form. Results showed that broilers spent 2.9% more time eating at NT than at HT in the last three weeks of age and showed 34.6% more panting at HT than at NT. High temperature decreased feed intake and BW gain. Control- and wet-fed birds had a higher feed intake, BW gain and water intake than HE- and dry-fed birds. At HT, similar BW gain between wet-HE-fed birds and dry-control- or wet-control-fed birds was observed especially in wk 4 to 6. At d 21, temperature did not affect the GIT development. Relative length of all GIT segments and empty weights of gizzard were higher in HE-fed birds than in control-fed birds. At d 42, the length and empty weight of most intestinal segments at NT were longer and heavier than at HT. Relative length of the duodenum, jejunum and ileum were shorter and weight of the empty gizzard was lighter in control-fed than those in HE-fed birds. Body temperature and heterophil/lymphocyte ratio were higher at HT than at NT at d 20 and 41. It can be concluded that a wet diet has potential positive effects to improve performance at both temperature regimens, although it cannot reduce temperature effects on heat stress and improve GIT development.


**Key words**: temperature, wet diet, performance, gastrointestinal tract, broiler

## Introduction

Several feeding strategies have been proposed to improve performance and health in modern broilers kept under temperate conditions. However, methods applied at temperate conditions may not be effective when the animals are kept under heat stress. It is expected that chickens will consume less diet and may choose a diet with the lowest heat production at temperature (T) above 30$^{o}$C (high temperature, HT). A reduction in feed intake may result in a too low intake of some essential nutrients. As a result, BW gain will be low compared with the birds kept at normal temperature (NT) of 21-22$^{o}$C.

Dietary management by adding water to feed at NT is known to alter some aspects during the grower period of broilers, e.g., high moisture feeds gave a higher feed efficiency compared with low moisture feeds (Moritz et al., 2001). During the starter period, such beneficial effects of wet feed are less clear. An increase in water content of feed by adding water to a pellet diet increased feed intake and BW gain (Yalda and Forbes, 1995, 1996). In studies with mash diets, feeding a wet mash diet increased feed intake and BW gain of broilers as well (Yasar and Forbes, 1999; Preston et al., 2000; Khoa, 2007). Broilers with access to water that were given a choice between a dry and a wet diet mostly chose a dry diet; in the absence of drinking water, they predominantly chose the wet diet (Shariatmadari and Forbes, 2005). The birds fed a dry diet had a reduced rate of gain due to a lower dry matter feed intake than with a wet diet (Shariatmadari and Forbes, 2005). At HT, a wet mash diet increased feed intake and live weight gain compared with a dry mash diet (Awojobi and Meshioye, 2001; Kutlu, 2001).

At NT, water addition to a diet has positive effects on the development of the gastrointestinal tract (GIT). Irrespective of the diet conformation (wet or dry) fed during the grower phase, relative empty weight of all foregut segments at the end of grower phase were highest for birds that had been fed with a wet diet as compared to a dry diet in the starter phase (Khoa, 2007). The empty fresh weights of the upper part of intestine segments were also heavier in birds fed wet diet, but the lengths of these organs were similar compared to in birds fed dry diet (Yasar and Forbes, 1999). In another study, similar empty fresh weights of these segments in wet diet compared to dry diet were found (Yasar and Forbes, 2000). Under tropical conditions, gizzard weight was highest in birds fed a dry mash (Awojobi and Meshioye, 2001). The effects of wet diet on GIT development both at NT and HT have not been studied extensively.

Most studies with a wet diet have been done at NT and used a standard diet formulation for broilers. Also, studies on a wet pellet diet with a high energy to protein ratio (HE) at HT were not found in literature. It is not known whether the effects of wet and dry form at NT and HT remain the same between different diet formulations. We hypothesized that birds will eat more of a wet diet at HT; a high energy to protein ratio may reduce heat production and therefore partly alleviate the negative effects of a high ambient temperature (T) at the same intake.

The objectives of this experiment were to test if a wet pellet diet with a high energy-to-protein ratio will alleviate panting behavior and improve feed intake, performance, and GIT development in broiler chickens kept at HT as compared to NT.

## Materials and Methods

### *Animal Ethics*

All the procedures involving animals in this experiment were approved by the Animal Experimental Committee of Wageningen University, the Netherlands.

### *Bird, Housing and Care*

A total of 216 one day old male broiler chickens (Ross 308) were purchased from a commercial hatchery (Morren Breeders B.V., Lunteren, The Netherlands). After arrival, each bird was weighed and wing-tagged for identification. After that, each bird was randomly allotted to one of 24 floor pens with 9 birds each. Unique colouring of each bird within a pen was made to monitor the feed intake and panting behaviors.

Twelve identical pens were made in each of two identical T controlled rooms. One room was designed for NT and the other for HT. Each pen had three drinking nipples, with a cup underneath, connected to a water tank of 10 litres capacity. The height of nipple was adjusted twice a week at approximately the height of the back of the broiler as the birds grew. Each pen was equipped with one feed trough. Wood shavings were used as litter and were added to each pen as necessary to maintain good litter conditions. Pen dimensions were 1.75 m x 1.15 m and 0.80 m (W x L x H). All birds were exposed to a 23-h light (L) and 1-h dark (D) cycle for the first three days. Thereafter, a schedule of 12-h L (between 07:00 and 19:00 h) and 12-h D (19:00 to 07:00 h) per day was applied. This lighting scheme resembles the natural situation in countries near the equator. Light intensity was maintained at 20 lux during the light periods throughout the experiment.

Two perches were placed in each pen. The wooden slat of a perch measured 5 cm x 5 cm x 50 cm (wide x height x length) and had rounded angles. The slat was fixed on two 5 cm high structures. Each perch is about 10 cm above the floor.

The T in the room was initially kept at 32±2$^0$C with relative humidity (RH) of 70 to 80% from day 0 to 7. This setting allowed the birds to develop thermotolerance and prevent mortality (May and Lott, 2000). The T and RH cycles were set up for a day and night rhythm. After 7 d of age, the T in each room was set according to the experimental design.

### *Experimental Design and Treatments*

This study was performed in a 2x2x2 factorial arrangement in completely randomized design. The factors were two levels of temperature (NT and HT), two diet formulations (control and high energy to protein ratio diet; (HE)) and two diet conformations (dry and wet). Each dietary combination was assigned to three replicate pens in each T room with six birds each. Thus, pen was the experimental unit.

One room was maintained at HT and the other room was maintained at NT. The HT regimen was set at 32±2$^o$C during the day (from 07:00 to 19:00 h) and at 25±2$^o$C during the night (from 19:00 until 07:00 h) with RH 70 to 80% from 8 to 42 d of age. The NT regimen was set at 20$^o$C during the day and at 18$^o$C during the night with RH of 40 to 50% from 21 to 42 d of age. From days 8 to 20 onwards, a step down decrease to a T of 20$^o$C was applied by 0.5$^o$C per day (d 8 to13) and 1$^o$C per day (d 14 to 20) for NT. Relative humidity was maintained about constant with no more than 5% variation at the level of the chickens. Ventilation rates were similar in both rooms.

The control diet contained the recommended nutrient levels with regard to CP, essential amino acids, and ME level (NRC, 1994) for broiler chickens for the entire growing period (CP: 215.0 g/kg; ME: 2,895 kcal/kg). The HE diet contained 194.7 g of CP/kg and 3,067 kcal of ME/kg. The ingredients were ground in a hammer mill and pelleted (2 mm). Dietary formulations of the experimental diets are presented in Table 1. Within each of the two ambient T rooms (NT and HT), the four dietary treatment combinations were offered. The birds in each room had ad libitum access to their dry or wet diet, the latter which was made by adding one part of water to one part of dry diet. The preparation and mixing of the diet was done outside the experimental shed to prevent

**Table 1**. The ingredient (g/kg) and calculated nutrient composition of the broiler diets.

| Ingredients | Diet formulation | |
|---|---|---|
| | Control diet | HE diet |
| Maize | 368.8 | 398.4 |
| Soybean meal, crude fiber < 50 | 260.0 | 209.0 |
| Soybean, heat treated | 100.0 | 100.0 |
| Wheat | 200.0 | 200.0 |
| Fat/Oil plan origin hg VC[1] | 35.0 | 55.0 |
| Limestone | 14.0 | 14.0 |
| Salt (NaCl) | 2.3 | 2.3 |
| Premix (maize)[2] | 5.0 | 5.0 |
| Monocalcium phosphate | 9.0 | 9.0 |
| $NaHCO_3$ | 2.0 | 2.0 |
| DL-Methionine | 2.1 | 2.1 |
| L-Lysine HCl | 1.3 | 2.0 |
| L-Threonine | 0.4 | 0.6 |
| Natuphos 5000G[3] | 0.1 | 0.1 |
| Total | 1,000.0 | 1,000.0 |
| Calculated composition (/kg) | | |
| ME (kcal/kg) | 2,895.0 | 3,067.0 |
| CP (g) | 215.0 (230.4)[4] | 194.6 (207.9) |
| Moisture (g) | 119.5 | 117.0 |
| Crude fat (g) | 76.0 (75.0) | 96.0 (94.6) |
| Crude fibre (g) | 28.2 (25.0) | 27.0 (24.1) |
| Total lysine | 12.2 | 11.3 |
| Digestible lysine | 10.5 | 9.8 |
| Total methionine | 5.3 | 5.0 |
| Digestible methionine | 4.9 | 4.6 |
| Total methionine+cystine | 8.8 | 8.2 |
| Digestible methionine+cystine | 7.7 | 7.2 |
| Total threonine | 8.2 | 7.6 |
| Digestible threonine | 6.9 | 6.3 |
| Total tryptophan | 2.5 | 2.2 |
| Digestible tryptophan | 2.2 | 1.9 |
| Calcium (g) | 8.8 | 8.7 |
| Phosphorus (g) | 5.5 | 5.4 |
| Available phosphorus (g) | 3.0 | 3.0 |

[1]Highly digestible vegetable oil (soya oil). [2]Composition of 1 kg premix:vitamin A (retinyl acetate), 12,000 IU; vitamin $D_3$ (cholecalciferol), 2,400 IU; vitamin E (dl-α-tocopherol), 30 mg; vitamin $K_3$ (menadion), 1.5 mg; vitamin $B_1$ (thiamin), 2.0 mg; vitamin $B_2$ (riboflavin), 7.5 mg; vitamin $B_6$ (pyridoxine-HCl), 3.5 mg, vitamin $B_{12}$ (cyanocobalamin), 20 µg; niacin, 35 mg; vitamin $B_5$ (d-pantothenic acid), 10 mg; choline chloride, 460 mg; folic acid, 1.0 mg, vitamin B-complex, 0.2 mg; iron, 80 mg; copper, 12 mg; manganese, 85 mg; zinc, 60 mg; cobalt, 0.4 mg; iodine, 0.8; selenium, 0.1 mg and antioxidant 125 mg. [3]BASF, Ludwigshafen, Germany. [4]Values in parenthesis were analyzed values.

any disturbance to the chickens. The wet diet was made and offered at 07:00 and 12:00 h every day. During each feeding time, all feeders were taken out of the pens and weighed, and new, pre-weighed feeders were placed back.

***Measurements***

Feed intake behavior and panting behavior were recorded by direct observation during a 5 to 7 min walk through each room. Each bird in each of the 12 pens in each room was observed instantaneously for a moment during this period (a scan). After 5 to 7 min, the behavior of each bird in the room was scanned at a single moment. This procedure was repeated with 25 min pauses several times between 09:00 to 12:00 h and between 14:00 to 17:00 h. In total, one day scan sampling lasted 3 h in the morning and 3 h in the afternoon. This was done to ensure a representative sample for a 12 h daylight period. Scan sampling was done two days per wk. Observations were conducted from wk 1 to 6 by an experienced assessor standing in front of the pen. Based on these observations (instantaneous scan sampling), we calculated the percentage of time budget the chickens in a certain pen spent on eating and panting behaviors relative to other behaviors. Eating was defined as a chicken having its head in or above the feeder, and panting was defined as a chicken breathing rapidly with short gasps with open beak and split wing feather alignment. All other behaviors were recorded as described by Bokkers and Koene (2003). Previous experience showed that making more than one scan per observation of a pen increased behavioral activity of the birds as a reaction to the presence of the observer (Bokkers and Koene, 2003). Therefore, one scan per observation was made. All scan data per pen per week were pooled.

For measuring feed intake of each meal, the offered quantity of feed was corrected for the weight of the feed residues in the feeder. Feed intake of the wet diet was calculated on the basis of the quantity of dry feed offered to the birds. The real 'dry' intake of the wet diet was corrected for water loss due to evaporation which was estimated by placing an extra feed trough with a wet diet outside the pen each time the feed was refreshed and weight loss was measured. Weighing the dry feed was done every three to four days. We did not correct for loses of feed from the feeder.

Fresh water intake was recorded in each pen by subtracting the remaining water from offered water and correcting for evaporation. Total water intake of wet diet fed birds was calculated from fresh water intake plus the water content of the wet diet consumed. BW gain was determined per bird/week. Weights of birds that died or had to be culled were recorded, and its BW gain was included in the calculation of the feed conversion ratio (FCR). Feed intake, BW gain and water intake were calculated per bird/week.

Blood samples, collected from the wing veins of birds from each pen, were taken at 7, 20, and 41 days of age. Three birds were randomly taken out of the pen and blood was collected within 2 min after the chicken was caught. Birds were marked and the same birds were sampled again at the next sampling day. Blood samples of approximately one ml per bird were collected into syringe-needle assemblies that had been flushed with a solution of EDTA. One blood smear for each broiler was prepared and fixed with methanol before immediately being stained with Wright's stain 100% rinsed with distilled water and ran air dry. Heterophil/Lymphocyte (H/L) ratio was counted from 100 cells per slide and classified using oil immersion microscopy at 100X (Dumonceaux and Harrison, 1994). Body T was measured using a digital thermometer just before blood sampling of those birds which were used also for H/L ratio determination. H/L and body T data of the three birds per pen were averaged.

GIT developments were measured in euthanized birds at d 21 and 42. In total two birds per pen per treatment at d 21 and three birds per pen at d 42 of each treatment group were dissected. Each bird was euthanized immediately by injection of T61 and dissected to separate the GIT from the body. A flexible tape was used to determine the lengths of each intestinal segment on a flat surface. The length of the duodenum (from the pyloric junction to the distal most point of insertion of the duodenal mesentery), the jejunum (from the distal most point of insertion of the duodenal mesentery to the junction with Meckel's diverticulum), the ileum (from junction with Meckel's diverticulum to ileo-caecal junction) and the sum of the lengths from the ostium to the tip of caeca and colon were determined. The full and empty weight (±0.1 g) of the crop, proventriculus and gizzard were determined. Data of length and empty weight of GIT segments were expressed per 100 g BW with data of the two birds per pen averaged.

***Statistical Analysis***

Birds were randomly allocated to pens representing different dietary treatments in each room. All performance data were repeatedly taken on the same experimental units during 6 wk. Therefore, a mixed model can be used to account for the covariance structure among repeated observations (Littell et al., 1998; Wang and Goonewardene, 2004). In the analysis, the repeated statement with PROC MIXED in SAS (version 9.1; SAS Inst. Inc., Cary, NC) was used adding the factor time ("W" as the time factor) and pen was considered as an additional random effect. After testing all the performance data with a higher hierarchy in the model, only feed intake was significant with three way interactions. Therefore, it was decided to exclude three way interactions from the model. The following statistical model was used:

$$Y_{ijklm} = \mu + T_i + F_j + C_k + T_i \times F_j + T_i \times C_k + F_j \times C_k + W_l + T_i \times W_l + F_j \times W_l + C_k \times W_l + e_{ijklm}$$

where $Y_{ijklm}$ is dependent variable, $\mu$ is overall mean effect, $T_i$ is the *i*th fixed T effect ( *i*=1 is NT and *i*=2 is HT), $F_j$ is the *j*th fixed diet formulation effect (*j*=1 is control diet and *j*=2 is HE diet), $C_k$ is the *k*th fixed diet conformation effect (*k*=1 is dry and *k*=2 is wet), $W_l$ is the *l*th random week effect when the measurement was taken (*l*=1..6), $T_i \times F_j$ is the fixed interaction effect between T and diet formulation, $T_i \times C_k$ is the fixed interaction effect between T and diet conformation, $F_j \times C_k$ is the fixed interaction effect between diet formulation and diet conformation, $T_i \times W_l$ is the random interaction effect between T and week, $F_j \times W_l$ is the random interaction effect between diet formulation and week, $C_k \times W_l$ is the random interaction effect between diet conformation and week and $e_{ijklm}$ is a random error associated with the *j*th diet formulation and *k*th diet conformation assigned to the *i*th T at wk*l*, $e_{ijklm} \sim NID(0, \sigma^2_e)$.

Differences were considered significant at a probability level of $P < 0.05$. If significant main effects or their interactions were detected, means were compared using least squares means comparison. Mean separation ($P < 0.05$) was produced with PDMIX800 in SAS, which is a macro for converting mean separation output to letter groupings (Saxton, 1998). The Kenward-Roger method was used for computing the denominator degrees of freedom for the tests of main effects.

The best covariance structure was based on the corrected Akaike Information Criteria (AICC). The first-order ante-dependence covariance structure [ANTE (1)] fitted the data best for feed intake. The heterogenous autoregressive covariance structure [ARH(1)] fitted the data best for water intake and BW gain. The unstructured covariance structure (UN) fitted the data best for water-to-feed ratio and compound symmetry covariance structure fitted the data best for feed conversion ratio (FCR).

Body T, heterophil/lymphocyte ratio and GIT development data were analyzed by PROC MIXED in SAS. After testing all the performance data with a higher hierarchy in the model, only body T at d 20 showed to be significant as a three way interaction.

Therefore, we decided to exclude three way interactions from the model. The following linear model was used:

$$Y_{ijkl} = \mu + T_i + F_j + C_k + T_i \times F_j + T_i \times C_k + F_j \times C_k + e_{ijkl}$$

where $Y_{ijkL}$ is measurement of response of the *l*th bird kept on *i*th T having the *j*th diet formulation with *k*th diet conformation, μ is overall mean effect, $T_i$ is the *i*th fixed T effect (*i*=1 is NT and *i*=2 is HT), $F_j$ is the *j*th fixed diet formulation effect (*j*=1 is control diet and *j*=2 is HE diet), $C_k$ is the fixed diet conformation effect (*k*=1 is dry and *k*=2 is wet), $T_i \times F_j$ is the fixed interaction effect between T and diet formulation, $T_i \times C_k$ is the fixed interaction effect between T and diet conformation, $F_j \times C_k$ is the fixed interaction effect between diet formulation and diet conformation and $e_{ijk}$ is the residual error. $e_{ijkl} \sim NID(0, \sigma^2_e)$.

Behavior data were analyzed with replicated observations per pen per week. Feed intake behavior and panting behavior were analyzed using the Kruskal-Wallis test and post hoc with the Wilcoxon two sample test by non-linear procedure of SAS.

## Results

***Temperature and Relative Humidity.*** The T and RH are given as the average minimum and maximum ± SD for the respective interval. The T in the HT room during the first week was between 30.6±1.4°C and 33.2±1.4°C and the RH was between 59.0±11.8% and 81.3±10.8%, whereas the T in the NT room was between 31.3±1.1°C and 32.7±0.6°C and the RH was between 58.1±7.9% and 74.2±5.5%. The T in the HT room from wk 2 to 6 was between 26.5±2.5°C and 31.7±2.5°C and the RH was between 58.2±10.9% and 84.9±10.8%. The T in the NT room from wk 4 to 6 was between 19.6±0.9°C and 21.5±0.6°C and the RH was between 61.3±9.8% and 71.6±8.8%.

***Eating and Panting Behavior***. Results on the percentage time spent on eating and panting behaviors per wk are given in Figures 1 and 2, respectively. Eating time declined from wk 1 to 6 in both temperatures, both dietary formulations and both conformations. Time spent on eating was higher in birds kept at HT than at NT in the second wk of age, but higher at NT than at HT in the sixth wk of age (Figure 1, panel A). Time spent on eating was similar between control diet and HE diet at all ages (Figure 1, panel B). Birds fed a wet diet spent more time on eating than birds fed a dry diet in the second wk of age (Figure 1, panel C). Panting time was higher at HT than at NT after wk 1 onwards (Figure 2, panel A) and was similar in both diets and conformations at all ages (Figure 2, panels B and C).

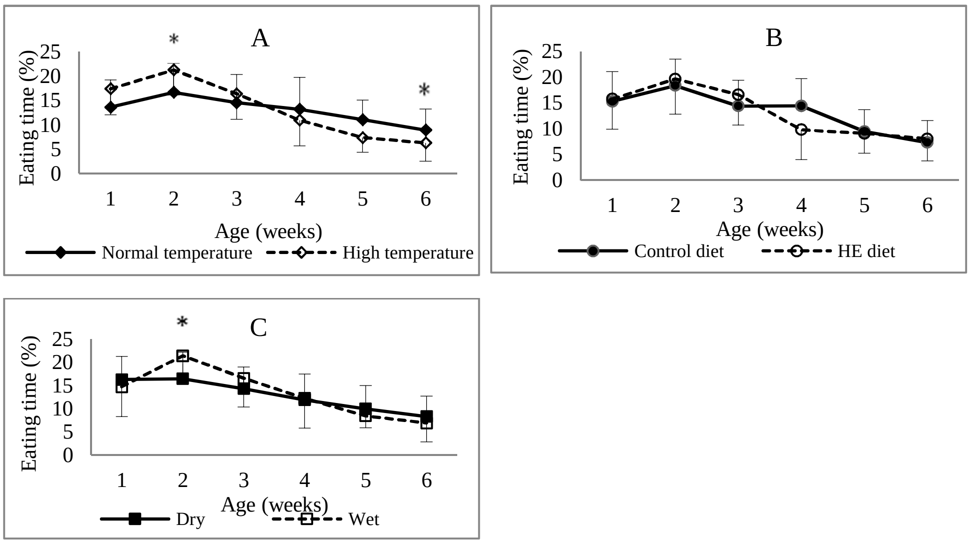


**Figure 1**. Eating time (expressed as a percentage within observation time) at different temperatures (panel A), on different diet formulations (panel B) and on different diet conformations (panel C). Vertical bars represent SD. The asterisk (*) means a significant difference of at least *P* < 0.05 at a certain age. The T and relative humidity (RH) in the high-T (HT) room during the first week were 31.9±1.4°C and 70.2±11.3%, whereas the T and RH in the normal-T (NT) room were 32.0±0.8°C and 66.2±6.7%. For the NT, the T ranged from 19.6±0.9°C to 21.5±0.6°C and the RH ranged from 61.3±9.8% to 71.6±8.8% from wk 4 to 6. For the HT, the T ranged from 26.5±2.5°C to 31.7±2.5°C and the RH ranged from 58.2±10.9% to 84.9±10.8% from wk 4 to 6. Control diet = 215 g of CP/kg and 2,895 kcal of ME/kg; HE diet = 194.6 g of CP and 3,067 kcal of ME/kg. Dry diet; Wet diet (adding 1 part of water to 1 part of dry diet).

***Bird Performance.*** Mortality in this study was very low (1%). All performance data in the tables are data corrected for mortality by wk. Probability values for all parameters are presented in Table 2. Differences in performance of the broilers in each wk at different temperatures, diets, and conformations are presented in Table 3. Significant two-way interactions are summarized in Figures 3, 4 and 5.

*Feed Intake*. Temperature and diet formulation had a major effect on feed intake, but diet conformation did not influence feed intake (Table 2). On average, feed intake at NT (670.1 g/bird) was 13.3% higher than at HT (591.3 g/bird). Feed intake of control-fed birds (671.6 g/bird) was 13.8% higher than of HE-fed birds (589.9 g/bird).

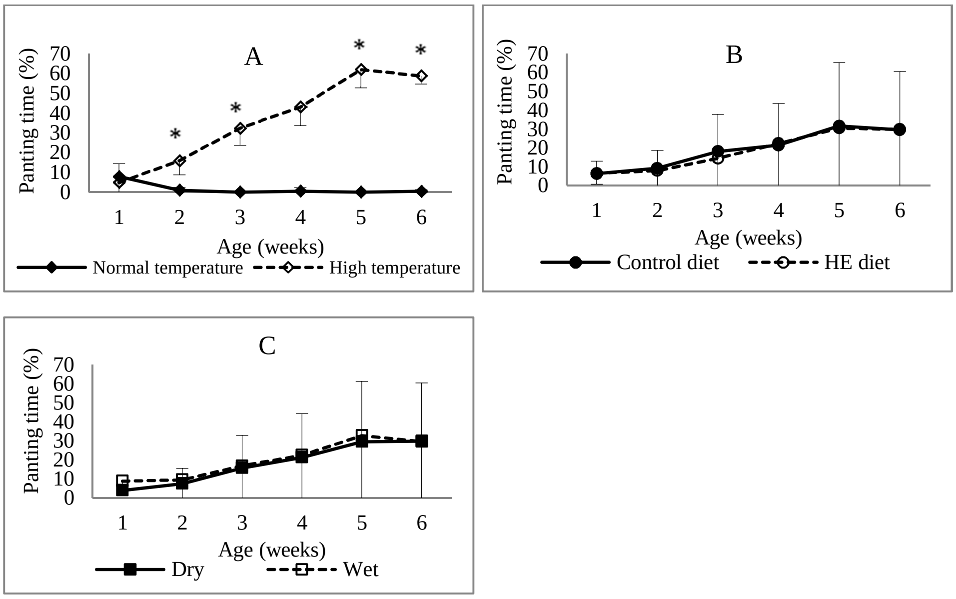


**Figure 2**. Panting behavior (expressed as a percentage within observation time) at different temperatures (panel A), on different diet formulations (panel B) and on different diet conformations (panel C). Vertical bars represent SD. The asterisk (*) means a significant difference of at least $P <$ 0.05 at a certain age. The T and relative humidity (RH) in the high-T (HT) room during the first week were 31.9±1.4°C and 70.2±11.3%, whereas the T and RH in the normal-T (NT) room were 32.0±0.8°C and 66.2±6.7%. For the NT, the T ranged from 19.6±0.9°C to 21.5±0.6°C and the RH ranged from 61.3±9.8% to 71.6±8.8% from wk 4 to 6. For the HT, the T ranged from 26.5±2.5°C to 31.7±2.5°C and the RH ranged from 58.2±10.9% to 84.9±10.8% from wk 4 to 6. Control diet = 215 g of CP/kg and 2,895 kcal of ME/kg; HE diet = 194.6 g of CP and 3,067 kcal of ME/kg. Dry diet; Wet diet (adding 1 part of water to 1 part of dry diet).

Feed intake of wet-control-fed birds was for most weeks similar to that of dry-control-fed birds at NT and HT (Table 3). At NT, feed intake of wet-HE-fed birds was for most weeks similar to that of dry-HE-fed birds. At HT, feed intake of wet-HE-fed birds was numerically higher in wk 1 to 4 and even significantly higher in wk 5 and 6 than of dry-fed birds (Table 3).

An interaction between T and week (Table 2) showed that feed intake was lower at HT than at NT in wk 5 and 6 (Figure 3). An interaction between diet formulation and week showed that feed intake was higher in wk 2 to 6 for the control-fed birds as compared with that for the HE-fed birds (Figure 4). An interaction between diet conformation and week showed that feed intake was higher in wk 2 to 3 for the wet-fed birds as compared with that for the dry-fed birds (Figure 5).

**Table 2**. Probability values of main effects and interaction between temperature (T), diet formulation (F), diet conformation (C) and week (W) for different traits[1].

| Main effect[2] | Feed intake (g/bird per wk) | BW gain (gr/bird per wk) | Water intake (gr/bird per wk) | Water-to-feed ratio (g/g) | FCR (g/g) |
|---|---|---|---|---|---|
| T | **<0.001** | **<0.001** | 0.197 | **0.036** | **<0.001** |
| F | **<0.001** | **<0.001** | **<0.001** | **0.005** | **0.019** |
| C | 0.095 | **0.005** | **<0.001** | **0.005** | **0.043** |
| W | **<0.001** | **<0.001** | **<0.001** | **<0.001** | **<0.001** |
| T x F | 0.903 | 0.174 | 0.502 | 0.239 | **0.003** |
| T x C | 0.376 | **0.022** | 0.055 | **0.001** | **0.001** |
| F x C | 0.864 | 0.959 | 0.689 | **0.022** | **<0.001** |
| T x W | **<0.001** | **<0.001** | **<0.001** | **0.002** | **<0.001** |
| F x W | **<0.001** | **<0.001** | **<0.001** | 0.951 | 0.158 |
| C x W | **0.036** | **0.001** | **0.014** | 0.163 | 0.693 |

[1]The T and relative humidity (RH) in the high-T (HT) room during the first week were 31.9±1.4°C and 70.2±11.3%, whereas the T and RH in the normal-T (NT) room were 32.0±0.8°C and 66.2±6.7%. For the NT, the T ranged from 19.6±0.9°C to 21.5±0.6°C and the RH ranged from 61.3±9.8% to 71.6±8.8% from wk 4 to 6. For the HT, the T ranged from 26.5±2.5°C to 31.7±2.5°C and the RH ranged from 58.2±10.9% to 84.9±10.8% from wk 4 to 6. Control diet= 215 g of CP/kg and 2,895 kcal of ME/kg; HE diet= 194.6 g of CP and 3,067 kcal of ME/kg. Dry diet; Wet diet (adding 1 part of water to 1 part of dry diet).
[2]T x F = interaction between T and F; T x C = interaction between T and C; F x C = interaction between F and C; T x W = interaction between T and W; F x W = interaction between F and W; C x W = interaction between C and W.

*Body Weight Gain***.** Temperature, diet formulation and diet conformation affected BW gain (Table 2). On average, BW gain at NT (475.2 g/bird) was 23.7% higher than at HT (384.1 g/bird). BW gain of control-fed birds (449.1 g/bird) was 9.5% higher than that of HE-fed birds (410.2 g/bird). BW gain of wet-fed birds (442.1 g/bird) was 6.0% higher than that of dry-fed birds (417.2 g/bird).

BW gain of wet-control-fed birds was significantly higher than that of dry-control-fed birds until wk 3 and similar from wk 4 onwards, at NT (Table 3). At HT, BW gain of wet-control-fed birds was for most weeks similar to that of dry-control-fed birds. BW gain of wet-HE-fed birds was significantly higher than that of dry-HE-fed birds in wk 2 to 5, at NT. At HT, BW gain of wet-HE-fed birds was, although statistically similar, numerically higher than that of dry-HE-fed birds for all week (Table 3).

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**Table 3**. Least square means of performance parameters in broiler chicken from 0 to 6 week of age as affected by temperature (T), diet formulation (F) and diet conformation (C)[1].

| Parameters | T | F | C | Wk 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|---|---|
| Feed intake (g/bird per wk) | | | | | | | | | |
| | NT | Control | Dry | 112.6$^{ab}$ | 279.8$^{a}$ | 466.8$^{bc}$ | 758.4$^{ab}$ | 1117.8$^{a}$ | 1464.3$^{a}$ |
| | | | Wet | 120.3$^{a}$ | 290.5$^{a}$ | 517.1$^{a}$ | 815.6$^{a}$ | 1194.5$^{a}$ | 1497.4$^{a}$ |
| | NT | HE | Dry | 114.2$^{ab}$ | 247.0$^{bc}$ | 415.3$^{e}$ | 693.3$^{cd}$ | 977.8$^{b}$ | 1352.6$^{b}$ |
| | | | Wet | 117.2$^{ab}$ | 228.4$^{c}$ | 430.1$^{cde}$ | 660.5$^{d}$ | 937.1$^{b}$ | 1274.1$^{b}$ |
| | HT | Control | Dry | 118.7$^{ab}$ | 239.7$^{c}$ | 488.9$^{ab}$ | 750.2$^{bc}$ | 987.5$^{b}$ | 1117.7$^{c}$ |
| | | | Wet | 110.0$^{b}$ | 286.0$^{a}$ | 524.7$^{a}$ | 769.9$^{ab}$ | 987.4$^{b}$ | 1101.6$^{cd}$ |
| | HT | HE | Dry | 110.9$^{ab}$ | 235.8$^{bc}$ | 421.0$^{de}$ | 654.4$^{d}$ | 831.1$^{c}$ | 948.5$^{e}$ |
| | | | Wet | 110.7$^{ab}$ | 266.4$^{ab}$ | 460.6$^{bcd}$ | 692.0$^{cd}$ | 950.2$^{b}$ | 1028.1$^{d}$ |
| | SEM | | | 3.3 | 10.6 | 15.0 | 20.2 | 34.2 | 26.5 |
| BW gain (g/bird per wk) | | | | | | | | | |
| | NT | Control | Dry | 104.7$^{cd}$ | 211.9$^{bcd}$ | 335.0$^{cde}$ | 568.7$^{ab}$ | 773.2$^{ab}$ | 908.5$^{a}$ |
| | | | Wet | 118.9$^{a}$ | 241.2$^{a}$ | 389.6$^{ab}$ | 567.3$^{ab}$ | 812.0$^{a}$ | 874.1$^{a}$ |
| | NT | HE | Dry | 106.9$^{bcd}$ | 205.9$^{cd}$ | 300.5$^{e}$ | 499.8$^{cd}$ | 651.1$^{c}$ | 828.7$^{a}$ |
| | | | Wet | 113.9$^{ab}$ | 229.4$^{ab}$ | 370.8$^{abcd}$ | 553.7$^{abc}$ | 744.9$^{b}$ | 894.0$^{a}$ |
| | HT | Control | Dry | 111.7$^{abc}$ | 229.0$^{ab}$ | 395.6$^{ab}$ | 503.8$^{cd}$ | 635.0$^{c}$ | 584.6$^{b}$ |
| | | | Wet | 109.3$^{bcd}$ | 242.7$^{a}$ | 380.2$^{abc}$ | 521.5$^{bcd}$ | 642.0$^{c}$ | 516.7$^{b}$ |
| | HT | HE | Dry | 102.3$^{d}$ | 199.9$^{d}$ | 329.9$^{de}$ | 415.4$^{e}$ | 516.9$^{d}$ | 492.9$^{b}$ |
| | | | Wet | 104.6$^{cd}$ | 219.9$^{bc}$ | 348.8$^{bcd}$ | 465.9$^{de}$ | 603.2$^{c}$ | 545.4$^{b}$ |
| | SEM | | | 2.7 | 6.6 | 16.0 | 20.5 | 21.8 | 39.5 |

*Continued*

**Table 3**. (Continued). Least square means of performance parameters in broiler chicken from 0 to 6 week of age as affected by temperature (T), diet formulation (F) and diet conformation (C)[1].

| Parameters | T | F | C | Wk 1 | Wk 2 | Wk 3 | Wk 4 | Wk 5 | Wk 6 |
|---|---|---|---|---|---|---|---|---|---|
| Water intake (g/bird per wk) | | | | | | | | | |
| | NT | Control | Dry | $270.0^{bc}$ | $533.3^{bcd}$ | $981.5^{bc}$ | $1647.6^{bc}$ | $2257.1^{b}$ | $3100.0^{b}$ |
| | | | Wet | $372.1^{a}$ | $603.0^{ab}$ | $1138.3^{a}$ | $1757.4^{ab}$ | $2589.9^{a}$ | $3386.0^{a}$ |
| | NT | HE | Dry | $281.5^{bc}$ | $451.9^{d}$ | $800.0^{e}$ | $1366.7^{d}$ | $1833.3^{c}$ | $2604.8^{e}$ |
| | | | Wet | $334.9^{ab}$ | $496.5^{cd}$ | $949.0^{cd}$ | $1513.7^{cd}$ | $2242.3^{b}$ | $2858.0^{c}$ |
| | HT | Control | Dry | $281.5^{bc}$ | $638.9^{a}$ | $1100.0^{ab}$ | $1795.2^{ab}$ | $2400.0^{ab}$ | $2971.4^{bc}$ |
| | | | Wet | $291.1^{bc}$ | $584.2^{abc}$ | $1134.6^{a}$ | $1888.9^{a}$ | $2533.4^{ab}$ | $2846.4^{cd}$ |
| | HT | HE | Dry | $225.9^{c}$ | $477.8^{d}$ | $851.9^{de}$ | $1433.3^{d}$ | $1795.2^{c}$ | $2276.2^{f}$ |
| | | | Wet | $251.1^{c}$ | $532.0^{bcd}$ | $944.4^{cd}$ | $1543.1^{cd}$ | $2241.0^{b}$ | $2522.7^{e}$ |
| | SEM | | | 25.5 | 30.0 | 42.5 | 70.7 | 98.0 | 79.0 |
| Water-to-feed ratio (g/g) | | | | | | | | | |
| | NT | Control | Dry | $2.4^{b}$ | $1.9^{b}$ | $2.1^{abc}$ | $2.2^{ab}$ | $2.0^{c}$ | $2.1^{e}$ |
| | | | Wet | $3.1^{a}$ | $2.1^{b}$ | $2.2^{ab}$ | $2.2^{ab}$ | $2.2^{b}$ | $2.3^{de}$ |
| | NT | HE | Dry | $2.5^{b}$ | $1.8^{b}$ | $1.9^{c}$ | $2.0^{b}$ | $1.9^{c}$ | $1.9^{f}$ |
| | | | Wet | $2.9^{ab}$ | $2.2^{b}$ | $2.2^{ab}$ | $2.3^{a}$ | $2.4^{ab}$ | $2.2^{e}$ |
| | HT | Control | Dry | $2.4^{b}$ | $2.7^{a}$ | $2.3^{ab}$ | $2.4^{a}$ | $2.4^{a}$ | $2.7^{a}$ |
| | | | Wet | $2.6^{ab}$ | $2.0^{b}$ | $2.2^{ab}$ | $2.5^{a}$ | $2.6^{a}$ | $2.6^{ab}$ |
| | HT | HE | Dry | $2.0^{b}$ | $2.0^{b}$ | $2.0^{bc}$ | $2.2^{ab}$ | $2.2^{b}$ | $2.4^{cd}$ |
| | | | Wet | $2.3^{b}$ | $2.0^{b}$ | $2.1^{abc}$ | $2.2^{ab}$ | $2.4^{ab}$ | $2.5^{bc}$ |
| | SEM | | | 0.2 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |

*Continued*

**Table 3**. (Continued). Least square means of performance parameters in broiler chicken from 0 to 6 week of age as affected by temperature (T), diet formulation (F) and diet conformation (C)[1].

| Parameters | T | F | C | Week (W) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 1 | 2 | 3 | 4 | 5 | 6 |
| FCR (g/g) | | | | | | | | | |
| | NT | Control | Dry | 1.08 | 1.32[a] | 1.39[a] | 1.33[cd] | 1.45[b] | 1.61[c] |
| | | | Wet | 1.01 | 1.20[a] | 1.33[ab] | 1.44[bc] | 1.47[ab] | 1.72[c] |
| | NT | HE | Dry | 1.07 | 1.20[a] | 1.38[ab] | 1.39[bc] | 1.51[ab] | 1.64[c] |
| | | | Wet | 1.03 | 1.00[b] | 1.16[c] | 1.19[d] | 1.26[c] | 1.43[d] |
| | HT | Control | Dry | 1.06 | 1.05[bc] | 1.24[bcd] | 1.49[ab] | 1.55[ab] | 1.92[b] |
| | | | Wet | 1.01 | 1.18[ac] | 1.40[ab] | 1.48[ab] | 1.55[ab] | 2.16[a] |
| | HT | HE | Dry | 1.09 | 1.18[ac] | 1.28[abcd] | 1.59[ab] | 1.61[ab] | 1.95[b] |
| | | | Wet | 1.06 | 1.21[a] | 1.32[abd] | 1.49[ab] | 1.57[ab] | 1.89[b] |
| | SEM | | | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |

Means within a week between treatments without a common superscript letters (a-e) differ significantly ($P < 0.05$); no superscript means nonsignificant differences.
[1]The T and relative humidity (RH) in the high-T (HT) room during the first week were 31.9±1.4°C and 70.2±11.3%, whereas the T and RH in the normal-T (NT) room were 32.0±0.8°C and 66.2±6.7%. For the NT, the T ranged from 19.6±0.9°C to 21.5±0.6°C and the RH ranged from 61.3±9.8% to 71.6±8.8% from wk 4 to 6. For the HT, the T ranged from 26.5±2.5°C to 31.7±2.5°C and the RH ranged from 58.2±10.9% to 84.9±10.8% from wk 4 to 6. Control diet= 215 g of CP/kg and 2,895 kcal of ME/kg; HE diet= 194.6 g of CP and 3,067 kcal of ME/kg. Dry diet; Wet diet (adding 1 part of water to 1 part of dry diet).

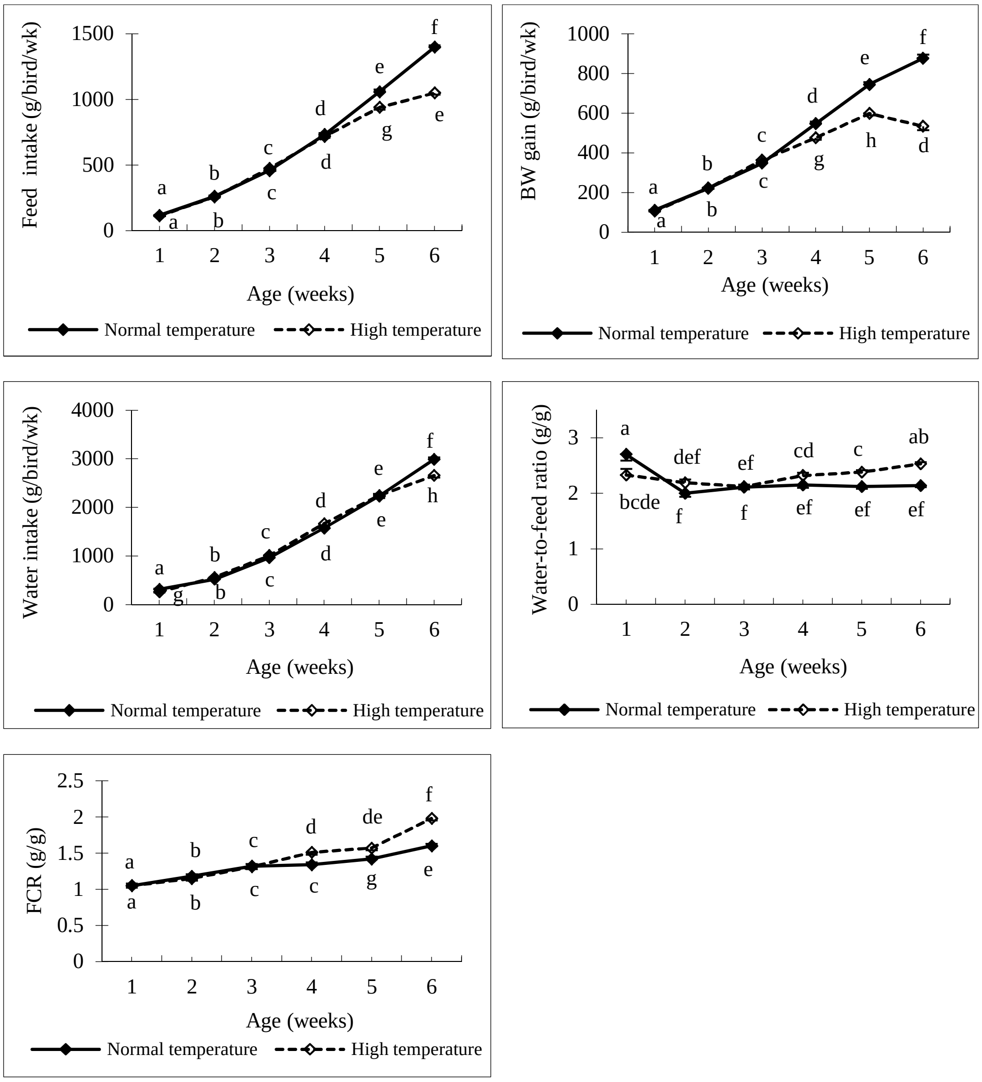


**Figure 3**. Least square means for traits that show a significant T and week interaction. Means within and between lines without common letters (a-g) differ significantly ($P < 0.05$). The T and relative humidity (RH) in the high-T (HT) room during the first week were 31.9±1.4°C and 70.2±11.3%, whereas the T and RH in the normal-T (NT) room were 32.0±0.8°C and 66.2±6.7%. For the NT, the T ranged from 19.6±0.9°C to 21.5±0.6°C and the RH ranged from 61.3±9.8% to 71.6±8.8% from wk 4 to 6. For the HT, the T ranged from 26.5±2.5°C to 31.7±2.5°C and the RH ranged from 58.2±10.9% to 84.9±10.8% from wk 4 to 6.

An interaction between T and diet conformation showed that at NT, the difference in BW gain between wet- and dry-fed birds was 30 g/bird (490.2 vs. 460.2 g/bird, respectively), whereas at HT, this difference was only 20 g/bird (394.0 vs. 374.1 g/bird, respectively). An interaction between T and week showed that BW gain was lower at HT than at NT in wk 4 to 6 (Figure 3). An interaction between diet formulation and week and between diet conformation and week showed that BW gain was higher for the control- and the wet-fed birds as compared with that for the HE- and the dry-fed birds in wk 1 to 5, respectively (Figures 4 and 5).

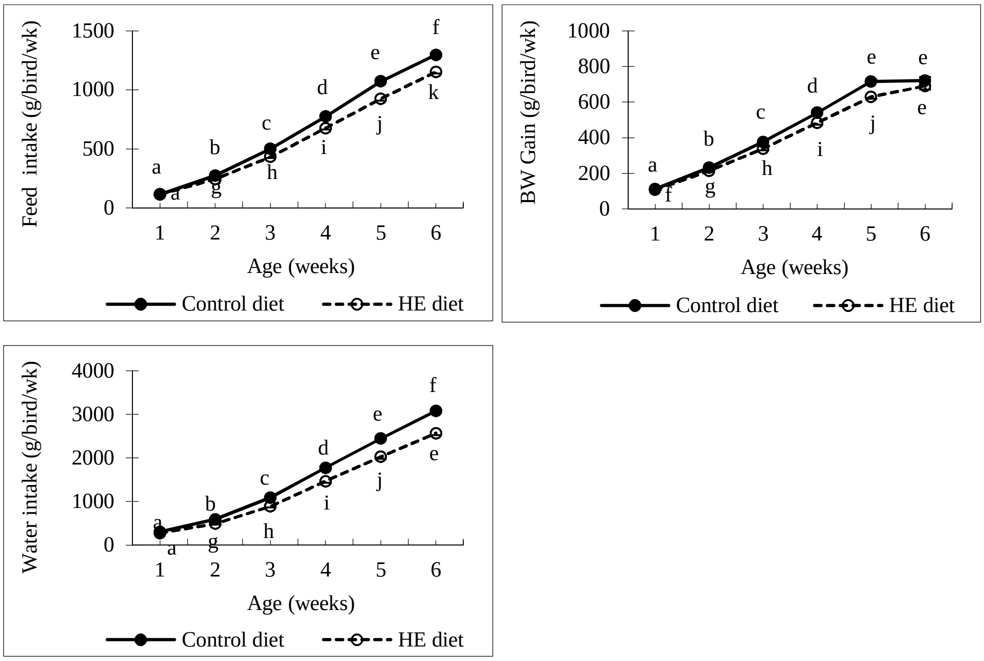


**Figure 4**. Least square means for traits that show a significant diet and week interaction. Means within and between lines without common letters (a-k) differ significantly ($P < 0.05$). Control diet= 215 g of CP/kg and 2,895 kcal of ME/kg); HE diet= 194.6 g of CP and 3,067 kcal of ME/kg).

*Water Intake and Water-to-Feed Ratio*. Temperature did not significantly affect water intake, but it affected water-to-feed ratio. Diet formulation and diet conformation did significantly affect water intake and water-to-feed ratio (Table 2). Water-to-feed ratio at HT (2.31 g/g) was higher than at NT (2.20 g/g). Water intake of control-fed birds (1545.9 g/bird) was 20.4% higher than that of HE-fed birds (1284.5 g/bird). Water intake of wet-fed birds (1481.4 g/birds) was 9.8% higher than that of dry-fed birds (1349.0 g/bird). Water-to feed ratio of control-fed birds (2.33 g/g) was 6.9% higher than that of HE-fed birds (2.18 g/g), respectively. Water-to-feed ratio of wet-fed birds (2.33 g/g) was 6.8% higher than that of dry-fed birds (2.18 g/g).

At NT, water intake of wet-control-fed birds was numerically higher in wk 1 to 4 and even significantly higher than that of dry-control-fed birds in wk 5 to 6 (Table 3). At HT, water intake of wet-control-fed birds was, for most weeks, similar to that of dry-control-fed birds. Water intake of wet-HE-fed birds was numerically higher in wk 1 to 4 and even significantly higher than that of dry-HE-fed birds in wk 5 to 6 at both T (Table 3).

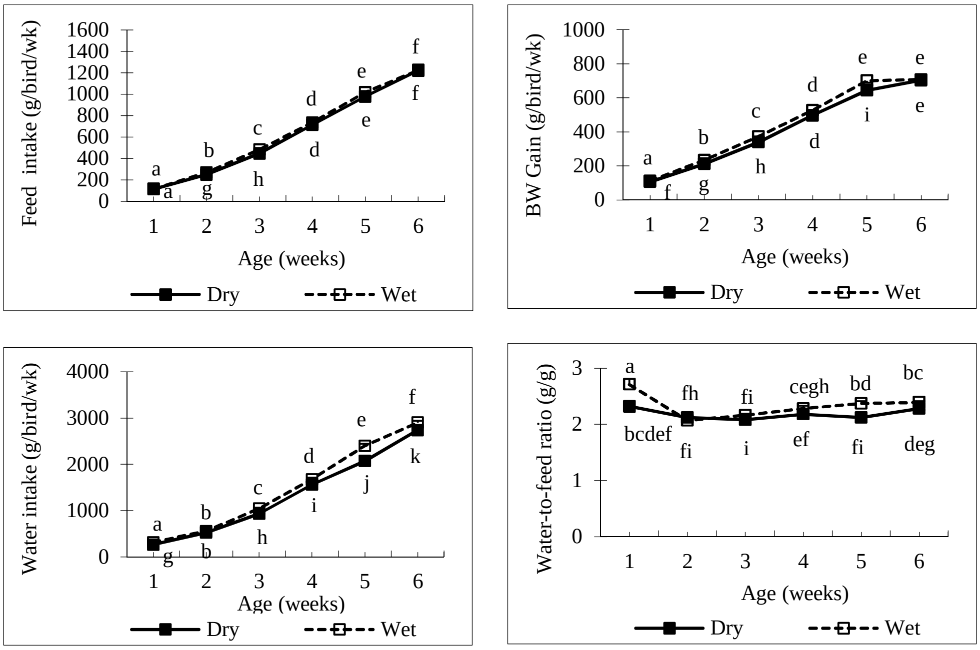


**Figure 5**. Least square means for traits that show a significant diet conformation and week interaction. Means within and between lines without common letters (a-k) differ significantly ($P < 0.05$). Dry diet; Wet diet (adding 1 part of water to 1 part of dry diet).

Water-to-feed ratio of wet-control-fed birds was, for most weeks, similar to that of dry-control-fed birds at NT and HT. At NT, water-to-feed ratio of wet-HE-fed birds was numerically higher in wk 1 and 2 and even significantly higher than that of dry-HE-fed birds in wk 3 to 6. At HT, water-to-feed ratio of wet-HE-fed birds was for most weeks similar to that of dry-HE-fed birds (Table 3).

An interaction between T and diet conformation showed that at NT, the difference in water-to-feed ratio between wet- and dry-fed birds was 0.24 g/g (2.34 vs. 2.07 g/g, respectively), whereas at HT, this difference was 0.02 g/g (2.32 vs. 2.30 g/g, respectively).

An interaction between diet formulation and diet conformation showed that the difference in water-to-feed ratio between wet-control- and dry-control-fed birds was 0.06 g/g (2.36 vs. 2.30 g/g, respectively), whereas difference between wet-HE and dry-HE-fed birds was 0.24 g/g (2.30 vs. 2.06 g/g, respectively).

An interaction between T and week showed that water intake was only much lower at HT than at NT in wk 1 and 6, whereas water-to-feed ratio was much lower at NT than at HT in wk 1 and wk 4 to 6 (Figure 3). An interaction between diet formulation and week showed that water intake of control-fed birds was higher than that of HE-fed birds in wk 2 to 6 (Figure 4). An interaction between diet conformation and week showed that water intake was higher in wk 1 and wk 3 to 6 for the wet-fed-birds as compared with that the dry-fed birds. Water-to-feed ratio was higher in wk 1 and wk 5 to 6 for the wet-fed birds as compared with that the dry-fed birds (Figure 5).

*Feed Conversion Ratio*. Temperature, diet formulation and diet conformation influenced FCR (Table 2). On average, FCR at NT (1.32 g/g) was 7.7% lower than at HT (1.43 g/g). FCR of control-fed birds (1.39 g/g) was 3.0% higher than that of HE-fed birds (1.35 g/g). FCR of wet-fed bird (1.36 g/g) was 2.2% lower than that of dry-fed birds (1.39 g/g).

FCR of wet-control-fed birds was similar to that of dry-control-fed birds at NT. At HT, FCR of wet-control-fed birds was significantly higher than that of dry-control-fed birds only in wk 2 and 3. FCR of wet-HE-fed birds was lower than that of dry-HE-fed birds in wk 2 to 5, at NT. At HT, FCR of wet-HE-fed birds was similar to that of dry-HE-fed birds in all week (Table 3).

An interaction between T and diet formulation showed that at NT, the difference in FCR between control- and HE-fed birds was 0.09 g/g (1.36 vs. 1.27 g/g, respectively), whereas at HT, this difference was 0.02 g/g (1.42 vs. 1.44 g/g, respectively). An interaction between T and diet conformation showed that at NT, the difference in FCR between wet- and dry-fed birds was 0.09 g/g (1.27 vs. 1.36 g/g, respectively), whereas at HT, this difference was 0.02 g/g (1.44 vs. 1.42 g/g, respectively). An interaction between diet formulation and diet conformation showed that the difference in FCR between wet-control- and dry-control-fed birds was 0.04 g/g (1.41 vs. 1.37 g/g, respectively), whereas difference between wet-HE- and dry-HE-fed birds was 0.11 g/g

(1.30 vs. 1.41 g/g, respectively). An interaction between T and week showed that FCR at HT was higher than at NT after wk 3 (Figure 3).

***Gastrointestinal Tract Development.*** Temperature did not significantly affect the GIT development at d 21 except for empty weight of ileum. Diet formulation affected relative lengths of all GIT segments and empty weight of gizzard. The relative lengths of duodenum, jejunum, ileum, caeca and colon of control-fed birds were 14.2, 13.8, 11.8, 15.0 and 22.0% shorter than those of HE-fed birds. The relative empty weight of the gizzard of control-fed birds was 12% lower than that of HE-fed birds. Diet conformation only affected relative length of duodenum and jejunum. Dry-fed birds had a 9.3 and 8.0% higher duodenum and jejunum than wet-fed birds, respectively (Table 4).

At day 42, temperature affected relative lengths of jejunum, ileum and colon and relative empty weights of proventriculus, duodenum, jejunum, ileum and colon. The relative lengths of jejunum, ileum and colon of birds housed at NT were 12.5, 8.7 and 14.2% shorter than those of birds housed at HT. The empty weights of proventriculus, duodenum, jejunum, ileum and colon at NT were 9.2, 24.0, 18.0, 23.6 and 18.6% heavier than at HT (Table 5).

Diet formulation affected relative lengths most of GIT segments and empty weight of gizzard. The relative lengths of duodenum, jejunum and ileum of control-fed birds were 6.8, 8.0 and 6.7% shorter than those of HE-fed birds. The relative empty weight of the gizzard of control-fed birds was 15.6% lower than that of HE-fed birds. Relative length of ileum of wet-fed birds was 6.5% shorter than that of dry-fed birds (Table 5).

There was an interaction between T and diet formulation in empty weight of proventriculus at day 21 and of caeca at day 42. At day 21, an interaction showed that at NT, the difference in empty weight of proventriculus between control- and HE-fed-birds was 0.03g/100 g BW (0.54 vs. 0.51 g/100 g BW, respectively), whereas at HT, this difference was 0.11 g/100 g BW (0.41 vs. 0.52 g/100 g BW, respectively) (Table 4). At day 42, an interaction showed that at NT, the difference in empty weight of caeca between control- and HE-fed-birds was 0.06g/100 g BW (0.33 vs. 0.39 g/100 g BW, respectively), whereas at HT, this difference was 0.01 g/100 g BW (0.34 vs. 0.33 g/100 g BW, respectively) (Table 5).

**Table 4**. Effects of temperature (T), diet formulation (F) and diet conformation (C) on GIT development of male broiler chickens at d 21[1].

| Gastrointestinal segment | Normal Temperature | | | | High Temperature | | | | SEM | Source of variation (*P*-value)[3] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control-dry | Control-wet | HE-dry | HE-wet | Control-dry | Control-wet | HE-dry | HE-wet | | T | F | C | T x F | TxC | FxC[2] |
| Length (cm/100 g BW) | | | | | | | | | | | | | | | |
| Duodenum | 3.28 | 3.03 | 3.76 | 3.40 | 3.24 | 2.91 | 3.81 | 3.55 | 0.10 | 0.925 | **<0.001** | **0.020** | 0.265 | 0.946 | 0.938 |
| Jejunum | 8.27 | 7.60 | 9.32 | 8.45 | 8.02 | 7.82 | 9.95 | 9.06 | 0.30 | 0.323 | **<0.001** | **0.047** | 0.324 | 0.739 | 0.466 |
| Ileum | 7.98 | 7.12 | 8.21 | 7.96 | 7.60 | 7.27 | 9.19 | 8.61 | 0.30 | 0.157 | **<0.001** | 0.650 | 0.086 | 0.888 | 0.763 |
| Caecum | 1.64 | 1.59 | 2.02 | 1.68 | 1.58 | 1.53 | 1.89 | 1.87 | 0.10 | 0.756 | **<0.001** | 0.860 | 0.445 | 0.208 | 0.338 |
| Colon | 0.79 | 0.64 | 0.94 | 0.85 | 0.61 | 0.71 | 0.89 | 0.85 | 0.04 | 0.444 | **<0.001** | 0.332 | 0.649 | 0.086 | 0.660 |
| Empty weight (g/100 g BW) | | | | | | | | | | | | | | | |
| Crop | 0.68 | 0.63 | 0.67 | 0.61 | 0.61 | 0.58 | 0.71 | 0.74 | 0.03 | 0.679 | 0.167 | 0.347 | 0.660 | 0.462 | 0.597 |
| Proventriculus | 0.57 | 0.50 | 0.51 | 0.51 | 0.41 | 0.41 | 0.52 | 0.58 | 0.03 | 0.069 | 0.170 | 0.543 | **0.033** | 0.585 | 0.553 |
| Gizzard | 2.75 | 2.44 | 2.75 | 2.73 | 2.20 | 2.09 | 2.67 | 2.64 | 0.10 | 0.053 | **0.017** | 0.307 | 0.174 | 0.793 | 0.415 |
| Duodenum | 0.51 | 0.61 | 0.61 | 0.61 | 0.56 | 0.45 | 0.68 | 0.67 | 0.10 | 0.915 | 0.051 | 0.911 | 0.223 | 0.319 | 0.973 |
| Jejunum | 0.99 | 1.34 | 0.99 | 1.13 | 1.05 | 1.00 | 1.09 | 1.15 | 0.10 | 0.509 | 0.869 | 0.095 | 0.147 | 0.108 | 0.733 |
| Ileum | 1.10 | 1.10 | 1.04 | 0.93 | 0.96 | 0.83 | 0.99 | 0.90 | 0.10 | **0.028** | 0.488 | 0.136 | 0.123 | 0.613 | 0.781 |
| Caecum | 0.40 | 0.46 | 0.51 | 0.46 | 0.43 | 0.41 | 0.45 | 0.42 | 0.02 | 0.199 | 0.130 | 0.489 | 0.489 | 0.625 | 0.284 |
| Colon | 0.13 | 0.13 | 0.15 | 0.15 | 0.14 | 0.11 | 0.14 | 0.17 | 0.02 | 0.538 | 0.185 | 0.867 | 0.788 | 0.859 | 0.424 |

[1]Mean values are expressed as an average of 3 replicate pens. The T and relative humidity (RH) in the high-T (HT) room during the first week were 31.9±1.4°C and 70.2±11.3%, whereas the T and RH in the normal-T (NT) room were 32.0±0.8°C and 66.2±6.7%. For the NT, the T ranged from 19.6±0.9°C to 21.5±0.6°C and the RH ranged from 61.3±9.8% to 71.6±8.8% from wk 4 to 6. For the HT, the T ranged from 26.5±2.5°C to 31.7±2.5°C and the RH ranged from 58.2±10.9% to 84.9±10.8% from wk 4 to 6. Control diet= 215 g of CP/kg and 2,895 kcal of ME/kg; HE diet= 194.6 g of CP and 3,067 kcal of ME/kg. Dry diet vs. Wet diet (adding 1 part of water to 1 part of dry diet).

[2]T x F = interaction between T and F; T x C = interaction between T and C; F x C = interaction between F and C.

[3]Significant effects ($P < 0.05$) are printed in bold.

**Table 5**. Effects of temperature (T), diet formulation (F) and diet conformation (C) on GIT development of male broiler chickens at d 42[1].

| Gastrointestinal segment | Normal Temperature | | | | High Temperature | | | | SEM | Source of variation (*P*-value)[3] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control -dry | Control -wet | HE- dry | HE- wet | Control -dry | Control -wet | HE- dry | HE- wet | | T | F | C | T x F | TxC | FxC[2] |
| Length (cm/100 g BW) | | | | | | | | | | | | | | | |
| Duodenum | 1.36 | 1.24 | 1.44 | 1.32 | 1.37 | 1.36 | 1.49 | 1.47 | 0.04 | 0.080 | **0.048** | 0.128 | 0.765 | 0.268 | 0.938 |
| Jejunum | 3.06 | 3.15 | 3.53 | 3.23 | 3.44 | 3.67 | 4.02 | 3.70 | 0.10 | **<0.001** | **0.011** | 0.480 | 0.855 | 0.767 | **0.033** |
| Ileum | 3.27 | 3.10 | 3.70 | 3.23 | 3.50 | 3.58 | 3.93 | 3.56 | 0.10 | **<0.001** | **0.006** | **0.008** | 0.641 | 0.271 | **0.030** |
| Caecum | 1.04 | 0.67 | 0.79 | 0.72 | 0.72 | 0.73 | 0.77 | 0.74 | 0.10 | 0.431 | 0.660 | 0.167 | 0.404 | 0.192 | 0.434 |
| Colon | 0.33 | 0.31 | 0.35 | 0.34 | 0.40 | 0.36 | 0.42 | 0.37 | 0.10 | **0.004** | 0.461 | 0.102 | 0.596 | 0.445 | 0.928 |
| Empty weight (g/100 g BW) | | | | | | | | | | | | | | | |
| Crop | 0.51 | 0.56 | 0.49 | 0.53 | 0.52 | 0.52 | 0.57 | 0.50 | 0.03 | 0.866 | 0.896 | 0.966 | 0.487 | 0.208 | 0.471 |
| Proventriculus | 0.36 | 0.34 | 0.37 | 0.36 | 0.33 | 0.32 | 0.34 | 0.32 | 0.01 | **0.041** | 0.492 | 0.124 | 0.841 | 0.865 | 0.953 |
| Gizzard | 1.36 | 1.39 | 1.88 | 1.61 | 1.31 | 1.40 | 1.56 | 1.42 | 0.08 | 0.101 | **0.006** | 0.379 | 0.177 | 0.572 | 0.115 |
| Duodenum | 0.65 | 0.61 | 0.68 | 0.59 | 0.53 | 0.51 | 0.58 | 0.42 | 0.04 | **0.004** | 0.922 | 0.055 | 0.738 | 0.768 | 0.210 |
| Jejunum | 1.01 | 1.07 | 1.18 | 1.14 | 0.89 | 0.94 | 0.99 | 0.91 | 0.05 | **0.003** | 0.141 | 0.912 | 0.411 | 0.775 | 0.254 |
| Ileum | 0.98 | 0.89 | 0.98 | 0.97 | 0.78 | 0.76 | 0.77 | 0.78 | 0.02 | **<0.001** | 0.315 | 0.242 | 0.409 | 0.323 | 0.259 |
| Caecum | 0.34 | 0.32 | 0.39 | 0.39 | 0.34 | 0.35 | 0.34 | 0.32 | 0.01 | 0.179 | 0.061 | 0.538 | **0.017** | 0.716 | 0.734 |
| Colon | 0.13 | 0.12 | 0.13 | 0.13 | 0.12 | 0.10 | 0.11 | 0.10 | 0.01 | **0.001** | 0.956 | 0.179 | 0.511 | 0.314 | 0.577 |

[1]Mean values are expressed as an average of 3 replicate pens. The T and relative humidity (RH) in the high-T (HT) room during the first week were 31.9±1.4°C and 70.2±11.3%, whereas the T and RH in the normal-T (NT) room were 32.0±0.8°C and 66.2±6.7%. For the NT, the T ranged from 19.6±0.9°C to 21.5±0.6°C and the RH ranged from 61.3±9.8% to 71.6±8.8% from wk 4 to 6. For the HT, the T ranged from 26.5±2.5°C to 31.7±2.5°C and the RH ranged from 58.2±10.9% to 84.9±10.8% from wk 4 to 6. Control diet= 215 g of CP/kg and 2,895 kcal of ME/kg; HE diet= 194.6 g of CP and 3,067 kcal of ME/kg. Dry diet vs. Wet diet (adding 1 part of water to 1 part of dry diet).

[2]T x F = interaction between T and F; T x C = interaction between T and C; F x C = interaction between F and C.

[3]Significant effects ($P < 0.05$) are printed in bold.

There was an interaction between diet formulation and diet conformation in lengths of jejunum and ileum at day 42. An interaction showed that the difference in relative length of jejunum and ileum between dry-control- and wet-control-fed birds were 0.16 (3.25 vs. 3.41, respectively) and 0.05 (3.39 vs. 3.34, respectively), whereas the difference between dry-HE- and wet-HE-fed birds were 0.31 (3.78 vs. 3.47, respectively) and 0.39 (3.81 vs. 3.40, respectively).

***Body Temperature and Heterophil/Lympocyte Ratio.*** Temperature had a major effect on body T and heterophil/lymphocyte (H/L) ratio at d 20 and 41. Body T and H/L ratio were higher at HT than at NT at these ages. Neither diet formulation nor diet conformation influenced on body T and H/L ratio. There was an interaction between T and diet conformation in H/L ratio at d 20. An interaction showed that at NT, the difference in H/L ratio between wet- and dry- fed-birds was 0.03 (0.16 vs. 0.19, respectively), whereas at HT, this difference was 0.08 (0.27 vs. 0.19, respectively) (Table 6).

## Discussion

***Eating and Panting Behavior.*** During the first three wk of age, the birds housed at HT on average needed about 3.4% more time to consume their feed than the birds housed under
NT. It seems that the birds housed at HT had a reduced meal size per unit of time than the birds at NT had because their feed intake was similar but they showed more panting activities during these weeks. Panting behavior may therefore induce a reduction in meal size by frequently interrupting eating behavior in heat-stressed birds (Chapter 3). During the last three wk of age, the time spent on eating by the birds at HT was about 2.9% less than birds at NT, which could be attributed to their lower average feed intake. The birds kept at HT showed 34.6% more time on panting behavior than the birds kept at NT (Figure 2). This is in close agreement with the study of McLean et al. (2002) who found a lower feed intake and a higher panting activity at high stocking density (40 kg m-2) than at low stocking density (28 kg m-2) because high stocking density was not confine when the birds grew older.

***Feed Intake and BW gain***. Broilers exposed to HT consumed about 11.8% less feed than those housed at NT during the 6 wk experimental period. The negative effect of HT on feed intake increased when the birds became older. The difference was -1.6% from wk 2 to 3 and 12.7% from wk 4 to 6. This corresponds with a 1.2% decrease in

**Table 6**. Effect of ambient temperature (T), diet treatment (F) and diet conformation (C) on body temperature and heterophyl/lymphocyte (H/L) ratio[1].

| Treatment[2] | | | Body temperature (°C) Day | | | Heterophil/lymphocyte ratio Day | | |
|---|---|---|---|---|---|---|---|---|
| T | F | C | 7 | 20 | 41 | 7 | 20 | 41 |
| NT | Control | Dry | 41.19 | 41.22 | 40.89 | 0.09 | 0.19 | 0.20 |
| | | Wet | 41.38 | 41.18 | 41.12 | 0.09 | 0.18 | 0.18 |
| | HE | Dry | 41.20 | 41.11 | 41.16 | 0.11 | 0.19 | 0.30 |
| | | Wet | 41.28 | 41.29 | 41.29 | 0.10 | 0.14 | 0.28 |
| HT | Control | Dry | 41.26 | 41.74 | 42.56 | 0.09 | 0.22 | 0.37 |
| | | Wet | 41.46 | 42.01 | 42.70 | 0.10 | 0.30 | 0.38 |
| | HE | Dry | 41.31 | 42.00 | 42.68 | 0.10 | 0.17 | 0.32 |
| | | Wet | 41.28 | 41.67 | 42.53 | 0.08 | 0.25 | 0.39 |
| | | SEM | 0.08 | 0.08 | 0.10 | 0.01 | 0.03 | 0.04 |
| Source of variation (*P* value)[3] | | | | | | | | |
| T | | | 0.294 | **<0.001** | **<0.001** | 0.662 | **0.023** | **<0.001** |
| F | | | 0.384 | 0.774 | 0.198 | 0.490 | 0.140 | 0.126 |
| C | | | 0.084 | 0.829 | 0.224 | 0.557 | 0.268 | 0.743 |
| T x F | | | 0.889 | 0.774 | 0.118 | 0.289 | 0.544 | 0.048 |
| T x C | | | 0.677 | 0.520 | 0.224 | 0.889 | **0.026** | 0.282 |
| F x C | | | 0.163 | 0.232 | 0.198 | 0.338 | 0.703 | 0.639 |

[1]The T and relative humidity (RH) in the high-T (HT) room during the first week were 31.9±1.4°C and 70.2±11.3%, whereas the T and RH in the normal-T (NT) room were 32.0±0.8°C and 66.2±6.7%. For the NT, the T ranged from 19.6±0.9°C to 21.5±0.6°C and the RH ranged from 61.3±9.8% to 71.6±8.8% from wk 4 to 6. For the HT, the T ranged from 26.5±2.5°C to 31.7±2.5°C and the RH ranged from 58.2±10.9% to 84.9±10.8% from wk 4 to 6. Control diet= 215 g of CP/kg and 2,895 kcal of ME/kg; HE diet= 194.6 g of CP and 3,067 kcal of ME/kg. C=Diet conformation: Dry diet; Wet diet (adding 1 part of water to 1 part of dry diet).
[2]T x F = interaction between T and F; T x C = interaction between T and C; F x C = interaction between F and C;
[3]Significant effects (*P* < 0.05) are printed in bold.

feed intake for each degree C increase within a T range from 21.5 to 31.7°C. This negative response is similar to an observation in an earlier study in this thesis (Chapter 3) with male broilers exposed to ambient T ranging from 21.0 to 31.5°C (a 1.7% decrease in feed intake per degree C). The reduction in feed intake at HT was due to the cumulative effect of heat stress and associated effects of a lowering BW gain. Overall, BW gain after 6 wk of age was about 19% lower at HT than at NT. Therefore, the effect of T was larger on BW gain than on feed intake, indicating a potential change in body composition of the birds at HT compared with the birds at NT.

Intake of the control-fed birds was, on average, 12.2% higher than the intake of HE-fed birds. The higher feed intake of control-fed birds is logic because broiler needs more energy supply with increasing age at NT (Lopez and Leeson, 2005) and at HT (Sakomura et al., 2005). This energy demand can be met by consuming more feed. The lowest intake with a HE diet is partly due to the higher energy content of this diet compared with a control diet. A higher energy concentration in the diet decreased the feed intake (NRC, 1984), but the difference did not fully disappear. Another explanation is possibly due to deficient levels of CP and amino acids in a HE diet. Aftab et al. (2006) reported that the concentration and balance of dietary amino acids could affect feed intake in broilers. Deficiencies or excesses of certain essential amino acid cause to decline in feed intake by influencing the area in the brain controlling feed intake (NRC, 1984). Metabolic pathways and metabolites produced by these pathways integrate to the regulation of feed intake and energy metabolism (Richards, 2003). The balance in the activity of anabolic and catabolic circuits within the hypothalamic melanocortin system determines the energy status and BW (Richards and Proszkowiec-Weglarz, 2007). BW gain of the control-fed birds was higher probably due to higher intake of protein and, as a consequence, a higher feed intake to meet the energy demands.

Several reports in literature concluded that there are benefits of using wet diets in the birds' life with regard to the enhancement of feed intake and BW gain (Yalda and Forbes, 1995, 1996; Yasar and Forbes, 1999; Preston et al., 2000; Khoa, 2007). These studies, however, show some variation in results due to differences in experimental conditions. Generally, feed intake of wet-fed birds was not significantly higher than that of dry-fed birds. The difference was 7.0% from wk 2 to 3 and 2.1% from wk 4 to 6, respectively. It seems that the advantage of feeding a wet diet on feed intake is larger for young birds than for older birds. High feed intake in wet-fed birds has been reported in studies at NT (Yalda and Forbes, 1995, 1996; Yasar and Forbes, 1999; Preston et al., 2000; Khoa, 2007) and HT (Awojobi and Meshioye, 2001; Kutlu, 2001; Awojobi et al., 2009). In the present experiment, this effect was not apparent. However, birds fed a wet-HE diet showed a significantly higher feed intake in wk 5 and 6 at HT. This result confirms partly the positive effect of feeding a wet diet at HT in broilers (Awojobi and Meshioye, 2001; Kutlu, 2001; Awojobi et al., 2009). The reason for the higher intake of wet-HE-fed birds at HT is probably that the heat load of the birds could be lowered as a consequence of extra water intake and therefore ability to cope at HT.

BW gain of birds kept at NT was 2.6% lower than that of birds kept at HT from wk 2 to 3, but was 25.7% higher than that of birds kept at HT from wk 4 to 6, respectively. This means than the effect of HT on BW gain becomes larger when the birds get older. These results were in line with the previous study (Chapter 3), and with Cheng et al. (1997) and May et al. (1998).

Overall, BW gain of HE-fed birds was 8.8% lower than that of control-fed birds at both T regimens. The lower BW gain of HE-fed birds was probably the cumulative lower feed intake of this diet. Furlan et al. (2004) reviewed that low protein diets have negative effects on broiler performance when the ambient T is high due to low feed intake and amino acid deficiency.

BW gains of wet-fed birds from wk 2 to 3 and from wk 4 to 6 were 8.9% and 4.7% higher than those of dry-fed birds, respectively. The effect of wet diet on BW gain seems better when the birds are young. Overall, BW gain of wet-fed birds in this study was 6% higher than that of dry-fed birds. Other studies reported much higher differences in BW gain between wet- and dry-fed birds (50% in Preston et al., 2000; 85% in Khoa, 2007). The smaller difference between dry- and wet-fed birds in the present study as compared with other reports may be associated with the lighting schedule of this experiment. Feed offered in this study was based on *ad libitum* meal feeding at 12L:12D during the entire trial whereas it was at 16L:8D in the other studies. As such there less time available for the birds to consume their diet as compared with the two other studies. Extra feed intake and BW gain can be expected with a wet diet although the birds had a limited time for eating and tended to reduce the time on eating the wet diet after 4 wk of age.

BW gains of wet-HE-fed birds were 12.0% and 11.1% higher compared with that of dry-HE-fed birds at NT and HT, respectively. Whereas BW gains of wet-control-fed birds were 3.3% higher and 1.7% lower compared with that of dry-control-fed birds at NT and HT, respectively. At HT, similar BW gain between wet-HE-fed birds and dry-control- or wet-control-fed birds was noted especially in wk 4 to 6 (Table 3). Therefore, it is beneficial to exploit a wet feeding at HT especially with a HE diet.

The higher BW gain of wet-fed birds in this study agrees with the earlier studies of broilers fed a wet mash by Yalda and Forbes (1995, 1996), Yasar and Forbes (1999), Awojobi and Meshioye (2001), Kutlu (2001), and Khoa (2007) and a wet pellet by Moritz et al. (2001). This effect may be explained by an improvement in feed digestibility (Forbes, 2003; Dei and Bumbie, 2011). Therefore, this study seems to support the hypothesis that an increased passage rate with wet diet through the GIT may have occurred.

***Feed Conversion Ratio.*** FCR at NT was on average 7.7% lower than at HT. On the HE-fed birds, it was on average 2.9% lower than that on control-fed birds. FCR of the wet-fed birds was on average 2.2% lower than that of dry-fed birds. Lower FCR at NT and wet-fed birds confirm results of other studies in broilers at different ambient T (Cheng et al., 1997) and in wet diet study under tropical T (Awojobi et al., 2009). These studies showed that broilers exposed to NT, HE- and wet-fed birds converted the diet into BW gain more efficiently than broilers exposed to HT and control- and dry-fed birds. Irrespective T, the efficiency of HE-fed birds can be increased by wetting the diet because it produced the lowest FCR and the same BW gain as compared with dry- and wet-control-fed birds.

***Water Intake and Water-to-Feed Ratio.*** Water intake rises as ambient T rises (North and Bell, 1990; NRC, 1994) as the birds seek to manage heat stress by increasing water consumption and then increasing urinary production (Borges et al., 2004). However, total water consumption in this study was similar for both T regimens. It may be that taking water from nipples at HT was a problem during panting (Chapter 3), although we followed the suggestion of May et al. (1997) to set the optimal height of the nipples at the height of the back of a bird.

Total water consumption in this study was higher in control-fed birds than in HE-fed birds. As a consequence, water-to-feed ratios in control-fed birds were higher than in HE-fed birds. Water intake was increased over time and water intake of control-fed birds was always higher than that of HE-fed birds after the second week onwards. The high water intake in control-fed birds was probably related to the higher feed intake and protein intake. It is clear from literature that there is a high positive correlation between feed and water consumption (Lott et al., 2003). Increasing the protein level in the diet increases water intake and the water-to-feed ratio (Marks and Pesti, 1984).

The higher water intake in wet-fed birds was derived from drinking water plus water contained in the wet diet consumed. These findings are in accordance with those of Yasar and Forbes (1999). Increasing water intake will benefit the birds because it is a source of evaporation (Belay and Teeter, 1993). This may limit the increase in body T at the same feed intake (Furlan et al., 2004; Ahmad et al., 2005). The results, from this study, confirm that the volume of water consumed by the birds is influenced by various factors of feed and environment (Manning et al., 2007). The higher water-to-feed ratio at HT may indicate that the birds will probably use the water to evaporate during panting (Figure 2).

***Gastrointestinal Tract Development.*** In the present study, temperature had a major effect on relative lengths and empty weight of GIT segments on day 42 only. By recalculating on the result reported by Gariga et al. (2006) also showed that relative length of jejunum in birds housed to NT (20$^{o}$C) was 9% shorter than that in birds housed at HT (30$^{o}$C).

Relative lengths of most GIT segments were affected by diet formulation at day 21 and 42, but not relative empty weight. Control-fed birds had shortened relative lengths than HE-fed birds, suggesting that the higher BW gain of control-fed birds (vs. HE-fed) were not accompanied by a similar increase in length.

At d 42, proventriculus, duodenum, jejunum, ileum and colon empty weights for birds kept at HT were lower than at NT. Gizzard empty weight of control-fed birds was 15.6% lighter compared with that of HE-fed birds. Wetting the diet did not increase empty weight of intestines both at d 21 and d 42. These results agree with the observation in wet-fed broilers during the wet season under tropical climate that weight of gizzard, intestine and the proventriculus were similar in size compared with dry-fed birds (Awojobi et al., 2009). These discrepancies show that differences in T and diet formulation in this study were the most crucial factors determining intestinal development rather than diet conformation.

***Body Temperature and Heterophil/Lymphocyte Ratio***. A high T resulted in a significant increase in body T. The association between high ambient T and increased body T agrees with other studies (Yahav, 2000; De Basilio et al., 2003). The effect of heat stress was more pronounced in broilers with a high growth potential than in slow-growing broilers (Ahmad and Sarwar, 2006). Comparing the results of feed intake and BW gain showed that feed intake and BW gain were significantly lower, while body T was significantly higher at HT. When broilers are exposed to a heat stress environment, there is a strong negative correlation between body T and traits of economic importance (Tao et al., 2006).

A high T also resulted in a significant increase in H/L ratio at d 20 and d 41. H/L ratio is often used as a stress indicator, and its ratio was positively correlated with ambient T and body T and negatively correlated with BW gain (Puvadolpirod and Thaxton, 2000a; Borges et al., 2004; Al-Murrani et al., 2006). The results suggest that wetting the diet was not able to reduce the heat stress of the birds due to detrimental effects of HT when they got older.

## Conclusion

It can be concluded that a wet diet has potential positive effects to improve feed intake and BW gain at both NT and HT. Water addition to a HE diet has a more pronounced effect to improve growth at NT and HT, although it cannot fully reduce the adverse effect of T on heat stress and an improve GIT development.

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# CHAPTER 6

# Effects of Wet and/or High Energy to Protein Ratio Diets on Performance and Gastrointestinal Tract Development of Indigenous and Broiler Chickens Reared under Tropical Conditions in Jambi Province, Indonesia

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## Abstract

The effects of feeding a wet diet and a high dietary energy to protein ratio (HE) on performance of slow-growing indigenous chickens (Experiment 1) and fast-growing broilers (Experiment 2) under tropical climatic conditions were evaluated in this study. One-day old unsexed indigenous (n=144) and male Ross 308 broilers (n=205) were randomly allocated to 12 and 16 pens, respectively. Two dietary energy to protein ratios (normal versus HE mash diet) were tested in wet or dry form. The wet diet was made by adding one part of water to one part of dry diet. Temperature and relative humidity (RH) were not pre-set and just followed climatic conditions in Jambi Province, Indonesia. In Experiments 1, the temperature and RH varied between 24.9-32.9$^{o}$C and 54-89% while in Experiment 2 it ranged from 22.8-35.4$^{o}$C and 58-94%, respectively. The light followed the natural day light (12L:12D) except for the first 3 days when a 23L:1D scheme was employed. Results showed that fast-growing broilers spent 1% more time on eating and 22% more time on panting than Kampung chickens. In Experiment 1, feed intake (in g/d) was numerically lower in wet-control- and wet-HE-fed birds (0.6% and 5.3%, respectively) as compared with the same diets in dry form. BW gain per d was numerically higher in wet-control (6.3%) and lower in wet-HE fed birds (0.8%) compared to the respective controls. FCR was not significantly improved in wet-control- and in wet-HE-fed birds. In Experiment 2, feed intake (in g/d) was 19.9 and 24.0% higher in wet-control- and wet-HE-fed birds compared to the respective dry diet. BW gain (in g/d) was 20.2 and 18.2% higher in wet-control- and in wet-HE-fed birds compared to the respective controls. FCR was not significantly improved in wet-control- and wet-HE- fed birds. The gastrointestinal tract of Kampung chickens grew proportionally with body weight but this was not the case for broilers.


**Key words**: tropical climate, wet diet, performance, gastrointestinal tract, chicken

## Introduction

Poultry shows a reduced feed intake under heat stress conditions. This may lead to a lower intake of energy and some essential nutrients, and is the main reason for a reduction in growth under hot environmental conditions. Attempts have been made to ensure sufficient feed intake under heat stress conditions (Lin et al., 2006; Borges et al., 2007). It is assumed that this can be achieved with a feeding strategy where birds produce a smaller amount of heat per unit of feed consumed. Another way is to facilitate the emission of the heat produced from the body of the bird. Dietary manipulation by adding water to the feed is well known to have a positive effects on birds at normal temperature (Yalda and Forbes, 1995, 1996; Yasar and Forbes, 1999; Preston et al., 2000; Moritz et al., 2001; Scott, 2002; Khoa, 2007). The advantage of wet feeding can not only be found for layers but also broilers under tropical conditions. Wet diets have been proven to increase feed intake, live weight, feed efficiency, weights of heart, weight of the crop, and abdominal fat deposition (Awojobi and Meshioye, 2001). These effects are noted in experiments by either giving the birds access to drinking water or without drinking water but providing a wet feeding (Awojobi et al., 2009).

Under normal temperature (NT), feed intake and water intake of birds fed a wet diet are increased (Yasar and Forbes, 1999, 2000). The increased feed dry matter intake with a wet diet is probably due to the moisture of feed particles in the crop that provides a quicker passage rate from the crop into the proventriculus (Yasar and Forbes, 2000). Moreover, it could be due to the slowdown in increasing body temperature at the same feed dry matter intake when a wet diet is fed. During the hottest period of the day, feed dry matter intake may be sufficiently high, especially for an animal with a high metabolic rate, when the body temperature can be maintained within a normal range.

A well-developed and healthy gastrointestinal tract (GIT) is important for proper growth of chickens. The development and physiology of the avian digestive tract is known to be influenced by dietary particle size and feed form (Nir and Ptichi, 2001; Engberg et al., 2002). Many studies have shown that coarseness of a broiler diet is related to a heavy gizzard (Hetland and Svihus, 2001; Nir and Ptichi, 2001; Engberg et al., 2002; Erener et al., 2003; Hetland et al., 2004; Khoa, 2007). The gizzard is the food mechanical processing organ in avian species.

It has been shown that water excretion in excreta was reduced when the proportion of coarse particles in the diet increased (Carré et al., 2002) and that there is a decreased transit time of chyme through the GIT (Carré et al., 2002). This prolonged time in the GIT may allow more water to be reabsorbed compared with fine particle diets and less water in excreta. In addition, birds can use more of the consumed water for evaporative purposes and as such the decreased transit time may reduce peak heat production after feeding.

Most of the studies investigating wet-feeding of poultry have been conducted at controlled ambient temperatures using mostly fast-growing broilers. Indigenous chickens (e.g. Kampung chickens, Indonesia) differ in relative feed intake and growth rate compared to fast-growing broilers. Little research however has been conducted comparing the influence of wet feeding of indigenous and broiler chickens under tropical climate conditions. In a previous experiment, it was shown that birds under hot climatic conditions preferred a diet containing a decreased CP intake (Syafwan et al., in press). Whether the effects of wet and dry diets for the different breeds remain the same when dietary compositions change is unknown. The present study investigated the impact of a control diet and a high energy to protein ratio diet both in wet and dry form (with similar particle sizes) on the performance of slow-growing indigenous Indonesian chickens and fast-growing broilers under tropical conditions in Muaro Jambi District, Jambi Province of Indonesia.

## Materials and Methods

### *Bird, Housing and Care*

All procedures and protocols were approved by the ethical committee of Jambi University. In Experiment 1 (April to July, 2010), 144 one-day old indigenous unsexed chickens were purchased from a local hatchery in Jambi city, Indonesia. In Experiment 2 (July to August, 2010), 205 one-day old male broilers (Ross 308) were purchased from PT Vista Agung Kencana, South Sumatera Province, Indonesia. Each bird was weighed and wing-tagged for identification. All chickens in Exp. 1 and 2 were randomly allocated to 12 and 16 pens with 12 and 12-13 birds per pen, respectively. Each bird was coloured using a permanent marker on the head for identify during the behavior observations. Birds were housed in a floor system in an open sided house under tropical climatic conditions. There was one bell shaped drinker with a 3.3 L capacity and one feeding trough in each pen. The birds in each pen were provided their diet ad libitum as dry or wet. Wood shavings (Exp. 1) and rice husks (Exp. 2) were used as litter with new bedding material regularly added to each pen. The pens were made of wire with pen dimensions of 1.75 x 1.2 x 2.0 m (l x w x h) in Exp. 1 and 1.75 x 1.2 x 1.0 m in Exp. 2.

Two wooden slat perches measuring 5 cm x 5 cm x 50 cm (w x h x l) with rounded angles were provided in each pen. The slat was fixed on two 5 cm high structures and placed about 10 cm above the floor.

Temperature (T) and relative humidity (RH) were recorded every day using a minimum-maximum thermo-hygrometer in Exp. 1 and a wireless weather centre (La Crosse Technology, type WS2-550) in Exp. 2. During the first week of age, the house was covered with black plastic hanging at half-height of the wall during the entire day to ensure an appropriate T at chicken level. The week thereafter, it was only covered during the night (from 07:00 to 19:00 h). Temperature inside each pen from 1 to 7 d of age was increased by a 75 Watt bulb which was positioned under a bluish white metal with Chinese shaped-head. After d 7, T followed the outside temperature. Artificial light was provided as to have 23-h light (L) and 1-h dark (D) per day for the first three days whereafter the lighting schedule followed the natural day light (12L:12D). The dark hours were created by covering the bulb with a tin which was hung closely to the Chinese shaped-head.

### *Experimental Design and Treatments*

Experiment 1 lasted 12 weeks and Exp. 2 lasted 6 weeks. Each of the two experiments was conducted as a 2x2 factorial arrangement in a completely randomized design. The factors were two dietary formulations (control diet and a high energy to protein ratio diet; HE) and two conformations (dry and wet). Each dietary treatment combination was assigned to three (Exp. 1) and four (Exp. 2) replicated pens.

The control diet contained the NRC (1994) recommended nutrient levels for CP and metabolizable energy (ME) contents of broiler chickens for the entire growing period in both experiments. The control diet contained 215.0 g of CP/kg and 2,942 kcal of ME/kg. In Exp. 1, the HE diet contained 181.0 g of CP/kg and 3,179 kcal of ME/kg. In Exp. 2, the HE diet contained 195.0 g of CP/kg and 3,063 kcal of ME/kg. The protein sources were soybean meal and fish meal. The energy sources were rice bran, maize, palm kernel meal and vegetable oil. Dietary compositions of the experimental diets are presented in Table 1. Each diet was supplied as mash. The wet diet was made by adding one part of water to one part of dry feed. The preparation and mixing of the diets was done outside the experimental shed to prevent any disturbance to the chickens. The wet diet was offered and weighed at 07:00 and at 12:00 h every day. Before each meal time, all feeders were taken out of the pens, weighed, and new filled feeders were placed back.

**Table 1**. The ingredients (g/kg) and calculated nutrient composition of the Kampung chickens dietary treatments.

| Ingredients | Control diet | HE diet Experiment 1 | HE diet Experiment 2 |
|---|---|---|---|
| Rice bran | 350.0 | 300.0 | 284.0 |
| Maize | 246.7 | 290.0 | 290.0 |
| Soybean meal | 240.0 | 160.0 | 165.0 |
| Palm kernel meal | 80.0 | 112.0 | 143.0 |
| Fish meal | 50.0 | 50.0 | 65.0 |
| Vegetable oil | 0.0 | 55.0 | 20.0 |
| Limestone | 14.0 | 14.0 | 14.0 |
| Salt | 2.3 | 2.3 | 2.3 |
| Top Mix[1] | 5.0 | 5.0 | 5.0 |
| Dicalcium phosphate | 8.0 | 8.0 | 8.0 |
| DL-Methionine | 2.7 | 2.7 | 2.7 |
| L-lysine HCl | 1.3 | 1.0 | 1.0 |
| Total | 1000.0 | 1000.0 | 1000.0 |
| Calculated nutrient content (/kg) | | | |
| Metabolisable energy (kcal) | 2942.0 | 3178.7 | 3063.2 |
| Crude protein (g) | 21.5 | 18.1 | 19.5 |

[1]Composition of 1 kg Top Mix: vitamin A (retinyl acetate), 12,000 IU; vitamin $D_3$ (cholecalciferol), 2,000 IU; vitamin E (dl-α-tocopherol), 8.0 mg; vitamin K, 2.0 mg; vitamin $B_1$ (thiamin), 2.0 mg; vitamin $B_2$ (riboflavin), 5.0 mg; vitamin $B_6$ (pyridoxine-HCl), 0.5 mg, vitamin $B_{12}$ (cyanocobalamin), 12 mg; vitamin C, 25 mg; niacin, 40 mg; vitamin $B_5$ (d-pantothenic acid), 6.0 mg; choline chloride, 10 mg; methionine, 30 mg; lysine, 30 mg; iron, 20 mg; copper, 4 mg; manganese, 120 mg; zinc, 100 mg; cobalt, 0.2 mg; iodine, 0.2; zinc bacitracin, 21 mg and santoquin (antioxidant), 10 mg.

A coarse diet structure was chosen because highly processed fine diets may not be optimal for the functionality of the gastrointestinal tract. Particle size distribution was measured by sieve analysis (Goelema, 1999), and the results are shown in Figure 1. The proportion of particles that could not pass a 1.4 mm sieve was about 30% in both experiments.

***Measurements***

Eating and panting behavior were measured as describe by Syafwan et al. (in press). In brief, an experienced observer walked during a 5 to 7 min period through the room and applied scan sampling. Each bird in each pen was observed instantaneously for a single moment during this scan (instantaneous scan sampling). This procedure was repeated with 25 min pauses several times between 09:00 to 12:00 h and between 14:00 to 17:00 h to ensure a representative scanning for a 12 h daylight period. Scan sampling was done two days per wk with in total 12 scan samplings done per observation day. Based on these observations, the percentage of time the chickens in a certain pen spent on eating and panting behaviors relative to other behaviors were determined. Eating was defined as a chicken having its head in or above the feeder, and panting was defined as a chicken breathing rapidly with short gasps with open beak and split wing feather alignment. All scan data per pen per wk were pooled.

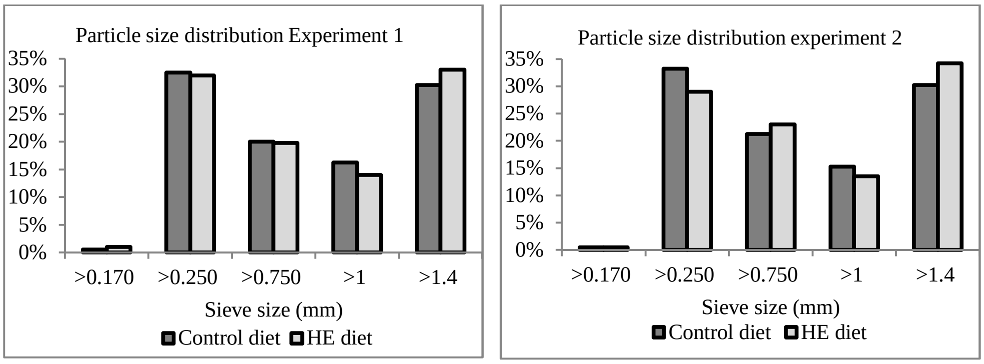



**Figure 1**. Particle size distribution of dietary treatments in experiments 1 and 2.

For measuring feed intake, the offered quantity of feed was corrected for the weight of the feed residues in the feeder. Feed intake of the wet diet was calculated on the basis of the quantity of dry feed offered to the birds. The real 'dry' intake of the wet diet was corrected for water loss due to evaporation which was estimated by placing an extra feed trough with a wet diet outside the pen each time the feed was refreshed and weight loss was measured. Weighing the dry feed was done every three to four days. We did not correct for loses of feed from the feeder.

Fresh water intake was recorded in each pen by subtracting the remaining water from offered water. Total water intake of wet-fed birds was calculated from fresh water intake plus the water content of the wet diet consumed. BW was determined per pen and expressed as BW gain per bird/wk. Weights of birds that died or had to be culled were determined, and their BW gain included in the calculation of the feed conversion ratio (FCR). Feed intake, BW gain, water intake, water-to-feed ratio and FCR were calculated per bird for a 14-d period in Exp. 1 and per bird for a 7-d period in Exp. 2. Body T was measured from three birds in each pen using a digital thermometer just before weighing the birds and body T data per pen were averaged.

GIT development was measured by dissecting two birds per pen per treatment at d 28, 56 and 84 in Exp. 1 and at d 22 and 42 in Exp. 2. Each bird was weighted, euthanized by cervical dislocation and dissected to separate the GIT from the body. A flexible tape was used to determine the lengths of each intestinal segment on a flat surface. The length of the duodenum (from the pyloric junction to the distal most point of insertion of the duodenal mesentery), the jejunum (from the distal most point of insertion of the duodenal mesentery to the junction with Meckel's diverticulum), the ileum (from junction with Meckel's diverticulum to ileo-caecal junction) and the sum of the lengths from the ostium to the tip of caeca and colon were determined. The full and empty weight (±0.1 g) of the crop, proventriculus and gizzard were determined. Data of length and empty weight of GIT segments were expressed per 100 g BW with data of the two birds per pen averaged.

### *Statistical Analysis*

Pen was considered as the experimental unit. A mixed model was used to account for the covariance structure among repeated observations (Littell et al., 1998; Wang and Goonewardene, 2004). In the analysis, the repeated statement PROC MIXED in SAS (version 9.1; SAS Inst. Inc., Cary, NC) was used adding the factor time ("p" as the time factor) and pen was considered as an additional random effect. The following statistical model was used for both experiments:

$$Y_{ijkl} = \mu + F_i + C_j + F_i \times C_j + P_k + F_i \times P_k + C_j \times P_k + F_i \times C_j \times P_k + e_{ijkl}$$

where $Y_{ijkl}$ is the dependent variable, μ is the overall mean effect, $F_i$ is the *i*th fixed diet formulation effect (*i*=1 for control diet or *i*=2 for HE diet), $C_j$ is the *j*th fixed diet conformation effect (*j*=1 for dry or *j*=2 for wet), $P_k$ is the *k*th random period of measurement (*k*=1..6), $F_i \times C_j$ is the fixed interaction effect between diet formulation and diet conformation, $F_i \times P_k$ is the random interaction effect between diet formulation and period, $C_j$ x $P_k$ is the random interaction effect between diet conformation and period, $F_i$ x $C_j$ x $P_k$ is the random interaction effect between diet formulation, diet conformation and period and $e_{ijkl}$ is a random error associated with the *i*th diet formulation and *j*th diet conformation at period *k*, $e_{ijkl} \sim$ NID (0, $\sigma^2_e$).

Differences were considered significant at a probability level of $P < 0.05$. If significant main effects or their interactions were detected, means were compared using least squares means comparison. Mean separation ($P < 0.05$) was produced with PDMIX800 in SAS, which is a macro for converting mean separation output to letter groupings (Saxton, 1998). The Kenward-Roger method was used for computing the denominator degrees of freedom for the tests of main effects.

The best covariance structure was based on the corrected Akaike Information Criteria (AICC). In Exp. 1, the heterogenous autoregressive covariance structure [ARH(1)] fitted the data best for feed intake, BW gain, water intake and FCR. The autoregressive [AR(1)] covariance structure fitted the data best for water-to-feed ratio. In Exp. 2, the first-order ante-dependence covariance structure [ANTE (1)] fitted the data best for all performance data.

GIT development and body T were analyzed by PROC MIXED in SAS with the following linear model:

$$Yijk = \mu + Fi + Cj + Fi \times Cj + eijk$$

Where $Y_{ijk}$ is dependent variable, $\mu$ is overall mean effect, $F_i$ is the *i*th fixed diet formulation effect (*i*=1 for control diet or *i*=2 for HE diet), $C_j$ is *j*th fixed diet conformation effect (*j*=1 for dry or *j*=2 for wet), $F_i$ x $C_j$ is the fixed interaction effect between diet formulation and diet conformation, and $e_{ijk}$ is the residual error, $e_{ijk} \sim$ NID (0, $\sigma^2_e$).

Behavior data were analysed with replicated observations per pen. Eating and panting behaviors were analysed with the Kruskal-Wallis test and post hoc with the Wilcoxon two sample test by non-linear procedure of SAS.

## Results

***Temperature and Relative Humidity.*** The average T and RH inside the house during Exp. 1 (12 weeks duration) ranged from 24.9 to 32.9$^o$C and 54 to 89%, respectively. The average T and RH during Exp. 2 (6 weeks duration) ranged from 22.8

to 35.4°C and 58 to 94%, respectively. During both experiments (April to July, 2010 for Exp. 1 and July to August, 2010 for Exp. 2), the season in Muaro Jambi Regency, Jambi Province of Indonesia was a dry season with normal rainfall (MCGA, 2010).

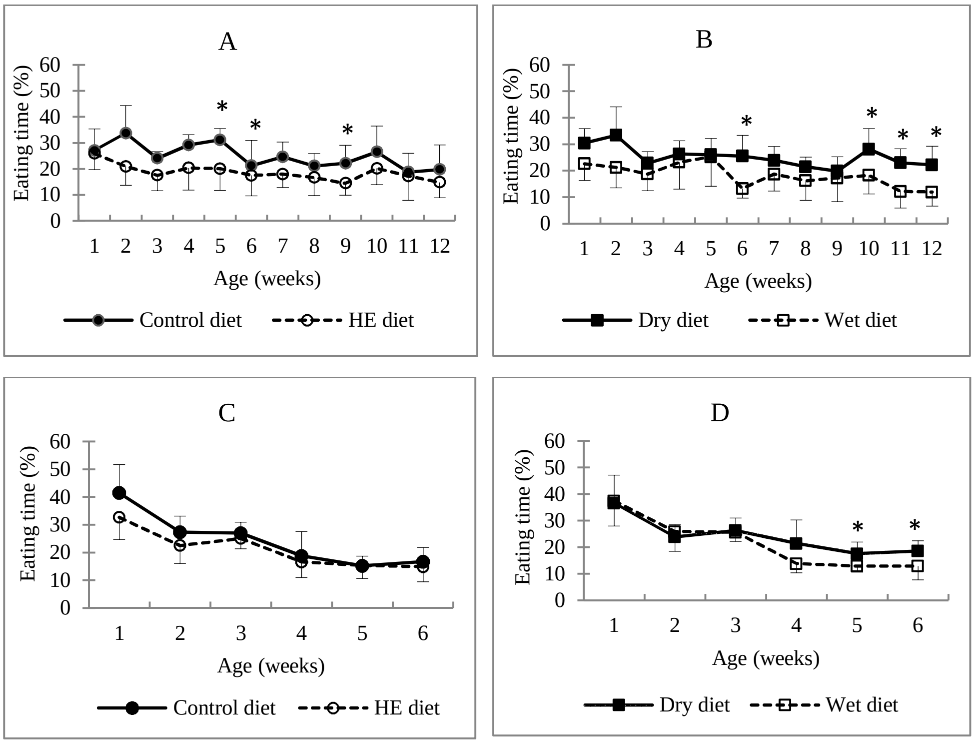



**Figure 2**. Eating time (expressed as a percentage within observation time in Experiment 1; panel A and B, and in Experiment 2; panel C and D on different diet formulations and different diet conformations. Vertical bars represent SD. Values with an asterisk (*) are significant difference ($P < 0.05$). Control diet in Experiments 1 and 2 = 215.0 g of CP/kg and 2,942 kcal of ME/kg. HE diet in experiments 1 = 181.0 g of CP/kg and 3,178.7 kcal of ME/kg and HE diet in Experiment 2 = 195.0 g of CP/kg and 3,063.2 kcal of ME/kg. Conformation = Dry diet; Wet diet (adding 1 part of water to 1 part of dry diet).

***Eating and Panting Behavior.*** Results on the percentage time spent on eating and panting behavior are given in Figures 2 and 3. Eating time decreased from wk 1 to 12 for the Kampung chickens. A decrease was also observed for the broiler chickens but the decrease was more pronounced from wk 1 to 6. In Exp. 1, the control-fed birds spent more time on eating than the HE-fed birds in weeks 5, 6 and 9 (Figure 2 panel

A). Dry-fed birds spent more time on eating than wet-fed birds in weeks 6, 10, 11 and 12 (Figure 2 panel B). In Exp. 2, time spent on eating was similar between diet formulations (Figure 2, panel C). Dry-fed birds spent more time on eating than wet-fed birds in weeks 5 and 6 (Figure 2, panel D).

In Kampung chickens (Exp. 1), panting behavior was not significantly affected by diet formulation and diet conformation (Figure 3, panel A and B). In broilers (Exp. 2), time spent on panting increased with age in both diet formulations and diet conformations. Control-fed birds spent more time on panting than HE-fed birds in weeks 1, 3 and 5 (Figure 3, panel C). Wet-fed birds spent more time on panting than dry-fed birds in weeks 5 and 6 (Figure 3, panel D).

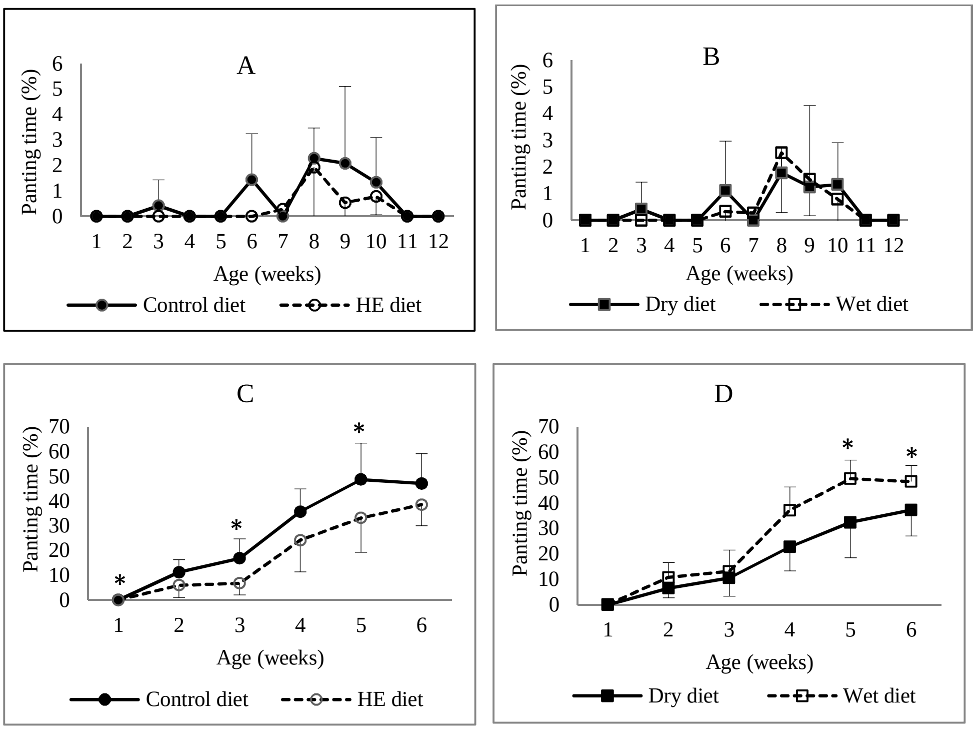



**Figure 3**. Panting behavior (expressed as a percentage within observation time in Experiment 1; panel A and B, and in Experiment 2; panel C and D on different diet formulations and different diet conformations. Vertical bars represent SD. Values with an asterisk (*) are significantly difference ( $P < 0.05$). Control diet in Experiments 1 and 2 = 215.0 g of CP/kg and 2,942 kcal of ME/kg. HE diet in Experiments 1 = 181.0 g of CP/kg and 3,178.7 kcal of ME/kg) and HE diet in Experiment 2 = 195.0 g of CP/kg and 3,063.2 kcal of ME/kg).

***Bird Performance.*** Mortality was 7.6% in Exp. 1 and 8.3% in Exp. 2. Probability values for all performance parameters are presented in Table 2. Differences in performance of the birds in each period at different diet formulations and diet conformations are presented in Table 3a (Exp. 1) and Table 3b (Exp. 2). The effect of period was significant for all parameters and is discussed only in relation to the interaction with other factors.

**Table 2**. Probability values of main effects and interaction between diet formulation (F), diet conformation (C) and period (P) for different traits (Exp. 1 and 2)[1].

| Parameters | Source of variance[2] | | | | | | |
|---|---|---|---|---|---|---|---|
| | F | C | FxC | P | FxP | CxP | FxCx P[3] |
| Experiment 1 | | | | | | | |
| Feed intake (g/bird/period) | **<0.001** | 0.396 | 0.516 | **<0.001** | **<0.001** | 0.299 | 0.914 |
| BW gain (g/bird/period) | 0.108 | 0.611 | 0.518 | **<0.001** | 0.361 | 0.150 | 0.794 |
| Water intake (g/bird/period) | **0.007** | 0.335 | 0.115 | **<0.001** | **0.001** | **0.004** | 0.273 |
| Water-to-feed ratio (g/g) | 0.248 | **<0.001** | **0.048** | **<0.001** | **0.005** | **<0.001** | 0.916 |
| FCR (g/g) | 0.221 | 0.094 | 0.658 | **<0.001** | **0.008** | **0.014** | 0.338 |
| Experiment 2 | | | | | | | |
| Feed intake (g/bird/period) | **<0.001** | **<0.001** | 0.948 | **<0.001** | **0.002** | **<0.001** | 0.195 |
| BW gain (g/bird/period) | **<0.001** | **<0.001** | 0.372 | **<0.001** | **<0.001** | **<0.001** | 0.141 |
| Water intake (g/bird/period) | **<0.001** | **<0.001** | 0.819 | **<0.001** | **0.001** | **<0.001** | 0.523 |
| Water-to-feed ratio (g/g) | **0.009** | 0.480 | 0.784 | **<0.001** | 0.094 | **0.024** | 0.159 |
| FCR (g/g) | **<0.001** | 0.979 | 0.126 | **<0.001** | 0.225 | **0.045** | 0.597 |

[1]Control diet in experiments 1 and 2 = 215.0 g of CP/kg and 2,942 kcal of ME/kg. HE diet in Experiment 1 = 181.0 g of CP/kg and 3,178.7 kcal of ME/kg and HE diet in Experiment 2 = 195.0 g of CP/kg and 3,063.2 kcal of ME/kg. Conformation = Dry diet vs. Wet diet (adding 1 part of water to 1 part of dry diet). Period: six 14-d periods in Experiment 1 and six 7-d periods in Experiment 2.
[2]Significant effects ($P$ < 0.05) are printed in bold.
[3]F x C = interaction between F and C, C x P = interaction between C and P; F x C x P = interaction between F, C and P.

*Feed Intake.* Diet formulation had a major effect on feed intake in both experiments, but diet conformation only affected feed intake in Exp. 2 (Table 2). On average, feed intake was 16.2% higher in control-fed birds (449.9 g/bird) than in HE-fed birds (387.3 g/bird) in Exp. 1, whereas it was 16.5% higher in control-fed birds (540.5 g/bird) than in HE-fed birds (464.0 g/bird) in Exp. 2. In Exp. 1, feed intake of wet-control- and wet-HE-fed birds were similar to that of dry-control-fed and dry-HE-

**Table 3a**. Least square means of performance parameters in Kampung chickens from 1 to 6 periods as affected by diet formulation (F) and diet conformation (C) (Experiment 1)[1].

| Parameters | F | C | Period | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 |
| Feed intake (g/bird/period) | | | | | | | | |
| | Control | Dry | $118.3^{a}$ | $254.7^{a}$ | $387.5^{a}$ | $499.2^{ab}$ | 636.4 | 812.1 |
| | | Wet | $96.8^{b}$ | $232.2^{ab}$ | $394.8^{a}$ | $529.7^{a}$ | 615.7 | 821.5 |
| | HE | Dry | $116.0^{a}$ | $212.1^{bc}$ | $300.4^{b}$ | $461.2^{ab}$ | 575.9 | 723.5 |
| | | Wet | $92.1^{b}$ | $192.5^{c}$ | $301.9^{b}$ | $456.6^{b}$ | 534.3 | 677.7 |
| | SEM | | 3.9 | 7.3 | 15.5 | 21.0 | 36.6; 44.8 | 41.0; 50.2 |
| Body weight gain (g/bird/period) | | | | | | | | |
| | Control | Dry | $50.0^{a}$ | $83.8^{ab}$ | $137.6^{a}$ | 163.2 | 158.4 | 208.3 |
| | | Wet | $50.5^{a}$ | $94.0^{a}$ | $137.6^{a}$ | 184.8 | 152.1 | 227.5 |
| | HE | Dry | $42.8^{b}$ | $67.0^{b}$ | $121.6^{ab}$ | 159.9 | 170.9 | 198.1 |
| | | Wet | $42.6^{b}$ | $71.2^{b}$ | $108.3^{b}$ | 155.2 | 133.5 | 243.7 |
| | SEM | | 0.9 | 5.4 | 8.7 | 12.6 | 21.6; 26.5 | 13.7; 16.8 |
| Water intake (g/bird/period) | | | | | | | | |
| | Control | Dry | $253.8^{bc}$ | $452.3^{b}$ | $798.6^{ab}$ | $1153.1^{ab}$ | 1537.5 | 1923.3 |
| | | Wet | $325.2^{a}$ | $621.2^{a}$ | $857.7^{a}$ | $1306.3^{a}$ | 1818.3 | 2069.1 |
| | HE | Dry | $226.3^{c}$ | $365.9^{c}$ | $776.6^{ab}$ | $1106.2^{ab}$ | 1404.5 | 1742.4 |
| | | Wet | $281.0^{ab}$ | $427.4^{b}$ | $684.5^{b}$ | $1002.6^{b}$ | 1425.0 | 1623.8 |
| | SEM | | 15.1 | 14.2 | 38.6 | 72.8 | 150.0; 183.7 | 167.6; 205.3 |
| Water to feed ratio (g/g) | | | | | | | | |
| | Control | Dry | $2.15^{b}$ | $1.78^{c}$ | $2.06^{b}$ | 2.31 | $2.42^{b}$ | 2.37 |
| | | Wet | $3.36^{a}$ | $2.68^{a}$ | $2.17^{b}$ | 2.46 | $2.94^{a}$ | 2.52 |
| | HE | Dry | $1.96^{b}$ | $1.73^{c}$ | $2.58^{a}$ | 2.39 | $2.43^{b}$ | 2.39 |
| | | Wet | $3.06^{a}$ | $2.23^{b}$ | $2.28^{ab}$ | 2.20 | $2.65^{ab}$ | 2.38 |
| | SEM | | 0.12 | 0.12 | 0.12 | 0.12 | 0.12; 0.15 | 0.12; 0.15 |
| Feed conversion ratio (g/g) | | | | | | | | |
| | Control | Dry | $2.37^{ab}$ | $3.05^{a}$ | 2.83 | 3.14 | 4.01 | $3.90^{a}$ |
| | | Wet | $1.92^{c}$ | $2.48^{b}$ | 2.87 | 2.87 | 4.34 | $3.60^{a}$ |
| | HE | Dry | $2.71^{a}$ | $3.19^{a}$ | 2.49 | 2.88 | 3.42 | $3.68^{a}$ |
| | | Wet | $2.17^{bc}$ | $2.74^{ab}$ | 2.82 | 2.94 | 4.16 | $2.79^{b}$ |
| | SEM | | 0.10 | 0.14 | 0.16 | 0.15 | 0.44; 0.55 | 0.13; 0.16 |

Means within a period (column) between treatments without a common superscript letters (a-c) differ significantly ($P < 0.05$); no superscript letter means non significant differences.

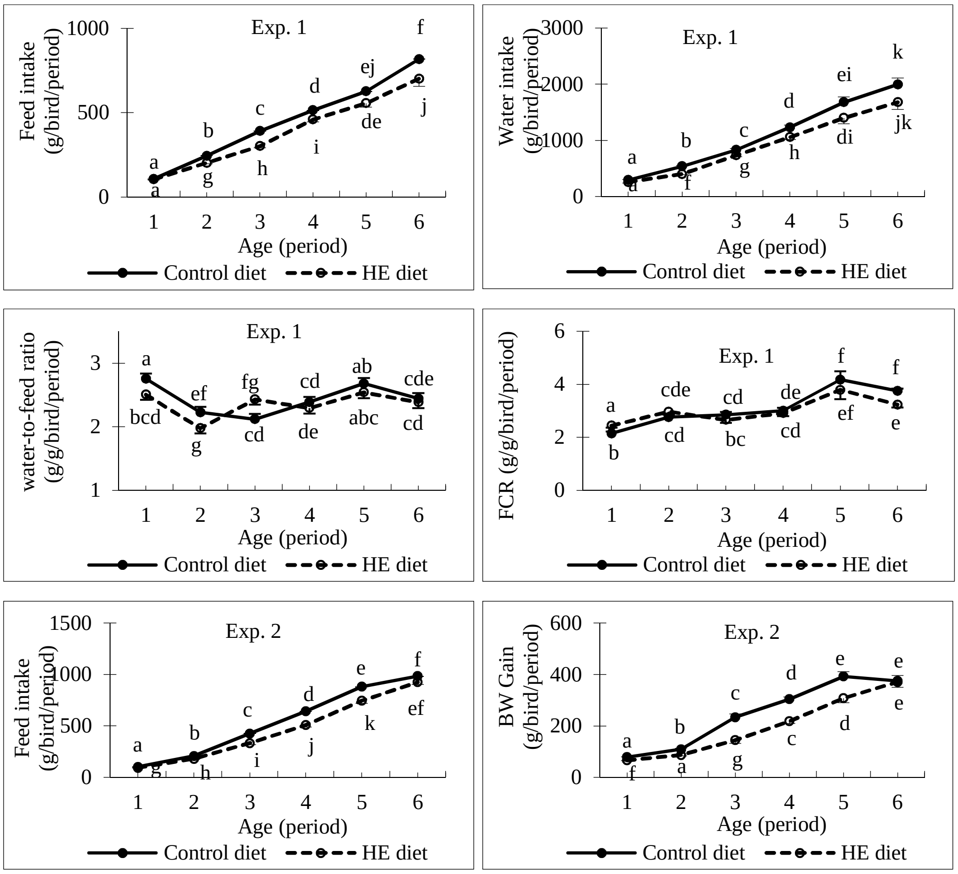
[1]Control diet in Experiment 1 = 215.0 g of CP/kg and 2,942 kcal of ME/kg. HE diet in Experiment 1 = 181.0 g of CP/kg and 3,178.7 kcal of ME/kg. Conformation = Dry diet; Wet diet (adding 1 part of water to 1 part of dry diet). Period: six 14-d periods.

**Table 3b**. Least square means of performance parameters in broiler chicken from 1 to 6 periods as affected by diet formulation (F) and diet conformation (C) (Experiment 2)[1].

| Parameters | F | C | Period | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 |
| Feed intake (g/bird per period) | | | | | | | | |
| | Control | Dry | 96.3[ab] | 213.8[a] | 402.2[b] | 561.7[b] | 778.9[b] | 896.6[b] |
| | | Wet | 105.6[a] | 202.6[ab] | 449.9[a] | 723.2[a] | 983.7[a] | 1070.9[a] |
| | HE | Dry | 91.2[b] | 176.5[c] | 286.6[c] | 446.5[c] | 627.5[c] | 856.1[c] |
| | | Wet | 94.5[b] | 183.4[bc] | 377.9[b] | 570.7[b] | 862.6[ab] | 994.4[ab] |
| | SEM | | 3.3 | 6.3 | 22.8 | 28.5 | 39.2 | 28.6 |
| BW gain (g/bird per period) | | | | | | | | |
| | Control | Dry | 76.3[a] | 118.1[a] | 210.1[a] | 252.3[b] | 331.6[b] | 337.3 |
| | | Wet | 81.3[a] | 100.8[ab] | 258.1[a] | 355.9[a] | 453.3[a] | 413.3 |
| | HE | Dry | 67.3[b] | 85.6[b] | 137.2[b] | 188.1[c] | 250.5[c] | 349.0 |
| | | Wet | 65.7[b] | 89.0[b] | 152.7[b] | 249.0[b] | 365.6[b] | 392.7 |
| | SEM | | 2.1 | 5.7 | 17.5 | 13.1 | 25.8 | 28.7 |
| Water intake (g/bird per period) | | | | | | | | |
| | Control | Dry | 254.8[a] | 546.0[a] | 988.8[a] | 1532.2[b] | 2203.8[b] | 2878.4[b] |
| | | Wet | 286.4[a] | 487.6[a] | 1,066.4[a] | 1994.2[a] | 2762.9[a] | 3459.4[a] |
| | HE | Dry | 206.3[b] | 405.8[b] | 794.2[b] | 1257.5[b] | 1526.7[c] | 2338.3[c] |
| | | Wet | 264.4[a] | 379.4[b] | 754.8[b] | 1457.6[b] | 2193.4[b] | 2988.4[b] |
| | SEM | | 14.3 | 22.4 | 47.0 | 180.6 | 131.8 | 115.6 |
| Water to feed ratio (g/g) | | | | | | | | |
| | Control | Dry | 2.67 | 2.55[a] | 2.48[ab] | 2.72 | 2.83[a] | 3.21[a] |
| | | Wet | 2.71 | 2.41[ab] | 2.36[ab] | 2.75 | 2.81[a] | 3.24[a] |
| | HE | Dry | 2.30 | 2.29[b] | 2.81[ab] | 2.82 | 2.43[b] | 2.74[b] |
| | | Wet | 2.80 | 2.07[c] | 2.03[b] | 2.55 | 2.54[ab] | 3.00[ab] |
| | SEM | | 0.20 | 0.06 | 0.16 | 0.09 | 0.11 | 0.12 |
| Feed conversion ratio (g/g) | | | | | | | | |
| | Control | Dry | 1.26[b] | 1.81[b] | 1.92[b] | 2.23[b] | 2.38[a] | 2.72 |
| | | Wet | 1.30[b] | 2.01[b] | 1.80[b] | 2.04[bc] | 2.18[ab] | 2.59 |
| | HE | Dry | 1.35[ab] | 2.06[ab] | 2.09[ab] | 2.38[ab] | 2.52[a] | 2.47 |
| | | Wet | 1.44[a] | 2.12[a] | 2.47[a] | 2.29[ab] | 2.37[ab] | 2.60 |
| | SEM | | 0.03 | 0.10 | 0.13 | 0.08 | 0.08 | 0.18 |

Means within a period (column) between treatments without a common superscript letters (a-c) differ significantly ($P < 0.05$); no superscript letter means non significant differences.

[1]Control diet in Experiment 2 = 215.0 g of CP/kg and 2,942 kcal of ME/kg. HE diet in Experiment 2 = 195.0 g of CP/kg and 3,063.2 kcal of ME/kg. Conformation = Dry diet; Wet diet (adding 1 part of water to 1 part of dry diet). Period: six 7-d periods.



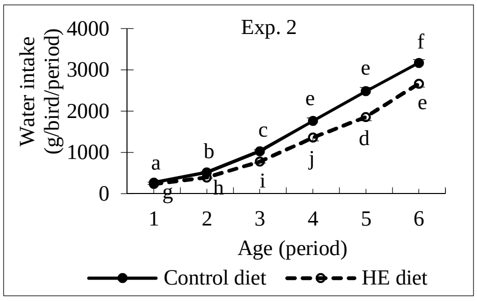




**Figure 4**. Least square means for feed intake, water intake, water-to-feed ratio and FCR (Exp.1), and feed intake, BW gain and water intake (Exp. 2) for diet formulation and periods interaction. Means within and between lines without common superscript letters (a-k) differ significantly ($P < 0.05$). Control diet in experiments 1 and 2 = 215.0 g of CP/kg and 2,942 kcal of ME/kg. HE diet in Experiment 1 = 181.0 g of CP/kg and 3,178.7 kcal of ME/kg and HE diet in Experiment 2 = 195.0 g of CP/kg and 3,063.2 kcal of ME/kg. Period: six 14-d periods in Experiment 1 and six 7-d periods in Experiment 2.

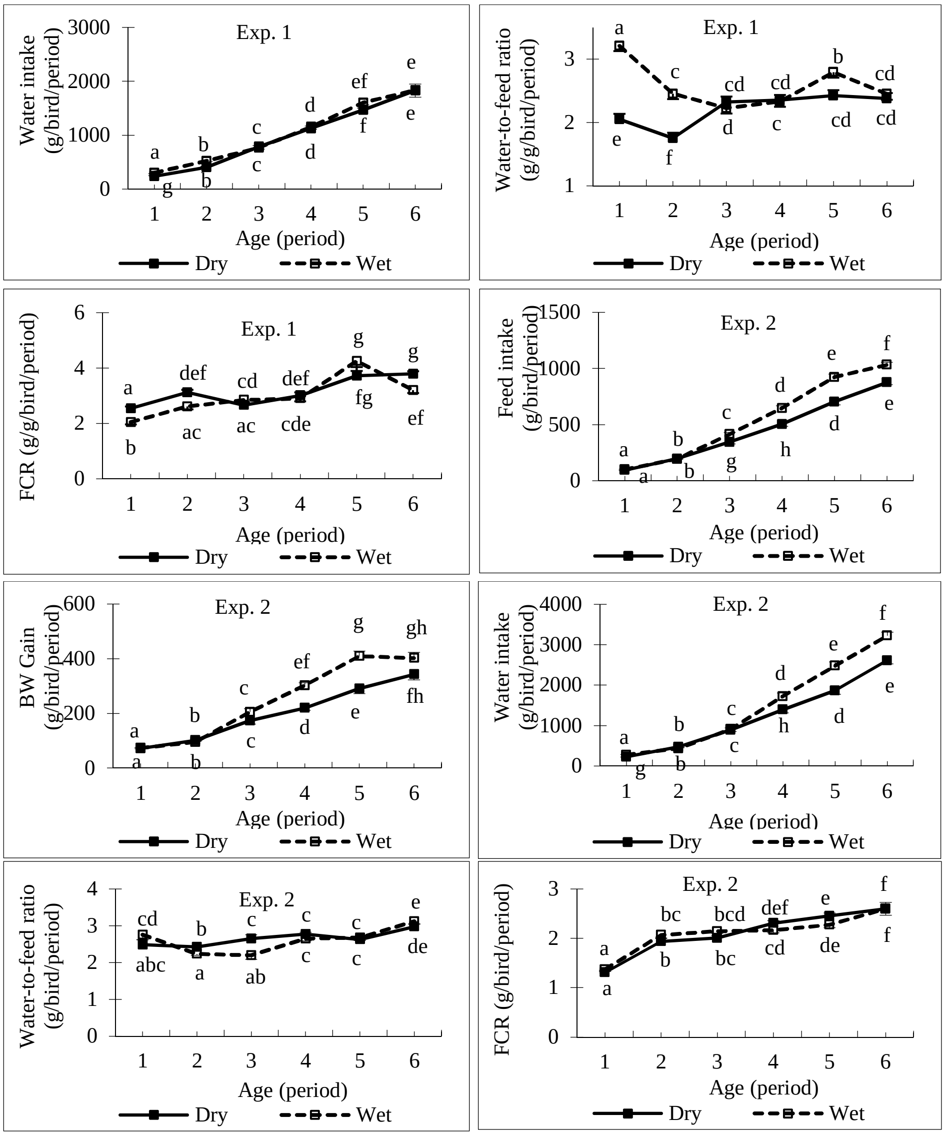



**Figure 5**. Least square means for water intake to feed ratio, water intake and FCR (Exp.1), and feed intake, BW gain, water intake, water-to-feed ratio and FCR (Exp. 2) for diet conformation and periods interaction. Means within and between lines without common superscript letters (a-h) differ significantly ($P < 0.05$). Dry diet; Wet diet (adding 1 part of water to 1 part of dry diet). Period: six 14-d periods in Experiment 1 and six 7-d periods in Experiment 2.

fed birds for most periods, respectively (Table 3a). In Exp. 2, feed intakes of wet-control- and wet-HE-fed birds were significantly higher than those of the respective dry-fed birds in periods 3 to 6 (Table 3b).

An interaction between diet formulation and period showed that feed intake was higher in periods 2 to 4 and 6 (Exp. 1) and in periods 1 to 5 (Exp. 2) for the control-fed birds as compared to the HE-fed birds, respectively (Figure 4).

In Exp. 2, an interaction between diet conformation and period showed that feed intake was higher in periods 3 to 6 for the wet-fed birds compared to the dry-fed birds (Figure 5).

*Body Weight Gain*. Neither dietary formulation nor conformation affected BW gain in Exp. 1, but both factors affected BW gain in Exp. 2 (Table 2). On average, BW gain of control-fed birds (249.1 g/bird) was 24.9% higher than that of HE-fed birds (199.4 g/bird), whereas BW gain of wet-fed birds (248.1 g/bird) was 23.9% higher than that of dry-fed birds (200.3 g/bird) in Exp. 2.

Body weight gains of wet-control- and wet-HE-fed birds were numerically higher in periods 1 to 3 and even significantly higher than that of the respective dry-fed birds in periods 4 to 5 in Exp. 2 (Table 3b).

In Exp. 2, an interaction between diet formulation and period showed that BW gain was higher in periods 1 to 5 for the control-fed birds as compared with the HE-fed birds (Figure 4). An interaction between diet conformation and period showed that BW gain was higher in periods 4 to 5 for the wet-fed birds as compared with the dry-fed birds (Figure 5).

*Water Intake.* Water intake was clearly affected by diet formulation in both experiments, but it was affected by diet conformation in Exp. 2 only (Table 2). In Exp. 1, water intake of control-fed birds (1093.0 g/bird) was, on average, 18.4% higher than that of HE-fed birds (923.4 g/bird). In Exp. 2, water intake of control-fed birds (1538.5 g/bird) was 26.7% higher than that of HE-fed birds (1214.1 g/bird), and water intake of wet-fed birds (1508.2 g/bird) was 21.2% higher than that of dry-fed birds (1244.4 g/bird).

In Exp. 1, water intakes of wet-control- and wet-HE-fed birds were, for most periods, similar to that of dry-control- and dry-HE-fed birds, respectively (Table 3a). In Exp. 2, water intakes of wet-control- and wet-HE-fed birds were higher than that of dry-control- and dry-HE-fed birds in periods 4 to 6 and periods 5 to 6, respectively (Table 3b).

An interaction between diet formulation and period on water intake showed that water intake was higher in control-fed birds than in HE-fed birds in periods 2 to 4 (Exp. 1) and periods 1 to 6 (Exp. 2), respectively (Figure 4). There was an interaction between diet conformation and period for water intake (Table 2). In Exp. 1, water intake was increased over time for both wet-fed and dry-fed birds. In Exp. 2, water intake was higher in wet-fed birds than that in dry-fed birds in periods 1, and 4 to 6 (Figure 5).

*Water-to-Feed Ratio.* Water-to-feed ratio was significantly affected by diet conformation in Exp. 1, and affected by diet formulation in Exp. 2. In Exp. 1, the water-to-feed ratio was 16.7% higher in wet-fed birds (2.58 g/g) than in dry-fed birds (2.21 g/g). In Exp. 2, the water-to-feed ratio was 7.9% higher in control-fed birds (2.73 g/g) than that in HE-fed birds (2.53 g/g). In Exp. 1, water-to-feed ratios of wet-control- and wet-HE-fed birds were significantly higher than those of dry-control- and dry-HE-fed birds in periods 1, 2 and 5 and periods 1 to 2, respectively (Table 3a). In Exp. 2, water-to-feed ratios of wet-control- and wet-HE-fed birds were similar to that of the respective dry-fed birds for most periods (Table 3b).

There was an interaction between diet formulation and diet conformation on the water-to-feed ratio in Exp. 1 (Table 2). The difference in the water-to-feed ratio between wet-control- and dry-control-fed birds was 22.7% (2.70 vs. 2.20 g/g, respectively), whereas the difference between wet-HE- and dry-HE-fed birds was 8.7% (2.50 vs. 2.30 g/g, respectively).

In Exp. 1, an interaction between diet formulation and period on water-to-feed ratio showed that the ratio was higher in control-fed birds than that in HE-fed birds in periods 1 and 2 and lower in period 3 (Figure 4). There was an interaction between diet conformation and period for water-to-feed ratio in both experiments (Table 2). Water-to-feed ratio of wet-fed birds was higher than that of dry-fed birds in periods 1, 2 and 5 (Exp. 1) and period 1 (Exp. 2), but lower in periods 2 and 3 (Exp. 2), respectively (Figure 5).

Feed Conversion Ratio. FCR was only affected by diet formulation in Exp. 2 (Table 2). On average, FCR of control-fed birds (2.02 g/g) was 7.3% lower than that of HE-fed birds (2.18 g/g). In Exp. 2, FCR of wet-control- and wet-HE-fed birds were similar to that of dry-control- and dry-HE-fed birds, respectively (Table 3b).

Interactions were observed between diet formulation and period (Exp. 1) and between diet conformation and period (Exp. 1 and 2). On average, FCR increased over time in both experiments (Figures 4 and 5).

***Gastrointestinal Tract Development.*** Gastrointestinal tract data in Exp. 1 at d 28, 56 and 84 are presented in Tables 4 and 5, respectively. Data in Exp. 2 at d 28 and d 42 are presented in Table 6, respectively. Relative lengths of ileum (day 28) and colon (days 28 and 56) were affected by diet formulation in Exp. 1: control-fed birds had a 13% shorter ileum (day 28) and a 22 and 18% shorter colon (days 28 and 56) than HE-fed birds, respectively. Relative lengths of jejunum and ileum (day 42) were affected by diet formulation in Exp. 2: control-fed birds had a 14 and 15% shorter jejunum and ileum than HE-fed birds, respectively.

In Exp. 1 at d 56, empty weight of proventriculus in dry-fed birds was 15% heavier than that in wet-fed birds. Empty weights of duodenum, jejunum and ileum in control-fed birds were respectively 18, 19 and 17% lower than those in HE-fed birds. Empty weights of jejunum and ileum in the wet-fed birds were respectively 17 and 24% higher than those in the dry-fed birds at d 84. In Exp. 2 at d 42, empty weight of proventriculus in control-fed birds was 28% lower than in HE-fed birds. Empty weights of gizzard, jejunum and ileum in wet-fed birds were respectively 14, 15 and 15% lower than those in dry-fed birds.

***Body Temperature.*** Body T was measured every week between 11:00 to 12:00 h and results are presented in Table 7 for both experiments. In Exp. 1, body T was higher in dry-fed birds than in wet-fed birds at week 4 and it was higher in control-fed birds than in HE-fed birds in weeks 6 and 7. In Exp. 2, body T was similar in all groups.

## Discussion

***Eating and Panting Behavior.*** Kampung chickens required about 6.3% (24.9 vs. 18.6%) and 7.0% (25.2 vs. 18.2%) more time to consume the control and dry diets as compared with the HE and wet diets, respectively. Broilers needed about 3.2% (24.4 vs. 21.2%) and 2.6% (24.1 vs. 21.5%) more time to consume the control and dry diets as compared with the HE and wet diets, respectively. As such, the Kampung chickens seem more sensitive to differences in diet formulation and conformation than broilers. For broilers, the extra eating time spent to consume the dry diet did not lead to a higher feed intake compared to the wet diet. It appears that the control- and the wet-fed birds had a larger meal size per unit of time than the HE- and the dry-fed birds.

**Table 4**. Effects of diet formulation (F) and diet conformation (C) on gastrointestinal tract development of Kampung chickens at d 28 and 56 (Experiment 1)[1].

| Gastrointestinal segment | Control diet | | SEM | HE diet | | SEM | Source of variation ($P$-value)[2] | | |
|---|---|---|---|---|---|---|---|---|---|
| | Dry | Wet | | Dry | Wet | | F | C | F x C[3] |
| **Day 28** | | | | | | | | | |
| Length (cm/100 g body weight) | | | | | | | | | |
| Duodenum | 11.68 | 11.81 | 0.79 | 13.24 | 11.52 | 0.79 | 0.448 | 0.343 | 0.276 |
| Jejunum | 22.08 | 22.16 | 1.38 | 27.53 | 22.71 | 1.38 | 0.061 | 0.113 | 0.114 |
| Ileum | 19.80 | 19.67 | 1.06 | 24.27 | 20.91 | 1.06 | **0.027** | 0.137 | 0.164 |
| Caeca | 5.09 | 4.97 | 0.25 | 5.72 | 5.34 | 0.25 | 0.084 | 0.361 | 0.620 |
| Colon | 2.08 | 2.20 | 0.21 | 2.90 | 2.55 | 0.21 | **0.028** | 0.605 | 0.303 |
| Empty weight (g/100 g body weight) | | | | | | | | | |
| Crop | 0.83 | 0.89 | 0.06 | 0.93 | 0.73 | 0.06 | 0.681 | 0.303 | 0.070 |
| Proventriculus | 0.90 | 1.03 | 0.09 | 0.86 | 0.81 | 0.09 | 0.224 | 0.693 | 0.378 |
| Gizzard | 4.84 | 4.40 | 0.26 | 5.06 | 4.36 | 0.26 | 0.734 | 0.057 | 0.610 |
| Duodenum | 1.37 | 1.49 | 0.10 | 1.51 | 1.48 | 0.10 | 0.563 | 0.672 | 0.483 |
| Jejunum | 1.75 | 1.88 | 0.13 | 2.27 | 1.87 | 0.13 | 0.090 | 0.342 | 0.078 |
| Ileum | 1.36 | 1.28 | 0.13 | 1.39 | 1.38 | 0.13 | 0.660 | 0.762 | 0.789 |
| Caeca | 0.51 | 0.73 | 0.10 | 0.72 | 0.74 | 0.10 | 0.302 | 0.258 | 0.349 |
| Colon | 0.18 | 0.20 | 0.03 | 0.21 | 0.16 | 0.03 | 0.897 | 0.695 | 0.246 |
| **Day 56** | | | | | | | | | |
| Length (cm/100 g body weight) | | | | | | | | | |
| Duodenum | 3.57 | 3.94 | 0.84 | 4.02 | 4.91 | 0.84 | 0.424 | 0.479 | 0.769 |
| Jejunum | 8.72 | 7.99 | 0.59 | 8.94 | 9.25 | 0.59 | 0.245 | 0.730 | 0.405 |
| Ileum | 7.69 | 7.48 | 0.38 | 8.35 | 8.51 | 0.38 | 0.055 | 0.959 | 0.601 |
| Caeca | 2.34 | 2.49 | 0.18 | 2.51 | 2.41 | 0.18 | 0.797 | 0.902 | 0.520 |
| Colon | 1.03 | 0.87 | 0.06 | 1.20 | 1.11 | 0.06 | **0.014** | 0.089 | 0.664 |
| Empty weight (g/100 g body weight) | | | | | | | | | |
| Crop | 0.60 | 0.53 | 0.04 | 0.64 | 0.56 | 0.04 | 0.413 | 0.092 | 0.953 |
| Proventriculus | 0.60 | 0.49 | 0.02 | 0.62 | 0.57 | 0.02 | 0.068 | **0.006** | 0.137 |
| Gizzard | 3.56 | 3.57 | 0.21 | 4.17 | 3.49 | 0.21 | 0.240 | 0.152 | 0.146 |
| Duodenum | 0.92 | 0.89 | 0.08 | 1.07 | 1.15 | 0.08 | **0.034** | 0.735 | 0.475 |
| Jejunum | 1.19 | 1.33 | 0.08 | 1.60 | 1.52 | 0.08 | **0.004** | 0.700 | 0.172 |
| Ileum | 0.84 | 0.98 | 0.08 | 1.10 | 1.08 | 0.08 | **0.047** | 0.500 | 0.336 |
| Caeca | 0.50 | 0.62 | 0.05 | 0.59 | 0.59 | 0.05 | 0.569 | 0.236 | 0.270 |
| Colon | 0.19 | 0.16 | 0.01 | 0.19 | 0.19 | 0.01 | 0.345 | 0.183 | 0.168 |

[1]Mean values are expressed as an average of 3 replicate pens. Control diet in Experiment 1 = 215.0 g of CP/kg and 2,942 kcal of ME/kg. HE diet in Experiment 1 = 181.0 g of CP/kg and 3,178.7 kcal of ME/kg. Conformation = Dry diet; Wet diet (adding 1 part of water to 1 part of dry diet).
[2]Significant effects ($P < 0.05$) are printed in bold.
[3]F x C = interaction between F and C.

**Table 5**. Effects of diet formulation (F) and diet conformation (C) on gastrointestinal tract development of Kampung chickens at d 84 (Experiment 1)[1].

| Gastrointestinal segment | Control diet | | SEM | HE diet | | HE diet | | Source of variation (*P*-value)[2] | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Dry | Wet | | Dry | SEM | Wet | SEM | F | C | F x C[3] |
| Length (cm/100 g body weight) | | | | | | | | | | |
| Duodenum | 3.07 | 2.86 | 0.23 | 2.91 | 0.23 | 3.12 | 0.28 | 0.854 | 0.990 | 0.426 |
| Jejunum | 6.41 | 5.56 | 0.56 | 6.21 | 0.56 | 6.64 | 0.69 | 0.459 | 0.772 | 0.298 |
| Ileum | 6.13 | 5.50 | 0.57 | 5.85 | 0.57 | 6.05 | 0.70 | 0.829 | 0.729 | 0.517 |
| Caeca | 1.72 | 1.45 | 0.12 | 1.63 | 0.12 | 1.74 | 0.14 | 0.444 | 0.514 | 0.174 |
| Colon | 8.76 | 0.70 | 0.07 | 0.78 | 0.07 | 0.73 | 0.09 | 0.745 | 0.513 | 0.947 |
| Empty weight (g/100 g body weight) | | | | | | | | | | |
| Crop | 0.56 | 0.57 | 0.04 | 0.52 | 0.04 | 0.48 | 0.05 | 0.411 | 0.877 | 0.954 |
| Proventriculus | 0.50 | 0.57 | 0.04 | 0.44 | 0.04 | 0.48 | 0.05 | 0.249 | 0.615 | 0.975 |
| Gizzard | 3.00 | 3.12 | 0.27 | 3.49 | 0.27 | 3.65 | 0.40 | 0.134 | 0.696 | 0.996 |
| Duodenum | 0.68 | 0.84 | 0.05 | 0.78 | 0.05 | 0.84 | 0.07 | 0.243 | 0.061 | 0.730 |
| Jejunum | 1.11 | 1.34 | 0.05 | 1.24 | 0.05 | 1.42 | 0.06 | 0.055 | **0.004** | 0.719 |
| Ileum | 0.79 | 1.06 | 0.06 | 0.91 | 0.06 | 1.04 | 0.06 | 0.442 | **0.014** | 0.236 |
| Caeca | 0.43 | 0.50 | 0.05 | 0.55 | 0.05 | 0.58 | 0.07 | 0.146 | 0.447 | 0.672 |
| Colon | 0.16 | 0.19 | 0.02 | 0.16 | 0.02 | 0.16 | 0.02 | 0.589 | 0.360 | 0.462 |

[1]Mean values are expressed as an average of 3 replicate pens. Control diet in Experiment 1 = 215.0 g of CP/kg and 2,942 kcal of ME/kg. HE diet in Experiment 1 = 181.0 g of CP/kg and 3,178.7 kcal of ME/kg. Conformation = Dry diet; Wet diet (adding 1 part of water to 1 part of dry diet).
[2]Significant effects ($P < 0.05$) are printed in bold.
[3]F x C = interaction between F and C.

Increasing panting activity may be a reliable indicator of thermal discomfort and compromised welfare (McLean et al., 2002). Kampung chickens fed a control diet and a wet diet showed similar times spent on panting behavior as birds fed a HE diet and a dry diet, respectively. Broilers fed a control diet and a wet diet showed 8.3% (26.6 vs. 18.3%) and 8.2% (26.5 vs. 18.3%) more time on panting behavior than the broilers fed a HE diet and a dry diet, respectively. It is clear from these experiments that broilers consumed more feed and thus may have had a greater difficulty to cope at high T (HT). This is clear from the data as broilers showed more time spent on panting (22.8 vs. 0.5%; $P < 0.001$) compared to the Kampung chickens.

**Table 6.** Effects of diet formulation (F) and diet conformation (C) on GIT development of broilers at d 22 and 42 (Experiment 2)[1].

| Gastrointestinal segment | Control diet | | HE diet | | SEM | Source of variation (*P*-value)[2] | | |
|---|---|---|---|---|---|---|---|---|
| | Dry | Wet | Dry | Wet | | F | C | F x C[3] |
| **Day 22** | | | | | | | | |
| Length (cm/100 g body weight) | | | | | | | | |
| Duodenum | 6.05 | 6.66 | 7.51 | 7.98 | 0.91 | 0.153 | 0.568 | 0.941 |
| Jejunum | 14.44 | 16.10 | 19.09 | 19.48 | 2.29 | 0.105 | 0.662 | 0.786 |
| Ileum | 12.66 | 15.68 | 17.04 | 16.88 | 1.88 | 0.164 | 0.461 | 0.415 |
| Caeca | 2.68 | 2.71 | 3.57 | 3.44 | 0.45 | 0.097 | 0.916 | 0.854 |
| Colon | 1.08 | 1.20 | 1.28 | 1.20 | 0.14 | 0.504 | 0.894 | 0.523 |
| Empty weight (g/100 g body weight) | | | | | | | | |
| Crop | 0.69 | 1.29 | 0.99 | 0.86 | 0.17 | 0.726 | 0.206 | 0.061 |
| Proventriculus | 0.79 | 1.05 | 0.86 | 0.74 | 0.15 | 0.440 | 0.676 | 0.243 |
| Gizzard | 3.57 | 4.68 | 4.61 | 3.81 | 0.64 | 0.899 | 0.807 | 0.164 |
| Duodenum | 1.39 | 1.79 | 1.46 | 1.48 | 0.20 | 0.555 | 0.305 | 0.356 |
| Jejunum | 2.77 | 3.09 | 2.47 | 2.63 | 0.40 | 0.353 | 0.560 | 0.845 |
| Ileum | 1.76 | 1.96 | 1.69 | 1.87 | 0.23 | 0.733 | 0.438 | 0.964 |
| Caeca | 0.55 | 0.68 | 0.62 | 0.82 | 0.14 | 0.473 | 0.260 | 0.795 |
| Colon | 0.39 | 0.31 | 0.16 | 0.23 | 0.07 | 0.059 | 0.869 | 0.316 |
| **Day 42** | | | | | | | | |
| Length (cm/100 g body weight) | | | | | | | | |
| Duodenum | 2.59 | 2.23 | 2.84 | 2.64 | 0.20 | 0.125 | 0.199 | 0.702 |
| Jejunum | 6.79 | 5.62 | 7.41 | 7.03 | 0.42 | **0.030** | 0.085 | 0.358 |
| Ileum | 6.39 | 5.23 | 7.14 | 6.46 | 0.43 | **0.040** | 0.053 | 0.591 |
| Caeca | 1.43 | 1.25 | 1.56 | 1.44 | 0.12 | 0.201 | 0.229 | 0.783 |
| Colon | 0.66 | 0.63 | 0.63 | 0.57 | 0.03 | 0.215 | 0.239 | 0.494 |
| Empty weight (g/100 g body weight) | | | | | | | | |
| Crop | 0.65 | 0.64 | 0.69 | 0.64 | 0.04 | 0.852 | 0.879 | 0.848 |
| Proventriculus | 0.65 | 0.57 | 0.81 | 0.89 | 0.08 | **0.011** | 0.990 | 0.351 |
| Gizzard | 2.61 | 2.24 | 2.87 | 2.45 | 0.11 | 0.057 | **0.004** | 0.799 |
| Duodenum | 1.03 | 0.90 | 1.15 | 0.99 | 0.08 | 0.256 | 0.107 | 0.876 |
| Jejunum | 1.70 | 1.49 | 1.99 | 1.63 | 0.12 | 0.093 | **0.030** | 0.520 |
| Ileum | 1.30 | 1.18 | 1.51 | 1.21 | 0.70 | 0.132 | **0.014** | 0.270 |
| Caeca | 0.56 | 0.55 | 0.59 | 0.51 | 0.05 | 0.939 | 0.410 | 0.539 |
| Colon | 0.31 | 0.15 | 0.20 | 0.15 | 0.09 | 0.513 | 0.269 | 0.531 |

[1]Mean values are expressed as an average of 4 replicate pens. Control diet in Experiment 2 = 215.0 g of CP/kg and 2,942 kcal of ME/kg. HE diet in Experiment 2 = 195.0 g of CP/kg and 3,063.2 kcal of ME/kg. Conformation = Dry diet; Wet diet (adding 1 part of water to 1 part of dry diet).
[2]Significant effects ($P < 0.05$) are printed in bold.
[3]F x C = interaction between F and C.

**Table 7**. Body temperature ($^oC$) of Kampung (Experiment 1) and broiler chickens (Experiment 2)[1].

| Experiment | Week | Control diet | | HE diet | | SEM | Source of variation[2] | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Dry | Wet | Dry | Wet | | F | C | F x C[3] |
| Experiment 1 | | | | | | | | | |
| | 1 | 41.12 | 40.98 | 40.76 | 40.83 | 0.12 | 0.064 | 0.787 | 0.426 |
| | 2 | 41.12 | 41.17 | 41.08 | 40.86 | 0.20 | 0.390 | 0.661 | 0.515 |
| | 3 | 41.12 | 40.99 | 40.77 | 40.83 | 0.12 | 0.064 | 0.787 | 0.426 |
| | 4 | 41.82 | 41.47 | 41.66 | 41.31 | 0.09 | 0.126 | **0.006** | 0.954 |
| | 5 | 41.88 | 41.94 | 41.90 | 41.90 | 0.08 | 0.892 | 0.685 | 0.685 |
| | 6 | 41.41 | 41.51 | 40.76 | 40.02 | 0.10 | **0.001** | 0.100 | 0.423 |
| | 7 | 41.66 | 41.56 | 41.04 | 41.36 | 0.17 | **0.048** | 0.560 | 0.270 |
| | 8 | 41.32 | 41.00 | 41.04 | 41.10 | 0.31 | 0.781 | 0.678 | 0.558 |
| | 9 | 41.61 | 41.29 | 41.48 | 41.54 | 0.10 | 0.564 | 0.244 | 0.092 |
| | 10 | 41.73 | 41.54 | 41.51 | 41.70 | 0.12 | 0.781 | 1.000 | 0.141 |
| | 11 | 41.39 | 41.48 | 41.51 | 41.19 | 0.14 | 0.576 | 0.438 | 0.188 |
| | 12 | 41.08 | 41.17 | 41.69 | 40.57 | 0.39 | 1.000 | 0.215 | 0.160 |
| Experiment 2 | | | | | | | | | |
| | 1 | 41.11 | 41.07 | 40.94 | 40.78 | 0.13 | 0.106 | 0.442 | 0.642 |
| | 2 | 41.40 | 41.33 | 41.19 | 41.07 | 0.11 | 0.060 | 0.391 | 0.828 |
| | 3 | 41.11 | 41.07 | 40.94 | 40.78 | 0.13 | 0.106 | 0.442 | 0.642 |
| | 4 | 41.23 | 41.69 | 41.38 | 41.70 | 0.23 | 0.721 | 0.111 | 0.747 |
| | 5 | 41.54 | 41.33 | 41.29 | 41.27 | 0.22 | 0.487 | 0.584 | 0.663 |
| | 6 | 42.19 | 41.98 | 42.01 | 41.77 | 0.18 | 0.287 | 0.234 | 0.928 |

[1]Mean values are expressed as an average of 3 replicate pens. Control diet in experiments 1 and 2 = 215.0 g of CP/kg and 2,942 kcal of ME/kg. HE diet in experiments 1 = 181.0 g of CP/kg and 3,178.7 kcal of ME/kg and HE diet in experiment 2 = 195.0 g of CP/kg and 3,063.2 kcal of ME/kg. Conformation = Dry diet; Wet diet (adding 1 part of water to 1 part of dry diet).
[2]Significant effects ($P < 0.05$) are printed in bold.
[3]F x C = interaction between diet formulation and diet conformation.

***Feed Intake and Body Weight gain***. The differences in energy density were 236.7 and 121.1 kcal of ME/kg between the HE and the control diet for Exp. 1 and 2, respectively. The feed intake in g by the control-fed birds was 14% higher than the feed intake of the HE-fed birds in both experiments. The higher feed intake of the control-fed birds is logical because birds needed to consume more lower energy-containing feed to meet their energy requirement (Lopez and Leeson, 2005; Sakomura et al., 2005). But the lower intake (in terms of energy and protein) of the HE-fed birds is contrary to expectations as a HE diet will yield less heat per unit of ME. It seems that the birds did not consume more energy with the high energy diet under HT, and this resulted in less CP and ME intake. However, the energy intake in kcal of ME per g BW gain was numerically higher in the HE-fed Kampung chickens and significantly higher in the HE-fed broilers than in the respective control-fed birds (see Table 8). The lower CP intake of HE-fed birds did not lead to a significantly retarded growth rate in Kampung chickens, but in broilers a depressed growth rate was observed.

**Table 8**. Feed intake (FI), crude protein (CP) and ME intake and ME/BW gain of birds in the different diets and conformations per day.

| Type of birds | Dietary treatments | FI (g/bird/d) | CP (g/bird/d) | ME (kcal/bird/d) | ME/BW gain/d |
|---|---|---|---|---|---|
| Kampung chickens | Control-dry diet | 32.2[a] | 6.9[a] | 94.5[a] | 9.4[ab] |
| | Control-wet diet | 32.0[a] | 6.9[a] | 94.2[a] | 8.8[b] |
| | HE-dry diet | 28.4[b] | 5.1[b] | 90.4[a] | 9.7[a] |
| | HE-wet diet | 26.9[b] | 4.8[b] | 85.5[a] | 9.3[ab] |
| Broiler | Control-dry diet | 70.2[b] | 15.1[b] | 206.6[b] | 6.0[b] |
| | Control-wet diet | 84.2[a] | 18.1[a] | 247.7[a] | 5.8[b] |
| | HE-dry diet | 59.2[c] | 11.5[c] | 181.2[c] | 6.6[a] |
| | HE-wet diet | 73.4[b] | 14.3[b] | 224.9[b] | 6.8[a] |

Means within a column for each bird type without common superscript letters (a-c) differ significantly ($P < 0.05$).
[1]Control diet in experiments 1 and 2 = 215.0 g of CP/kg and 2,942 kcal of ME/kg. HE diet in Experiment 1 = 181.0 g of CP/kg and 3,178.7 kcal of ME/kg and HE diet in Experiment 2 = 195.0 g of CP/kg and 3,063.2 kcal of ME/kg). Conformation = Dry diet; Wet diet (adding 1 part of water to 1 part of dry diet).

Increasing energy in the diet has been thought to improve feed intake, as a result of a reduced heat increment. This effect is thought to be more pronounced in birds at heat-stress conditions because protein has a higher caloric increment than carbohydrate and fat (Black, 1995; Musharaf and Latshaw, 1999). The hypothesis that the strategy of the birds would be to avoid an increased heat production by consuming more of the HE diet was not confirmed, although the decreased CP intake of the HE diet may have helped to reduce heat production. Therefore, the lower feed intake of the HE diet is possibly due to an imbalance of amino acids which is mostly the case with the HE diet (Aftab et al., 2006) because feed intake of the control-fed birds was higher. Aftab et al. (2006) reported that the concentration and balance of dietary amino acids can influence feed intake in broilers. NRC (1984) also reports that the contents of essential amino acids can influence feed intake either through their concentrations in blood and affecting brain receptors, or by influencing hepatic amino acid metabolism. In addition, metabolic pathways and metabolites produced by these pathways integrate to the regulation of feed intake and energy metabolism (Richards, 2003).

It can be seen from Table 8 that feed intake of birds fed a wet diet increases especially in broilers, and the increase was more pronounced with the HE diet. The higher intake of the wet diet by broilers was in line with the previous study at HT reported in Chapter 5. However, the effect of wetting the diet on feed intake in Kampung chickens and broilers was opposite. The results in this study confirm a higher feed intake of a wet diet at HT in broilers (Awojobi and Meshioye, 2001; Kutlu, 2001; Awojobi et al., 2009). The reason for the higher intake of wet-fed broilers at HT is probably that the birds try to cope with HT by consuming extra water and ME intake. The Kampung chickens are more acclimatized to tropical climatic conditions and do not need to make use of the wet diet for thermoregulation.

Body weight gains of Kampung chickens and broilers fed with a control diet were 8.7% (9.8 vs. 9.0 g/d) and 25% (35.6 vs. 28.5 g/d) higher than when fed with a HE diet, respectively. The lower BW gain of HE-fed birds is undoubtedly due to the cumulative lower energy and protein intake with this diet (Table 8). This is in agreement with the review of Furlan et al. (2004) who concluded that low protein diets have negative effects on broiler performance when the ambient temperature is high due to a low feed intake and amino acid deficiency.

Body weight gains of Kampung chickens and broilers fed a wet-control diet were 6.3% (10.1 vs. 9.5 g/d) and 20.2% (39.6 vs. 31.6 g/d) higher than the two types of birds fed a dry-control diet, respectively. Body weight gains of Kampung chickens and broilers fed a wet-HE diet were 0.8% lower (8.9 vs. 9.1 g/d) and 18.2% higher (31.3 vs. 25.6 g/d) than these birds fed a dry-HE diet, respectively. Moreover, a similarity in BW gain between wet-HE- and dry-control-fed birds was noted, especially in broilers (Table 3b). This indicates that CP and energy intake due to wetting the HE diet in broilers was similar to the dry-control diet (Table 8). Therefore, it is beneficial to exploit a wet feeding strategy for broilers raised under tropical climatic conditions. The higher BW gain of wet-fed birds in this study agrees with the finding from earlier studies in broilers fed a wet mash diet at NT (Yalda and Forbes, 1995, 1996; Yasar and Forbes, 1999; Khoa, 2007) and HT (Awojobi and Meshioye, 2001; Kutlu, 2001; Awojobi et al., 2009). This effect may be explained by extra ME intake and some improvement in digestibility (Forbes, 2003; Dei and Bumbie, 2011). This study supports the hypothesis that an increased passage rate with wet diet through the GI tract may have occurred in chickens (Yasar and Forbes, 2000).

***Water Intake and Water-to-Feed Ratio.*** Total water consumption in this study was higher in control-fed birds than in HE-fed birds. As a consequence, water-to-feed ratio in control-fed birds was higher than in the HE-fed birds, especially in broilers (high intake of feed and water). It is clear from literature that there is a high correlation between feed intake and water consumption (Lott et al., 2003). An increasing protein concentration in the diet increases water intake and also the water-to-feed ratio (Marks and Pesti, 1984). The higher water intake in wet-fed birds was obtained from drinking and additional water contained in the wet diet consumed. These findings are in accordance with those of Yasar and Forbes (1999). Increasing water intake will benefit the birds at HT because it can be used for evaporative heat loss (Belay and Teeter, 1993) especially in broilers. The higher water-to-feed ratio in control-fed broilers may indicate that the broilers will probably use the water for several purposes including digestion and heat evaporation (Figure 3). The high water-to-feed ratio of control-fed broilers can only come from water intake.

***Gastrointestinal Tract Development.*** In Kampung chickens, effects of diet formulation on GIT development disappeared when the birds grew older, suggesting that this type of bird adapts easily to changes in dietary nutrient content. Only a positive effect of the relative weight of the intestine of birds fed a wet diet could be observed. In general, it seems that the Kampung chickens grow proportionally. In broilers, effects of diet formulation and diet conformation on GIT development were absent at day 21 and could only be observed at day 42. This in partly unexpected because Khoa (2007) found that wetting a diet had a more pronounced effects during the starter phase on GIT development.

If data on BW and GIT development are compared, it seems that the broiler carcass is growing faster than its GIT in control- and wet-fed birds, which may have detrimental effects on the digestive properties for this supply organ when feeding such diets. The heavier gizzard was also found in dry-fed broilers under tropical climate (Awojobi and Meshioye, 2001).

***Body Temperature.*** In general, body T was similar between diet formulations and diet conformations for both Kampung and broiler chickens. These results were similar to the previous study reported in Chapter 5 that there were no effects of diet formulation and diet conformation on body T within normal T and high T.

## Conclusion

Both Kampung and broiler chickens spent more time consuming the control and dry diet compared to a high energy to protein ratio and wet diet. Broilers spent more time in panting behavior than Kampung chickens when raised under tropical climatic conditions. Providing a high energy to protein ratio diet to Kampung and broiler chickens housed under tropical conditions results in a lower growth rate compared to feeding a control diet. This effect is more pronounced for broiler compared to Kampung chickens. Providing a wet, high energy to protein ratio diet will result in similar body weight gain as a dry, control diet. The positive effect of a wet diet in gastrointestinal tract development was greater in later life of broilers. At a later age, an increase in body weight gain was not followed by an increase in gastrointestinal tract development.

## Acknowledgements

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# CHAPTER 7

# General Discussion

## Introduction

Poultry meat production has increased drastically during the past 35 years especially in developing countries, even exceeding production in developed countries since 2000. In 2005, five of the 10 leading countries with regard to poultry meat production were developing countries and four were located in Asia (Windhorst, 2006) and included most of the hot regions of the world including Indonesia. The hot regions of the world have probably the largest potential for further growth since the level of poultry meat consumption is still low. Figure 1 shows that the poultry meat consumption per capita in hot temperature countries is on average lower than in countries from the temperate regions.

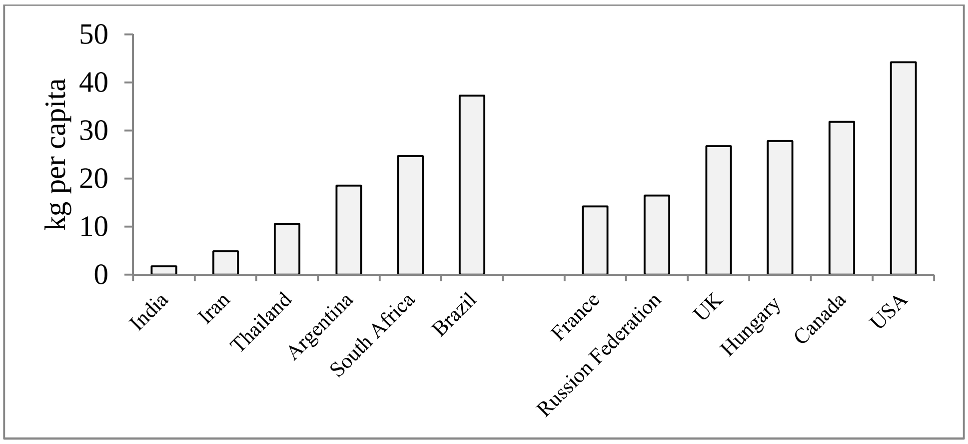


**Figure 1**. Poultry meat consumption per capita (kg) in hot and temperate region in 2005 (Windhorst, 2006).

Increasing poultry meat consumption in Indonesia, which is still below Indonesia's neighbouring countries in Asia (3.7 kg/capita/year) (Meylinah, 2007), is an important aim for improving the nutrition of the population. Poultry production in Indonesia is characterized by traditional and small-scale farming systems and is predominantly done by small farmers with a relatively low income. The bulk of the national supply of poultry meat and eggs in Indonesia is derived from the small and subsistence-type of farms rather than from large commercial operations (Daghir, 2008c). Indonesia has a large population and there is probably great potential for expansion of poultry production. In order to increase poultry production, the development of small- and medium-scale commercial operations is needed. Success of these operations depends on the adoption of modern practices of poultry production in order to be economically viable.

Broilers have a large growth potential, a high metabolic rate and as such increasingly used for poultry meat production (Havenstein et al., 2003). These birds perform very well at normal temperatures (NT; 21-22$^{o}$C). However, broilers have a poorer performance under hot climatic conditions. Due to economics, farmers do not apply control devices of ambient temperature in the broiler house (Deeb and Cahaner, 2002).

There is little or no information available regarding the temperature range for optimal production of the predominant type of bird used in Indonesia, the Kampung chicken. Under hot climatic conditions, the animal's physiological, anatomical and behavioral mechanisms will facilitate heat loss, or minimizing heat gain from the environment to maintain normal body temperature (Etches et al., 2008). In addition, animals will reduce heat production by lower their feed intake. The primary consequence of heat stress, therefore, is that animals will reduce feed intake progressively when ambient temperature increases (May and Lott, 1992). This lower feed intake (energy intake) reduces heat production of the chicken (Koh and MacLeod, 1999a), but body weight (BW) gain is also decreased (Cheng et al., 1997).

Lowering the heat load in birds during periods of high ambient temperatures will allow an animal to maintain their performance or minimise the reduction in growth rate. The concentration of some dietary nutrients (e.g. protein) will give a relatively high heat increment and the intake of these nutrients should be adjusted to optimal levels as to minimise excess heat production. The nutrient requirements for birds at high ambient temperatures (HT) are predominantly based on the international feeding tables such as those of the NRC (1994). These tables are mainly derived from experiments that have been conducted under moderate climatic conditions with an abundant feed availability. In addition, these tables are mainly based on corn-soybean based diets and fast-growing chickens. NRC (1994) stated that some nutrient requirement estimates should be adjusted to fit to the wide variety of ages, sexes, strains of broiler chickens and temperatures. Nutrient adjustments are proposed for broilers at high temperatures in the USA, but the hot climate conditions in the USA may not be the same as the climate conditions in Indonesia. As such, the NRC (1994) tables do not account for differences in nutrient requirements and intake levels for different strains of chickens at HT.

Allowing birds to select from a set of nutrients may be an option to optimise the nutrient intake that generates the lowest amount of heat (at a given production level) for the birds. This strategy can provide insight into the nutrient requirements which are appropriate to meet the physiological demand of chickens at HT. Kampung chickens are scavenger chickens which have been raised for many centuries (and still are being used) by local farmers under the HT conditions in Indonesia, where they are kept in (semi) intensive-outdoor-system. Although raised under sub-optimal conditions, Kampung chickens still have a capability to produce meat and eggs under these farming conditions. Kampung chickens, therefore, may have a better capability to select a diet compared to modern broilers, although it is unknown how Kampung chickens select a diet if allowed to choose.

The studies reported in this thesis focus on how chickens (Kampung and broilers) deal with high ambient temperatures and how they respond to self-selection of a diet. In addition, studies reported here were aimed at determining the response of chickens to a wet diet, with the emphasis on performance parameters.

## Eating and Panting Behavior

In poultry meat-production, fast- and slow-growing chickens can be discriminated. Fast-growing chickens are used in conventional, commercial broiler production systems, kept in large flocks and grown to a slaughter weight of 2 kg in about 42 days. Slow-growing chickens are used in free-range systems and treated less intensive in management. Behavior of fast-growing chickens have been studied extensively at NT (Bokkers and Koene, 2003), but hardly at HT. The behavior of chickens may differ between NT and HT and could explain the bird's performance.

In this thesis, behavior was observed as the time budget the bird spent on eating and panting behavior relative to other behaviors. It was found here that there were substantial changes in time budget of chickens raised at NT and HT. At NT, the birds spent more time on eating and less time on panting behavior compared with birds raised at HT (Chapters 3 and 5). If breeds are compared, broilers spent a similar amount of time on eating as the Kampung chickens (Chapter 6). However, the time that broilers spent on panting was significantly higher at HT than at NT (Chapters 3 and 5). In addition, broilers spent more time on panting than Kampung chickens (Chapter 6). The larger time spent on eating at NT was attributed to a higher feed intake. It was also observed that feed intake of broilers was higher than that of Kampung chickens and thus they may have had a problem to cope at the HT of the tropical climatic conditions. These results are in close agreement with lower feed intake and higher panting activity observed at high stocking density compared to low stocking density in broilers, particularly in 6 weeks of age (McLean et al., 2002). Panting activity is mainly a route to dissipate heat at HT (Syafwan et al., 2011) and panting can be used also as a reliable indicator of thermal discomfort (McLean et al., 2002).

## Ambient Temperature During the Growing Period

Five different studies were conducted with two studies at HT and NT (Chapters 3 and 5). The three studies reported in Chapter 4 (with Kampung chickens) and in Chapter 6 (with both Kampung and broiler chickens) were done under tropical climatic conditions.

It was clear that feed intake and BW gain of broilers were much lower when there was a chronic high temperature for a longer period of time. Figures 2 and 3 show that broilers had a higher feed intake per kg metabolic body weight after 2 weeks of age when they were raised at wide ranges of temperature (T) mainly due to day-night differences. The T and relative humidity (RH) were maintained relatively constant in the studies reported in Chapters 3 and 5. In Chapters 4 and 6 (performed in Indonesia), the T and the RH were completely dependent on the climatic conditions because birds were raised in an open-sided house. Temperature and RH of the broiler houses in Chapter 3 were maintained at 18.5-21.0$^{o}$C with 40-49% RH and at 25.2-31.5$^{o}$C with 61-79% RH. In Chapter 5, the room with NT was maintained at 19.6-21.5$^{o}$C with 61-72% RH and the room with HT was maintained at 26.5-31.7$^{o}$C with 58-85% RH. These ambient T were chosen in order to mimic practical conditions in Europe and the tropical climatic conditions in Indonesia. In Chapter 6, the T under tropical climatic condition during this experiment ranged between 21.8 and 35.4$^{o}$C while RH ranged between 58 and 94%. Based on data in the literature the thermal effects on feed intake and metabolism can be expected to be more severe under a high constant T than under diurnal, cycling T conditions. Cheng at al. (1999) found that BW gain was higher, and feed conversion ratio (FCR) was lower in birds raised under diurnally cycling T (26.6$^{o}$C during 16 h and 35$^{o}$C during 8h) than birds continuously kept at a constantly high (32.2$^{o}$C) ambient T. In the literature, no negative effects of a moderate regular warm cyclic temperature fluctuation on BW gain of broilers were found (Segura et al., 2006). However, here it was shown that high regular warm cyclic temperature fluctuations give a large depression in performance (Figure 3 and Chapter 6). The very high RH range (58-94%) combined with the extremely large HT ranges employed in the study reported in Chapter 6 as compared with Chapter 3 and 5 may have caused more difficulties for the birds to lose heat. The result is a lower BW of the birds than BW in the studies reported in Chapters 3 and 5 in which the range of HT was smaller. Yahav (2000) reported that broiler chickens exposed to 30°C and 28°C during 4 to 8 weeks of age, BW gain and feed intake responded in a bell-shaped fashion to RH. The response of both variables to RH was maximal at RH=60% to 65%.

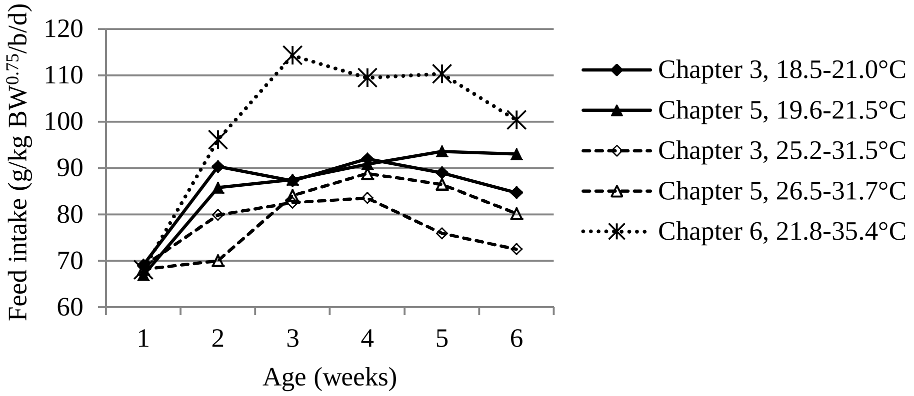


**Figure 2**. Feed intake of male broilers fed a control diet from 1 to 6 weeks of age at normal and high temperature over time.

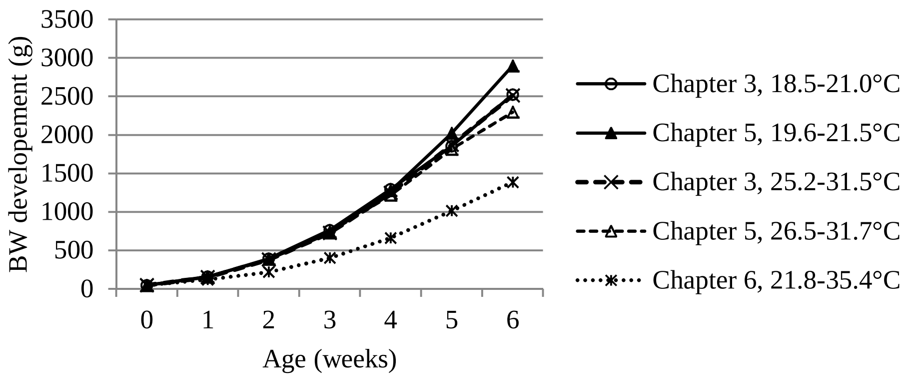


**Figure 3**. Body weight (BW) of male broilers fed a control diet from 0 to 6 weeks of age at normal and high temperature.

It can be derive also from Figures 2 and 3 that feed intake was higher and BW gain was much lower in the broilers studied in Chapter 6 resulting in a decreased feed efficiency. Therefore, the performance of these birds which were kept at HT and the RH range of Chapter 6 was lowered. High producing birds do not appear to cope well with these environmental conditions.

Feed intake was decreased by 1.7% and 1.2% for each degree Celsius increase within the temperature ranges from 21.0 to 31.5°C and 21.5 to 31.7°C, respectively. Apparently, temperatures above 21°C have a large effect on feed intake in broilers. Cheng et al. (1997) reported that feed intake was decreased with about 2.7% per degree Celsius increase from 21.0 to 32.2°C. Temim et al. (1999) reported a decreased feed intake of 2.9% for a control diet and 2.3% for a high-protein diet per degree Celsius increase from 22 to 32°C (See Figure 4). From this figure it is clear that studies in the literature which were conducted over a wider range of HT compared to the studies reported here resulted in a larger depression in feed intake.

Chapters 3 and 5, the decrease in feed intake resulted in a decreased BW gain in broilers of 1.4 and 1.9%, respectively for each degree Celsius increase in temperature. The negative effect of HT on feed intake and BW gain increased with increasing age or with increasing weight. The decrease in feed intake per degree Celsius increase was less than the decrease in BW gain. Therefore, broilers kept at HT had a lower feed efficiency. This can be explained by the fact that broilers at HT with a lowered feed intake will use the same amount of energy intake for maintenance, and thus all the reduction in feed intake means that less energy remains for processes of protein and lipid accretion.

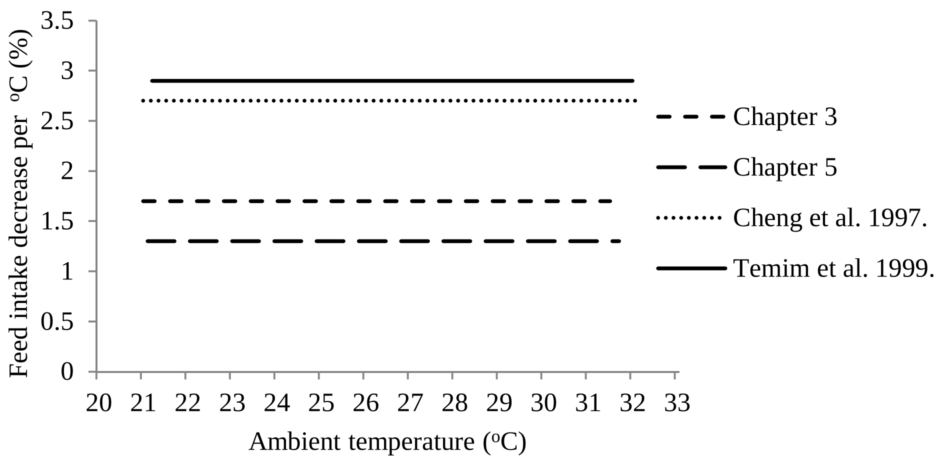


**Figure 4**. Percentage decrease in feed intake of broilers with increasing ambient temperature as observed in different studies.

## Protein and Energy Selection

Birds select feed with the purpose of ingesting a sufficient amount of energy and nutrients. This selection becomes clear if their requirements change over time when these changes are reflected in adjustment of the proportion of different feeds or feed ingredients chosen. The changes over time will occur naturally during the growing period in addition to an imposed change in conditions such as ambient temperature (Forbes and Shariatmadari, 1994).

As the bird grows, its rate of protein deposition is relatively constant during a few weeks, whereas its energy requirement increases, so that the concentration of protein required in the feed declines with age (NRC, 1994). If self-selection for certain nutrients reflects the requirement of birds, then it can be expected that the ratio of intake of those nutrients to total feed intake would decrease or increase with age. In this way, protein and energy content in the selected diet can deviate as compared with a control diet. Indeed the studies reported here these responses are observed. Data in Chapters 3 and 4 were examined to see whether these changes occurred during growth and compared with data reported by Forbes and Shariatmadari (1993) in broilers raised at temperature of 20 to 24$^{o}$C. These authors offered a high-protein (HP) and very low-protein (VLP) approximately isoenergic diet in Experiment 1, and diets with different levels of protein in Experiment 2 (Table 1).

It can be seen from Table 1 that when HP and LP diets are given, the animal will choose a balanced diet, and there is indeed a significant decrease in the CP content of the diet chosen at a higher age indicating that changes occur. This is in line with the decrease in protein-to-energy ratio requirements as birds grow older. In the work of Delezie et al. (2009), it was shown that broilers consumed a higher proportion of low-protein diet (55-60%) in the second half of the growing period compared to the first half of growing period. Moreover, when broilers are offered two diets with different CP content, they consume a ratio between both diets that matches best to their actual requirement (see Experiment 2 of Shariatmadari and Forbes, 1993). If the birds grow older, the CP content of the chosen diet will decrease due to a higher feed intake, but never below the lowest CP level of the offered diets. The results from the choice feeding study with broilers in Chapter 3 and with Kampung chickens in Chapter 4 indeed showed that when diets which differ in CP and ME contents were offered, the preference for the HE diets increased as the birds grew. Therefore, there is clear evidence that the broilers and

Kampung chickens are able to meet their requirement for both energy and protein by appropriate selection from two or more feeds at any ambient temperature condition.

**Table 1**. Crude protein (CP) content of diets chosen by birds by selecting from different diets at different ages.

| Study | CP content of feeds offered (g/kg) | | | CP content of diet chosen (g/kg) (days of age ) | | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | High CP | Low CP | 28 | 42 | 63 | 70 | 84 |
| Shariatmadari and Forbes (1993) | | | | | | | | |
| Experiment 1 (Broiler) | | | | | | | | |
| Free choice (22 to 24°C) | | 280 | 65 | 229 | | 192 | | |
| Experiment 2 (Broiler) | | | | | | | | |
| Free choice (22 to 24°C) | | 115 | 65 | 110 | | 98 | | |
| Free choice (22 to 24°C) | | 225 | 65 | 194 | | 184 | | |
| Free choice (22 to 24°C) | | 225 | 115 | 219 | | 219 | | |
| Free choice (22 to 24°C) | | 280 | 225 | 234 | | 234 | | |
| Free choice (22 to 24°C) | | 320 | 280 | 284 | | 280 | | |
| Chapter 3 (Broiler) | | | | | | | | |
| Free choice (18.5 to 21°C) | 215 | 300 | 151 | 180.1 | 170.7 | | | |
| Free choice (25.2 to 31.5°C) | 215 | 300 | 151 | 195.2 | 188.2 | | | |
| Chapter 4 (Kampung chicken) | | | | | | | | |
| Free choice (21.8 to 35.4°C) | 216 | 304 | 142 | 185.7 | 183.0 | 180.3 | 175.4 | 142.5 |

### *Growth Potential*

Broilers have been selected for several decades (Havenstein et al., 2003) while Kampung chickens have not been deliberately selected for growth potential (Argono and Iskandar, 2005). Therefore, there are considerable differences in the potential for growth between these two chicken strains. Feed intake also varies accordingly. It can also be expected that the slow-growing birds will choose a lower ratio of protein to total feed than fast-growing birds. This is logic because the latter eat more feed for synthesis of body protein gain. As the protein requirements for growth are much higher than the need for maintenance, protein requirement in the diet will increase with protein gain.

Broilers kept at NT selected their diet to contain 59% from the high-energy diet, 25% from the control diet and 16% from the high-protein diet. This resulted in an overall CP and ME content in the selected diet of 190 g/kg and 3,084 kcal/kg, respectively. Broilers kept at HT selected choice diet to contain 52% from the high-energy diet, 27% from the control diet and 20% from the high-protein diet. This resulted in an overall CP and ME content in the selected diet of 198 g/kg and 3,054 kcal/kg, respectively. Some differences in the chosen diet can occur when temperatures are high or low. The Kampung chickens composed their diet by selecting 47% from the high-energy diet, 33% from the control diet and 20% from the high-protein diet. This means that the overall CP and ME content in the selected diet was 185 g/kg and 3,186 kcal /kg, respectively. So, indeed the Kampung chickens did select a diet with numerically lower CP compared to broilers. Shariatmadari and Forbes (1993) compared layers with broiler cockerels and also found a significantly lower ratio of CP to total feed intake in the slow-growing layers. The result reported here also confirmed the observation by Brody et al. (1984) who found that broilers selected for high BW gain consumed more protein than those selected for low BW gain when given a choice of high-protein and low-protein diets compared with the intake when given a complete control diet. It can be concluded that the selection from complete, high-protein and high-energy diets to match the protein and energy requirements are different between genetic strains of chickens.

***Ambient Temperature***

Feed intake of birds fed a single complete diet is depressed as a result of an increase in ambient temperature above the thermoneutral range (Cheng et al., 1997; Koh and MacLeod, 1999a). When the birds are allowed to choose between diets differing in composition, the intake would change towards the intake of those nutrients that are most needed and at the same time reduce the intake of those nutrients that yield excessive heat during digestion and metabolism (and in this way reducing the heat load). The evidence from the literature that birds are able to select a proper mix of dietary ingredients to compose a diet that reduces heat load at HT is scarce and conflicting.

Choice-fed birds were found to consume significantly lower amounts of CP at NT (15%) and HT (13%) than control-fed birds that had no choice. They consumed, however, significantly more energy at NT (9.9%) and had a numerically higher energy intake at HT compared with birds fed a complete control diet. These findings suggest that the birds somehow prefer to consume the nutrient that provides a low heat increment at all temperatures. The higher energy intake at NT also suggests that the energy level provided in the complete control diets, is lower than what is preferred by the birds. A high energy intake at HT in the present study may support the hypothesis that high level energy densities under high temperature conditions are preferred to reduce the heat load (Balnave and Brake, 2005). The CP concentration in the selected diet at HT was higher than at NT, but this seemed insufficient to prevent a reduction in performance caused by HT. In choice feeding studies at HT with diurnally cyclical-temperature between 25$^{o}$C and 35$^{o}$C, broilers preferred to consume less protein and more energy compared to a complete diet (Sinurat and Balnave, 1986). Also, Japanese quail preferred to consume more energy and less protein if they were offered a choice between diets compared to a single complete diet between 20$^{o}$C and 35$^{o}$C (MacLeod and Dabutha, 1997). The results here confirm these observations. The lower intake of protein at HT can be explained by the fact that the birds try to avoid a high heat increment from protein at HT indicating that birds are able to adjust the intake of certain nutrients.

## Wet Feeding

In many parts of the world, particularly in warm tropical regions, subtropical regions, the southern part of Europe and during summer time in temperate climates, HT can have detrimental effects on broilers production. This is especially the case when the birds are approaching market weight. In contrast to studies in broilers, the effects of HT on performance of tropical Kampung chickens have been studied much less. The effects of HT on broiler production are mediated by a high level of heat production and thus animals will reduce feed intake. The high heat production of broilers is a result of genetic selection for rapid growth which is accompanied by a high metabolic rate (Havenstein et al., 2003). Reduced feed intake under heat stress conditions may lead to a low intake of essential nutrients. Increasing feed intake however will increase heat production (Koh and MacLeod, 1999a). To ensure a high feed intake with relative less heat production under heat stress, a wet feeding strategy may be advantageous. It may be assumed that a wet diet provides a lower peak in heat production as well as a lower heat increment due to a more effective digestion. Forbes (2003) suggested that wet feeding could be useful to reduce some of the adverse effect of HT on feed intake and growth of broiler chickens.

So far, to the best of our knowledge, there has been no study done using a wet diet with different protein and energy content and which uses different breeds of chickens at HT conditions as reported in Chapters 5 and 6. A summary of the data on feed intake and BW gain of broilers and Kampung chickens fed with different diet formulations and diet conformations are presented in Table 2.

It can be seen in Table 2 that broilers fed a control diet (Chapter 5 and 6) clearly had a higher feed intake at NT than when fed a HE diet. At HT, broilers fed a wet-HE diet had a higher feed intake than those fed a dry-HE diet: the feed intake from the HE diet in wet form was 9.6 and 24% higher than in dry form, whereas the feed intake of the control diet in wet form was only 2.0 and 20% higher than when fed in a dry from at HT, respectively.

The most interesting result of the feed intake data of broilers is the similarity in feed intake between wet-HE diet and dry-control diet at HT in both experiments. This means that the detrimental effect of HT on feed intake can be partly compensated by wet feeding a HE diet. However, in Kampung chickens, the wet diet did not improve feed intake. Probably the need for reducing heat load is much less in Kampung chickens due to their lower feed intake (Table 2). The results presented here with broilers confirm the results of a higher feed intake with a wet diet at HT by Awojobi and Meshioye (2001), Kutlu (2001) and Awojobi et al. (2009). An adequate feed intake of birds under heat stress conditions is facilitated by providing a wet feed. This suggests that wet feeding can provide some cooling and/or will produce less heat or less high heat peaks which may prevent an increase in body temperature. Extra water in the metabolism will facilitate heat loss by means of evaporation through panting activity. In Chapters 5 and 6, panting behavior was significantly higher in broilers fed a wet diet than broilers fed a dry diet under heat stress conditions. Panting behavior in broilers was significantly higher than in Kampung chickens.

Overall, BW gain in broilers fed a HE diet was lower than for broilers fed a control diet at both temperature regimens. However, BW gain of wet-HE fed birds was similar to the BW gain of dry-control-fed birds at both NT and HT. This phenomenon was also observed when broilers were kept at tropical climatic conditions. The similarity in BW gain between control-fed at NT and wet-HE-fed birds at HT indicates that the differences in protein and energy intake by using this strategy becomes small (Table 3). It also shows that birds fed a wet-HE diet had similar CP and ME intakes as birds fed a normal CP and ME in dry form. This is particularly the case in broilers raised at tropical climatic conditions.

Under controlled room conditions, CP intake of birds fed a wet-HE diet was slightly lower than birds fed a dry-control diet, but they showed similar BW gains. This is probably compensated by the similarity in energy intake. When raised under the normal climatic conditions at Jambi Province in Indonesia, growth rate in Kampung chickens did

**Table 2**. Feed intake and BW gain of broilers and Kampung chickens fed with different diets and conformations.

| Temperature | Diet formu-lation | Diet confor-mation | Feed intake (g/bird/d) | | | Body weight gain (g/bird/d) | | |
|---|---|---|---|---|---|---|---|---|
| | | | Broiler | Broiler | Kampung chicken | Broiler | Broiler | Kampung chicken |
| | | | Chapter 5 | Chapter 6 | | Chapter 5 | Chapter 6 | |
| NT | Control | Dry | 100.0$^{a}$ | | | 69.1$^{a}$ | | |
| | | Wet | 105.6$^{a}$ | | | 71.5$^{a}$ | | |
| | HE | Dry | 90.5$^{b}$ | | | 61.7$^{b}$ | | |
| | | Wet | 86.8$^{bc}$ | | | 69.2$^{a}$ | | |
| HT | Control | Dry | 88.2$^{bc}$ | 70.2$^{b}$ | 32.3$^{a}$ | 58.5$^{bc}$ | 31.6$^{b}$ | 9.5$^{a}$ |
| | | Wet | 90.0$^{b}$ | 84.2$^{a}$ | 32.0$^{a}$ | 57.4$^{bc}$ | 39.6$^{a}$ | 10.1$^{a}$ |
| | HE | Dry | 76.2$^{d}$ | 59.2$^{c}$ | 28.4$^{b}$ | 49.0$^{d}$ | 25.7$^{c}$ | 9.1$^{a}$ |
| | | Wet | 83.5$^{c}$ | 73.4$^{b}$ | 26.9$^{b}$ | 54.5$^{c}$ | 31.3$^{b}$ | 9.0$^{a}$ |

**Table 3**. CP and ME intake of broilers and Kampung chickens fed with different diet formulations and diet conformations.

| Temperature | Diet formu-lation | Diet confor-mation | CP intake (g/bird/d) | | | ME intake (kcal/kg/bird/d) | | |
|---|---|---|---|---|---|---|---|---|
| | | | Broiler | Broiler | Kampung chicken | Broiler | Broiler | Kampung chicken |
| | | | Chapter 5 | Chapter 6 | | Chapter 5 | Chapter 6 | |
| NT | Control | Dry | 21.5$^{b}$ | | | 289.5$^{ab}$ | | |
| | | Wet | 22.7$^{a}$ | | | 305.7$^{a}$ | | |
| | HE | Dry | 17.6$^{d}$ | | | 277.5$^{bc}$ | | |
| | | Wet | 16.9$^{de}$ | | | 266.4$^{cd}$ | | |
| HT | Control | Dry | 18.9$^{c}$ | 15.1$^{b}$ | 6.9$^{a}$ | 255.2$^{d}$ | 206.6$^{b}$ | 94.9$^{a}$ |
| | | Wet | 19.3$^{c}$ | 18.1$^{a}$ | 6.9$^{a}$ | 260.5$^{d}$ | 247.7$^{a}$ | 94.2$^{a}$ |
| | HE | Dry | 14.8$^{f}$ | 11.5$^{c}$ | 5.1$^{b}$ | 233.0$^{e}$ | 181.2$^{c}$ | 90.4$^{a}$ |
| | | Wet | 16.3$^{e}$ | 14.3$^{b}$ | 4.9$^{b}$ | 256.2$^{d}$ | 225.0$^{b}$ | 85.5$^{a}$ |

Means within a column without a common superscript letters (a-c) differ significantly (*P* < 0.05).
HE = high energy; NT = normal temperature; HT = high temperature. For the actual temperature ranges and nutrients composition of all experimental diets, see Chapters 5 and 6.

not differ when fed a wet or dry diet. Therefore, it is beneficial to exploit wet feeding at HT with broilers especially in conjunction with HE diet.

The higher BW gain of the wet-fed broilers in this study agrees with the finding from earlier studies in broilers fed wet mash of Yalda and Forbes (1995, 1996), Yasar and Forbes (1999), Awojobi and Meshioye (2001), Kutlu (2001), and Khoa (2007) and a study with wet pellets by Moritz et al. (2001). Adding water to the feed reduces digesta viscosity (Yasar and Forbes, 2000), stimulates pre-digestion and may also facilitate absorption. These positive effects of a wet diet are possibly due to a faster penetration of digestive enzymes into feed particles than with the ingestion of dry food. As a result, rate of digestion and also digestibility may be enhanced (Forbes, 2003; Dei and Bumbie, 2011). The later, however, remains to be proven. In addition, the energy required for the digestive purposes may be somewhat lower. Thus the wet diet could reduce the adverse effect of high temperature on feed intake and growth, especially in broilers.

Irrespective of temperatures, broilers that could only eat a wet diet did not increase feed intake at different diet formulations. Broilers that could only eat a wet HE diet however had a higher BW gain and lowered FCR (Table 4) compared to a dry diet. Therefore, there is potential for reducing the protein content in the diets when birds are fed with a wet feeding system. Our results confirm the potential that exists for reducing the protein content of feeds if these are fed in wet systems (Forbes, 2003).

**Table 4**. Performance of broilers from 1 to 6 weeks fed different diet formulations and diet conformations.

| Diet | Conformation | Feed intake (kg) | BW gain (kg) | FCR |
|---|---|---|---|---|
| Control | Dry | 3.95[a] | 2.68[a] | 1.47[a] |
| | Wet | 4.11[a] | 2.71[a] | 1.52[a] |
| High energy | Dry | 3.50[b] | 2.33[b] | 1.51[a] |
| | Wet | 3.58[b] | 2.60[a] | 1.38[b] |

Means within a column without a common superscript differ significantly ($P < 0.05$). FCR= feed conversion ratio.

Total water intake at HT was similar to that at NT as shown by the results of studies in Chapters 3 and 5. Water intake was more determined by the amount of feed intake and crude protein intake than by temperature. This is in agreement with data of Marks and Pesti (1984), who reported that water intake increased with increasing dietary protein level.

A wet diet did not only increase feed intake but also water intake at both temperatures. The higher water intake with wet feeding was contributed to the extra water intake due to the wet diet. The advantages of this water intake at HT are facilitating the release of heat from the body and trigger the birds to adapt at HT by panting behavior. This is particularly seen in broilers rather than in Kampung chickens. So the performance of broilers at HT can be increase by wet feeding.

## Gastrointestinal Tract Development

Changes in feed management such as adding water to a diet are well known for their positive effects at NT on GIT development compared to birds fed a dry feed (Yasar and Forbes, 2000; Moritz et al., 2001; Shariatmadari and Forbes, 2005; Khoa, 2007). At HT, however, the effects of wet diets on GIT development have hardly been studied. Awajobi and Meshioye (2001) reported that gizzard weights were higher in broilers fed a dry diet compared to birds fed a wet diet.

In the study reported in Chapter 5, the relative lengths and empty weights of each segment of GIT at day 21 were not affected by temperature. At day 42, however, relative lengths of GIT segments were shorter in birds kept at NT than in birds kept at HT. The shorter lengths of these segments are certainly related to the higher BW of birds housed at NT. Previous findings also reported that the jejunum of birds housed at NT was relatively shorter than the jejunum of birds housed at HT at 42 d of age (Garriga et al., 2006). No large positive effects of a wet diet in the empty weights of GIT segments were found in the present work. It seems that a wet diet cannot fully reduce the adverse effect of high temperature on GIT development. These results agree with observation in wet-fed broilers during the wet season under tropical climate conditions that relative weight of gizzard, intestine and the proventriculus were similar in size compared with dry-fed birds (Awojobi et al., 2009).

A positive effect of feeding a wet diet was seen in later life of the broilers when they were kept at the same high ambient temperature, as reported in Chapter 6. A better development of intestinal segments in later life by wet feeding at high ambient temperature may guarantee more area available for absorbing nutrients. That will also be beneficial for high feed intake and BW. However, relative length of GIT in broilers fed a wet diet was shorter than birds fed a dry diet. This indicates that the development of this organ was not proportionally with the development of BW. The opposite was true for Kampung chickens.

The empty weight of gizzard was higher in dry fed birds than in wet fed birds, and the difference was more pronounced in the birds fed a HE diet. This is in agreement with Khoa (2007) who reported that the combined empty weight of the proventriculus together with gizzard in dry-coarse fed birds was higher than for wet-coarse fed birds. The heavier gizzard was also found in dry fed broilers under tropical climate (Awojobi and Meshioye, 2001). An explanation for this observation could be that the feed with the added water resulted in softer ingredient particle. The softening of ingredient particles in a wet diet may require less work for the gizzard to grind the feed particles compared to birds fed a dry diet.

## Effects of Chronic Heat-Stress on Body Temperature and Heterophil/ Lympochyte Ratio

There are differences between breeds and strains of poultry in response to a high ambient temperature (Gowe and Fairfull, 2008). Reports indicate that fast-growing broilers are more susceptible to heat stress than slow-growing strains (Berrong and Washburn, 1998; Gowe and Fairfull, 2008). Indigenous breeds of chickens have been considered to possess some tolerance and adaptation capacity to high ambient temperatures (Gowe and Fairfull, 2008). Two stress indicators were studied in poultry in the present work: body T and heterophil/lymphocyte (H/L) ratio.

High temperature resulted in a significantly higher body T and an increased H/L ratio in the blood compared to broilers raised at NT. Yahav (2000) reported that body T of broilers at HT was significantly higher compared to broilers housed at low temperature. Puvadolpirod and Thaxton (2000a) also found a significantly higher H/L ratio in broilers that were continuously exposed to adrenocorticotropin (ACTH). Neither self-selection nor wet feeding strategies resulted in lower body T in broiler and Kampung chickens and lower H/L ratio in broilers at HT. It seems that a high water intake when feeding a wet diet at HT was just sufficient to facilitate heat dissipation by means of evaporation. This idea is supported by the higher percentage of time spent on panting by wet-fed bird compared to dry-fed birds.

Body T and H/L ratio can be used to indicate the production level of the bird. At moderate temperature conditions, the heat can be lost relatively easily from the body. Therefore, the birds have relatively little difficulty to dissipate their body heat and can still increase feed intake and heat production while maintaining normal body T. At HT, however, the heat load in birds is derived partly from the environment in addition to the heat increment from feed digestion and nutrient metabolism. Therefore, under these conditions the birds will reduce their feed intake to avoid a further increase in heat production. This adaptation results in a lower production performance. In addition, at HT, additional copying strategies are employed such as panting, stand with their wings drooped and lifted slightly from the body which require additional energy which cannot be used for BW gain. When the birds have a chance to dissipate the heat at HT e.g. wet feeding strategy and therefore become less stress, they will increase feed intake and performance.

## Practical Implications

Based on the results of this thesis, it can be concluded that birds are able to adjust the protein and energy requirements by selecting diets that are different in protein and energy contents. Broilers kept at HT adapt to heat stress by lowering their feed intake and body weight. But this adaptation means that production will also be less. These adaptations contribute to the survival of the birds.

Nutritional information in feeding commercial broilers at temperate regions is available from several sources, including NRC (1994), breeder companies' information, technical leaflets, research institutions and feed manufacturers. However, specific nutritional information for hot regions is far from complete.

When chickens have an opportunity to select from a set of diets differing in protein and energy concentrations, they respond similarly under NT and HT. They will choose a diet with a high energy content both at NT and HT. The preferred energy level in the diet however, differs between NT and HT and this preference depends on age. The increased consumption of the energy-rich diet may continue to increase until 6 weeks of age at NT but only until 3 weeks of age at HT. Based on the studies reported here, it can be concluded that the NRC (1994) recommendations do not completely match with the nutrient levels that birds would choose when several diets differing in nutrient composition and energy concentration are provided.

To enable an appropriate growth of broilers at NT and HT, it is save to recommend a feed with a dietary protein and energy level below NRC (1994) recommendations. However, energy contents of feed for Kampung chickens are in accordance with NRC

(1994) recommendations (Table 5). It should be noted that, the protein content in the control diet (formulated on the basis of Dutch practice in 2007) used in the present studies was 7% above and energy content 8.8-9.5% below NRC (1994) recommendations. This may mean that the NRC (1994) recommendations for dietary energy content are close to what the animal would chose.

**Table 5**. Indication of protein and energy adjustments for increased performance of male broilers and Kampung chickens relative to NRC (1994) recommendations, as obtained from this thesis.

| Age (wk) | NRC (1994) | | Syafwan et al. (2012) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Normal Temperature | | High Temperature | | Tropical climate | |
| | CP (%) | ME (kcal/kg) | CP (%) | ME (kcal/kg) | CP (%) | ME (kcal/kg) | CP (%) | ME (kcal/kg) |
| | Broilers | | Broilers | | | | Kampung chickens | |
| 1 | 23 | 3,200 | 21.4 | 2,990 | 21.8 | 2,976 | 21.2 | 3,071 |
| 2 | 23 | 3,200 | 21.0 | 3,005 | 19.7 | 3,056 | 20.1 | 3,095 |
| 3 | 23 | 3,200 | 18.9 | 3,086 | 18.8 | 3,091 | 18.3 | 3,142 |
| 4 | 20 | 3,200 | 18.0 | 3,122 | 19.5 | 3,071 | 18.6 | 3,224 |
| 5 | 20 | 3,200 | 17.4 | 3,144 | 20.2 | 3,047 | 19.3 | 3,194 |
| 6 | 20 | 3,200 | 17.1 | 3,157 | 18.8 | 3,086 | 18.3 | 3,212 |
| 7 | | | | | | | 17.8 | 3,212 |
| 8 | | | | | | | 17.9 | 3,208 |
| 9 | | | | | | | 18.0 | 3,207 |
| 10 | | | | | | | 17.5 | 3,212 |
| 11 | | | | | | | 17.5 | 3,214 |
| 12 | | | | | | | 14.3 | 3,218 |

For farmers with insufficient knowledge regarding feed formulation, it is save to feed the birds by a self-selection strategy. As such these farmers only need to feed their chickens by providing several feed ingredients during the day, perhaps in different feed troughs.

In this thesis, it is clear that performance is adversely affected when ambient temperatures are above 21$^{o}$C. Increased dietary energy density above the energy level in the control diet did not improve performance at HT. However, adding water to feed at a 1:1 ratio improved broiler performance and GIT development in grower phase of life at either NT or HT. The beneficial effects of a wet diet on improving feed-to-gain ratio are more pronounced at low than at high temperatures. The wet diet effects were also positive with HE diet at either temperature. In Kampung chickens, feeding a wet diet did not show any major effects on performance. Wet feeding can be easily applied in small-scale broiler farms by using family workers. The wet feeding can also be used in a large-scale broiler farms or industries although special equipment is required such as an automatic mixing device and wet feed transportation equipment.

## Conclusions

1. Broiler and Kampung chickens are able to compose an appropriate diet by selecting more from an energy-rich diet than from a protein-rich diet.
2. Broiler continued to consume a higher proportion of an energy-rich diet with increasing age at normal temperature, but reached a plateau at 3 weeks of age at high temperature.
3. Broilers adapted to chronic heat stress effectively. They decreased their feed intake and consumed relatively less protein and more energy in a choice feeding situation. Low feed and protein intake will help them to survive due to low heat production.
4. The negative effects of high temperature on feed intake and BW gain of broilers increases with age.
5. Adding one part of water to one part of dry diet improved broilers performance at either temperature, especially with a high energy to protein diet.
6. Feeding a wet diet did not reduce body temperature and heterophil/lymphocyte ratio of chickens at high temperature.
7. Feeding a wet diet did not improve the performance of Kampung chickens under hot tropical conditions.
8. Metabolizable energy intake per g body weight gain was lower in broilers than in Kampung chickens.
9. An increase in body weight gain of broilers was not accompanied by an increase in gastrointestinal tract development in later age.

**Suggestions for further research**

1. The thermal comfort zone of Kampung chickens needs to be determined.
2. Energy and protein including amino acid requirements of broilers and Kampung chickens raised at high temperatures should be investigated.
3. The size of tissue layers in the proventriculus and gizzard, as well as the height of villi, depth of crypts and thickness of tunica muscularis of intestinal tissues in broilers and Kampung chickens fed wet diets at high temperature should be investigated; in other words: effects of wet feeding on gastrointestinal tract development need to be studied.
4. The water content in excreta and litter of poultry fed wet diets at HT should be investigated.
5. Nutrient digestibility of wet diet fed to poultry at HT should be investigated.
6. Temperature is an important factor determining the performance of broilers. Therefore, it is crucial to report the ambient temperatures that have been used in any experiment published in peer reviewed journals or used to derive feeding tables.

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SUMMARY

Worldwide poultry meat production has increased fast between 1970 and 2005. However, poultry meat production systems and market shares vary considerably between continents. North and Central America and Europe have lost some share of the world market in the period 1970 to 2005. Asia and South America have increased their relative contribution to global production considerably. In 2005, the latter contributed almost 50% to the global poultry meat production, whereas their contribution was less than 24% in 1970. In 2005, five of the ten leading countries were developing countries and four were located in Asia. The United States were still the largest producer with a share of 22.9%, but China, Brazil and Indonesia are now ranked number two, three and nine.

In Western European countries, the meat-type birds are mostly kept in confined systems in temperate zones. They show high feed intakes and thus high metabolic rates. They are able to regulate their heat balance relatively well and do not spend much energy on activity. In many other parts of the world, however, particularly in warm tropical and subtropical regions as well as in the southern part of Europe, poultry are kept in (semi)-intensive-outdoor-systems. In these systems, high ambient temperatures can have detrimental effects on production efficiency because high temperature (HT) reduces feed intake.

A reduction in heat production at high ambient temperatures (T) can be obtained by decreasing the level of heat production, by changing the heat production pattern within a day or by increasing the possibilities to dissipate the heat load. To reduce the heat production at high ambient T a specific feeding strategy can be used e.g. by feed components which produce less heat. Diurnal feeding patterns, self-selection and wet feeding are the most promising ways to reduce the adverse effects of heat stress. Chickens fed restricted for 2 h prior to a hot period of the day gained 2.8% more BW and showed a lower heterophil/lymphocyte ratio than ad libitum fed heat-stressed birds. Self-selection allows the birds to regulate their protein and energy intake to adjust the heat production. Wet diets may facilitate the dissipation of the heat load.

Despite high ambient temperatures, our lack of knowledge concerning nutrient requirements of birds at HT is a basic constraint for productivity of broilers and for indigenous chickens. In general, most diets for chickens are nowadays formulated with feeding table values based on trials carried out under temperate conditions. These table values may not provide the optimal diet for the birds at HT. Experiments described in

this thesis were designed to address how birds subjected to chronic heat stress select nutrients, especially crude protein and energy. From that a feeding strategy can be designed to alleviate the adverse effects of heat stress.

The main objective of this study was to investigate how male broilers and unsexed Kampung chickens reared at HT select nutrients from a set of diets which differ in protein and energy contents. It was hypothesized that animals are able to compose a diet which is best suited to their needs. From the chosen composition appropriate protein and energy requirements can be derived to optimize production and limit the adverse effects of heat stress on performance.

In Chapter 2, a literature review is provided based on experiments reported in literature. There is evidence that both wild and domesticated fowl can choose their nutrient intake by selecting from various feed ingredients and compose a diet that matches their physiological requirements. In this way, they are also able to reduce heat production during the hot period of the day. The bird will try to maintain its body T and dissipate excess metabolic body heat. The birds can change behavior and physiologically adjust it at both normal T (NT) and high temperature (HT). At NT, birds increase feed consumption, mostly from energy rich diets. At HT, birds decrease feed intake, move away from each other, apply split wing feather alignment and increase panting to dissipate heat production.

In the first experiment described in Chapter 3, a self-selection study was conducted to determine the crude protein (CP) and energy requirements of broilers at NT and HT. At each temperature, control birds were fed a standard control diet (CP: 215 g/kg; ME: 2,895 kcal/kg) formulated for broiler chickens during the grower period. The choice-fed birds could choose between the control diet, a high-protein diet (CP: 299 g/kg; ME: 2,780 kcal/kg) or a high-energy diet (CP: 150.7 g/kg; ME: 3,241 kcal/kg). The diets had a similar pellet size and color. The choice-fed birds consumed most feed from the energy-rich diet (59% at NT and 52% at HT). The amount chosen as second choice was the control diet (25% at NT and 27% at HT), and the third choice was the high-protein diet (16% at NT and 20% at HT) as a proportion to total feed intake.

High temperature decreased feed intake, protein intake, energy intake and BW gain. Within temperature, feed intake and BW gain were similar between control- and choice-fed birds. Protein intake was higher for control-fed birds than for choice-fed birds. Energy intake was lower for the control-fed birds than for the choice-fed birds but at NT only. The ratio of feed-to-gain of broilers at HT was higher than at NT at 6 weeks of age (1.91 vs. 1.84) due to less BW gain. The ratio of feed-to-gain was similar between control- and choice-fed birds.

In this experiment, body T and heterophil/lymphocyte ratio were determined to study the effects of choice feeding on stress indicators. It was assumed that the stress level of choice-fed birds would be lower than control-fed birds because they have an opportunity to adjust their heat production. No differences in body T or heterophil/lymphocyte ratio between the choice-fed birds compared to the control-fed birds was found. Apparently, a choice feeding strategy does not help to reduce the effects of high ambient temperature on body T and heterophil/lymphocyte ratio or the stress level was not sufficiently high.

The preferences of birds to different diets resulted in lower protein and higher energy concentrations in the chosen diet than the control diet at both temperatures. At NT, the CP and energy contents in the chosen diet decreased from 214 to 171 g/kg and increased from 2,990 to 3,157 kcal/kg, respectively. At HT, the CP and energy contents decreased from 218 to 188 g/kg and increased from 2,976 to 3,086 kcal/kg, respectively.

In the second experiment reported in Chapter 4, the same self-selection study was conducted with indigenous Kampung chickens under tropical climatic conditions at Jambi Province, Sumatera, Indonesia in an intensive cafeteria system. The T ranged from 25.6±1.0$^{o}$C to 31.2±1.4$^{o}$C and the RH ranged from 63±8% to 89±5%. The control animals were fed with the standard control diet for broiler chickens. The choice-fed birds could choose from a) control diet, b) a high-protein diet or c) a high-energy diet. Birds had free access to each diet in separate feeding troughs. Diets were offered as mash from 1 to 21 d of age: control diet (CP: 233 g/kg; ME: 2,999 kcal/kg), a high-protein diet (CP: 290.6 g/kg; ME: 2,895 kcal/kg), or a high-energy diet (CP: 138 g/kg; ME: 3,258 kcal /kg), and as crumbles from 22 to 84 d of age: control diet (CP 216 g/kg; ME: 3,104 kcal/kg), a high-protein diet (CP: 303.6 g/kg; ME: 3,072 kcal/kg), or a high-energy diet (CP: 142.4 g/kg; ME: 3,293 kcal/kg).

The choice-fed birds consumed about 47% feed from the energy-rich diet, 33% from the control diet and 20% from the high-protein diet as a proportion of total feed intake. Feed intake and ME intake were similar between the two dietary treatments. Protein intake was 22% higher in control-fed birds than in choice-fed birds. BW gain was 20% higher in control- than in choice-fed birds. Feed conversion ratio (FCR) was 18% lower in control- than in choice-fed birds. Based on the results of this study, the crude protein concentration was lower and the energy concentration was higher in the chosen diet than in the control diet. When the ME/CP ratios in the diet were considered, the protein and the energy concentration in the choice diet eaten by the birds varied from 212 to 145 g CP/kg and from 3,071 to 3,217 kcal of ME/kg between 1 to 12 wk of age. The ratio of feed-to-gain increased with increasing age in all dietary treatments. However, the increase was more pronounced in choice-fed Kampung chickens from week 10 onwards. Body T was similar between the two dietary treatments.

In the third experiment reported in Chapter 5, dry diets were diluted 1:1 with water to obtain so-called wet diets. In addition, a high energy to protein ratio (HE) diet was investigated as compared to a control diet. This HE-diet was formulated based on the choices of the birds in the previous choice feeding experiment (Chapter 3). It was hypothesized that birds will eat more of a wet diet at HT and this may partly alleviate the negative effects of HT. At NT, the wet-control-fed birds had a somewhat higher feed intake, and similar BW gain and FCR compared to the dry-control-fed birds. The wet-HE-fed birds had a lower feed intake, a higher BW gain and a lower FCR than the dry-HE-fed birds. At HT, the wet-control-fed birds had a higher feed intake and a lower BW gain which resulted in a higher FCR. On the other hand, the wet-HE-fed birds had a higher feed intake and BW gain than the dry-HE-fed birds. Water intake and water-to-feed ratio were higher in control- and wet-fed birds than HE- and dry-fed birds. Potential positive effects to improve the broilers performance with wet diet at both NT and at HT are discussed.

Temperature did not significantly affect gastrointestinal tract (GIT) development at d 21 except for empty weight of the ileum. At d 21, the relative lengths of all GIT segments and empty weight of gizzard were mainly affected by diet formulation. The relative lengths of duodenum, jejunum, ileum, caeca and colon of control-fed birds were 11 to 22% shorter than those of HE-fed birds. The relative empty weight of the gizzard of control-fed birds was 12% lower than that of HE-fed birds. Diet conformation only affected relative length of the duodenum and jejunum.

At day 42, temperature and diet formulation were major factors affecting relative lengths of the GIT. The relative lengths of jejunum, ileum and colon of birds housed at NT were shorter than those of birds housed at HT. The empty weights of proventriculus, duodenum, jejunum, ileum and colon at NT were between 9 and 23.6% heavier than at HT. Diet formulation affected relative lengths of most GIT segments and empty weight of the gizzard. The relative lengths of duodenum, jejunum, ileum and colon of control-fed birds were about 5% shorter than those of HE-fed birds. Temperature and diet formulation in this study were the most crucial factors determining intestinal development rather than diet conformation.

In the fourth experiment (Chapter 6), it was investigated whether and how a wet diet and/or a HE diet would affect performance of both slow-growing indigenous chickens (Kampung chickens, Experiment 1) and fast-growing chickens (broiler, Experiment 2) under tropical climatic conditions. Feed intake of the control-fed birds was 14% higher than of the HE-fed birds for both experiments.

In Experiment 1, feed intake was decreased by 0.6 and 5.3% in wet-control-fed birds and wet-HE-fed birds as compared with the respective dry-fed-birds. In Experiment 2, feed intake was increased by 19.6 and 24.3% in wet-control-fed birds and wet-HE-fed birds as compared with the respective dry-fed-birds. Feed intake was increased by a wet diet especially for broilers, and the increase was more pronounced with a wet-HE diet. The results of these studies confirm that a high feed intake can be reached by a wet diet at HT in broilers. Broilers apparently cope at HT by consuming extra water and ME with the wet HE diet. Kampung chickens appeared to have been acclimatized to tropical climatic conditions and did not make use of a wet diet.

The final Chapter provides a discussion (Chapter 7) of the results reported in the Chapter 2 to 6. Prospects for further research are suggested and practical implications for feeding strategies of indigenous and broiler chickens are presented.

In conclusion, the studies described in this thesis clearly show that broiler and Kampung chickens are able to compose an appropriate diet by selecting more from an energy-rich diet than from a protein-rich diet, both at NT and HT. Moreover, the negative effects of a high ambient temperature on feed intake and BW gain of broiler chickens can be partly alleviated by wet diets. This effect was not observed in Kampung chickens.

# SAMENVATTING

De productie van pluimveevlees in de wereld is zeer sterk toegenomen tussen 1970 en 2005. Deze toename verschilt zeer sterk tussen de verschillende continenten van de wereld omdat het productiesysteem en de markt zeer verschillend is. Sinds 1970 hebben Europa en Noord en Centraal Amerika een kleiner aandeel gekregen in de wereldmarkt van pluimvee. Azie en Zuid Amerika daarentegen hebben hun aandeel in de wereldproductie aanzienlijk vergroot in deze periode. In 2005 was de bijdrage van Zuid Amerika aan de wereldproductie ongeveer 50%. Ter vergelijking in 1970 was het aandeel minder dan 24%. In 2005 waren 5 van de 10 landen met de grootste productie ontwikkelingslanden en vier daarvan liggen in Azie. De Verenigde Staten hadden in 2005 nog steeds de grootste productie met een wereldaandeel van 22,9%, maar China, Brazilie en Indonesie waren nummer twee, drie en negen.

In West Europa wordt pluimvee dat bestemd is voor de vleesproductie meestal in speciale huisvestingsystemen gehouden. De vleeskuikens hebben een zeer hoge voeropname en een zeer snelle groei. Als gevolg hiervan is hun warmteproductie zeer hoog. Dit betekent echter ook dat ze hun warmteafgifte goed moeten kunnen reguleren vooral bij hoge omgevingstemperaturen. Bovendien spenderen ze niet al te veel energie aan fysieke activiteit. In veel streken van de wereld en speciaal in de tropen en subtropen zoals in Zuid Europa wordt pluimvee vaak gehouden in (semi) intensieve systemen en soms in de buitenlucht. In deze systemen kunnen hoge luchttemperaturen sterk negatieve effecten hebben op productieresultaten.

Een verlaging van de warmteproductie van de dieren bij deze zeer hoge temperaturen kan bereikt worden door de dieren minder voer te laten opnemen of voer aan te bieden en ook door de dieren meer mogelijkheden te geven tot het kwijtraken van de geproduceerde warmte. Minder warmteproductie door dieren kan bereikt worden door toepassen van een strategie die gebruik maakt van voercomponenten die minder warmte produceren. Ook kan men door voeren op andere tijden van de dag en door zelfselectie (dieren kiezen zelf uit verschillende voerbronnen) de warmtebelasting uit voer verminderen. Zelfselectie en nat voeren bleken in dit onderzoek het meest veelbelovend om negatieve effecten van een hoge omgevings-temperatuur te verminderen. Kippen die, 2 uur voorafgaand aan een hitteperiode, beperkt gevoerd werden hadden 2.8% meer groei en ze hadden ook een lagere heterophil/lymphocyte (H/L) ratio in hun bloed in vergelijking met dieren die voor en tijdens hittestress ad libitum gevoerd werden. De H/L ratio wordt wel gezien als een maat voor langdurige

stress. Zelfselectie geeft dieren de mogelijkheid om eiwit- en energieopname zelf te reguleren en zo dus ook invloed te hebben op de warmteproductie. Nat voeren kan het warmteproductie-patroon binnen de dag mogelijk veranderen en dus ook warmteafgifte faciliteren.

Ondanks het feit dat in gebieden waar veel kippen worden gehouden vaak hoge omgevingstemperaturen voorkomen is onze kennis over de voedingsbehoefte bij hoge omgevingstemperaturen nog verre van compleet. En deze beperking in kennis draagt ook bij aan de lage productiviteit van vleeskuikens (broilers) en van lokale kippen in tropische gebieden. In het algemeen worden de meeste rantsoenen berekend op grond van tabellen die gebaseerd zijn op studies in de gematigde klimaatzones. Het is duidelijk dat de waarden in deze tabellen vaak niet het optimale dieet opleveren voor kippen bij een hoge omgevingstemperatuur. De proeven in deze dissertatie waren opgezet om na te gaan welk rantsoen de dieren zelf zouden samenstellen met betrekking tot eiwit- en energiegehalte, indien zij relatief langdurig blootgesteld zouden worden aan hoge temperaturen. De gedachte hierachter is dat de gekozen samenstelling de hittestress kan verminderen in vergelijking met controlerantsoenen.

Het hoofddoel van de studies was om na te gaan hoe mannelijke broilers en niet gesexte lokale vleeskippen (Kampung kippen in Indonesie) die gehouden werden bij hoge temperaturen (HT) hun rantsoen zouden samenstellen via keuzes uit een drietal voeders die verschilden in eiwit- en energiegehalte. De hypothese was dat dieren kunnen selecteren en zelf een optimaal dieet kunnen samenstellen om in hun behoefte aan eiwit en energie te voorzien. Een tweede hypothese die getoetst werd is dat men de door de dieren zelf gekozen samenstelling kan gaan gebruiken om een meer optimale voersamenstelling voor warme omgevingscondities te kunnen maken. En dat op deze manier wellicht een meer optimale productie bereikt worden bij hittestress.

Men heeft aangetoond dat zowel gedomesticeerde als niet gedomesticeerde kippen ook bij hittestress een goed voer kunnen samenstellen door te selecteren uit een veelheid van voer ingredienten. En deze gekozen voersamenstelling voldoet aan hun fysiologische behoefte. De verwachting is dat een voer dat op deze manier is samengesteld een wat lagere warmteproductie bij de dieren zal geven tijdens de heetste periode van de dag.

De kip als homeotherm dier zal de lichaamstemperatuur binnen nauwe grenzen moeten handhaven door de extra warmte uit metabolisme via waterverdamping kwijt te raken. Een kip zal naar verwachting in de hitte ten opzichte van een normale temperatuur bij keuze meer van een voer opnemen dat veel goed verteerbare en benutbare componenten bevat en dat dan dus minder extra warmte per eenheid voer oplevert.

Met betrekking tot gedrag bij hoge omgevingstemperaturen (HT) bewegen de dieren verder weg van elkaar. Ze tonen ook minder eetgedrag, zetten vleugelveren verder uit elkaar en gaan hijgen. Dit alles om beter warmte kwijt te raken en/of er minder van te produceren. Hoofdstuk 2 bevat een overzicht van de literatuur met bevindingen uit studies op dit terrein.

Twee experimenten werden uitgevoerd waarbij de kuikens werden gehouden bij ofwel een constante lage (normale) ofwel een constant hoge temperatuur. De resultaten zijn weergegeven in de hoofdstukken 3 and 5. De omgevingstemperatuur had zoals verwacht een zeer grote invloed op de voeropname en dus ook op de groei. De voeropname daalde respectievelijk met 1.7% en 1.2% voor elke graad Celsius toename tussen 21 en 31.5$^{o}$C en tussen 21.5 en 31.7$^{o}$C.

Dit had als consequentie een afname in groei van respectievelijk 1.4% en 1.9% voor elke graad Celsius toename in beide temperatuurranges. De negatieve effecten van temperatuur op voeropname en groei werden sterker bij het ouder worden van de dieren (of een toename in gewicht). De afname in groei was sterker dan die in voeropname. Gevolg was dat de voederconversie bij HT wat minder goed was. Broilers hadden bij HT een lagere voer efficientie. Dit is logisch omdat bij een lage voeropname een groter deel van het rantsoen bestemd is voor onderhoud en er dus minder ‘overblijft’ voor eiwit- en vetaanzet.

In de eerste proef die beschreven is in hoofdstuk 3 was een zelfselectie behandeling opgenomen waarbij de dieren uit drie voersoorten konden kiezen en dus zelf hun rantsoen konden samenstellen met betrekking tot ruw eiwit (RE)- en Omzetbare Energie (ME)-gehalte (‘keuzedieren’) bij zowel normale temperatuur (NT; 18-24$^{o}$C) als bij HT (25-30$^{o}$C). Bij elke temperatuur was er ook een behandeling waarbij dieren een normaal standaard voer voor broilers kregen (RE: 215 g/kg; ME: 2895 kcal/kg) (‘controledieren’). De keuzedieren hadden dus drie voersoorten tot hun beschikking namelijk het controlevoer, een hoog eiwitvoer (RE: 299 g/kg; ME: 2780 kcal/kg) en een hoog energievoer (RE: 150,7 g/kg; ME: 3241 kcal/kg). Alle pellets hadden hetzelfde formaat en kleur. De keuzedieren namen het grootste deel van hun keuzevoer op van het energierijke voer (59% bij NT en 52% bij HT). Op de tweede plaats stond het controlevoer (25% bij NT en 27% bij HT) en het eiwitrijke voer maakte 16% uit van het uiteindelijk rantsoen bij NT en 20% bij HT.

De hoge temperatuur deed de voeropname, eiwitopname, energieopname en groei afnemen. Binnen temperatuur waren voeropname en groei bij controle- en keuzedieren gelijk. Bij NT was de eiwitopname hoger voor de controledieren dan voor de keuzedieren, terwijl de energieopname voor de controledieren lager was dan bij de keuzedieren. Op 6 weken leeftijd was de voederconversie (voer/groei) bij HT hoger dan bij NT (1,91 vs. 1,84) en tussen keuze- en controledieren was er geen duidelijk verschil.

In deze proef werden ook de lichaamstemperatuur en de H/L ratio bepaald om te zien of het feit dat dieren uit voerbronnen konden kiezen deze stressparameters zou beïnvloeden. De veronderstelling was dat de keuzedieren een lager stressniveau (op grond van deze beide parameters) zouden hebben dan de controledieren. We vonden echter geen verschil tussen keuze- en controledieren.

Er werd gevonden dat keuzes leidden tot een lager RE-gehalte in het opgenomen keuzevoer dan in het controlevoer bij beide temperaturen. Bij NT daalde het RE-gehalte in het keuzevoer van 214 tot 171 g/kg en steeg het energiegehalte van 2,990 tot 3,157 kcal/kg. Bij HT was er een daling in het RE-gehalte van 218 tot 188 g/kg en de energieconcentratie in het voer steeg van 2,976 naar 3,086 kcal/kg.

In de tweede proef in hoofdstuk 4 werd een soortgelijke zelfselectie proef als in hoofdstuk 3 uitgevoerd met lokale Kampung kippen onder tropische condities in de Jambi Provincie op Sumatra in Indonesie onder een intensief cafetaria systeem. De temperatuur varieerde van 25.6$^{o}$C bij aanvang van de proef tot 31.2$^{o}$C op het eind en de relatieve luchtvochtigheid (RV) steeg van 63 tot 89%. De controledieren kregen ook nu weer het standaardvoer voor broilers. De keuzedieren konden weer kiezen uit een controlevoer, een eiwitrijk voer en een energierijk voer en de keuzevoeders werden weer aangeboden in drie aparte voerbakken. Het voer werd verstrekt als meel van 0 tot 21 dagen met als samenstellingen: controlevoer (RE: 233 g/kg; ME: 2,999 kcal/kg), hoog eiwitvoer (RE: 291 g/kg; ME: 2,895 kcal/kg), en hoog energievoer (RE: 138 g/kg; ME: 3,258 kcal /kg). Vanaf 22 tot 84 dagen werd uit praktische overwegingen het voer in kruimelvorm aangeboden. De samenstellingen waren nu: controlevoer (RE: 216 g/kg; ME: 3,104 kcal/kg), hoog eiwitvoer (RE 304 g/kg; ME: 3,072 kcal/kg), en hoog energievoer (RE: 142 g/kg; ME: 3,293 kcal/kg).

De keuzedieren namen 47% van hun opname uit de energierijke voerbak, 33% uit de controle voerbak en 20% uit de hoog eiwit voerbak. De voeropname en ME opname waren gelijk bij de keuzedieren en bij de controledieren. De eiwitopname was echter

22% hoger bij de controledieren dan bij de keuzedieren. En de groei was 20% hoger bij de controledieren. Gevolg was dat de voederconversie 18% beter was bij de controledieren. Gevolg van de keuze was ook dat de ruw eiwitconcentratie in het keuzedieet veel lager was dan in het controlevoer terwijl de energieconcentratie juist weer hoger was in het gemaakte keuzerantsoen. Wanneer men het energie- en eiwitgehalte vergelijkt valt op dat het RE-gehalte in het keuzevoer veel lager is, van 212 tot 145 g RE/kg, terwijl het energiegehalte juist verhoogd is bij het keuzevoer van 3,071 tot 3,217 kcal ME/kg tijdens de groeiperiode van 1 tot 12 weken. Lichaamstemperatuur was niet verschillend tussen de dieren op de voerbehandelingen.

In het 3e experiment (hoofdstuk 5) werd voor het controle voer uitgegaan van het door de dieren zelf gekozen rantsoen bij HT uit de eerdere experimenten. De rantsoenen werden nu aangeboden in droge vorm of als brijvoer. Het is bekend dat door watertoevoeging aan het kuikenvoer ook bij NT al verbeteringen in productie kunnen ontstaan. Een brijvoer heeft volgens de literatuur een gunstige invloed op de ontwikkeling van het maagdarmkanaal. Daarbij zijn bijna alle studies uitgevoerd bij NT en met een normale voersamenstelling. Studies met een brijvoer en met een hoog energie- en laag eiwitgehalte (HE) werden in de literatuur niet gevonden. De geformuleerde hypothese testte of er een hogere opname is met brijvoeding bij HT. Als dit het geval is kan dat misschien ook (deels) de negatieve effecten van HT verminderen. Het brijvoer had toegevoegd water in de verhouding 1:1. Bij NT hadden de controledieren op brijvoer een hogere opname en een gelijkwaardige groei en voederconversie als de controledieren op droog voer hadden. De kuikens die HE-voer in brijvorm gevoerd kregen hadden een lagere opname, een hogere groei en een lagere voederconversie dan de HE-dieren op droog voer. Bij HT hadden de controledieren op brijvoer een hogere voeropname en een lagere groei dan bij NT. Aan de andere kant hadden HE-dieren op brijvoer een hogere voeropname en een hogere groei dan de HE-dieren op droog voer bij HT en dus ook een lagere voederconversie. Wateropname en de water-voerverhouding waren verhoogd bij de controledieren op brijvoer t.o.v. de HE-dieren op droog voer. Dit betekent dat men met brijvoer zowel bij NT als bij HT een betere productie kan bereiken.

In deze proeven werden ook relatieve (t.o.v. het lichaamsgewicht) lengtes en gewichten van de spijsverteringsorganen gemeten. Op een leeftijd van 21 dagen had de omgevingstemperatuur geen invloed op de ontwikkeling van het maagdarmkanaal, behalve die van het ileum. Echter, de voersamenstelling beïnvloedde op die leeftijd wel de relatieve lengtes van darmsegmenten en het gewicht van de spiermaag.

De relatieve lengtes van duodenum, jejunum, ileum en ceca van de dieren op controlevoer was tussen de 11 en 22% korter dan die van de dieren op HE-voer. En het relatieve leeg gewicht van de spiermaag was bij controledieren 12% minder. Droogvoer of brijvoedering beïnvloedde alleen de relatieve lengte van duodenum en jejunum.

Op dag 42 beïnvloedde zowel de omgevingstemperatuur als de rantsoensamenstelling de relatieve lengte van het maagdarmkanaal. Met name de lengten van jejunum, ileum en colon waren langer bij HT en de relatieve gewichten van kliermaag, duodenum, jejunum, ileum en colon waren duidelijk zwaarder bij NT. Een HE-voer verhoogde de relatieve lengtes van de meeste delen van het maagdarmkanaal en het lege gewicht van de kliermaag. De relatieve lengtes van duodenum, jejunum, ileum en colon in dieren op controlevoer waren rond de 5% korter dan bij HE-dieren. Dus zowel voersamenstelling als omgevingstemperatuur kunnen darmontwikkeling beïnvloedden.

In het 4e experiment (hoofdstuk 6), is de proef met brijvoer uitgevoerd onder tropische condities in Indonesië met zowel inheemse Kampung kippen als met broilers. Er werd onderzocht of en hoe een brijvoer met een hoog energie- en laag eiwitgehalte (HE-voer) de opname en groei van zowel de langzaam groeiende Kampung kippen (experiment 1) als de snelgroeiende broilers (experiment 2) zou kunnen beïnvloeden. Bijna alle studies die in de literatuur zijn vermeld zijn uitgevoerd bij gecontroleerde temperaturen en bij snelgroeiende broilers. In de eerdere proeven in deze dissertatie werd gevonden dat dieren bij een HT een voer met een lager RE-gehalte prefereerden. Daarom werd in deze proef het eiwitgehalte verlaagd in het HE-rantsoen. Omdat Kampung kippen langzaamgroeiende dieren zijn, werd voor deze dieren een groeiperiode van 12 weken gekozen en voor broilers een periode van 6 weken.

De voeropname van de controledieren van beide typen kuikens was 14% hoger dan van de HE-dieren. Er werd verwacht dat HE-dieren een hogere opname zouden laten zien dan controledieren in termen van energie en eiwit, gezien de lagere warmteproductie met minder RE. Dat bleek dus niet zo te zijn.

Gevolg van deze invloed op de voeropname is een lagere opname van zowel energie als eiwit bij deze dieren onder tropische condities. De ME-opname per g groei bij Kampung kippen was gelijk in beide behandelingen terwijl bij broilers de ME-opname per g groei bij dieren op het HE-voer duidelijk hoger was dan op het controlevoer.

Voeropname bij Kampung kippen was afgenomen met respectievelijk 0,6% en 5,3% bij controle- en HE-dieren op brijvoer in vergelijking met dieren op deze voeders in droge vorm. Bij broilers echter was de voeropname verhoogd met 16,6% en 19,3% bij controle- en HE-dieren op brijvoer in vergelijking met de dieren op deze voeders in droge vorm. Dus wederom bij broilers meer voeropname met brijvoer speciaal bij het HE-dieet. De resultaten van deze studie laten zien dat het aanbieden van brijvoer bij HT kan leiden tot een hogere voeropname. Broilers kunnen de brijvoeding gebruiken voor extra wateropname en eventueel ook voor extra ME-opname. Kennelijk zijn de lokale kippen reeds goed aangepast en gebruiken ze deze mogelijkheid niet of veel minder.

Groei van Kampung kippen en broilers op controle brijvoer was verhoogd met respectievelijk 5,9% (10,1 vs. 9,5 g/d) en 20,3% (39,6 vs. 31,6 g/d) in vergelijking met droog controlevoer. Op HE brijvoer was de groei bij Kampung kippen en broilers respectievelijk 0,8% lager (8,98 vs. 9,05 g/d), en 18,2% hoger (31,3 vs. 25,6 g/d) dan op droog HE voer. Bovendien was er een gelijke groei bij HE-dieren op brijvoer en bij controledieren op droog voer, speciaal bij broilers. Bij broilers zijn de verschillen in RE-opname (14,3 vs. 15,1 g) en in ME-opname tussen brij- en droog HE-voer in vergelijking met controle dieren erg klein in vergelijking met dieren op droog controlevoer. Daarom kan brijvoeding bij broilers onder HT condities zoals in de tropen worden gebruikt om een meer optimale productie te behalen. De iets snellere ontwikkeling van het maagdarmkanaal in het begin van de groeiperiode is in elk geval gunstig voor een optimale vertering en absorptie van nutriënten in de dunne darm. Kippen kunnen dus baat hebben bij brijvoer, speciaal op heel jonge leeftijd. Op een leeftijd van 6 weken is het voordeel van het nat maken van het voer op voeropname en darmontwikkeling niet meer zo duidelijk aanwezig.

In hoofdstuk 7 worden de resultaten van de verschillende studies van Hoofdstukken 2 tot 6 bediscussieerd met het oog op de consequenties van zelfselectie en met het oog op de toepassing van brijvoeders bij kuikens in warme gebieden. Er worden suggesties gedaan voor verdere studies en er worden implicaties aangegeven voor voerstrategieën in de praktijk die gebaseerd zijn op de bevindingen bij broilers en bij lokale kippen.

Er kan geconcludeerd worden uit de studies van deze dissertatie dat broilers en ook Kampung kippen in staat zijn om een rantsoen samen te stellen uit een energierijk en een eiwitrijk voer zowel bij hoge als bij lage omgevingstemperatuur. Door het rantsoen als nat voer aan te bieden kunnen de negatieve effrecten van hoge temperatuur verminderd worden bij broilers echter niet bij Kampung kippen.

# RINGKASAN

Produksi daging ungas dunia meningkat pesat diantara tahun 1970 dan 2005. Akan tetapi, sistem produksi daging unggas dan jumlah penjualan sangat bervariasi diantara benua. Amerika Utara dan Amerika Pusat dan Eropa kehilangan jumlah penjualan pasar dunia dalam periode 1970 dan 2005. Asia dan Amerika Selatan meningkat kontribusinya terhadap produksi global. Pada tahun 2005, kedua negara terakhir berkontribusi hampir 50% terhadap produksi daging unggas global, sementara kontribusi mereka kurang dari 24% di tahun 1970. Pada tahun 2005, lima dari sepuluh negara terbesar dalam produksi unggas adalah negara berkembang dan empat diantaranya terletak di Asia. Amerika Serikat masih pada posisi teratas dengan penjualan 22,9%, tapi Cina, Brazil dan Indonesia berada pada peringkat nomor dua, tiga dan sembilan.

Di negara-negara Eropa bagian barat, unggas produksi daging kebanyakan dipelihara dalam sistim yang terbatas di daerah bermusim. Mereka menunjukkan konsumsi pakan yang tinggi dan juga laju metabolik yang tinggi. Mereka mampu mengatur keseimbangan panasnya dengan relative baik dan tidak banyak menghabiskan energi untuk aktivitas. Dibagian dunia lainnya, akan tetapi, khususnya di daerah tropik yang panas, daerah subtropik dan juga daerah Eropa bagian selatan, unggas dipelihara dengan sistim semi-intensif diluar kandang. Dalam sistim ini, suhu lingkungan yang tinggi dapat memberikan efek yang membahayakan terhadap efisiensi produksi karena suhu lingkungan yang tinggi menurunkan konsumsi pakan.

Penurunan produksi panas di suhu lingkungan yang tinggi dapat dilakukan dengan penurunan tingkat produksi panas, dengan perubahan pola produksi panas harian atau dengan peningkatan peluang untuk membuang beban panas. Untuk menurunkan produksi panas dalam suhu lingkungan yang tinggi strategi pemberian pakan yang khusus dapat digunakan, misalnya dengan komponen-komponen pakan yang menghasilkan panas yang rendah. Pola pemberian pakan harian, memilih sendiri dan pakan basah adalah cara-cara yang lebih menjanjikan untuk menurunkan pengaruh jelek cekaman panas. Ayam-ayam yang dibatasi makanan selama 2 jam sebelum periode panas dalam suatu hari pertambahan berat badan (PBB) naik lebih dari 2,8% dan menunjukkan ratio heteropil/limposit (H/L) yang lebih rendah dari pada ayam-ayam diberikan ad libitum dengan cekaman panas. Memilih sendiri memungkinkan ayam-ayam untuk mengatur konsumsi protein dan energinya untuk menyesuaikan produksi panas. Pakan basah dapat memfasilitasi pembuangan beban panas.

Disamping suhu lingkungan yang tinggi, kekurangan pengetahuan tentang kebutuhan nutrisi unggas di suhu tinggi adalah pembatas yang dasar terhadap produktifitas broiler dan ayam asli. Secara umum, kebanyakan pakan untuk ayam sekarang ini diformulasi dengan nilai-nilai tabel nutrisi yang didasarkan pada penelitian yang dilakukan dibawah kondisi lingkungan bermusim. Nilai-nilai tabel ini mungkin tidak menyediakan pakan yang optimal bagi ayam di suhu tinggi. Penelitian-penelitian yang diuraikan dalam tesis ini dirancang untuk menjawab bagaimana ayam-ayam dikenakan cekaman panas yang lama memilih nutrisi, khususnya protein kasar dan energy, dan merancang sistim pemberian pakan untuk mengurangi pengaruh jelek cekaman panas.

Tujuan utama studi ini adalah untuk menyelidiki bagaimana ayam jantan broiler dan Ayam Kampung (tanpa membedakan jenis kelamin) dipelihara di bawah suhu tinggi memilih nutrisi-nutrisi dari suatu seri pakan yang berbeda kandungan protein dan energy. Dihepotesakan bahwa ayam-ayam mampu untuk menyusun suatu pakan yang sangat cocok untuk kebutuhan mereka. Dari komposisi pakan yang dipilih kesesuaian kebutuhan protein dan energy dapat diperoleh untuk mengoptimalkan produksi dan mengurangi pengaruh jelek cekaman panas terhadap performans.

Dalam Bab 2, tinjauan pustaka telah diberikan berdasarkan penelitian-penelitian yang dilaporkan dalam pustaka. Terdapat bukti bahwa unggas liar dan yang sudah didomistikasi mampu memilih konsumi nutrisi mereka dengan memilih dari berbagai bahan pakan yang memenuhi kebutuhan fisiologi mereka. Dengan cara ini, mereka mampu menurunkan produksi panas selama periode panas dalam suatu hari. Unggas akan berusaha untuk menjaga suhu tubuhnya dan membuang kelebihan panas tubuh metabolik. Ayam dapat merobah tingkah laku dan menyesuaikan secara fisiologi di suhu normal dan suhu tinggi. Di suhu normal, ayam meningkatkan konsumsi pakan, sebagian besar dari pakan kaya energi, untuk menjaga suhu tubuh. Di suhu tinggi, ayam menurunkan konsumsi pakan, berjauhan satu sama lain, membuka bulu sayap dan meningkatkan laju pernafasan utnuk membuat kelebihan produksi panas.

Pada penelitian pertama yang diuraikan dalam Bab 3, studi pakan bebas memilih dilakukan untuk menentukan jumlah kebutuahn protein kasar (PK) and energi pada broiler di suhu normal dan suhu tinggi. Pada setiap suhu, ayam sebagai kontrol diberi pakan standar (PK: 215 g/kg; ME:2.895 kcal/kg) untuk broiler periode pertumbuhan. Ayam yang sebagai pakan bebas memilih dapat memilih antara pakan kontrol, pakan tinggi protein (PK: 299 g/kg; ME: 2.780 kcal/kg) atau pakan tinggi energy (PK: 150,7 g/kg: ME: 3.241 kcal/kg). Semua pakan sama dalam ukuran pelet dan warnanya. Ayam yang sebagai pakan bebas memilih mengkonsumsi kebanyakan pakan dari pakan tinggi energi (59% pada suhu normal dan 52% pada suhu tinggi). Jumlah yang dipilih sebagai pilihan ke dua adalah pakan kontrol (25% pada suhu normal dan 27% pada suhu tinggi), dan pilihan ke tiga adalah pakan tinggi protein (16% pada suhu normal dan 20% pada suhu tinggi) sebagai proporsi terhadap total konsumsi pakan.

Suhu tinggi menurunkan konsumsi pakan, konsumsi protein, konsumsi energi dan PBB. Dalam setiap suhu, konsumsi pakan dan PBB adalah sama diantara ayam kontrol dan ayam yang pakan bebas memilih. Konsumsi protein lebih tinggi pada ayam kontrol dari pada ayam yang pakan bebas memilih. Konsumsi energi lebih rendah pada ayam kontrol dari pada ayam yang pakan bebas memilih pada suhu normal saja. Rasio pakan terhadap PBB (FCR) di suhu tinggi adalah lebih tinggi dari pada di suhu normal pada umur 6 minggu (1,91 berbanding 1,84) karena penuruan PBB. FCR adalah sama diantara ayam kontrol dan bebas milih.

Pada penelitian ini, suhu tubuh dan H/L rasio diukur untuk mempelajari efek pakan memilih terhadap indikator stres. Diasumsikan bahwa tingkat stres ayam yang pakan bebas memilih mungkin lebih rendah dari pada ayam kontrol karena mereka mempunyai kesempatan untuk menyesuaikan produksi panas. Tidak ditemukan adanya perbedaan dalam suhu tubuh dan H/L rasio diantara ayam yang memilih sendiri dan ayam kontrol. Tampaknya, strategi memilih sendiri tidak banyak membantu untuk menurunkan efek suhu lingkungan yang tinggi terhadap suhu tubuh dan H/L rasio atau tingkat stresnya tidak terlalu tinggi.

Kesukaan ayam terhadap pakan yang berbeda menhasilkan konsentrasi protein yang rendah dan energy yang tinggi dalam pakan yang dikonsumsi dari pada pakan kontrol di ke dua suhu. Pada suhu normal, protein kasar berkisar dari 214 sampai 171 g/kg dan energy berkisar dari 2.990 sampai 3.157 kcal/kg. Pada suhu tinggi protein kasar berkisar dari 218 sampai 188 g/kg dan energy berkisar dari 2.976 sampai 3.086 kcal/kg antara umur 1 sampai 6 minggu.

Pada penelitian kedua yang dilaporkan dalam Bab 4, studi memilih sendiri yang sama dilakukan pada Ayam Kampung dibawah kondisi iklim tropik di Provinsi Jambi, Sumatera, Indonesia dalam sistim yang intensif. Suhu berkisar dari 25,6±1,0$^{o}$C sampai 31,2±1,4$^{o}$C dan kelembaban berkisar dari 63±8% sampai 89±5%. Ayam sebagai kontrol dapat memilih dari a) pakan kontrol, b) pakan tinggi protein dan c) pakan tinggi energi. Ayam memiliki akses secara bebas terhadap semua pakann dalam tempat yang terpisah. Pakan diberi dalam bentuk mash dari umur 1 sampai 21 hari dengan pakan kontrol ( PK: 233 g/kg; ME: 2.999 kcal/kg), pakan tinggi protein (PK: 290.6 g/kg; ME: 2.895 kcal/kg), atau pakan tinggi energi (PK: 138 g/kg; ME: 3.258 kcal/kg) dan dalam bentuk krumbel dari umur 22 sampai 84 hari dengan pakan kontrol (PK: 216 g/kg; ME: 3.104 kcal/kg), pakan tinggi protein (PK: 303,6 g/kg; ME: 3.072 kcal/kg), atau pakan tinggi energi (PK: 142,4/6 g/kg; ME: 3.293 kcal/kg).

Ayam bebas memilih mengkonsumsi sebanyak 47% pakan tinggi energi, 33% pakan kontrol dan 20% pakan tinggi protein sebagai proporsi terhadap total konsumsi pakan. Konsumsi pakan dan konsumsi energy adalah sama antara dua pakan perlakuan. Konsumsi protein adalah 22% lebih tinggi pada ayam diberi pakan kontrol dari pada ayam yang bebas memilih. PBB adalah 20% lebih tinggi pada ayam kontrol dan FCR adalah 18% lebih rendah pada ayam kontrol dari pada ayam bebas memilih. Berdasarkan hasil dari penelitian ini, konsentrasi protein kasar lebih rendah dan konsentasi energi lebih tinggi dalam pakan yang dipilih dari pada pakan kontrol. Bila rasio ME/PK dalam pakan diperhatikan, konsentari protein dan energi dalam pakan yang dikonsumsi oleh ayam bebas memilih bervariasi dari 212 samapi 145 g PK/kg dan 3.071 sampai 3.217 kcal ME/kg antara umur 1 sampai 12 minggu. FCR meningkat dengan bertambahnya umur dalam semua pakan perlakuan. Akan tetapi, peningkatan lebih tampak pada ayam yang pakan bebas memilih dari umur 10 minggu ke atas. Suhu tubuh adalah sama diantara kedua pakan perlakuan.

Pada penelitian ke tiga yang dilaporkan dalam Bab 5, pakan kering dicampurkan dengan air dengan perbandingan 1:1 untuk mendapatkan pakan basah. Sebagai tambahan, pakan tinggi rasio energi terhadap protein diselidiki dibandingkan pakan kontrol. Pakan tinggi rasio energi terhdapa protein disusun berdasarkan pilihan oleh ayam dalam penelitian pakan bebas memilih sebelumnya (Bab 3). Dihipotesakan bahwa ayam-ayam akan banyak memakan pakan basah di suhu tinggi dan hal ini dapat mengurangi sebagian pengaruh jelek suhu tinggi. Di suhu normal, ayam yang diberi pakan kontrol-basah memiliki konsumsi pakan dan PBB yang lebih tinggi dan FCR yang sama dari pada ayam yang diberi pakan kontrol-kering. Ayam yang diberi makan pakan tinggi energi-basah memiliki konsumsi pakan yang lebih rendah dan PBB yang lebih tinggi tapi FCRnya lebih rendah dari pada ayam yang diberi pakan tinggi energi-kering. Di suhu tinggi, ayam yang diberi makan pakan kontrol-basah memiliki konsumsi pakan yang lebih tinggi dan PBB yang lebih rendah. Karena itu FCRnya lebih tinggi. Dilain pihak, ayam yang diberi makan pakan tinggi energi-basah memiliki konsumsi pakan dan PBB yang lebih tinggi dari pada ayam yang diberi pakan tinggi energi-kering. Konsumsi air dan rasio air terhadap pakan lebih tinggi pada ayam yang diberi pakan kontrol dan pakan basah dari pada ayam yang diberi pakan tinggi energi dan pakan kering. Potensi efek positif untuk memperbaiki performans broiler dengan pakan basah di suhu normal dan suhu tinggi dibahas.

Suhu tidak nyata mempengaruhi pertumbuhan alat pencernaan pada umur 21 hari kecuali berat kosong relatif ileum. Pada umur 21 hari, panjang relatif semua bagian alat pencernaan dan berat kosong relatif tembolok terutama dipengaruhi oleh komposisi pakan. Panjang relatif duodenum, jejunum, ileum dan kolon ayam diberikan pakan kontrol adalah 11 sampai 22% lebih pendek dari pada ayam diberikan pakan tinggi rasio energi. Berat kosong relatif tembolok ayam kontrol adalah 12% lebih ringan dari pada ayam diberi pakan tinggi energi. Konformasi pakan hanya mempengaruhi panjang relatif duodenum dan jejunum.

Pada umur 42 hari, suhu dan komposisi pakan adalah faktor utama mempengaruhi panjang relatif alat pencernaan. Panjang relatif jejunum, ileum dan kolon broiler dipelihara di suhu normal lebih pendek dari pada broiler di suhu tinggi. Berat kosong proventriculus, duodenum, jejunum, ileum dan kolon di suhu normal berkisar 9 dan 23,6% lebih berat dari pada ayam di suhu tinggi. Komposisi pakan mempengaruhi panjang relatif kebanyakan bagian alat pencernaan dan berat kosong tembolok. Panjang relatif duodenum, jejunum, ileum dan kolon ayam kontrol adalah 5% lebih pendek dari pada ayam diberi pakan tinggi energi. Suhu dan komposisi pakan dalam penelitian ini merupakan faktor penting menentukan pertumbuhan alat pencernaan dari pada konformasi atau bentuk pakan.

Pada penelitian ke empat (Bab 6), diteliti apakah dan bagaimanakah pakan basah dan/atau pakan tinggi energi mungkin mempengaruhi performans ayam Kampung (penelitin 1) dan broiler (penelitian 2) dibawah kondisi iklim tropis. Konsumsi pakan pada ayam kontrol adalah 14% lebih tinggi dari pada ayam diberik pakan tinggi energi pada kedua penelitian tersebut.

Pada penelitian pertama, konsumsi pakan (g) menurun sebanyak 0,6% pada ayam yang diberi pakan kontrol-basah dan 5,3% pada ayam di beri pakan tinggi energi-basah dibandingkan dengan masing-masing pakan kering. Pada penelitian ke dua, konsumsi pakan meningkat sebanyak 19,6% dan 24,3% pada ayam yang diberi pakan kontrol-basah dan pakan tinggi energi-basah dibandingkan dengan masing-masing pakan kering. Konsumsi pakan meningkat dengan pakan basah terutama pada broiler dan peningkatan lebih jelas dengan pakan tinggi energi-basah. Hasil dari penelitian ini mengkonfirmasikan konsumsi pakan yang lebih tinggi dapat dicapai dengan pakan basah di suhu tinggi pada broiler. Broiler tampaknya dapat menyesuaikan diri di suhu tinggi dengan lebih banyak mengkonsumi air dan energi dengan pakan tinggi energi-basah. Ayam Kampung telah ber-aklimitasasi terhadap kondisi iklim tropik dan tidak mengambil manfaat efek pakan basah.

Bab terakhir menampilkan pembahasan umum (Bab 7) dari hasil-hasil yang dilaporkan dalam Bab 2 sampai 6. Prospek untuk penelitian lanjutan disarankan dan implikasi praktek bagi strategi pemberian pakan Ayam Kampung dan broiler diberikan.

Sebagai kesimpulan, penelitian-penelitian yang digambarkan dalam tesis ini secara jelas menunjukkan bahwa ayam broiler dan ayam Kampung dapat menyusun pakan yang sesuai dengan lebih banyak memilih dari pakan kaya energi dari pada pakan kaya protein di kedua suhu normal dan tinggi. Selanjutnya, pengaruh jelek suhu lingkungan yang tinggi terhadap konsumsi pakan dan pertambahan bobot badan ayam broiler dapat diturunkan sebagian dengan pakan basah. Pengaruh pakan basah tidak terlihat pada ayam Kampung.

.

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*Bismillah*, this thesis is the end of my five years flying from Indonesia to Netherlands and vice versa to obtain my PhD degree in the field of Animal Nutrition at Wageningen University. During this flying, I have not travelled with a clear mind. Likely, there are some people who eased the way with the right word of encouragement and suggestions to look to expand my work. Therefore, my last duty is to acknowledge all those people who have contributed to the work described in this thesis.

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Wageningen, 20 January 2012

Syafwan

## Curriculum Vitae

**Syafwan** was born on 7 February 1969 in Pl. Lintang village, Sarolangun, Jambi Province Indonesia. He has studied his high school (MAN Bangko) in 1986. He was invited to study at Jambi University, Jambi in 1986. He graduated from Animal Husbandry Faculty of Jambi University in 1991. He started his job as a lecturer at Animal Husbandry Faculty of Jambi University in 1993 as a consequence of scholarship he have been taken.

In 1998, he was admitted to Wageningen University to study Animal Science and obtained his MSc degree in 2000. His final thesis was about *the effect of heat stress on thyroid hormones production in sheep*. After he graduated, he returned back to University of Jambi to continue his job as a lecturer. Seven years later, he applied to Animal Nutrition Group, Wageningen University. The results of the research are presented in this thesis.

His present duties are teaching several courses and supervising researchers for undergraduate students. His research interests are on strategies to improve performance of broiler and Kampung chickens in relationship between temperature and nutrition. He can be reached by email at: wan.syafwan69@gmail.com

## List of Publications

### Refereed scientific journal

**Syafwan**, **S.,** R.P. Kwakkel and M.W.A. Verstegen (2011). Heat stress and feeding strategies in meat-type of chickens. *World's Poultry Science Journal* 67:653-674.

**Syafwan**, **S.,** G.J.D. Wermink, R.P. Kwakkel and M.W.A. Verstegen (2011). Dietary self-selection by broilers at normal and high temperature change feed intake behavior, nutrient intake and performance. *Poultry Science, accepted for publication.*

**Syafwan**, **S.,** R.P. Kwakkel and M.W.A. Verstegen (2011). Feed Intake Behavior, Nutrient Intake and Performance of Indigenous Chickens Fed a Choice Diet under Tropical Climatic Conditions in Jambi Province, Indonesia. *Asian-Australasian Journal of Animal Sciences, submitted.*

**Syafwan**, **S.,** N. Mayasari, R.P. Kwakkel, M.W.A. Verstegen and W. H. Hendriks. Effect of wet feeding and a high dietary energy to protein ratio on feed intake behavior, performance, and gastrointestinal tract development of broilers at normal and high ambient temperatures. *Poultry Science, submitted*

**Syafwan**, **S.,** R.P. Kwakkel, M.W.A. Verstegen and W. H. Hendriks. Effects of wet and/or high energy to protein ratio diets on performance and gastrointestinal tract development of indigenous and broiler chickens reared under tropical conditions in Jambi Province, Indonesia. *Asian-Australasian Journal of Animal Sciences, submitted.*

### Conference proceedings

**Syafwan**, Wermink, J. D, Kwakkel, R.P and Verstegen, M.W.A. (1998). Effects of diet self selection on nutrient intake and performance in broiler chickens, reared under normal and high temperatures. *Proceedings of XXXIII World's Poutlry Congress, 29 June-4 July, Brisbane, Australia.*

**Syafwan**, R.P. Kwakkel and M.W.A. Verstegen (2010). Performance and Feed Intake Behavior of indigenous chicken "Ayam Kampung"fed a diet by Choice Feeding in a Hot Tropical Climate in Indonesia. *Indonesian National Seminar, 23 June 2010, University of Jambi, Indonesia.*

Mayasari, N. , **S. Syafwan,** R.P. Kwakkel and M.W.A. Verstegen (2010). The Effect of Wet Feeding and High Energy to Protein Ratio on Performance of Broilers under Normal and High Temperature Regimes. *Proceedings of XIIIth European Poultry Conference, 23-27 August, Tours France.*

**Poster**

**Syafwan**, R.P. Kwakkel and M.W.A. Verstegen (2011). Effects of wet diet and composition on feed intake behavior, performance, GIT development on broiler chicken under tropical climate in Indonesia. *WIAS Science Day, Wageningen, The Netherlands.*

**Syafwan**, R.P. Kwakkel and M.W.A. Verstegen (2011). Effects of a wet and/or high energy to protein ratio diet on performance of indigenous and broiler chickens reared under tropical climatic conditions. *18th European Symposium on Poultry Nutrition, Çeşme - İzmir - Turkey*

## Training and Supervision plan

**Training and Supervision Plan**

| | |
|---|---|
| Name PhD student | Syafwan |
| Group | Animal Nutrition |
| Daily supervisor(s) | Dr. ir. R. P. Kwakkel |
| Supervisor(s) | Prof. dr. ir. M.W.A. Verstegen<br>Prof. dr. Ir. W. H. Hendriks |

**EDUCATION AND TRAINING**

| **The Basic Package** | year | credits * |
|---|---|---|
| WIAS Introduction Course , Wageningen, Sept 11-14 | 2007 | 1.5 |
| Philosophy of science and/or ethics, Wageningen, March 15,22,29 and Apr 12,19,26 | 2007 | 1.5 |
| Subtotal Basic Package | | **3.0** |
| **Scientific Exposure** (conferences, seminars and presentations) | | |
| ***International conferences*** | | |
| < full name of conference, place, start and end dates > | | |
| XXIII World's Poultry Congress <oral>, Brisbane, Australia, June 30-July 4 | 2008 | 1.5 |
| XIII European symposium on poultry nutrition <oral>, Tour, France, August 23-27 | 2010 | 1.5 |
| XIIIV European symposium on poultry nutrition <oral>, Izmir, Turkey, Oct 31-Nov 4 | 2011 | 1.5 |
| ***Seminars and workshops*** | | |
| WIAS Science Day, Wageningen, March 8 | 2007 | 0.3 |
| Genetic and immunology of insect bite hypersensitivity in horses, Wageningen, Oct 12 | 2009 | 0.3 |
| Indonesian Student Association seminar (food security issue in Asia-Afrika), Wageningen, Oct 19 | 2009 | 0.3 |
| Indonesian National seminar, Jambi, Indonesia, June 23 | 2010 | 0.3 |
| International Symposium (Highlights in Nutrition and Welfare in Poutlry Production), Wageningen, Nov10 | 2011 | 0.3 |
| WIAS Science Day, Wageningen, Feb 3 | 2011 | 0.3 |
| Scientific research in Animal Welfare: Do we make a difference, Wageningen, Jan 18 | 2011 | 0.2 |
| How to write a world-class paper, Wageningen, Apr 19 | 2011 | 0.2 |
| **Presentations** | | |
| XXIII World's Poultry Congress <oral>, Brisbane, Australia, June 30-July 4 | 2008 | 1.0 |
| Indonesian Student Association seminar (food security issue in Asia-Afrika) <oral>, Wageningen, Oct 19 | 2009 | 1.0 |
| Indonesian National seminar <oral>, Jambi, Indonesia, June 23 | 2010 | 1.0 |
| WIAS Science Day <poster>, Wageningen, Feb 3 | 2011 | 1.0 |
| Advances in feed evaluation science <oral>, Wageningen, Apr 14 | 2011 | 1.0 |

| | | |
|---|---|---|
| XIIIV European symposium on poultry nutrition, Izmir, Turkey, Oct 31-Nov 4 | 2011 | 1.0 |
| Subtotal International Exposure | | **12.6** |

| | | |
|---|---|---|
| **In-Depth Studies** | | |
| ***Disciplinary and interdisciplinary courses*** | | |
| The 5th Workshop on the Fundamental Physiology and Perinatal Development in Poultry, Wageningen, Aug 31-Sept 3 | 2011 | 0.6 |
| ***Advanced statistics courses*** | | |
| Statistics for the Life Sciences, Wageningen, May 30-31 and June 1,4,5 | 2007 | 1.5 |
| Design of Animal Experiment, Wageningen, Oct 14-16 | 2009 | 1.0 |
| Mixed Linear Model, Wageningen, June 20-21 | 2011 | 0.6 |
| ***MSc level courses*** *(only in case of deficiencies)* | | |
| Poultry- an Integrated Global Approach | 2007 | 3.0 |
| Subtotal In-Depth Studies | | **6.7** |
| **Professional Skills Support Courses** | | |
| Techniques for Writing and Presenting a Scientific Paper, Wageningen, Oct 16-19 | 2007 | 1.2 |
| Analytical work and possibilities within animal nutrition sciences, Wageningen, Nov 3-13 | 2009 | 1.0 |
| Mobilising your scientific network, Wageningen, June 1-8 | 2011 | 1.0 |
| Subtotal Professional Skills Support Courses | | **3.2** |
| **Research Skills Training** | | |
| Preparing own PhD research proposal | 2007 | 6.0 |
| Subtotal Research Skills Training | | **6.0** |
| **Didactic Skills Training** | | |
| ***Lecturing*** | | |
| Principles of animal nutrion (ANU-20306), Wageningen, Jan 14 | 2011 | 0.3 |
| ***Supervising theses*** | | |
| Dirkjan Wermink, MSc minor thesis | 2007 | 1.5 |
| Novi Mayasari, MSc major thesis | 2009 | 2.0 |
| Subtotal Didactic Skills Training | | **3.8** |
| **Total** | | **35.3** |

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