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FEEDING STRATEGIES TO ALLEVIATE THE EFFECT OF HEAT STRESS ON GROWTH PERFORMANCE IN BROILERS

A thesis presented in partial fulfilment of the requirements for the degree of

Master of Science

in

Animal Science

at Massey University, Manawatū,

New Zealand.

Heidi Anna Jack

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Abstract

Broiler production is seen as critical to socio-economic development within the tropics. With the higher and rising temperatures of the tropics, heat stress is a major challenge of the industry. Of the many approaches used to alleviate heat stress, nutritional strategies have been seen as more economically viable in comparison to non- nutritional strategies used to alleviate heat stress.

The current study was done to assess both the combined and specific impact of diet density (high fat versus low fat diets) and diet form (mash versus pelleted diets), on alleviating heat stress in broilers. Biological responses were monitored through growth performance and digestibility data.

The experimental design used in the study was a 2 x 2 x 2 factorial arrangement of treatments from day 10 to day 34 of the trial period. Birds were subject to one of eight treatments which included a combination of one of two different temperatures (normal or elevated), one of two different diet types (high fat or low fat) and one of two different diet forms (mash or pellet). These treatments included Low Fat Mash under Normal Temperature conditions (LMN), Low Fat Mash under Elevated Temperature conditions (LME), Low Fat Pellet under Normal Temperature conditions (LPN), Low Fat Pellet under Elevated Temperature conditions (LPE), High Fat Mash under Normal Temperature conditions (HMN), High Fat Mash under Elevated Temperature conditions (HME), High Fat Pellet under Normal Temperature conditions (HPN) and High Fat Pellet under Elevated Temperature conditions (HPE).

Pellet fed birds had a higher growth performance under elevated temperature and in phase 2 (day 21 to 34), had the highest (*P = 0.016*) feed intake (166.9 g/b/d) compared to other treatments which were all statistically equivalent. With respect to ileal nutrient intakes, the intake of fat for the overall period and the intake of AME for phase 2 was highest (P = 0.045 and P = 0.018 for fat and AME respectively) on pellet fed birds housed under elevated temperature. Also, these birds had the highest *(P = 0.048)* growth efficiency (16.8 MJ/kg per kilogram gain) compared to mash fed birds (18.8 MJ/kg per kilogram gain).

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LIST OF ABBREVIATIONS

AME	Apparent Metabolisable Energy
ADF	Acid Detergent Fibre
ADG	Average Daily Gain
AMEdg	AME require for a daily gain of one kilogram
CIAD	Coefficient of Ileal Apparent Digestibility
DM	Dry Matter
FCR	Feed Conversion Ratio
FI	Feed Intake
GE	Gross Energy
KJ	Kilo Joules
ME	Metabolisable Energy
MJ	Mega Joules
NDF	Neutral Detergent Fibre
NE	Net Energy
PDI	Pellet Durability Index
RO	Reverse Osmosis
cpNE	Net Energy obtained from protein
sNE	Net Energy obtained from starch
fNE	Net Energy obtained from fat
pNE	Potential Net Energy

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1 General Introduction

Broiler production throughout many parts of the developing world, most of which are within the tropics or hotter regions has become almost indispensable to socio-economic development (David Farrell, 2013; Permin, Pedersen, & Riise, 2001). The characteristically quick turn over and low investment requirements of the industry make it a suitable venture for the tropics. Apart from the quick turn over and low investments required, assessment of poultry production as a model for socio-economic development in rural communities of the developing tropics confirmed its potential as a source of income as well as a cost effective approach to animal protein production. Providing animal protein is essential and according to FAO there is an urgent need for protein in the human diet of the developing world where average consumption per capita is a mere 12.2g compared to countries of the developed world where consumption measures 47g per capita (D Farrell, 2013; Permin et al., 2001).

One of the major challenges of broiler production of the tropics is heat stress as a result of the high temperatures of these regions. Apart from other challenges including the scarcity of capital, reduced availability of grain and protein supplements, lack of supporting infrastructure and systems required to support the development of the industry as well as prevailing disease threats, heat stress from climate change and associated rising temperatures is seen as the most crucial and obvious threat to poultry production. With the threat of climate change and the projected heating up of the earth's surface between 1.1 and 2.9 in a less extreme scenario and 2.4 and 6.4 in a more extreme scenario within the 21st century and the associated increase in warm spells and heat wave events, the challenge of

heat stress in poultry will be more intensified within the tropics. Such conditions combined with the high heat load of rapidly growing modern day broilers have increased the need for strategies to alleviate the detrimental impact of heat stress.

Furthermore, within the context of the developing world of the tropics, one critical aspect of management, apart from genetic and environmental modifications, is nutritional interventions especially for the tropics where improvement of feed quality is needed. Coincidentally, there are various nutritional strategies used to alleviate heat stress. These strategies include the use of optimised diets, improved amino acid profiles, adjustment of energy and protein diet components, inclusion of vitamins and minerals, feed form and feed timing. In the following research specific emphasis was placed on strategies including nutrient modification (diet type) and diet form. With respect to high fat diets, the higher energy efficiency of fats compared to proteins or carbohydrates results in high fat diets being more energy efficient than low fat diets. It is also known that feed form impacts energy efficiency, with pelleted diets having higher energy efficiency than mash diets. The higher the energy efficiency of diets the lower the heat load of metabolic heat contributing to heat stress. The present study was done to assess both the combined and specific impact of diet type (high fat versus low fat diets) and diet form (mash versus pelleted diets), on alleviating heat stress in broilers. Biological responses were monitored through growth performance and digestibility data.

2 Literature Review

2.1 Introduction

One of the major challenges of the poultry industry of the tropics is heat stress (N. J. Daghir, 2008; Gous & Morris, 2005; Lin et al., 2006; Rosa et al., 2007). Apart from other challenges, heat stress from climate change and associated rising temperatures, is seen as the most crucial and obvious threat to poultry production (Borges et al., 2008; N. Daghir, 2009). With the already high temperatures of the hot regions of the world and the threat of further rise in temperatures, the rapidly growing broilers of today's production systems also known as chickens bred for meat, are subject to heat stress which has been the major cause of reduced productivity and rising mortalities in birds within the tropics (N. J. Daghir, 2008; Zohair et al., 2012). There are many strategies used to address the problem of heat stress and these include both non nutritional strategies (environmental modifications and genetic selection) and nutritional strategies (Gous & Morris, 2005; Lin et al., 2006). While all strategies are critical to addressing the problem of heat stress (Glatz & Pym, 2013), major emphasis will be placed on nutritional strategies used to alleviate the problem. In order to fully understand the challenge of heat stress and the use of nutrition as a strategy against such in broiler production, the objectives of the literature review are to

i. Describe the context of poultry production and management within the tropics
ii. describe the challenge of heat stress and its impact on broiler production within the tropics
iii. describe nutrition in broilers

iv. identify non nutritional and nutritional strategies used to address the issue of heat stress within the tropics

2.2 Poultry production within the tropics

2.2.1 Significance of the industry

Broiler production within many parts of the developing world, most of which are within the tropics or hotter regions is seen as significant for the socio-economic development of the regions of the tropics (D Farrell, 2013; Permin et al., 2001). The characteristically quick turn over and low investment requirements of the industry are characteristics that make it a suitable venture for the tropics (Permin et al., 2001). An assessment done by Permin et al. (2001) to measure the suitability of poultry production as a model for socio-economic development in rural communities of the developing tropics reflected it as a potential source of income and cost effective animal protein. Other benefits of the industry include the low demand for land space and refrigeration compared to other livestock production models within the rural poor population (D Farrell, 2013; Haitook, 2006). Such characteristics of poultry production make it a significant venture to be supported in the tropics.

2.2.2 Production trends

Poultry meat accounts for 30% of global meat produced and of this 30%, 87% is accounted for by broiler meat production (FAO, 2010). Such a high percentage of broiler meat of all poultry meats produced may be further supported by figures seen in Table 2.1. Broiler meat production is increasing and greater increase is being projected for the industry especially within the tropics (N. J. Daghir, 2008; FAO, 2010). This increase can be seen in Table 2.3 where there is an average percentage increase of 1042.5% from 1970 to 2005 for the hotter regions compared to a mere 226.8% increase for the temperate regions. The

expansion in production for the tropics can also be observed in Tables 2.2 - 2.4 and in Figure 2.1. With respect to consumption rates, developed countries based on Table 2.4 has a higher average consumption of 27% compared to developing nations with a mere 16% average consumption. Though the average consumption of the developing tropics is lower, FAO (2010) reports more pronounced increases in the percent change in consumption within the tropics when compared to those predicted for the developed countries of the temperate regions. The overall increase in support for poultry within the tropics may be attributed to the significance and suitability of the industry to the development of these parts of the world (FAO, 2010; Haitook, 2006).

Table 2.1. Production of different types of poultry meats within the major poultry producing countries (Data 1000 t) (Adapted from FAO (2010).

Country	Chicken meat	Turkey meat	Duck meat	Goose and Guinea fowl meat	Total poultry meat	Total meat production	% of global meats
US	16,211	3,397	83	n/a	19,691	42,020	47
China	10,617	4	2,329	2,092	15,042	70,464	21
Brazil	8,988	230	7	n/a	9,225	18,898	49
Mexico	2,542	22	21	n/a	2,585	5,548	47
India	2240	n/a	73	n/a	2313	6508	36

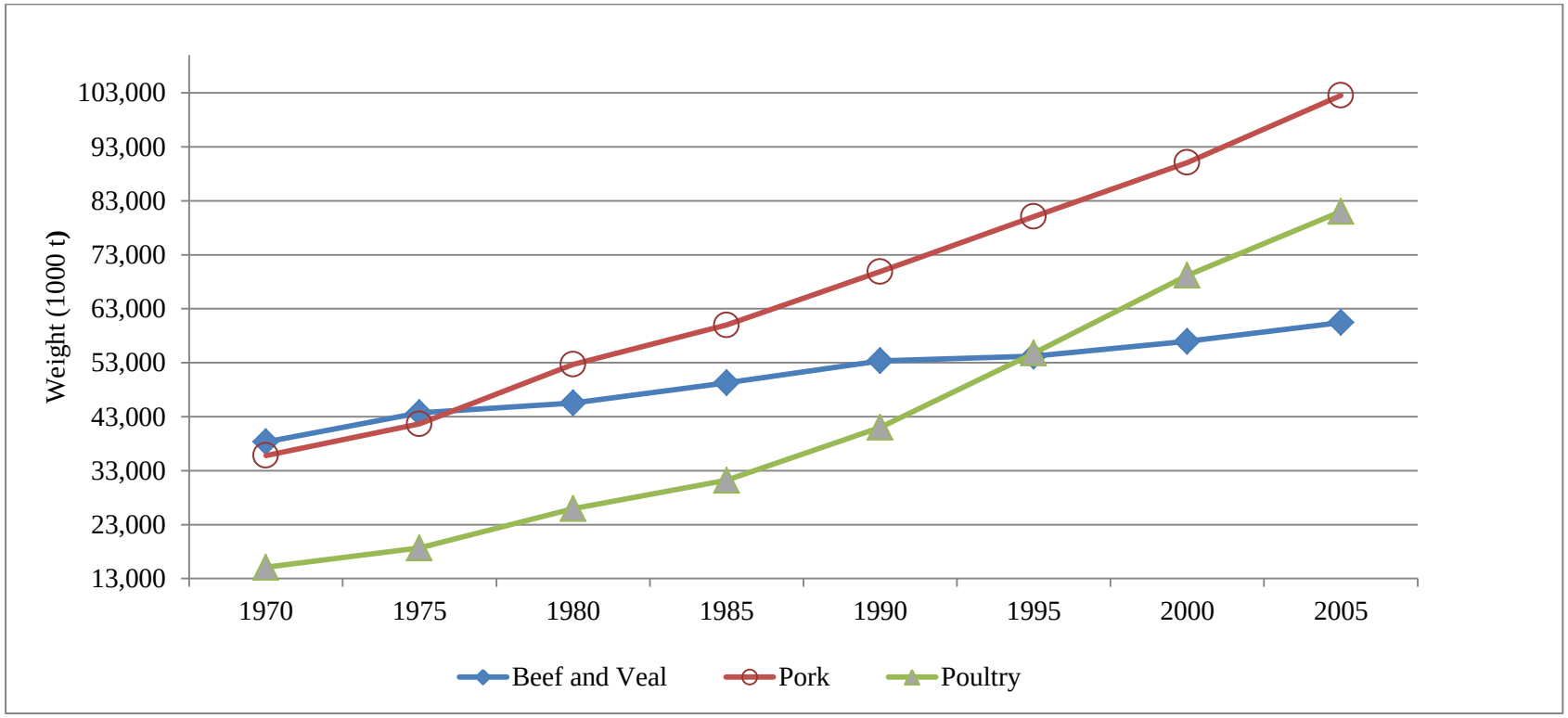

Figure 2.1 Comparison of global meat production within each year between years 1970 and 2005 (Data 1000 t) (Adapted from Windhorst (2006) cited by N. J. Daghir (2008)). Poultry meat appears to have a more pronounced rise between the years 1970 and 2005 indicating the greatest percentage increase (436.5%) followed by pork (186.4%), beef and veal (57.6%).

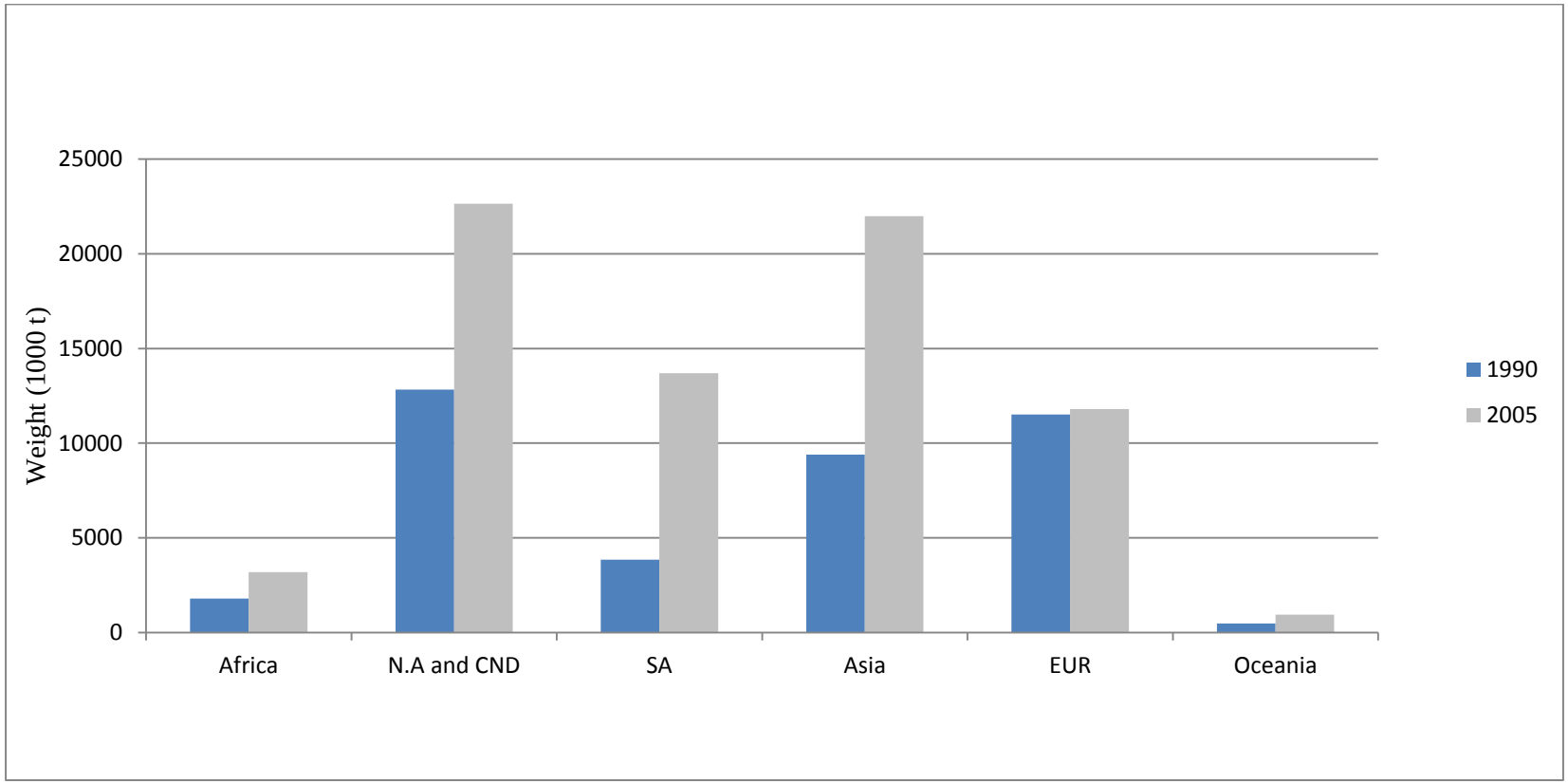

Figure 2.2 Changes in chicken meat production from the year 1990 to 2005 (Data 1000 t) (Adapted from FAOSTAT (2005a) cited by N. J. Daghir (2008)). Countries of the tropics including Africa South America (SA) and Asia display a greater difference in meat production between the two years. Such indicates higher rates of growth and expansion of poultry meat production within the tropics.

Table 2.2 Change in chicken meat production between the years 1990 and 2005 (Data 1000 t) (Adapted from FAOSTAT (2005a) cited by N. J. Daghir (2008)).

Continent	1990	2005	% Change
Africa	1790	3189	78
N.A and CND	12830	22653	77
SA	3850	13697	256
Asia	9390	21989	134
EUR	11520	11802	2
Oceania	480	942	96
World	39860	74272	86

Countries of the tropics including Africa South America (SA) and Asia display a greater difference in meat production between the two years measuring up to 256% and 134% respectively. Such indicates higher rates of growth and expansion of poultry meat production within the tropics

Table 2.3 Poultry meat production for developed and developing nations (Data 1000 t) (Adapted from Windhorst (2006) cited by N. J. Daghir (2008)).

Year	Developed nations	Developing nations	World
1970	11,219	3,882	15,101
1975	13,409	5,275	18,684
1980	17,986	7,279	25,265
1985	20,775	10,431	31,206
1990	25,827	15,214	41,041
1995	28,392	26,379	54,771
2000	32,708	36,483	69,191
2005	36,663	44,351	81,014
% increase	226.8	1042.5	436.5

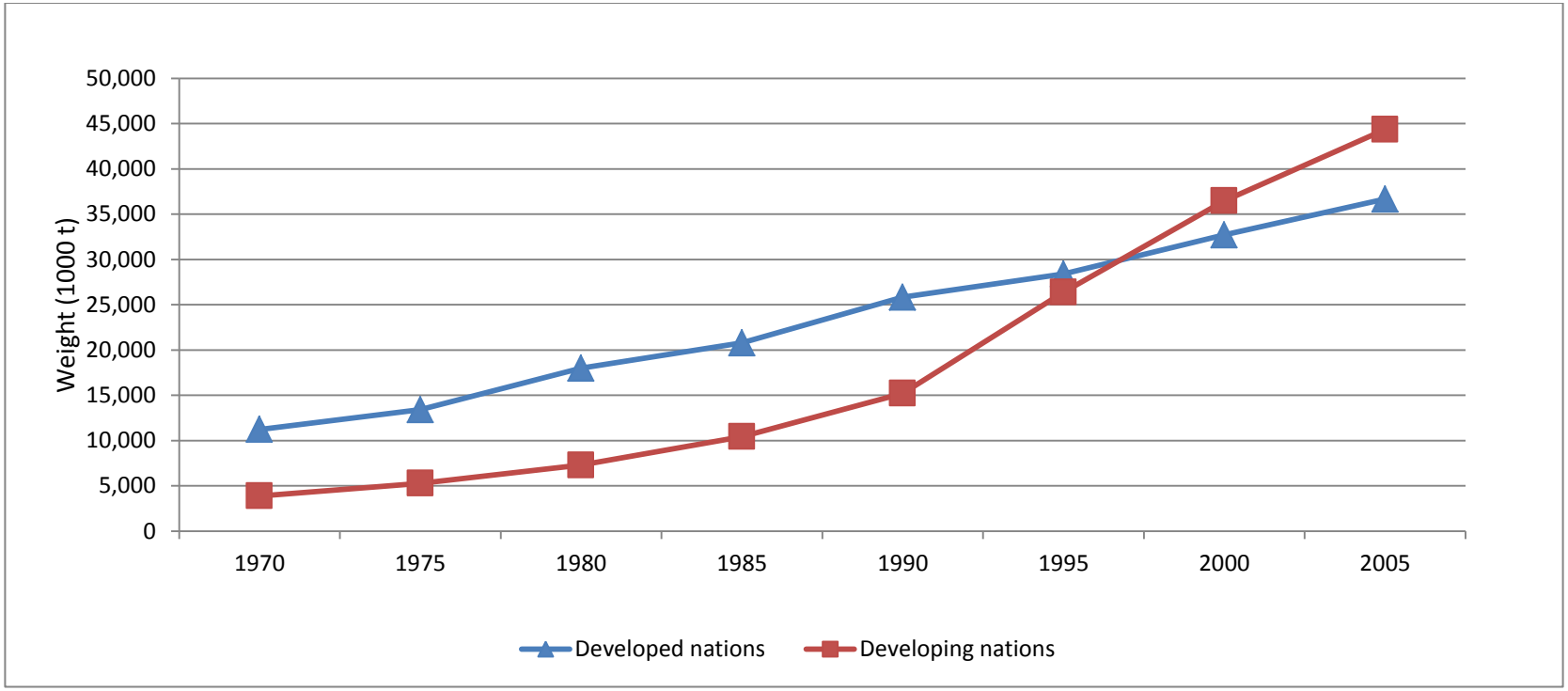

Figure 2.3 Poultry meat production for developed and developing nations between the years 1970 and 2005 (Data 1000 t) Adapted from (Windhorst, 2006) cited by N. J. Daghir (2008)). The figure demonstrates a steeper rise in poultry meat production within developing nations.

Table 2.4 The consumption per capita of broiler meat in tropical and temperate countries (Adapted from FAOSTAT (2005b) cited by N. J. Daghir (2008)).

Country	**Consumption (kg/capita)**
Tropics	
Argentina	18.54
Brazil	37.27
S. Africa	24.7
India	1.72
Iran	4.88
Thailand	10.54
Average consumption	**16.23**
Temperate	
Canada	31.82
France	14.24
Hungary	27.81
Russia Fed.	16.45
USA	44.24
UK	26.46
Average consumption	**26.78**

The Table indicates a higher average consumption of animal proteins in the more developed temperate regions versus the developing countries of the tropics.

2.2.3 Characteristics of the industry within the tropics

2.2.3.1 Broilers of the tropics

Most broiler breeds used within the tropics are developed within and exported from the temperate regions including Canada, France, Germany, Netherlands, the United Kingdom and United States of America (Elfick, 2011; Pym, 2013). Table 2.5 shows the performance index at day 34 (metric) for more popular broiler breeds used and exported by major export centres. The average lifespan of these birds is approximately six weeks at an average market weight of 2.5 kg. Obtaining this target weight may take longer depending on the environment birds are subject to including both diet and housing. With the higher temperatures and humidity of the tropics as well as the quality of diet management (see section 2. on feed resources) compared to that of the temperate regions (Table 2.6) where birds are bred and developed, the overall performance of birds is compromised and emphatically lower compared to that which is characteristic of the temperate regions (Pym, 2013). Consequently where modifications of the animal is concerned, much effort is currently being invested into developing strains that obtain progeny with the adaptability of indigenous breeds and the high performance of exotic breeds imported (Pym, 2013).

Table 2.5 Performance (metric) of broilers at 34 days of age (Adapted from Cobb (2012))

Male performance index					
Breed	**Age (days)**	**Weight for age (g)**	**Daily gain (g)**	**Daily feed intake (g)**	**Cumulative FCR**
Cobb avian 48	34	2100	99	208	1.53
Cobb500	34	2116	100	208	1.52
Cobb700	34	1932	90	190	1.55
Ross308	34	2075	98	188	1.55

Figures were calculated over a 34 day period – only day 34 was extracted to give an idea of standard performance at 34 days of age

Table 2.6 Performance of broilers subject to high temperatures

Temperature	**Growth performance**							
	Age (days)	LW (g/b)	Feed intake (g/b)	Average daily Gain (g/b)	Feed conversion ratio (g/g)	ME intake	Mortality (%)	Author
29-40°C (Modern housing)	42	1944	3454		2.03		25.96	Farhadi and Hosseini (2014)
29-40°C (Conventional housing)	42	1940	3526		2.06		27.52	
32°C (10% CP)	42	1681	124.2*	36.3*	3.44			S Temim et al. (2000)
32°C (15% CP)	42	1751	116.8*	40.6*	2.94			
32°C (20% CP)	42	1810	111.7*	44.6*	2.56			
32°C (28% CP)	42	1868	111.7*	49.2*	2.33			
32°C (33% CP)	42	1890	109.4*	50*	2.28			
25-35°C (diet 1)	22-45		2466	1059	2.33			Sinurat and Balnave (1985)
25-36°C (diet 2)	22-45		2502	1057	2.37			
25-37°C (diet 3)	22-45		2454	1071	2.29			
25-38°C (diet 4)	22-45		2425	1117	2.17			
32°C (Dietary crude protein content 160 g/kg)	44		2279	939	2.41			Alleman and Leclercq (1997)

32°C (Dietary crude protein content 200 g/kg)	44		2333	1118	2.19		
	35-56	1876	93.6*	22.29*	3.92	13	
34°C (Breed AA)							Lu et al. (2007)
34°C (Breed BJY)	35-56	713.6	43.1*	14.84*	2.91	0	
21-30°C (Variable natural temperature)	56	2050	3535	1358	2.59	3.62	
25°C (Constant chronic ambient temperature)	56	2107	3418	1544	2.19	0	Abu-Dieyeh (2006)
30°C (Constant chronic ambient temperature)	56	1847	3042	1178	2.6	12.9	
32°C (Breed PCLC)	42	1409			1.87		Rosa et al. (2007)
32°C (AgRoss 308)	42	1868			1.83		
32°C (na/na gene)[5]	49	2003	137*	58.1(2)	2.35+		Deeb and Cahaner

32°C (Na/na gene)	49	2051	141*	60.7*	0.431+		(1999)
32°C (Na/Na gene)	49	2010	139*	62.8*	0.453+		
25°C	49		3330	1680	1.71	45.4	Howlider and Rose (1992)
29°C	49		3050	1520	1.71	41.6	

[1] Basal Diet, [2] Basal Diet supplemented with animal tallow, [3] basal diet with amino acids, [4]basal diet with a 5% increase in metabolisable energy and amino acids, [5] (na/na), [6](Na/na), [7](Na/Na) represent the different genotypic expression of the naked neck gene.

* Means Feed intake and Average daily gain adjusted to g/d

+ Means FCR modified to feed to gain ratio

2.2.3.2 Feeds and feeds management within the tropics

2.2.3.2.1 Feed Resources

There is a diversity of feeds resources used within the tropics for broiler production which can be seen in Table 2.7 below. The first two groups of feeds including energy and protein supplements are commonly used within the tropics (N. J. Daghir, 2008; Ravindran, 2013). These feed resources most critically provide energy needs of broilers primarily through cereal grains and animal fats as well as the protein needs of broilers through protein supplements and animal meal products. As a result, of the 50-70% costs accounted for by feeds, energy and protein needs account for approximately 95% of feed costs (Ravindran, 2013). A high percentage of these grains are imported into the tropics and very little effort is being channelled towards the development and improvement in the quality of locally available feeds (Ravindran, 2013). Added to this challenge is the fact that grain is used as the main human staple within these countries and with the competing demand for grain from both biofuel production and other livestock production systems, the dependence on grain imports is becoming increasingly unreliable and costly (DJ Farrell, 2005; Ravindran, 2013). Through the appropriate development and investment of locally available feeds and novel feedstuffs identified as the third group in Table 2.7, dependence on imported feeds may be reduced (DJ Farrell, 2005). Such a venture has been proven to be critical for the developing tropics and further investment into research must take place to ensure the development of high quality feeds that can provide effective substitutes for imported feeds (Ravindran, 2013). Until such is done, the dependence on costly imports and the characteristically sub-quality feeds fed within the tropics will continue (Ravindran, 2013).

2.2.3.2.2 Diet types and form

For broilers reared under tropical conditions altogether energy, protein, amino acid, and mineral levels of starter, grower and finisher diets are different to those fed within temperate regions (N. J. Daghir, 2008). For instance protein is 1-2% lower with energy levels adjusted to suit protein. Also amino acid levels are raised as well as potassium levels which have been raised from 0.4% to 0.6%.

Birds within the tropics have a preference for large size particles and as a result diets are often offered in crumble or pellet form (Behnke & Beyer, 2002).With respect to the diets and feed forms, the starter which is normally in crumble form is given within the first 3 weeks of life (Behnke & Beyer, 2002). The grower diet which is often offered as pellets is given within the 3-6 weeks and finisher, also offered as pellets, is given from last 6 to 7 weeks of age (N. J. Daghir, 2008).

Table 2.7 Feedstuffs used within poultry production of the tropics and their recommended inclusion level (%) in broiler starter and finisher diets (Adapted from (N. J. Daghir, 2008)).

Feedstuff	Starter	Finisher	Benefits	Constraints
Plants				
Cereals				
Barley (Hordium sativum)	0-5	0-5	Source of energy	Anti-nutritional factors and over 20% inclusion leads to reduced performance in birds reduce palatability of diet if not in pelleted form
Millet (Setoris spp)	0-10	0-10	Source of energy Comparable to wheat and sorghum in its impact on production	
Sorghum (Sorghum bicolor)	0-20	0-40	Comparable to maize especially when grounded and treated properly	Anti-nutritional factors resulting in reduce performance and reduce availability of ME and amino acids

Rice paddy/Rice bran	0-5	0-5	Contains up to 12.91 KJ/g energy once properly processed Feed efficiency can be improved through enzyme supplementation	Rice bran oil is highly unsaturated and may become rancid easily
Triticale	0-20	0-30	This crop is a hybrid formed through the crossing of both wheat and rye. Productivity is comparable to that of wheat and its hardiness equivalent to that of rye Depending on variety used, can be used to replace a percentage of wheat and maize in diets	Certain varieties are lacking in both methionine and lysine. When fed must be supplemented with lysine Finely ground grain may result in reduce intake in older birds
Protein Supplements				
Coconut meal (Copra)	0-2	0-3	High protein content Oil content makes it a high energy ingredient	High fibre content Low in amino acids lysine and histidine Oil can become rancid if not treat with anti- oxidant

Cottonseed meal (Gossypium spp)	0-5	0-15	Rich source of protein	Anti-nutritional factors. These can be reduced through chemical treatments. Also there are varieties of plant that are low in anti- nutritional factors Low in lysine and methionine
Groundnut meal	0-5	0-10	Comparable impact on performance of birds to soybean AME of Groundnut meal can be increased through enzyme supplementation	Lacking in lysine and methionine and must be supplemented with these if used as a major source of protein in diets. Capable of producing aflatoxins if affected by aspergillus flavuus.
Sunflower seed (Helianthus annuus)	0-10	0-15	Good source of crude protein and ME Can replace up to 25, 50 and 75% of soybean in starter, grower and finisher diets respectively No anti nutritional factors common to other grains	

Sunflower meal (Helianthus annuus)	0-5	0-10	Good source of crude protein and ME Can replace up to 25, 50 and 75% of soybean in starter, grower and finisher diets respectively No anti nutritional factors common to other grains	Protein available is low in lysine
Safflower meal	0-5	0-10	Potential substitute for soybean in diets	Low in lysine and has a high fibre content - can be decorticated to reduce fibre
Sesame meal	0-5	0-10	Rich in methionine, cystine and trytophan May provide a potential substitute for soybean meal when inclusion is 15% or less	Deficient in lysine and cannot be used as the main source of protein Affects the availability of zinc and calcium. Calcium supplementation and autoclaving can be used to treat calcium and zinc deficiencies respectively
Linseed meal	0-2	0-3	Rich in n-3 fatty acids which are beneficial can improve nutritional value of meat	Anti-nutritional factors High quantities of indigestible mucilage Protein low in lysine and methionine

Mustard seed meal	0-3	0-5		Anti-nutritional compounds which can be reduced through chemical treatments
Single cell protein	0-3	0-5	Has a rapid turn-over within a short space of time Used effectively in a well-balanced broiler diet	Low palatability Low in methionine and lysine
Animal Feedstuffs				
Fishmeal	0-8	0-4	Good source of protein provides an abundance of minerals including calcium, phosphorus and trace minerals, B vitamins and essential fatty acids. Also contains an unidentified growth factor. Sometimes the only source of animal proteins used in most developing countries.	If fed above recommended levels may leave an unpleasant fishy taste in meat Poor processing in developing countries can lead to lower CP levels (40%-50%) compared to higher CP values of the temperate more developed countries (60%) A number of safety concerns regarding contamination of fisheries

Meat meal	0-10	0-10	High levels of protein, calcium and phosphorus	Lower quality protein source than soybean meal or fishmeal Low in lysine, methionine, tryptophan, amino acids Safety concerns of product after Spongiform encephalopathy crisis from feeding meat meal
Novel Feeds				
Plants				
Cassava root meal	0-10	0-20	Is a potential substitute for maize Rich source of highly digestible carbohydrates May be a potential substitute for maize in diets	Anti-nutritional compounds which may depress growth rates. Can be reduced through heat treatments
Dates and date by-products	0-2	0-3	Rich source of energy and fat	High in fibre Low in protein
Palm kernel meal	0-2	0-3	Fairly good source of protein Good balance of calcium and phosphorus No anti-nutritional factors	Unpalatable High fibre content

Mungbean	0-2	0-3	Good source of protein	
Bread fruit meal	0-5	0-10	Rich in carbohydrates mineral and vitamins Meal made from cook bread food results in more daily weight gain when compared to meal made from uncooked breadfruit A potential substitute for maize in diets	Anti-nutritional factors that may interfere with digestibility, absorption and utilisation of nutrients
Ipil Ipil leaf meal	0-2	0-3	Good source of protein	Anti-nutritional factors. Need for further investigation into different varieties and the impact they have on the composition and nutrient value of meals
Salseed	0-3	0-5	Source of energy Comparable to wheat and sorghum in its impact on production	Anti- nutritional factors which can be reduce through chemical treatments
Guar meal	0-2	0-3	Potential source of protein Rich in lysine and methionine	Anti-nutritional factors. Can be reduced through autoclaving or the use of enzymes

Jojoba meal	0-2	0-3	Potential source of protein	Anti-nutritional factors
Velvet beans	0-2	0-3	Amino acid comparable to that of soybean	Anti-nutritional factors. There is need for the development of processing techniques and methods of reducing these anti nutritional factors to ensure successful use of beans in poultry diets. Heat treatment via autoclaving has been successful
Animals				
Buffalo gourd meal (BGM)	0-2	0-3	Rich source of oil which has a high content of essential fatty acids Rich source of protein that is high in arginine, aspartic acid and glutamic acid	Low in lysine, threonine and methionine Toxins mainly in hull of seed which has adverse effects on chicks. There is a need for methods of detoxification to reduce or remove toxins in BGM
Dried poultry waste	0-2	0-3	Essential amino acids that can benefit birds Cost effective feed input once properly treated	Low in energy and can reduce performance in birds if not properly balanced in diets

2.2.3.3 Housing and environment

The objective of any efficient poultry housing should be to ensure that the safety and comfort of birds are secured (Glatz & Pym, 2013). One major component of their comfort is having environments that allow for birds to exist within their thermo neutral zone (Glatz & Pym, 2013; Lin et al., 2006). Poultry naturally have a body temperature range between 39.4 °C and 40 °C and for these birds the thermo neutral zone or suitable ambient temperature exists between 26 °C and 27 °C (Van Der Hel et al. (1991) cited by N. J. Daghir (2008)) and according to Lin et al. (2006) the ambient temperature for maximum performance lies within the range 18 °C to 22°C for growing broilers. For the higher temperatures of the tropics, the main challenge of housing is to ensure that the body temperature of birds is kept from exceeding the maximum thermo neutral limit where heat stress is inevitable. When birds are kept within their thermo neutral range energy expended is channelled towards growth and development and not expended towards thermoregulation (N. Daghir, 2009; Gous & Morris, 2005; Lin et al., 2006). When ambient temperatures are elevated above the thermo neutral range, energy intake is reduced so that the excess heat load of feed metabolism is lowered or when ambient temperature falls below the thermo neutral range energy intake is increased to generate the heat required to increase body temperature (Li et al., 1992; Van Kampen, 1988). In both cases there is less energy being utilised for growth and development as some is lost to thermoregulation and as a result the energy available for production (live weight gain) in birds is reduced (Howlider & Rose, 1992; Smith, 1973).

2.2.3.4 Housing systems within the tropics

There is a diversity of housing types used within the tropics ranging from housing types of the more complex high input commercial systems to housing types of the less complex low input semi- scavenging farming systems (N. J. Daghir, 2008; Glatz & Pym, 2013). These large scale commercial farms are costly and make up a mere 20 % of production systems. As a result with 80% production being within the medium to small scale production systems, housing type associated with these are more prevalent. Some of the main components of housing include ventilation, cooling and lighting systems which will be further described in the following section.

2.2.3.4.1 Housing within large scale commercial production systems

These complex systems usually involve a collaborative effort and integration between the farmers and company networks where there are component feed mills, breeding, hatchery and processing units (Glatz & Pym, 2013). Farmers normally provide both the rearing facilities and labour while companies provide all required input including feed, chicks, transportation and medicine (Glatz & Pym, 2013). Characteristic of such systems is the superior performance of birds within these systems. With computerized controlled housing environments the thermal needs of birds are more precisely and consistently met lending into higher performance in birds (Glatz & Pym, 2013). For cooling within these houses, automated fans are used with sensors to control when fans are switched on or off depending on the need for cooling. With respect to meeting the heating need required for young chicks, forced air furnaces and radiant heating systems are used (Glatz & Pym, 2013).

2.2.3.4.2 Housing within medium and small scale production systems

Such systems are less complex (Glatz & Pym, 2013). The main source of ventilation is natural airflow and heating of young birds are mainly done through radiant heating. For small scale production systems there are different types of housing made from timber, mud bricks and or bamboo. Such systems also depend on natural ventilation and birds are not kept confined for the entire day but are confined mainly during nights to prevent loss through predation or praedial larceny.

2.2.3.5 Components of housing systems within the tropics

2.2.3.5.1 Ventilation

The intent of any ventilation system must be to encourage convection heat loss as opposed to latent heat loss. Latent heat loss occurs through panting. Panting requires energy which reduces production through dehydration and respiratory acidosis. Convection cooling however involves the removal of heat from birds through the effect of airflow over birds and as a result less energy is required for thermoregulation. There are two broad categories of ventilation including natural ventilation and powered ventilation systems.

Naturally ventilated systems utilise the inflow of natural air to cool housing environment and birds (N. J. Daghir, 2008). With such systems different housing dimensions primarily being house width, side wall openings, local obstructions, housing orientation and roofing dimensions to ensure the effectiveness of the system. For instance recommended house length of 12 m or less for air circulation throughout the house compared to wider houses where circulation is not uniformed throughout the house. Side wall openings are usually adjusted through use of side wall curtains which can be adjusted depending on the

requirement for light and airflow. Local obstruction may include other poultry houses or building structures as well as vegetation that may impede airflow and circulation throughout houses. Based on the recommendation of N. J. Daghir (2008) housing structures must be properly spaced and positioned to avoid airflow obstruction to houses. With respect to vegetation obstructions vegetation must be low lying and if tall trees are present, branches must never fall below the eves of housing roof. The presence of grass insulates and deflects heat away from houses and large trees provide shade and protection for direct sunrays and its heating effect. The east to west orientation of housing is also critical for reducing the impact of direct sunlight on birds. Roofing dimensions including slope, overhang and colour are also important for cooling of houses.

With respect to power ventilated systems there are two types including both the negative and positive pressure systems (N. J. Daghir, 2008; Emery, 2004). Negative pressure systems are the more commonly used systems. These comprise a combination of both exhaust fans and air inlets which control the air into and out of houses and thus the airflow over birds. This automated airflow system is effective as it provides a consistent airflow over birds compared to the inconsistent and unpredictable nature of airflow from natural air in naturally ventilated systems. Two types of this negative pressure system include inlet ventilation and tunnel ventilation. With inlet ventilation there is an even distribution of fans and air inlets across the length of the house where with tunnel ventilation systems air inlets are on one end of the house and fans on the other. The tunnel ventilation has been proven to be more effective in cooling birds exposed to high temperature as it creates a greater air exchange and movement throughout the house compared to the outdated inlet systems.

2.2.3.5.2 Cooling systems commonly used in tropical poultry housing

Evaporative cooling systems are commonly used within the tropics and are often used in combination with ventilation systems (Butcher & Miles, 2011; N. J. Daghir, 2008; Haitook, 2006). There are three major types of cooling systems including (i) the fogging systems which can be applied in both natural and powered ventilation systems, (ii) pad systems which can only be used in the power ventilated system and the less commonly known (iii) sprinkler system. Of the three, fogging systems are more commonly used as the padding system has a more costly upkeep and the sprinkler system is mainly used within the arid topics. The combination of ventilation and cooling is important. Though cooling adds humidity to the already humid air, air movements of ventilation systems reduce humidity which can add greater stress under high temperatures.

2.2.3.5.3 Lighting management

Lighting is also another key component of poultry housing (Glatz & Pym, 2013). Low intensity lighting of between 5 and 10 lux is recommended as birds are kept calm. The two major types of lighting include incandescent and fluorescent lighting. Incandescent lighting has a more cost effective installation but lower efficiency and shorter lifespan of globes are shortfalls of this lighting system. Fluorescent globes however though less cost effective, globes last longer and bird engage in less energy exerting activity that produce heat. Within the tropics broilers are subject to 23 hours of light exposure which can be reduced if intermittent feeding programmes are used. However the prevalence of intermittent feeding programmes in hotter regions has led to less hours of light exposure for broilers. Lighting not only increases reproductive capacity but it also improves feed intake.

2.3 Heat stress and production systems

Heat stress is a major challenge of broiler production systems within the tropics (N. Daghir, 2009; Lin et al., 2006). Heat stress may either be acute where birds experience high intensity heat stress for short periods of time or chronic where birds are exposed to heat stress for a long period of time (Emery, 2004; Haitook, 2006; Lu et al., 2007). It occurs when the rate of body heat production exceeds the rate of heat released from the body (Emery, 2004). Because birds are homeotherms they are constantly maintaining their vital body temperature by either gaining or losing heat based on their ambient temperatures (Gonzalez-Esquerra & Leeson, 2006; Leeson, 1986; I. Sibbald, 1982). They produce body heat through the metabolism of food for maintenance and meat production or through physical activity (Emery, 2004; Li et al., 1992). Apart from body heat which is the dominant source of heat, other sources of heat may come from the surrounding housing surfaces including the litter, walls and roof (Emery, 2004). This heat can be lost through either sensible heat loss including radiation, convection and conduction which accounts for 75% of loss under regular temperature conditions or it can be lost through evaporative cooling or latent heat loss which accounts for 80% of heat loss when birds experience hyperthermia (Gous & Morris, 2005). With radiation, heat is lost to surrounding surfaces if temperatures are lower than the bird's body surface temperature. Heat loss through convection occurs when warm air is removed from around the body of birds through air flow and movement. Conduction is the loss of heat from the body of birds as it comes into contact with cooler surfaces. After these three means of sensible heat loss can no longer

add to heat lost by birds, heat is lost through the energy consuming evaporative cooling or latent heat loss by means of panting (see Figure 2.4).

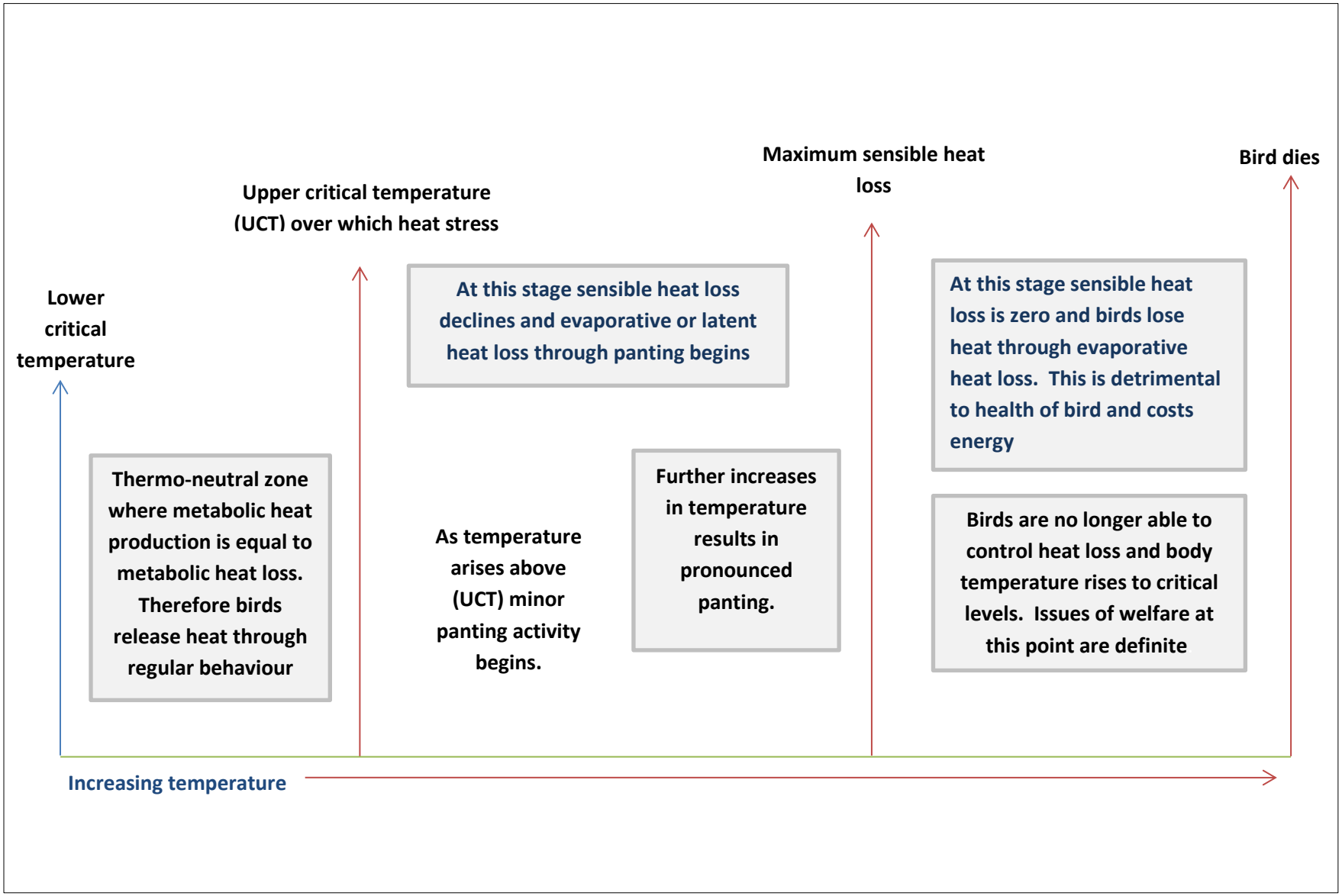

Figure 2.4 The stages of heat stress in broilers as temperature rises (Adapted from Emery (2004))

2.3.1 Impact of heat stress on the biological and production systems of broilers

Heat stress results in behavioural, physiological, neuroendocrine, molecular and immune impact on birds that are detrimental to production (Lara & Rostagno, 2013; Lin et al., 2006). The following section describes the impact of heat stress on both the biological and production systems of broilers.

2.3.1.1 Behavioural impact of heat stress

The behaviour of birds changes as they undergo heat stress (Howlider & Rose, 1992; Li et al., 1992; Smith, 1973; Tankson et al., 2001). These behavioural reactions to heat stress cost the birds less energy than physiological changes. When birds are subject to heat stress less time is spent on activities such as walking, standing, changing posture and social interaction, as these produce heat (Emery, 2004; Lara & Rostagno, 2013). Other behavioural changes include drooping of wings which are lifted away from the body to increase heat loss as well as searching for cooler spots in housing (Larbier & Leclercq, 1992).

2.3.1.2 Physiological impact of heat stress

Birds subject to heat stress undergo specific physiological changes (N. Daghir, 2009; Smith, 1973; Tankson et al., 2001). These changes are controlled by the integrated operations of organs and the endocrine system to ensure that the heat load is reduced. One physiological response includes acclimatisation to high ambient temperatures (Yalcin et al., 2001). According to Hillerman and Wilson (1955), broilers require 3-5 days to acclimatise to both hot and cold weather. During periods of high temperatures, broilers heat up from the outer peripheral tissues to the core inner tissues. Vo et al. (1978) cited by N. J. Daghir

(2008) indicated that the capacity to acclimatise depends on the nature of ambient temperature increase. For birds that are exposed to a rapid increase in ambient temperatures, the body temperature begins to increase at ambient temperatures above 30°C and for a slower increase in ambient temperature, birds maintain this temperature until ambient temperature reaches 33°C. Apart from rate of temperature rise Sahraei (2013) emphasised a greater acclimation in birds that were conditioned to high temperatures in their early life as well as those subject to restricted feeding programmes, compared to those who were not exposed to such practices.

Another physiological response includes changes in feed and water consumption (Donkoh, 1989; Hafez et al., 2011). Water consumption increases instantly when broilers are faced with heat stress as larger volumes of water is crucial to replacing water loss during latent heat loss. On the other hand, voluntary feed intake decreases and such a change is more gradual than the increase in water intake. This reduced feed consumption lowers the heat load caused by the metabolism of food. It is believed that such a reduction in feed intake accounts for 67% of reduced growth rate in birds (N. Daghir, 2009). Sensible heat loss is another physiological response of birds to heat stress. For instance specific anatomical features in birds allow for an increase in the blood flow to the surfaces of the legs and feet so that heat can be lost through conduction or radiation. This vascular system coupled with shunts mechanisms allows cooler venous blood to flow near to the warmer arterial blood resulting in heat exchange and loss through the uninsulated surfaces of the vascular system. The rete opthalmicum has also been seen as a heat loss mechanism which allows for heat loss through the cornea, the buccal cavity, the beak and the nasal passages. Sensible heat loss can also be increased through a reduction in feathers. Feather loss may take place from

the neck, back and breasts of chickens and may be as a result of genetics or as a result of environmental conditions including stocking density and temperature. Though feed efficiency can be decreased with increasing feather loss during low temperatures, under high temperatures, this increase in capacity to dissipate heat allows for higher performance levels under heat stress conditions (Merat (1986) cited by Lin et al. (2006).

Also, changes in respiration rate and blood pH are other physiological responses of broilers to high temperature (Scott & Balnave, 1988; Tankson et al., 2001). Elevated temperatures at or above that of the upper critical temperature may lead to panting in broilers which demands energy and results in blood alkalosis (Butcher & Miles, 2011; Hafez et al., 2011). During panting, blood CO_2 in broilers is lost through rapid respiration. In the attempts of the body to replaced lost CO_2, H+ ions in the blood is absorbed by bicarbonate in the presence of carbonic acid to produce and replace CO_2 lost. The reduction of H+ ions leads to the increase in blood pH also known as alkalosis. Alkalosis may also be as a result of the lost in monovalent ions including sodium and potassium through urination (Ahmad and Sarwar (2006) cited by N. Daghir (2009)). Often the regular acid-base or dietary electrolyte balance (DEB) measures 250 mEQ/kg and birds with a DEB that is very high (360 mEQ/kg) or low (0 mEQ/kg) are at risk of respiratory alkalosis. However apart from electrolyte therapy, the bird has natural mechanisms that assist them in dealing with such changes (N. Daghir, 2009). These include the Gular flutter or the thin floor of the bird's mouth which vibrates, enhancing heat loss. The air sacs which increase the passage of airflow over surfaces to increase the gas exchange between air and blood is also a natural heat loss mechanism (N. J. Daghir, 2008).

Change in the plasma concentration is another physiological response mechanism of birds during heat stress. In order for tissues to function well, the osmotic balance between intra and extra cellular fluid must be maintained. Major shifts in the concentration of the ions during heat stress may upset such a balance. For instance, exposing birds to 41°C resulted in increased plasma sodium and chloride and decreased plasma potassium and phosphate (Ait-Boulahsen et al. (1989) cited by N. J. Daghir (2008)).

Finally, heart rate, cardiac output, blood pressure and total peripheral resistance are other responses to heat stress (Darre & Harrison, 1987). The cardiovascular system works towards ensuring that body heat is transferred from the core to the peripheral tissues through the circulatory system. The heart rate of birds firstly decreases and the vasodilation in surface tissues takes place. Subsequently both blood pressure and surface resistance is such that cardiac output and heart rate is increased.

2.3.1.3 Neuroendocrine impact of heat stress

The neuroendocrine system controls the initiation and maintenance of natural mechanisms used by birds to dissipate heat during periods of heat stress (Haitook, 2006; Lara & Rostagno, 2013). It involves the activity of neohypophyseal (posterior-pituitary) hormones, mesotocin, growth hormone, hypothalamic-pituitary adrenal axis, catechoalmines, melatonin, reproductive and thyroid hormones.

Neohypophyseal hormones including arginine vasotocin (AVT) is one of the major hormones activated during heat stress (N. J. Daghir, 2008). This antidiuretic hormone is secreted by the posterior pituitary gland and is activated by the state of dehydration in birds. AVT stimulates the reabsorption of water by the kidneys back into the plasma and also

allows for the increased availability of free fatty acids that are critical for meeting the extra energy needs of muscles used for panting. As AVT increases, circulating thyroxin increases also, reduce triiodothyronine (T_3). T_3 speeds up metabolism and reducing levels of it decreases the extra heat load associated with increased metabolism during periods of heat stress.

Mesotocin (MT) decreases during heat stress (Wang & Bottje, 1989). Studies on the function of the diuretic hormone MT in birds is quite scarce, however increases in the concentration of AVT results in a decrease in the levels of MT in reptiles and may have a similar function in birds. Such a counter relationship between hormones may encourage the reabsorption of water. Conversely other studies have shown where an increase in MT during heat stress increases the respiratory rate which may be important for heat dissipation during heat stress (Robinzon et al., 1988).

Growth hormones increases during heat stress and plays an important role in the mobilization of fatty acids. This is key to supporting the high energy needs of muscles during panting (John et al., 1973).

With the Hypothalamic-pituitary adrenal axis, corticosterone stimulates the production of corticotrophin releasing factor which activates the production of adrenocorticotrophic hormone (ACTH) (N. J. Daghir, 2008). The production of ACTH leads to the release of hormones including corticosterone and aldosterone. The rise in corticosterone is rapid but ephemeral and decreases with time to levels lower than pre-stress levels. Low levels of the hormone are associated with high blood pH and low levels of circulating phosphate, glucose and sodium ions. The rapid decline in the hormone results in the proliferation of

conditions that lead to cardiovascular failure and death. Pre-treatment with resperine, propanol or dihydroergotamine has proven to sustain the adrenal response in birds and thus may sustain life during heat stress. Aldosterone works with AVT in the conservation of body fluids during heat stress.

Another set of hormones known as Catecholamines increase for broilers under heat stress (Marley & Nistico, 1972). These play a role in reducing temperature and studies have shown that pre-treatment with these lowered body temperatures of birds.

Also melatonin levels are impacted by heat stress (John & George, 1991; John et al., 1978). Melatonin is said to be a pineal hormone responsible for regulating body temperature. Studies have shown where elevated levels resulted in lower body temperatures and lower levels resulted in higher body temperatures (John et al., 1978). This hormone also enhances both the vasodilation and blood flow to surface tissue which encourages heat dissipation ((Jones & Johansen, 1972) cited by N. J. Daghir (2008)). Melatonin also increases the thermoregulatory capacity in birds through its influence on the hypothalamus (John & George, 1991).

With heat stress, there is a general inhibition of reproduction due to the reduced occurrence of reproductive hormones including Luteneizing hormone (LH) and Luteneizing hormone releasing hormone (LHRH) (Lara & Rostagno, 2013). The required pre-ovulatory surges of LH and progesterone are also reduced. Increase in hormones such as corticosterone during heat stress has a reductive impact on critical reproductive hormones such LH and LHRH in birds (Wilson & Follett, 1975).

Thyroid hormones are also affected by heat stress (Hahn et al., 1966; Mujahid, 2011). This hormone increases the metabolic rate and body temperature and as a result is reduced when birds undergo heat stress (Davison et al., 1980). Many studies have revealed where decreased levels or the absence of this hormone were associated with higher survival time under heat stress (Bowen et al., 1984).

2.3.1.4 Impact of heat stress on the molecular level

The most common response to heat stress is the increase in the production of heat stress proteins. During high temperatures birds undergo oxidative stress as a result of the proliferation of reactive oxygen species (ROS) produced to assist in homeostasis (Imik et al., 2012). In the midst of the increase in ROS, heat stress proteins or molecular chaperons protect heat sensitive proteins and reduce the damage to cells as a result of the precipitation of damaged proteins. Such a response may be breed specific and studies have shown where the dominant expression of the gene in native species when incorporated into the genetics of commercial breeds, improved their performance under heat stress (Marder, 1973).

2.3.1.5 Impact of heat stress on Immune function

Heat stress inhibits immune function (Mashaly et al., 2004; Mujahid, 2011). For instance the lower weight of the thymus, spleen, liver and increase in the heterophil to lymphocyte ratio are all indicators of this reduced immune response under heat stress.

The Thymus allows for the development and training of T-lymphocytes which defend the body against infection and disease (Lara & Rostagno, 2013). The weight of the spleen is also reduced which is an organ that not only filters blood from impurities but protects the body from infection (Taylor, 2013). The component white pulp tissue found in the spleen

is made up of lymphatic tissue comprising of macrophyl T-lymphocytes and B-lymphocytes that produce antibodies that destroy pathogens. Reduced weight of the spleen results in a reduction in the disease fighting capacity of the spleen. Also the reduced weight of liver as a result of heat stress also impacts negatively on the immunity of birds. Within the liver there are Kuffer cells which capture and digest the cells of fungi, parasites, bacteria, cell debris and dysfunctional blood cells. With a larger volume of blood flowing through the liver a greater volume of blood is cleansed and thus a reduction in the size of the liver may impede such a process. The bursa is also another organ that is affected negatively by the heat stress. This lymphoid organ specific to birds is responsible for the development of B cells which produce antibodies. With the reduced weight of the bursa, there is a lower level of circulating antibodies including Immunoglobulin G and M. Also the ratio of heterophils to lymphocytes is another indication of reduced immunity response to heat stressed birds. According to Prieto and Campo (2010), a higher heterophil-lymphocyte ratio is indicative of a suppressed and weakened immune response in birds experiencing heat stress.

2.3.1.6 Impacts of heat stress on production

The negative impacts of heat stress on broilers result in reduced production on farms (Cahaner et al., 1996; Scott & Balnave, 1988; Soleimani et al., 2010; Yunis & Cahaner, 1999). According to Lara and Rostagno (2013) and Yalcin et al. (2001), the reduction in production includes reduced meat production, reduced meat quality, increase in mortalities and reduced longevity in production.

The reduction of meat yield as a result of heat stress may be due to reduced feed intake and reduced quality of feed exposed to high temperatures (Attia et al., 2009; Gous & Morris, 2005). Dale and Fuller (1979) cited by N. J. Daghir (2008) identified a 63% reduction in growth rate attributed to reduced feed intake. With a lower feed intake less nutrients are being utilised towards growth and muscle yield and Yalcin et al. (2001) reported a 23% reduction in body weight as a result of a 15% reduction in food intake for birds exposed to high temperatures. Other causes of reduced nutrient intake and the associated reduction in meat yield may be a reduction in the digestibility of feeds exposed to high temperature (Yalcin et al., 2001). For instance Yalcin et al. (2001) reported reduced digestibility and greater ileal flow of proteins in heat stressed birds and Baião and Lara (2005) reported reduced quality of fats as a result of rancidification when exposed to high temperatures. With reduced digestibility and the wastage of nutrients, less is channelled towards meat production.

Not only is meat yield reduced but the quality of meat is also compromised in heat stressed birds (Imik et al., 2012; Tankson et al., 2001). The appearance of meat and texture are the two most critical indicators of meat quality (Imik et al., 2012). Many studies have shown where heat stress is especially related to increased cases of Pale Soft Exudative (PSE) meat in poultry which affects both the appearance and texture of meat. When birds are subject to heat stress, glycogen stores are rapidly depleted and as such post mortem levels are minimal. Lower glycogen levels post mortem is associated with high drip loss, reduced water holding capacity, reduced tenderness and a pale off colour in meats which are typical of PSE. The water holding capacity in meats is critical and with a lower water holding capacity as a result of protein denaturation in meats, texture is affected and meats are less

tender (Imik et al., 2012). Also under heat stress, the level of fat deposition is increased (Furlan et al., 2004; Lu et al., 2007; Rosa et al., 2007). Another negative impact of heat stress on meat quality is its impact on the type of meat proliferated by animals (Howlider & Rose, 1989; Imik et al., 2012; Tankson et al., 2001). When birds are subject to heat stress, there is a lower proliferation of breast meat which is of more economic value than thigh meat which increases under heat stress (Imik et al., 2012; Tankson et al., 2001).

High mortality is also another consequence of heat stress which impacts negatively on broiler production systems (Meremikwu et al., 2013; St-Pierre et al., 2003). Within the tropics, mortality rates measure up to 41% and may be more severe (61%) depending on the physiological state of the animal (the age, sex, breed) and farm management practices (Haitook, 2006). For instance many studies have shown where older animals with larger body weights are associated higher heat load and are more susceptible to heat stress than younger animals and male broilers with larger body masses are more susceptible to heat stress than female (Gous & Morris, 2005; St-Pierre et al., 2003; Yalcin et al., 2001). With respect to breeds Lu et al. (2007) as well as Yalcin et al. (2001) reported higher susceptibility of the rapidly growing strains of commercial broilers to heat stress compared to slow growing strains or strains crossed with indigenous breeds that have greater adaptability to high temperatures (Haitook, 2006; Rosa et al., 2007). Apart from the characteristics of animals, farm management practices such as acclimation and feed restriction in the earlier life of birds increase the tolerance and survival under high temperatures (Gous & Morris, 2005; Haitook, 2006; Lin et al., 2006).

Another impact of heat stress on broiler production systems is reduced longevity (Lara & Rostagno, 2013; Nardone et al., 2010). With the massive impact of heat stress on the

reproductive capacity of broilers, the proliferation and health of progeny can be negatively impacted thus reducing the long term productivity of farms (Attia et al., 2009; Smith, 1973). The depletive effect of high temperatures on reproductive hormones such as luteinising hormone (LH) and luteinising hormone releasing hormone (LHRH) or on pre-ovulatory surges of LH and progesterone reduces the reproductive capacity of breeding stock.

2.4 Understanding nutrition in broilers

In order to fully understand the significance of feed and its function in alleviating heat stress, the digestion of nutrients, their utilisation for energy, the partitioning of this energy to support the biological processes of the broilers and methods of measuring the energy utilisation of feeds will be first outlined and later followed by a description of different nutritional strategies used to alleviate heat stress.

2.4.1.1 Digestion and energy partitioning in birds

In birds food is apprehended by the beak and swallowed whole (Larbier & Leclercq, 1992). This food moves through the oesophagus and into the crop where it is stored and chemically broken down by lactic acid produced through bacterial fermentation. From the crop, the food enters into the proventriculus or true stomach where it is further broken down by hydrochloric acid. After digestion in the proventriculus, food moves to the gizzard where it is mechanically broken down through the contracting walls of the gizzard. This mechanical breakdown can be intensified in the presence of insoluble grit in the diet. After the gizzard the food moves through the small and large intestines where it is exposed to pancreatic juices and bile salts respectively. The nutrients from food are absorbed from the intestines that are layered with long finger like projections known as villi, to provide energy to support biological function in the bird.

The energy provided by food is in its chemical form (Emmans, 1994). This chemical energy yielded by the organic matter of food, primarily including carbohydrates, fats and proteins (NRC, 1994; Pirgozliev & Rose, 1999), is oxidised to produce gross energy, also known as heat of combustion. For the gross energy provided, both Metabolisable Energy

(ME) and Net Energy (NE) systems are used to describe the energy needs of birds, however the accuracy of both energetic systems in precisely describing the needs of broilers has been debated (De Groote, 1974; Pirgozliev & Rose, 1999). The total gross energy used by birds is known as ME which is more commonly referred to as apparent metabolisable energy (AME). AME is seen as the maximum dietary energy available to birds after the energy lost through faeces and urine is accounted for and NE is the metabolisable energy without the heat increment (see Figure 2.5) (Vohra, 1966). The energy obtained by birds is used for maintenance (ME_m) which is primarily expended to support the energy needs of basal metabolism, adaptive and dietary thermo-neogenesis as well as physical activity and measures between 42 to 44% of ME intake (MEi) (Emmans, 1994; Lopez & Leeson, 2005). The requirement for ME_m is calculated as a function of body weight raised to the power 0.60 as this more precisely estimate the maintenance requirements of birds compared to BW raised to the power of 0.75 (Lopez & Leeson, 2005). Apart from the energy used for maintenance, Fraps (1946) cited by Vohra (1966) defined net energy as that productive energy used for growth or the deposition of fat (k_f) and protein (k_p) and this accounts for 35 to 40 % of MEi. The energy costs associated with the deposition of fat includes the energy retained in fatty tissue which is 39.3 MJ/kg and the energy cost of depositing this fat which is 14 MJ/kg, giving a total requirement of 53.3 MJ/kg for k_f (Whittemore, 1997). For k_p the energy costs of depositing one kilogram of protein includes the energy retained in muscle tissue which measures 23.6 MJ/kg as well as the energy cost of protein deposition which measures 31 MJ/kg giving a total requirement of 54.6 MJ/kg for k_p (Whittemore, 1997).

The level of heat lost (heat increment) is dependent on the efficiency of available nutrients (Vohra, 1966). Nutrients are metabolised at different levels of efficiency for maintenance (K_m), and production or growth (fat (k_f) and protein (k_p) deposition). Based on the wide range of k values recorded in Table 2.8, fats generally have the highest energy efficiency followed by carbohydrate and then proteins (Emmans, 1994; Pirgozliev & Rose, 1999; Sakomura et al., 2005).

Table 2.8 A review of the various recorded efficiency of utilisation of carbohydrates, fats and proteins for different biological activities in animals

	CH[1]	Fats	Protein	K[2]	Species	Reference
K	0.9	0.7	0.6		Poultry (general)	Pirgozliev and Rose (1999)
Km[3]	0.78	0.88			Broilers	De Groote (1974)
km				0.76,0.8	Broilers	Sakomura 2005
km	0.75					De Groote (1971) cited by De Groote (1974)
km.g [4]			0.74-0.87		Broilers	MacLeod (1990)
kg[5]	0.9				Broilers	De Groote (1974)
kg				0.57	Broilers	Nijkamp et al. (1974)
kg				0.51-0.55	Broilers	I. R. Sibbald and Wolynetz (1986) cited by Nieto et al. (1995)
kg				0.74-0.78	Broilers	MacLeod (1990)
kg				0.57-0.66	Broilers	Nieto et al. (1995)
kg				0.6-0.65	Pigs	Birkett and Lange (2001)
kf[6]	0.70-0.74	0.9	0.707		Pigs	Emmans (1994)
kf				1.1-2.5	Taiwanese Chicken	Huang et al. (2007)
kf				0.96-1.11	Hubbard Hi Yield	Sakomura et al. (2003)
kf				0.64, 1.27	Broilers	Nieto et al. (1995)
kf	0.74	0.62-0.90	0.53		Pigs	Birkett and Lange (2001)
kf				0.75	Pigs	Whittemore (1997)
kf			0.52-0.53		Pigs	Whittemore (1997)
kf			0.56		Monogastrics	Birkett and Lange (2001)
kf				0.87	Broilers	Leclercq and Saadoun (1982) cited by McKinney and Teeter

						(2004)
kf				0.65	Broilers	Nieto et al. (1995)
kf				0.70-0.80	Broilers	Lopez and Leeson (2008)
kf	0.75	0.84	0.61	0.55,0.7,0.92	Broilers	Sakomura et al. (2005)
kp[7]				0.36,0.47, 0.58	Broilers	Sakomura et al. (2005)
kp				0.395	Pigs	Emmans (1994)
kp				0.41-0.58	Hubbard Hi Yield	Sakomura et al. (2003)
kp				0.40-0.58	Broilers	Nieto et al. (1995)
kp				0.6	Broilers	Noblet et al. (1989)
kp				0.44	Pigs	Whittemore (1997)
kp			0.47		Monogastrics	Birkett and Lange (2001)
kp				0.4	Broilers	Leclercq and Saadoun (1982) cited by McKinney and Teeter (2004)
kp				0.58	Broilers	Nieto et al. (1995)
kp				0.37-0.85	Broilers	Lopez and Leeson (2008)

[1]CH Carbohydrates, [2]K: efficiency of energy utilisation; [3]km efficiency of energy utilisation for maintenance; [4]km.g efficiency of energy utilisation for maintenance and growth; [5]kg: efficiency of energy utilisation for growth; [6]kf efficiency of energy utilisation for fat deposition; [7]kp efficiency of energy utilisation for protein deposition

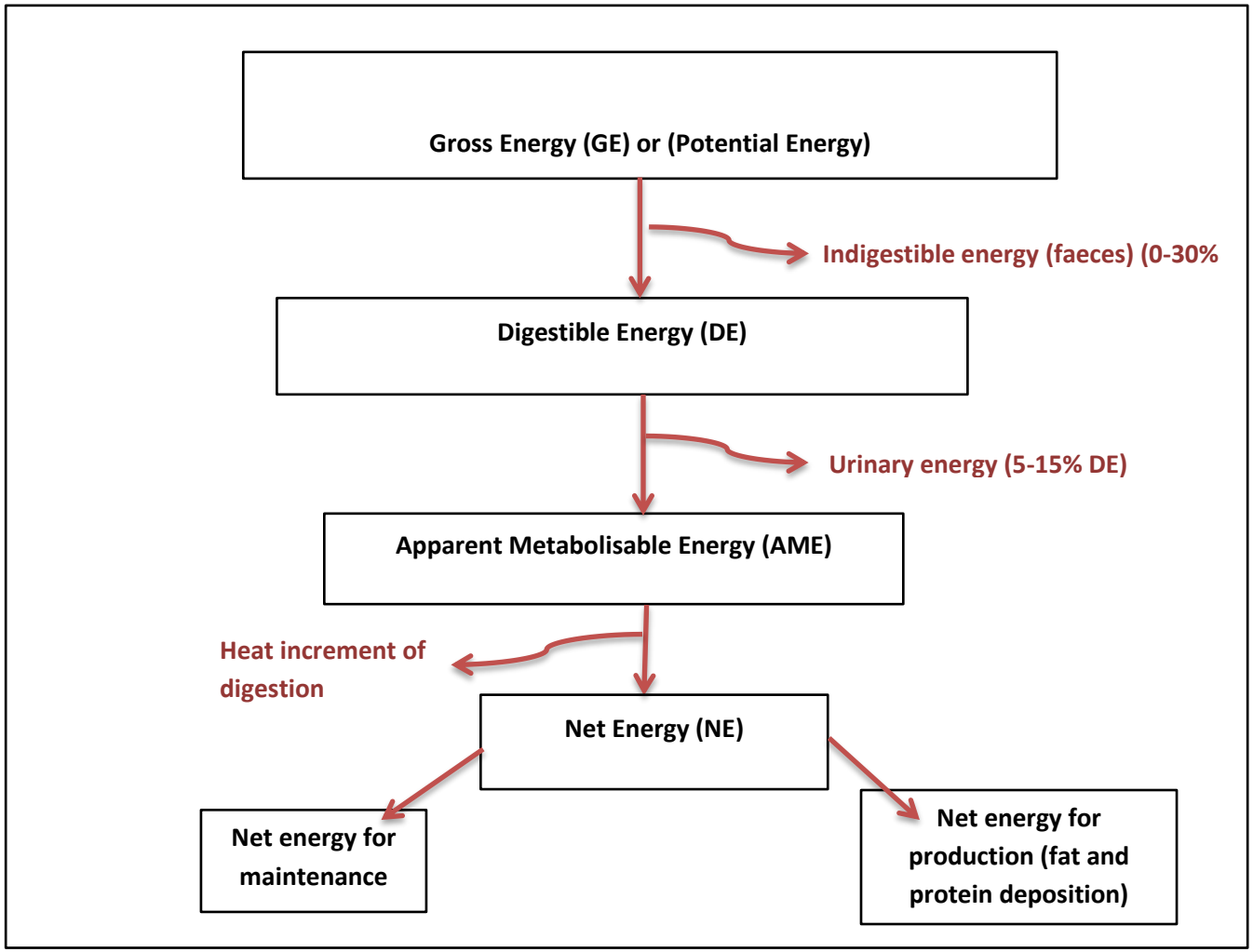

Figure 2.5 Energy partitioning in the broiler (Adapted from Smith and Leclercq (1990) and Birkett and Lange (2001)

2.4.1.2 Methods for measuring energy utilisation

The AME metabolisability and digestibility of feeds can be determined utilizing both in vivo and in vitro methods. One of the most popular reliable methods of measuring feed AME is the in vivo total collection method which involves the collection of digestibility data on faeces over a specific period of time as well as the collection of digestibility data on feeds which are both used to calculate the availability and utilisation of nutrients and energy in feeds (Dourado et al., 2010). Though such a method is highly recommended, there are many associated limitations to the method including the skewed excreta results as a consequence of contamination from feathers, feed and intestinal secretions in the excreta. Other limitations include loss of excreta that was not collected in collection trays, chemical changes in excreta due to fermentation during storage, variation in feed moisture content and storage space for collected excreta (Sales & Janssens, 2003). Another in vivo method used is the partial collection or marker method which involves the use of an inert material that is non- toxic, unchanged by processes of digestion taking place along the gastrointestinal tract, has no effect on the processes of digestion or no impact on the substrates or chemicals involve in digestive processes, has a high recovery rate in excreta and is simple to analyse ((Marais & D'Mello, 2000) cited by Dourado et al. (2010)). However according to Marais and D'Mello (2000) there is no such marker with all the desired features identified. This inert and indigestible substance is sometimes incorporated into diets in the form of an internal marker to track digestion and reflect the digestibility of nutrients in the diet. They include metal oxides such as chromic oxide, the mineral salt barium sulphate and titanium dioxide. With titanium dioxide, analyses are simple, accurate, requires a small sample size and results are more comparable to those of mass

collection when compared to results of other markers Sales and Janssens (2003). Analysis involves the digestion of samples in sulphuric acid and hydrogen peroxide which changes to an orange yellow colour that can be measured by a colorimeter. The recovery rate of Titanium dioxide measures 98% recovery versus chromium dioxide with a 94% recovery rate (Sales & Janssens, 2003). With the marker method, the limitation of contamination associated with the total collection method is eliminated (Dourado et al., 2010).

2.5 Strategies used to address the challenge of heat stress within the tropics

There are nutritional and non- nutritional strategies used to alleviate heat stress (Ahmad & Sarwar, 2006; Gous & Morris, 2005). The nutritional strategies range from nutrient density modification to timed feeding strategies while the non-nutritional strategies include both housing and genetic modification (Gous & Morris, 2005).

2.5.1 Nutritional strategies

There are various nutritional strategies used to alleviate heat stress (Gous & Morris, 2005; Lin et al., 2006). These strategies include use of feed form, optimizing the nutrient concentration and type of diet, improved amino acid profiles, modified energy and protein content of diets, inclusion of vitamins, adjustments of both the anion: cation and dietary electrolyte balance as well as feed timing (Gous & Morris, 2005).

2.5.1.1 Feed form

Feed form can also impact on the bird's capacity to tolerate heat stress (Behnke & Beyer, 2002; Hussar & Robblee, 1962; Ravindran, 2013). Almost all feeds used within commercial broiler production systems are in either crumble or pelleted form (Gous &

Morris, 2005). Processing feed into larger agglomerates of materials though costly, has compensated through the improved performance of birds (Behnke & Beyer, 2002). This improvement in performance has also been witnessed for birds subject to high temperature conditions and as a result has been put forward as a potential management strategy for reducing the impact of heat stress (Gous & Morris, 2005; Howlider & Rose, 1992). Pelleted diets or agglomerate feeds are more energy efficient than non-agglomerate feeds such as mash (M. Abdollahi et al., 2013; Behnke & Beyer, 2002; Reece et al., 1985). Such a characteristic of pelleted feeds may be as a result of decreased feed wastage, less time and energy expended in eating, reduced selective feeding, lower segregation of ingredients, improved palatability, improved digestibility and improved feed intake (Behnke & Beyer, 2002; Hussar & Robblee, 1962). With respect to decreased wastage, the larger sized more uniformed pellet units, birds are better able to prehend and consume food without wastage. The ease of prehension results in a faster rate of intake and less time and energy spent on gripping and eating food (Behnke & Beyer, 2002; Brickett et al., 2007; Engberg et al., 2002). Also with finely ground mix of ingredients compacted into larger pellet units, birds often have access to a balance of nutrients available in diets in a form that is palatable and much preferred by birds compared to tinier more segregated particles of mash diets. Additionally though the impact of the pelleting process on nutrient digestibility has been recently questioned (Bolton, 1960; Engberg et al., 2002; Lilly et al., 2011) it has been seen to be associated with the increased digestibility of some nutrients depending on different diets types used (Abdollahi et al., 2014; M. R. Abdollahi et al., 2013). These benefits of pelleted feeds contribute to the increased feed intake associated with this feed form (Behnke & Beyer, 2002; Engberg et al., 2002). In fact one of the major impacts of pelleted feed is the associated increase in feed intake and reports of up to 14% increase in feed

intake have been made (Abdollahi et al., 2011). The benefit of pelleted feed however is secured in the quality of the pellets which is impacted upon by the nature of processing (M. R. Abdollahi et al., 2013; Behnke & Beyer, 2002; Lilly et al., 2011). The better the quality of the pellets the longer they last and are not broken down into fines. This is critical and according to McKinney and Teeter (2004) 100% pellets adds 187kcal/kg to the diet and based on the findings of Behnke and Beyer (2002), a conversion to as little as 10% fines can result in a 0.01 increase in the FCR. Table 2.9 shows examples of the overall higher performance of pellet compared to mash fed birds.

Table 2.9 The impact of pellet versus mash diets on performance

	Mash/Meal	Pellet	Age (weeks)	Author
BW	1495.24	1647.15		
FI (g/bird/week)	638.27	660.37		
BWG (g/bird/week)	244.5	290.37	4-8	Jahan et al. (2006)
FCR/week	2.58	2.25		
Survivability (%)	98.33	98.33		
FI (kg/bird)	1.6	1.75		
BWG (kg/bird)	3.38	3.54		
FCR kg feed/kg WG	1.85	1.71		
AME intake (MJ/bird)	44	46		
BWG (kg) and Temperature				
17 °C	1.8	2.04		
21°C	1.72	2.04	7	Howlider and Rose (1992)
25°C	1.79	1.93		
29°C	1.58	1.76		
FI (kg) and Temperature				
17 °C	3.81	3.98		
21°C	3.55	4		
25°C	3.56	3.72		
29°C	3.29	3.44		
(g)	2857	3026		
BWG (g)	884	964	9	Bolton (1960)
FCR g feed/WG g	3.23	3.14		
Maize diets				
FI (g)	1024	1303		
BWG (g)	803	1023		

FCR g feed/WG g	1.278	1.28		
AME intake (MJ/bird)	12.95	16.55		
Wheat diets			3	M. Abdollahi et al. (2013)
FI (g)	1086	1318		
BWG (g)	856	1044		
FCR g feed/WG g	1.27	1.263		
AME intake (MJ/bird)	13.52	15.32		
FI (g)	976	1201		
BWG (g)	724	913		
FCR g feed/WG g	1.35	1.32	3	Abdollahi et al. (2014)
AME intake (MJ/bird)	13.02	15.19		
WG (g/bird)	875	965		
Feed intake (g/bird)	1150	1308	7	Abdollahi et al. (2011)
FCR g feed/WG g	1.317	1.368		
FI (g)	1840	2133		
BWG (g) (at 30 days)	1076	1361		
FCR g feed/WG g (at 0 days)	1.72	1.57	4	Svihus et al. (2004)
AME (MJ/kg) (at 24 days)	11.3	11.6		
BWG (g)	842	951		
FCR g feed/WG g	1.36	1.31		
32% Protein/3000 Kcal/kg			3	Jafarnejad et al. (2011)
BWG (g)	798	936		
FCR g feed/WG g	1.43	1.3		

FI (g)	1474	1562		
BWG (g)	941.53	1058.56	4	Zohair et al. (2012)
FCR g feed/WG g	0.78	0.66		

2.5.1.2 Optimizing the nutrient concentration and type of diet

The lower feed intakes associated with heat stress warrants high density diets that meet the need of birds at reduced levels of intake (Scott & Balnave, 1988). The optimizing of diets through increasing the nutrient density or through the manipulation of the types of nutrients used in diets maintains the benefit of diets to birds in spite of lower levels of intake at high ambient temperatures (Mujahid, 2011). Ghazalah et al. (2008) stated that increasing the fat content of diets to 5% improved average body weight, body weight gain, as well as FCR over the seven week trial. With increased levels of fats in the diet, the passage of feed through the gastrointestinal tract is slowed which is critical especially under high temperatures where the passage of feed is increased and the ability to break down and absorb nutrients is reduced as a result (Bonnet et al., 1997; Mujahid, 2011). Also the high energy efficiency of fats as a source of energy compared to carbohydrates and proteins allows fats to be a more suitable source of energy in diets for birds subject to high temperatures. With a higher energy efficiency there is a lower heat load carried by birds exposed to the high temperatures. Other studies have shown where the increase in the fat content of diets was coupled with the addition of amino acids including lysine and methionine which improved the performance of birds exposed to high temperatures (Reece et al., 1984; Sinurat & Balnave, 1986). Both the capacity of fats to slow the passage of feed and its characteristically high energy efficiency allows for the optimisation and improvement of the energy value and benefit of high fat diets offered to birds.

2.5.1.3 Improved amino acid profiles

Properly balanced amino acids is an essential strategy for reducing heat stress (Gous & Morris, 2005). During heat stress the ileal outflow of amino acids increases as a result of the decreased digestibility of certain amino acids (Soleimani et al., 2010). The deficiency of certain amino acids including arginine and lysine results in the increase in the T_3 hormone which increases the heat load of chickens (Furlan et al., 2004). Such a condition calls for the adjustment of the amino acid ratios and studies have shown where increasing the ratio of arginine and lysine coupled with the inclusion of sodium chloride in diets improved the digestibility and absorption of amino acids (Gous & Morris, 2005). With improvements in the balance of amino acids to meet the demands of the rapidly growing broiler, such wastage of amino acids through ileal flow is reduced. Not only is wastage reduced but the energy spent on and heat load associated with nitrogen excretion is reduced also (Gous & Morris, 2005). According to Waldroup et al. (1976) there was a significant improvement in the performance of birds fed on improved diets compared to birds fed on diets with improperly balanced amino acids. Such was opposed by Macleod (1997), as both groups of birds subject to high and low quality diets dissipated similar levels of heat under elevated temperatures. However according to Gous and Morris (2005) the overall lower performances of birds fed on poorly balanced diets in spite of no significant difference in heat dissipation for both groups, was an indication of the benefit of improved diets used by Macleod (1997).

2.5.1.4 Energy and protein requirements

The nutrient requirements of broilers reared under conditions of high temperature are different to that of broilers reared within their thermo neutral range. Such has been demonstrated through various studies reflecting differences in ME and protein requirements as well as differences in both protein to ME and amino acid to ME ratios incorporated in the diet of birds subject to differing ambient temperatures.

Ravindran (2013) reported differences for the metabolisable energy requirement as well as the protein requirements of birds reared under high versus low ambient temperatures.

The AME or ME requirement decreases with every rise in temperature above 21°C as a result of a lower maintenance requirement under high temperatures ((Hurwitz et al., 1980) cited by N. J. Daghir (2008)). Further, studies done by N. Daghir (1973) showed that there is a 10-15% lower energy requirement in birds subject to higher temperatures than birds subject to lower temperatures of the winter.

The protein requirement of broilers subject to heat stress is not clear and there is debate on such needs during heat stress. For instance increasing the protein content of the diet has been supported by many and justified by the increasing requirement for protein of the modern day broiler (Alleman & Leclercq, 1997; Gous & Morris, 2005; S Temim et al., 2000) (Gous & Morris, 2005). Other authors have disagreed with increasing the protein content of the diet under high ambient temperature even though the break-down of protein exceeds that of protein synthesis during heat stress. For these authors correction of reduced protein synthesis through the addition of dietary proteins cannot be accomplished. Also with the higher heat increment of protein digestion, increasing the content in diets of heat

stressed birds may exacerbate the heat load of the broilers (Cahaner et al., 1995; Lin et al., 2006; Soraya Temim et al., 2000). According to Furlan et al. (2004) and N. J. Daghir (2008), though increasing protein may be detrimental, supplementing poor quality protein and improving amino acid balances to meet the precise needs of birds is critical for heat stressed birds.

Also, the role of differing protein or amino acid to energy ratios and the impact on performance under heat stress has also been considered. For instance Sinurat and Balnave (1986) showed that birds preferred lower amino acid:ME (AA:ME) ratios when housed at elevated temperatures between 25° C and 35° C. Further, Sinurat and Balnave (1985) observed higher feed intake and body weight gain while Waldroup et al. (1976) saw improved growth rate and FCR when birds were given lower AA:ME under elevated ambient temperatures. Conversely Hurwitz et al. (1980) and Sinurat and Balnave (1985) saw the importance of increasing the AA:ME ratio. For Sinurat and Balnave (1985) however, the benefit of increasing the amino acid component at ambient temperatures above 30 ° C was negligible.

2.5.1.5 Inclusion of vitamins

The use of both vitamin A and E has proven to be effective in alleviating the impact of heat stress in broilers (Attia et al., 2009). Kutlu and Forbes (1993) cited by Gous and Morris (2005) reported a marked reduction in the levels of Malondealdehyde (MDA) in birds treated with vitamin C or ascorbic acid at a rate of 250 mg/kg feed. MDA is an indicator of lipid peroxidation due to cell damage caused by heat stress and this diminutive impact of vitamin C on MDA can be enhanced when combined with vitamin E which reduces tissue

damage as well as improves immunity response during heat stress. Other studies have shown where a combination of vitamin A and E at a rate of 15 000 IU retinol (vitamin A) and 250 mg Dl-alpha-tocopheryl acetate/kg of feed reduced MDA levels to more than twice that of other treatments. ZHANG et al. (2002) cited by Gous and Morris (2005) in their study demonstrated the benefit of combining ascorbic acid and chromium when an 11% increase in live weight was confirmed for this treatment.

2.5.1.6 Adjustments of both the anion: cation and dietary electrolyte balance

Adjustments to the anion: cation as well as electrolyte balance corrects alkalosis (Ahmad & Sarwar, 2006). For instance cation: anion interventions through the administration of ions in feed or water have been helpful to birds subject to high temperatures. Teeter et al. (1985) reported a reduction in the severity of alkalosis when calcium chloride was used to adjust sodium: chloride ratios. Also bicarbonate ions such as sodium bicarbonate and potassium bicarbonate are effective in replacing lost circulating bicarbonate ions as a result heat stress, with potassium bicarbonate resulting in greater weight gains (Gous & Morris, 2005). Apart from bicarbonates, ammonium chloride has also been effective in improving the blood pH of birds resulting in growth rate increases of up to 25% in heat stressed birds (Teeter et al., 1985). Water supply is also another way of adjusting the anion: cation balance. During heat stress 80% of heat is dissipated through latent heat loss where birds lose moisture. This moisture loss translates into an increase in water intake to compensate for moisture loss. The water given to birds must have a low temperature as this helps in the cooling of birds. Also the inclusion of potassium chloride to water at a rate of 5 g/l improves the feed intake and as a result improves weight gain under high temperatures.

With respect to the DEB, minerals including phosphorus, potassium, sodium, manganese and copper are lower during heat stress and with high levels of epinephrine and corticosteroids, magnesium and zinc levels are reduced (Ahmad & Sarwar, 2006). Such elicits the metabolic need for these mineral and both zinc and magnesium supplementation are linked to increased body weight and improved weight gain in birds subject to high temperatures. Zinc supplementation according to Ahmad and Sarwar (2006) is also associated with improved FCR, carcass quality and lower MDA concentrations in birds.

Other studies have shown where there are varying capacities of both anion : cation and DEB in explaining improvements in response to heat stress (Johnson & Karunajeewa, 1985). In fact Johnson reported a higher impact of electrolyte treatments on improving performance under high temperatures compared to adjusting the anion:cation ratio.

2.5.1.7 Feed Timing

There are different feeding regimes used within the tropics including *ad libitum* feeding and restricted feeding which includes intermittent feeding, feed withdrawal, dual feeding and early feed restriction programmes (Abu-Dieyeh, 2006; Lin et al., 2006). *Ad libitum* feeding is usually the most common feeding regime used in tropical broiler production systems and involves no feed restriction. Restricted feeding systems however involve the controlled administering of the amount or type of feed that is given to birds within specific periods of the day. When birds are not feeding they are left to rest and digest food (Brickett et al., 2007; Classen et al., 1991). In the humid tropics, periods of rest are important as it helps to reduce heat producing activity that can add to the heat load at high ambient temperatures (Yalcin et al., 2001). Within such feeding systems both food and water available are 20-30% more than non-intermittent systems. This allows birds to access as much feed and water desired within the restricted hours of feeding (N. J. Daghir, 2008). Though birds are offered more feed and water for feeding periods, the restrictive nature of the feeding systems is associated with a lower incidence of heart attacks, leg problems and ascites common to *ad libitum* feeding (Classen et al., 1991). Other benefits of intermittent feeding include lean meat and thus superior carcass quality as well as more uniformed production (Brickett et al., 2007). With feed withdrawal, feed is withdrawn from birds at specific times before the period of the day birds are most at risk of heat (Gous & Morris, 2005). Studies done have shown where withdrawn feeding has been associated with increased capacity to cope with heat stress. Francis et al. (1991) and Lin et al. (2006) witnessed longer survival times of fasted birds exposed to heat stress. Some of the disadvantages of such systems however is the longer growth period to obtain market weight and depletion of

intestinal mucus which can compromise intestinal function (Mujahid, 2011). Dual feeding is also another restricted feeding system which involves the manipulation of nutrient density during the hotter or cooler parts of the day. For instance the use of lower protein during the hotter periods of the day and higher protein levels during the cooler parts of the day (De Basilio et al., 2001). Though there are lower feed efficiency and growth rates, reduced mortality is a benefit of this system (De Basilio et al., 2001). Early feed restriction is also another form of restricted feeding (Zulkifli et al., 2000). Such involves restricted feeding in the early stages of the bird's life. Studies have shown where 4-5 day old birds under 60% feed restriction performed better, had stronger immune responses and had better survival rates when exposed to heat stress later on in life compared to those who were not on early restricted feeding programmes (Zulkifli et al., 2002).

2.5.2 Non- nutritional strategies

2.5.2.1 Genetic modifications

Genetic modification involves either selection for heat stress or the use of major genes including the naked neck gene (Na), frizzle gene (F), feather growth rate (K) or scale-less or featherless gene (Sc) (Yunis & Cahaner, 1999). Many of the broilers used within the regions of the tropics are not bred to perform well under the higher temperatures of the tropics and as a result selection for heat tolerance can assist in improving the tolerance of birds to high temperatures (Gous & Morris, 2005). For instance, studies on three different strains of broilers showed varying levels of heat tolerance in birds (Lin et al., 2006). Selection programmes for birds that have a high tolerance for high temperature can therefore be an option worth investigating. However, one challenge that must be addressed concerning selection for heat tolerance is its mutual exclusion with favourable traits such as high feed efficiency and growth rate in broilers. Many authors reported a negative correlation between both heat tolerance and traits linked to high growth rate and feed efficiency (Lin et al., 2006). With respect to the Na gene, heat dissipation is accelerated as there is a 20 to 40% reduction in feather mass associated with the hetero and homozygous genotypes respectively (Merat, 1986). This reduction in feather mass is especially around the neck region allowing for greater skin surface area from which heat can be released. Also with less plumage, there are less fatty deposits in the subcutaneous skin layers which also assist in easier heat dissipation. Birds having heterozygous and homozygous genotypes display higher growth rates, higher proliferation of breast meat and high quality carcass compared to feathered birds (Lin et al., 2006). The F gene is also effective as it reduces heat insulation by coding for feathers that curl outward and that are smaller than

regular sized feathers (Lin et al., 2006). The K gene allows for slowed or late feathering which is critical as with age, birds increase in size resulting in decreasing capacity to dissipate heat and with slower or late feather growth, heat dissipation can be enhanced. Gous and Morris (2005) emphasised the greater heat tolerance, faster growth rates and improved FCR exhibited by birds with slower late feather growth compared to their fast feather growth counterparts. The Sc gene is similar to that of the Na gene in reducing plumage, however with the Sc gene, birds are featherless. For such birds higher carcass yields as well as carcass quality with a characteristically high protein content and less fatty deposits were observed (Gous & Morris, 2005).

2.5.2.2 Environmental modifications

Environmental modifications to alleviate heat stress include lighting, humidity adjustments and early conditioning of birds (Lin et al., 2006). With respect to lighting, birds subject to intermittent lighting have shown reduced heat production. Studies done by Aerts et al. (2000) cited by Lin et al. (2006) showed reduced heat production and improved FCR for birds subject to cycles of 1 hour light to 3 hours of darkness. Humidity is also another factor of the environment to be controlled as with increasing levels of humidity, heat dissipation from birds becomes more challenging. With respect to evaporative cooling such a strategy is helpful in temperatures 28°C and above and for humidity levels below than 70% (Gous & Morris, 2005). Apart from lighting and humidity, early conditioning through early feed restriction and early thermal conditioning to increase acclimation of birds is also another environmental strategy. With the introduction of feed restriction at the early stages of life, birds are often found with higher levels of performance and immunity when subject to high temperatures later on in life. For instance, Zulkifli et al. (2000)

reported improved growth, better survival rates and reduced heterophil to lymphocyte ratio of birds subject to 60% restriction at the early stages of life. Studies conducted by Liew et al. (2003) demonstrated improved tolerance to high temperatures and higher resistance to disease in birds subject to both heat conditioning and feed restriction earlier in life.

2.6 Conclusion

The impact of heat stress is detrimental to broiler production within the tropics. However such can be abated by the implementation of various nutritional and non-nutritional strategies. While management is a holistic approach and all strategies must be held as critical, there is a great emphasis on nutritional strategies that can contribute to improving the liveability and tolerance of broilers subject to heat stress.

3 Materials and Methods

Experimental procedures described in this thesis were approved by the Massey University Animal Ethics Committee and complied with the New Zealand Revised code of Ethical Conduct for the use of live animals for research, testing and teaching.

3.1 Birds and Treatments

The experimental design was a 2 x 2 x 2 factorial arrangement of treatments from day 10 to day 34 with 12 replicates per treatment combination. A total of 288, one-day-old male broiler (Ross 308) chicks, obtained from a commercial hatchery, were used for the experimental trial. Day old chicks were separated into two different groups where they were housed in floor pens, subjected to temperature conditioning for elevated and normal temperature groups, fed a commercial diet and given access to water *ad libitum,* for the first 9 days of life.

On day 10 of the trial, birds were first weighed then separated into one of eight treatments as seen in Table 3.1 which included a combination of one of two different temperatures (normal or elevated), one of two different diet types (high fat or low fat) and one of two different diet forms (mash or pellet). Birds were placed into four normal temperature rooms and four elevated temperature rooms. Each room had 12 replicates (cages) of three birds and each replicate was randomly assigned one of four different diet treatments (form x type) namely low fat mash, low fat pellets, high fat mash or high fat pellets. Birds had access to water *ad libitum* via nipple drinkers. The composition of diets used is presented in Table 3.2.

From day 1 to day 9, the maximum temperature for elevated temperature birds was dropped from 30°C to 28°C and for normal temperature birds, temperature was dropped from 30°C to 26°C (see Figure 3.1). From day 10 to day 34, the maximum temperature in rooms with cages dropped from 30°C at day 10 of the trial to 23°C at day 34 of the trial and for normal temperature treatments, maximum temperatures was dropped from 25°C at day 10 of the trial to 21°C at day 34 of the trial. For elevated temperature treatments, temperatures were raised daily by an average of 1°C between 9 am and 4 pm during the week and for normal temperature treatments, temperatures were adjusted according to the recommendations of the breeding company (Ross Broiler Manual, 2009). Temperatures were maintained at 25°C on the weekends for elevated temperature groups (see Figure 3.1).

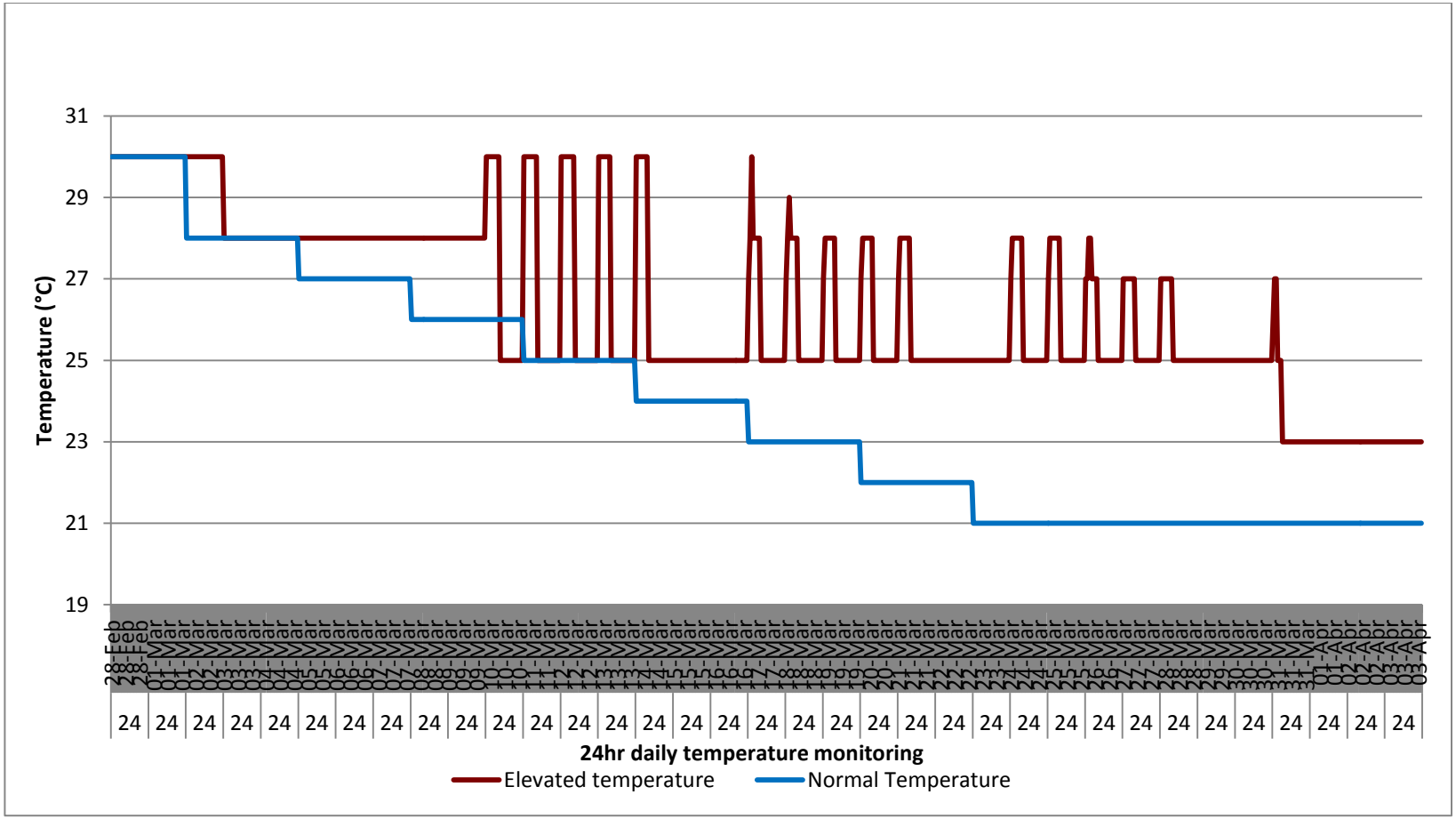

Figure 3.1 Daily temperature for both elevated and normal temperature treatments

Table 3.1 Treatments for 2 x 2 x 2 factorial design

Diet					
Type	**Form**	**Temperature**	**No. of cages**	**No. of birds**	**Total birds per treatment**
Low fat (L)	Mash (M)	Normal (N)	12	3	36
Low fat (L)	Mash (M)	Elevated (E)	12	3	36
Low fat (L)	Pellet (P)	Normal (N)	12	3	36
Low fat (L)	Pellet (P)	Elevated (E)	12	3	36
High fat (H)	Mash (M)	Normal (N)	12	3	36
High fat (H)	Mash (M)	Elevated (E)	12	3	36
High fat (H)	Pellet (P)	Normal (N)	12	3	36
High fat (H)	Pellet (P)	Elevated (E)	12	3	36

Table 3.2 Ingredients and calculated composition of low and high fat diets

	Diet	
Ingredients (%)%	**Low fat**	**High fat**
Maize	47.55	51.76
Maize starch	15	0
Soybean Meal 48%	24.1	32.18
Wheat bran	0	5.9
Maize gluten meal	2.79	0
Meat and bone meal	6.92	0
Soybean oil	1.62	6.01
Dicalcium Phoshate	0	1.78
Limestone	0.26	0.66
DL Methionine	0.34	0.33
Lysine	0.37	0.22
L Threonine	0.14	0.1
Salt	0.18	0.24
Sodium bicarbonate	0.2	0.29
Vitamin Premix	0.08	0.08
Min Premix	0.15	0.15
Titanium dioxide	0.3	0.3
Total	100	100
Calculated composition		
AME (Kcal/kg)	3150	3150
Starch (%)	44.2	33.8
Total protein	21	21
Digestible protein	16.6	17.17
Digestible Methionine	0.59	0.59
Digestible M+C	0.84	0.84
Digestible Lysine	1.1	1.1
Digestible Threonine	0.73	0.73
Digestible Arginine	1.14	1.25
Crude fat	4.3	8.08
Crude fibre	2.25	3.1
Calcium	0.9	0.9
Available Phosphorus	0.45	0.45
Na	0.19	0.19
Chloride	0.19	0.19

3.2 Growth and post growth data collection

At the start of the experiment on day 10 the birds were weighed and on days 14, 21, 28, and 34 the birds were weighed and body weight along with feed intake were recorded. Mortalities were recorded on a daily basis.

3.2.1 AME

For apparent metabolisable energy (AME) measurements, excreta were collected from each cage between days 31 and 34. Collected excreta were weighed, mixed and sub sampled and each sub sample freeze dried and ground to pass through a 0.5 mm sieve. Excreta samples were analysed for dry matter, ash, titanium dioxide and gross energy.

3.2.2 Ileal collection and analysis

Birds were euthanised by intravenous injection of pentobarbitone (1 ml/2kg body weight) and Ileal digesta was collected. The ileum which is defined as the portion of the intestines extending from the Meckel's diverticulum to a point ~40mm proximal to the ileocecal junction, was first divided into two halves and digesta was then flushed from the lower half of the ileum using reverse osmosis water (RO water). The ileal digesta collected from birds of the same cage were pooled together. The digesta were then freeze dried and ground to pass through a 0.5 mm sieve. Ground ileal samples from each cage was then paired and mixed with ileal from another cage with similar treatment combinations (temperature x form x type) giving six ileal replicates per combination. The mixed ileal samples were sent to Massey University Nutrition Laboratory for the analysis of dry matter, ash, nitrogen, fat, starch and titanium dioxide.

3.2.3 Diet

For the first phase which started on day 10 and ended on day 21, a total of 9000g of each diet was supplied to each cage. At the second phase from days 21 to 34, a total of 6000 grams of diet with a titanium dioxide marker was used to supply each cage for the partial collection data. For diets, 100 g of each diet was sent to the Massey University Nutrition Laboratory for analysis of dry matter, ash, nitrogen, fat, starch, neutral detergent fibre (NDF), acid detergent fibre (ADF), lignin and titanium dioxide.

Also samples of both the low fat and high fat pellet diets were submitted for measuring the Pellet Durability Index (PDI). Pellet durability was determined in a Holmen Pellet Tester (New Holmen NHP100 Portable Pellet Durability Tester, TekPro Ltd., Willow Park, North Walsham, Norfolk, UK) using the method described by Abdollahi et al. (2010). Clean pellet samples (100 g; six replicates per diet), with no fines, were rapidly circulated in an air stream around a perforated test chamber for 30 seconds. Fines were removed continuously through the perforations during the test cycle. After the test cycle, the subject pellets were ejected and weighed manually. The pellet durability index (PDI) was calculated as the percentage of the pellets not passing through the perforations at the end of the test.

3.3 Chemical Analysis

The determination of dry matter (DM) and gross energy (GE) was done for the diet, excreta and ileal samples. DM was done using the standard procedure (method 930.15, 925.10: AOAC) and GE determined using bomb calorimetry. Fat and protein content were determined for the diet and ileal samples using the Saxtec extraction method (991.36:

AOAC, 2014) for fat content and the Leco total combustion method (991.36: AOAC) for the nitrogen content. The starch content for both diet and ileal samples was measured using an assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland) based on thermostable alpha-amylase and amyloglucosidase (McCleary et al., 1997). For diet, excreta and ileal samples, the ash content was determined using the method 942.05: AOAC where samples were firstly ashed at 550°C. NDF, ADF and lignin were determined for the diet samples using the method by Robertson and Van Soest (1981) and the Tecator Fibreter System (method 2002.04: AOAC). Samples of the diet and ileal were assayed for titanium dioxide utilising sulphuric acid followed by colourimetric determination.

3.4 Calculations

The AME values of the diets were calculated using the following formula with appropriate corrections made for differences in the DM content.

$$\text{AME Coefficent} = \frac{(\text{Feed intake} \times GE_{diet}) - (\text{Excreta output} \times GE_{excreta})}{\text{Feed intake}}$$

AME in diet (MJ/kg DM) = AME Coefficient x GE in diet

AME intake (MJ/day) = Feed intake (kg DM/day) x AME in diet (MJ/kg DM)

Ileal apparent nutrient digestibility coefficients were calculated using the following formula:

$$\text{CIAD of the nutrient} = \frac{(\text{Nutrient in Diet}/\text{Titanium in diet}) - (\text{Nutrient in digesta}/\text{Titanium in digesta})}{}$$

(Nutrient in Diet /Titanium in Diet)

Ileal digestible nutrient content in Diet (g/Kg DM) = Nutrient CIAD x Nutrient in diet (g/Kg DM)

Nutrient intake (g/day) = Feed intake (kg DM/day) x Ileal digestible nutrient content in Diet (g/Kg DM)

Titanium Recovery (%) in the excreta was calculated as follows:

Titanium Recovery (%) = 100 x [(Titanium in excreta x excreta output)/ (Titanium in diet x feed input)]

The potential Net Energy (pNE) content of the diets was calculated as the sum of the ileal digestible energy obtained from protein, starch and fat multiplied by their corresponding k values.

cpNE Protein = crude protein in diet x ileal protein digestibility coefficient x 24 x 0.65

sNE Starch = starch in diet x ileal starch digestibility coefficient x 17 x 0.69

fNE Fat = fat in diet x ileal fat digestibility coefficient x 39 x 0.92

pNE (MJ/kg dry matter) = cpNE + sNE + fNE

This calculation does not take into account that some of the ileal digested protein are not used as an energy source by the animal and that some of the ileal undigested protein, ileal undigested starch and ileal undigested fat can be metabolised by bacteria in the hindgut, converted into volatile fatty acids and used as an energy source by the animal.

3.5 Statistical Analysis

The data was analysed using a 2 x 2 x 2 factorial design with the General Linear Model Procedure SAS (2012) and the differences were marked as significant at the $P < 0.05$ level using the following model:

$Y_{ijkl} = \mu + Type_i + Form_j + Temperature_k + Type_i \times Form_j + \textit{Type}_i \textit{ x Temperature}_k\text{*} + Form_j \times Temperature_k + \textit{Type}_i \textit{ x Form}_j \textit{ x Temperature}_k\text{*} + e_{ijkl}$

where

Y_{ijkl}: l^{th} observation in the i^{th} treatment group Type, jth observation in the treatment group Form and k^{th} observation in the treatment group Temperature

μ: General mean

$Type_i$: Fixed effect of diet type including high fat and low fat diets

$Form_j$: Fixed effect of diet form including pelleted and mash diets

$Temperature_k$: Fixed effect of temperature including normal and elevated temperatures

$Type_i \times Temperature_k$, $Form_j \times Temperature_k$, $Type_i \times Form_j \times Temperature_k$: interactions between fixed effects

e_{ijkl}: random residual error

*italicised interactions were not fitted into the final model as these interactions were not significant for the different parameters tested. The following reduced model was used $Y_{ijkl} = \mu + Type_i + Form_j + Temperature_k + Type_i \times Form_j + Form_j \times Temperature_k + e_{ijkl}$

Where applicable, significant differences between means were identified using the Least Significant Difference (LSD) test.

4 Results

Overall, the results were analysed using the reduced model " $Y_{ijkl} = \mu + Type_i + Form_j + Temperature_k + Type_i \times Form_j + Form_j \times Temperature_k + e_{ijkl}$", as both Type x Temperature as well as Type x Form x Temperature interactions were not significant for the different parameters tested.

The chemical analysis of diets used for treatments is presented in Table 4.1. The Pellet Durability Index (PDI) of both the low and high fat diets was measured. The PDI for the low fat diet was higher than the high fat diet (88.5% versus 77.1% respectively; (SE = 0.47, $P < 0.0001$).

Table 4.1 The chemical composition of low and high fat diets.

	Diet	
	Low Fat (Mash and Pellet)	**High Fat (Mash and Pellet)**
DM (g/kg)	894	897
Ash (g/kg DM)	54.0	63.0
Crude Protein (g/kg DM)	228.0	220
Fat (g/kg DM)	46.0	93.0
Starch (g/kg DM)	392.0	308.0
NDF (g/kg DM)	112.0	130.0
ADF (g/kg DM)	27.0	37.0
Lignin (g/kg DM)	9.0	9.0

TiO2(g/kg DM)	3.0	3.0
GE(KJ/g)	187.0	196.0

4.1 Mortality

Of the total 288 birds used, there was an overall 12% mortality rate (35 birds) with the majority of 11% mortality (32 birds) taking place between days 21 and 34 of the trial and a lower mortality of 1% (3 birds) taking place between days 10 and 21 of the trial. The diet type and form treatments both had significant effects on mortality. Based on Table 4.2, high fat diets had a higher *(P = 0.046)* mortality rate of 13.5% compared to mortality on low fat diets of 6.2%. Birds fed on pellet diets had a higher (*P <0.0001*) mortality rate of 18.5% compared to mortality on mash diets of 4.4%.

Table 4.2 The influence of the main effects of diet type, diet form and temperature on the rate of mortality in birds (Least square means + Standard Error (LSMEANS + SE)).

	n	LSMEANS[1]	SE	Mortality (%)[2]	Pr > ChiSq
Type					
High Fat	48	1.9	0.3	13.5	0.046
Low Fat	48	2.7	4	6.24	
Form					
Mash	48	3.1	0.4	4.4	<.0001
Pellet	48	1.5	0.2	18.5	
Temp					
Elevated	48	2.5	0.4	7.6	0.19
Normal	48	2.1	0.3	11.2	

[1] Logit values

[2] Back transformed values

The main effects including diet type and diet form as well as interactions between the diet type and form had significant influences on all growth and digestibility parameters examined.

4.2 Live weight

The live weight of the birds at the start of the experiment (day 10), at the end of the first period (day 21) and at the end of the experiment (day 34) are presented in Table 4.3.

At day 10 birds kept at the elevated temperature regimen were lighter than those kept at normal temperature (353 g vs 357 g, $p<0.01$).

Pellet fed birds had a higher (*P < 0 .0001*) average live weight of 1246 g on day 21 and higher (*P < 0 .0001*) average live weight of 2573 g on day 34 compared to that of mash fed birds where the average live weight was 1026 g on day 21 and 2306 g on day 34. At day 34, birds had a higher (*P = 0.036*) average live weight of 2476 g under normal temperature conditions compared to birds kept at elevated temperature where the average live weight was 353 g. Table 4.3 also showed that at day 21, low fat pellet diets resulted in the highest (*P <0.0001*) average live weight of 1264 g. Average live weight under low fat mash was the lowest (*P <0.0001*) measuring 986 g. At day 34, low fat pellets resulted in a higher (*P <0.0001*) average live weight of 2634 g than high fat pellets with an average live weight of 2511 g. When housed under elevated temperature pellet fed birds had a higher ($P = 0.0101$) average live weight of 2653 g compared to mash fed birds with an average live weight of 2297 g.

Table 4.3 The influence of diet type, diet form and temperature and their interactions on the live weight (g) of broilers at days 10, 21 and 34 (Least square means + Standard Error (LSMEANS + SE)).

		Live weight		
	n	**day 10**	**day 21**	**day 34**
Type				
High Fat	48	356	1147	2467
Low Fat	48	355	1125	2412
SE		0.893	8.092	23.871
P value		0.3329	0.0651	0.1038
Form				
Mash	48	356	1026	2306
Pellet	48	354	1246	2573
SE		0.893	8.092	23.871
P value		0.0489	<.0001	<.0001
Temp				
Elevated	48	353	1139	2476
Normal	48	357	1132	2404
SE		0.893	8.092	23.871
P value		0.0021	0.5372	0.0366
Type*Form				
High Fat Mash	24	357	1065c	2423b
High Fat pellet	24	354	1227b	2511b
Low Fat Mash	24	356	986d	2189c
Low Fat pellet	24	354	1264a	2634a
SE		1.263	11.443	33.759
P value		0.6334	<.0001	<.0001
Form*Temp				
Mash Elevated	24	354	1021	2297c
Mash Normal	24	359	1031	2314c
Pellet Elevated	24	352	1258	2653a
Pellet Normal	24	356	1233	2493b
SE		1.263	11.443	33.759
P value		0.6491	0.122	0.0101

Values with different superscripts [a, b, c, d] are significantly different from each other (LSD, P <0.05)

4.3 Feed intake

In Table 4.4 temperature, diet form as well as both type and form and form and temperature interactions had significant effects on feed intake of broilers ($P < 0.05$). Feed intake was higher on pellet versus mash diets for both phase 1 where average feed intake was 105.9 g/b/d on pellet diets and 87 g/b/d on mash diets and for phase 2 where intake was 160.3 g/b/d on pellet diets and 152.5 g/b/d on mash diets. Such was the same for the overall period where intake was higher ($P < 0.0001$) for pellet diets measuring 133.7 g/b/d compared to mash diets at 121.9 g/b/d. Feed intake for phase 2 was higher ($P = 0.007$) for elevated temperature (159.5 g/b/d) compared to the normal temperature treatment (153.3 g/b/d). Intake for phase 1 was higher ($P = 0.002$) on high fat (104.4 g/b/d) and low fat (107.5 g/b/d) pellets and lower ($P = 0.002$) on high fat (89.8 g/b/d) and low fat (84.1 g/b/d) mash diets. Low fat pellets resulted in the highest ($P < 0.0001$) average intake of 164.6 g/b followed by intakes of high fat mash (160.7kg/b/d) and high fat pellets (156 g/b/d) which were both comparable for phase 2. For the overall growth period, average intake for the low fat pellet diet was highest ($P < 0.0001$) at 137.5 g/b/d followed by comparable intakes of high fat mash and high fat pellet diets. Also intake for phase 2 was highest ($P = 0.016$) on the pellet diets fed under elevated temperature (166.9 g/b/d) with other treatments having similar intakes.

4.4 Average daily gain

Based on Table 4.4, the diet form, temperature as well as diet type and form interactions had significant effects on the average daily gain (ADG) of broilers ($P < 0.05$). For phase 1, the ADG for pellet diets was 81.1 g/b/d which was higher ($P < 0.0001$) than ADG 60.9

g/b/d for mash diets. ADG on pellet diets for the overall growth period was greater (*P < 0.0001*) at 91.8 g/b/d than mash diets where the gain was 80.9 g/b/d. Elevated temperatures resulted in higher gain for phase 2 and the overall period at 87.9 g/b/d and 102.8 g/b/d respectively compared to gain of 84.8 g/b/d and 97.8 g/b/d under normal temperature conditions. For phase 1, gain was highest (*P < 0.0001*) on pellet diets with the low fat pellet diet having the highest (*P < 0.0001*) average daily gain of 82.8 g/b/d which was followed by ADG of 79.4 g/b/d for high fat pellet diets. The lowest (*P < 0.0001*) gain of 57.3 g/b/d was recorded for the low fat mash diet. With respect to phase 2, the high fat pellet diet resulted in a lower (*P < 0.0001*) average gain of 98.8 g/b/d compared to the low fat pellet diet where average gain measured 105.4 g/b/d. Gain was lowest (*P < 0.0001, P < 0.0001 and P < 0.0001 for phase 1, 2* and overall growth period respectively) for low fat mash diets for phase 1 (57.3 g/b/d), phase 2 (92.5 g/b/d) and for the overall growth period (76.3 g/b/d). For the overall period, the average daily gain was highest (*P < 0.0001*) for the low fat pellet diets (94.8 g/b/d).

4.5 Feed conversion ratio

According to Table 4.4, the diet form, temperature, as well as type and form interactions had significant effects on the feed conversion ratio (FCR). For both phase 1 and for the overall growth period, mash diets had a higher (*P < 0.0001*) FCR of 1.43 and 1.51 respectively compared to pellet diets with a lower (*P < 0.0001*) FCR of 1.31 and 1.46 for phase 1 and overall growth period respectively. For phase 2 both diet forms had FCR values that were comparable to each other. FCR for elevated temperature conditions measured 1.35 and 1.48 for phase 1 and overall which were lower (*P = 0.021and P =*

0.047 for phase 1 and overall period respectively) than that for normal temperature which measured 1.39 and 1.49 for both phase 1 and for the overall growth period respectively.

Table 4.4 The influence of diet type, diet form and temperature and their interactions on feed intake (FI), average daily gain (ADG) and feed conversion ratio (FCR) of broilers for phase 1 (d 10 – d 21), phase 2 (d 21- d 34) and an average of performance parameter for the entire period (d 10 – d 34) (Least square means + Standard Error (LSMEANS + SE)).

		Phase 1			Phase 2			Overall		
	n	**FI (g/b/d)**	**ADG (g/b/d)**	**FCR**	**FI (g/b/d)**	**ADG (g/b/d)**	**FCR**	**FI (g/b/d)**	**ADG (g/b/d)**	**FCR**
Type										
High Fat	48	97.1	71.9	1.36	158.3	101.6	1.56	128.6	87.2	1.48
Low Fat	48	95.8	70.1	1.39	154.5	99	1.57	127.1	85.5	1.49
SE		0.99	0.74	0.01	1.98	1.59	0.01	1.36	1.01	0.006
P value		0.358	0.083	0.058	0.172	0.245	0.926	0.442	0.265	0.117
Form										
Mash	48	87	60.9	1.43	152.5	98.5	1.55	121.9	80.9	1.51
Pellet	48	105.9	81.1	1.31	160.3	102.1	1.58	133.7	91.8	1.46
SE		0.99	0.74	0.01	1.98	1.59	1.551	1.36	1.01	0.006
P value		<.0001	<.0001	<.0001	0.007	0.109	0.052	<.0001	<.0001	<.0001
Temp										
Elevated	48	95.8	71.5	1.35	159.5	102.8	1.56	129.2	87.9	1.48
Normal	48	97.1	70.5	1.39	153.3	97.8	1.57	126.4	84.8	1.49
SE		0.99	0.74	0.01	1.98	1.59	0.01	1.36	1.01	0.006
P value		0.335	0.337	0.021	0.029	0.029	0.22	0.154	0.032	0.047
Type*Form										
High Fat Mash	24	89.8^{b}	64.4^{b}	1.40^{c}	160.7^{b}	104.4^{ab}	1.54	127.3^{b}	85.5^{b}	1.48^{b}
High Fat Pellet	24	104.4^{c}	79.4^{c}	1.30^{a}	156.0^{b}	98.8^{a}	1.59	129.9^{b}	88.8^{b}	1.46^{ab}
Low Fat Mash	24	84.1^{a}	57.3^{a}	1.50^{d}	144.3^{a}	92.5^{ab}	1.56	116.6^{a}	76.3^{a}	1.52^{c}
Low Fat Pellet	24	107.5^{c}	82.8^{d}	1.30^{a}	164.6^{c}	105.4^{b}	1.57	137.5^{c}	94.8^{c}	1.45^{a}
SE		1.4	1.04	0.014	2.81	2.24	0.014	1.93	1.43	0.009

P value		0.002	<.0001	0.002	<.0001	<.0001	0.124	<.0001	<.0001	0.006
Form*Temp										
Mash Elevated	24	86.5	60.6	1.43	152.1[a]	98.2 [a]	1.55	121.3 [a]	80.5 [a]	1.51
Mash Normal	24	87.5	61.1	1.44	152.8 [a]	98.7 [a]	1.55	122.5 [a]	81.2 [a]	1.51
Pellet Elevated	24	105.1	82.4	1.28	166.9 [b]	107.3 [b]	1.56	137.1[c]	95.2 [c]	1.44
Pellet Normal	24	106.8	79.8	1.34	153.7 [a]	96.9 [a]	1.6	130.3 [b]	88.2 [b]	1.48
SE		1.4	1.04	0.014	2.81	2.24	0.014	1.93	1.43	0.009
P value		0.808	0.136	0.053	0.016	0.017	0.201	0.042	0.009	0.061

Values with different superscripts [a, b, c, d] are significantly different from each other (LSD, P <0.05)

4.6 Coefficient of ileal apparent digestibility (CIAD)

In Table 4.5 the diet type and form as well as type and form interactions had significant effects on the CIAD of ash, nitrogen, fat and starch ($P < 0.05$). The high fat diet had a higher ($P = 0.0031$, $P = 0.0001$, $P = 0.0004$ for ash, nitrogen and fat respectively) digestibility of 0.37, 0.81 and 0.91 for ash, nitrogen and fat respectively when compared to low fat diets having a lower ($P = 0.0031$, $P = 0.0001$ and $P = 0.0004$ for ash nitrogen and fat respectively) digestibility of 0.29, 0.78 and 0.89 for ash, nitrogen and fat respectively. There was a lower ($P < 0.0001$) digestibility of starch for high fat diets of 0.92 compared to low fat diets with a starch digestibility of 0.96. The digestibility of ash and nitrogen was higher ($P < 0.0001$) in mash diets measuring 0.38 and 0.82 respectively compared to pellet diets where digestibility was lower ($P < 0.0001$) at 0.28 and 0.78 for ash and nitrogen respectively. Digestibility of fats was higher ($P < 0.0001$) in pellet diets (0.93) compared to mash diets (0.87). The digestibility for ash was high ($P = 0.009$) on the high fat mash diet (0.38) which was comparable to digestibility for high fat pellet and low fat mash diets and lowest on the low fat pellet diet (0.21). For nitrogen, the high fat pellet diet had a higher ($P = 0.001$) digestibility of 0.81 compared to low fat pellet which had a digestibility of 0.74. Both high fat and low fat mash diets had nitrogen digestibility that was comparable to that of high fat pellet. With respect to fat, digestibility was comparable for both high fat (0.94) and low fat pellet diets (0.92) and lowest ($P < 0.005$) for low fat mash (0.84).

Table 4.5 The influence of diet type, diet form and temperature and their interactions on the coefficient of ileal apparent digestibility (CIAD) of ash, nitrogen (N), fat and starch (day 34) (Least square means + Standard Error (LSMEANS + SE).

		CIAD			
	n	**Ash**	**N**	**Fat**	**Starch**
Type					
High Fat	24	0.37	0.81	0.91	0.92
Low Fat	24	0.29	0.78	0.89	0.96
SE		0.016	0.005	0.005	0.005
P value		0.0031	0.0001	0.0004	<.0001
Form					
Mash	24	0.38	0.82	0.87	0.94
Pellet	24	0.28	0.78	0.93	0.93
SE		0.016	0.005	0.005	0.005
P value		<.0001	<.0001	<.0001	0.19
Temp					
Elevated	24	0.33	0.8	0.9	0.94
Normal	24	0.32	0.79	0.9	0.94
SE		0.016	0.005	0.005	0.005
P value		0.675	0.302	0.535	0.95
Type*Form					
High Fat Mash	12	0.38[b]	0.82[b]	0.89[b]	0.91
High Fat Pellet	12	0.34[b]	0.81[b]	0.94[c]	0.91
Low Fat Mash	12	0.38[b]	0.81[b]	0.84[a]	0.96
Low Fat Pellet	12	0.21[a]	0.75[a]	0.92[c]	0.95
SE		0.023	0.008	0.007	0.007
P value		0.009	0.001	0.005	0.571
Form*Temp					
Mash Elevated	12	0.38	0.83	0.87	0.94
Mash Normal	12	0.38	0.81	0.86	0.94
Pellet Elevated	12	0.29	0.78	0.93	0.93
Pellet Normal	12	0.27	0.78	0.93	0.94
SE		0.023	0.008	0.007	0.007
P value		0.649	0.213	0.619	0.19

Values with different superscripts [a, b, c, d] are significantly different from each other (LSD, P <0.05)

4.7 pNE and AME

According to Table 4.6 the diet type, diet form and temperature had significant effects on the energy content and digestibility of diets ($P < 0.05$). The digestibility coefficient for both indicator and total collection methods as well as the dry matter content of AME from the total collection method were higher ($P < 0.0001$) for low fat diets (0.75, 0.79 and 14.85 g/DM, respectively) than for high fat diets (0.7, 0.73 and 14.42 g/DM respectively). The digestibility and dry matter content of AME from the indicator method as well as the titanium recovery were higher ($P = 0.003$, $P = 0.003$ *and* $P = 0.001$ for digestibility and dry matter content for the indicator method and titanium recovery respectively) under elevated temperature conditions measuring 0.74, 14.2 and 0.95 respectively compared to normal temperature conditions where the digestibility, dry matter content and titanium recovery were 0.71, 13.62 and 0.83 respectively.

The pNE of both high fat mash and pellet diets were 9.07 MJ/kg DM and 9.21 MJ/kg DM respectively and were higher than those of the low fat diets which was 8.33 MJ/kg DM for mash and 8.47 MJ/kg DM for pellet (data not shown). The ratio between the pNE and the AME content reported in Table 4.6 were 0.63, 0.63, 0.56 and 0.57 for the high fat mash, high fat pellet, low fat mash and low fat pellet, respectively.

Table 4.6 The influence of diet type, diet form and temperature and their interactions on apparent metabolisable energy (AME) coefficient, contents and Titanium Recovery (TiRecovery) measured with either the Indicator or the Total Collection Method (d 31 – d 34) (Least square means + Standard Error (LSMEANS + SE)).

		AME				TiO2
		Digestibility coefficient (%)		Content (KJ/kgDM)		
	n	Indicator	Total Collection	Indicator	Total Collection	Recovery
Type						
High Fat	48	0.7	0.74	13.76	14.43	0.91
Low Fat	48	0.75	0.79	14.08	14.86	0.87
SE		0.007	0.002	0.141	0.045	0.023
P value		<.0001	<.0001	0.1133	<.0001	0.1759
Form						
Mash	48	0.72	0.76	13.86	14.57	0.9
Pellet	48	0.73	0.77	13.99	14.72	0.88
SE		0.007	0.002	0.141	0.045	0.023
P value		0.528	0.033	0.516	0.017	0.678
Temp						
Elevated	48	0.74	0.76	14.23	14.61	0.95
Normal	48	0.71	0.77	13.62	14.68	0.83
SE		0.007	0.002	0.141	0.045	0.023
P value		0.003	0.296	0.003	0.304	0.001
Type*Form						
High Fat Mash	24	0.7	0.73	13.64	14.31	0.91
High Fat Pellet	24	0.71	0.74	13.89	14.55	0.91

Low Fat Mash	24	0.75	0.79	14.08	14.83	0.88
Low Fat Pellet	24	0.75	0.79	14.09	14.89	0.85
SE		0.01	0.003	0.2	0.063	0.032
P value		0.576	0.105	0.56	0.16	0.611
Form*Temp						
Mash Elevated	24	0.75	0.76	14.33	14.55	0.98
Mash Normal	24	0.7	0.76	13.38	14.59	0.81
Pellet Elevated	24	0.74	0.77	14.13	14.68	0.91
Pellet Normal	24	0.72	0.77	13.85	14.76	0.85
SE		0.01	0.003	0.2	0.063	0.032
P value		0.093	0.721	0.096	0.722	0.103

Values with different superscripts [a, b, c, d] are significantly different from each other (LSD, P 0.05)

The comparison between the data collected for both the marker method and the total collection method showed a greater variability in the data collected from the marker method compared to the total collection method. With respect to the digestibility reported for both methods the coefficient of variation for the marker method measured 8.06% which more than doubles that of coefficient of variation for the total collection method which was 4.31%. Such variability between the results of both methods and within the partial collection method can be seen in Figure 4.1. For the marker method, the coefficient of variation for titanium recovery was high measuring 18.68 %. Also the graph also shows a slightly negative slope in the digestibility data for the indicator method.

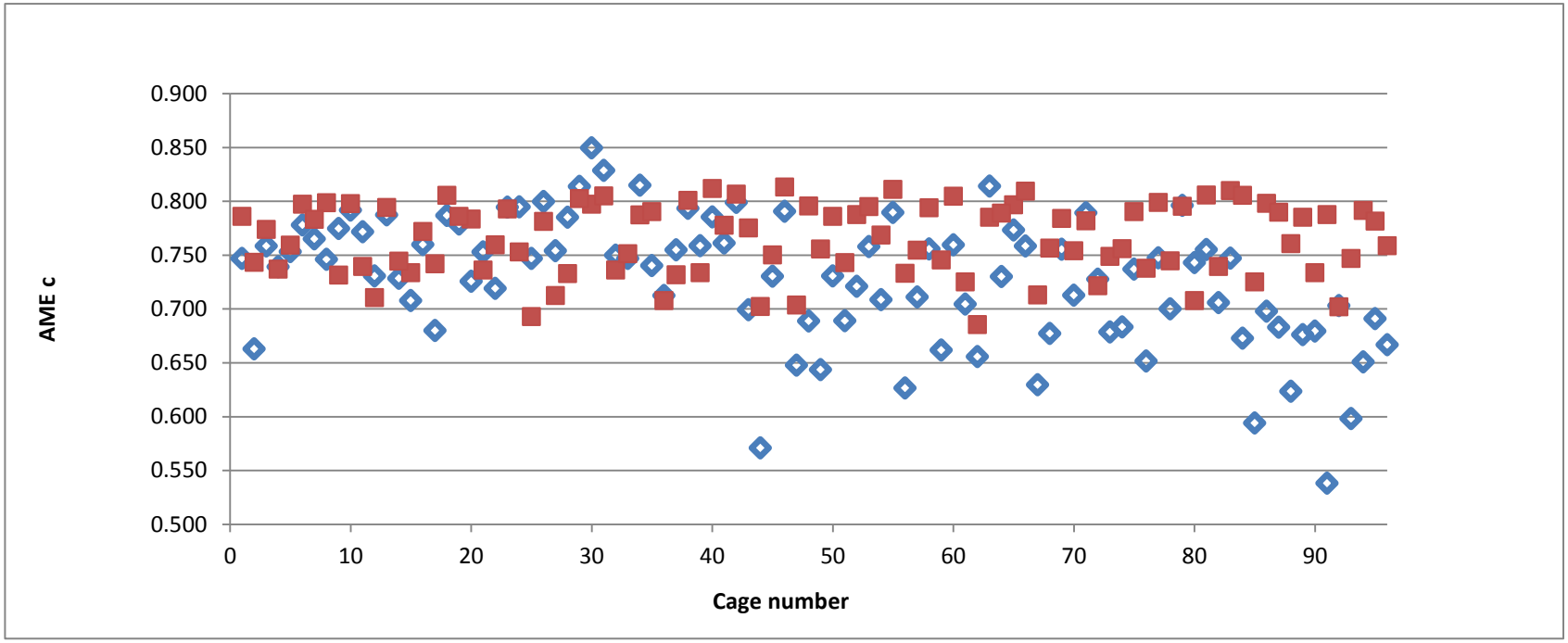

Figure 4.1 AME digestibility coefficients for the Marker and Total collection methods. AME coefficients measured either by the Indicator Method (◇) and the Total Collection Method (■) for each cage (1 to 96).

4.8 Ileal nutrient intakes and growth efficiency

Based on Table 4.7, the diet type, diet form as well as diet type and diet form interactions affected ileal nutrient intakes and growth efficiency (AMEdg) for phase 1 (*P < 0.05*). Intakes of ash and fat were higher (*P <0.0001*) in high fat diets measuring 2 g/b/d for ash and 74 g/b/d for fat compared to low fat diets where the average intake of ash was 1.3 g/b/d and that of fat 35.1 g/b/d. The intake of starch was higher (*P <0 .0001*) for the low fat diets (32.1 g/b/d) compared to high fat diets (24.5 g/b/d). On high fat diets there was a lower *(P <0.0001)* AMEdg of 17.6 MJ/kg compared to low fat diets with an AMEdg of 18.4 MJ/kg. On pellet diets, intakes of nitrogen, fat, starch and AME was higher (*P <0.0001*) measuring 16.5, 61.3, 31.0 (g/b/d) and 1369.82 KJ DM/b/d respectively, compared to mash diets with nitrogen, fat, starch and AME intakes of 14.3, 47.9 (g/b/d), 25.6 and 1133.1KJ DM/b/d respectively. Ash intake was higher (*P = 0.042*) on the mash diet (1.7 g/b/d) compared to pellet diet (1.6 g/b/d). AMEdg was lower (P <0.0001) for the pellet diet at 17.2 MJ/kg compared to the mash diet where intake was 18.7 MJ/kg. Ash intake was highest (*P = 0.002*) on the high fat pellet diet (2.1 g/b/d) and lowest *(P = 0.002)* on the low fat pellet diet (1.1 g/b/d). For starch, intake on the low fat pellet diet was higher (*P <0.0001*) (35.8 g/b/d) than that of the low fat mash diet (28.4 g/b/d) and for AME intake, the highest (*P = 0.001*) energy intakes were on the pellet diets with a lower (*P = 0.001*) intake on the high fat 1362.2 KJ/b/d versus low fat diet (1431.4 KJ/b/d). With respect to the AMEdg, birds on the high fat pellet had the lowest *(P = 0.001)* AMEdg of 17.2 MJ/kg and low fat mash the highest *(P = 0.001)* AMEdg at 19.5 MJ/kg. The pellet fed under elevated temperature had the lowest *(P = 0.048)* AMEgd of 16.8 MJ/kg followed by pellets fed under low temperatures. Both mash diets had comparable AMEdg of 18.7 MJ/kg for mash fed under

elevated temperature and 18.8 MJ/kg for mash fed under normal temperature conditions which were both higher than that recorded for pellet diets.

Table 4.7 The influence of diet type, diet form and temperature and their interactions on the Ileal nutrient intakes of ash (g /b/d), nitrogen (g/b/d) fat (g /b/d) starch (g /b/d), (AME KJ /b/d) and the AMEdg (MJ/kg) for phase 1 of the broiler growth cycle (d 10 -d 21) (Least square means + Standard Error (LSMEANS + SE)).

		Intakes					
	n	**Ash**	**Nitrogen**	**Fat**	**Starch**	**AME**	**AME dg[1]**
Type							
High Fat	48	2	15.6	74	24.5	1256.5	17.6
Low Fat	48	1.3	15.2	35.1	32.1	1273.4	18.4
SE		0.06	0.17	0.64	0.29	13.06	0.14
P value		<.0001	0.135	<.0001	<.0001	0.362	<.0001
Form							
Mash	48	1.7	14.3	47.9	25.6	1133.1	18.7
Pellet	48	1.6	16.5	61.3	31	1396.8	17.2
SE		0.06	0.17	0.64	0.29	13.06	0.14
P value		0.042	<.0001	<.0001	<.0001	<.0001	<.0001
Temp							
Elevated	48	1.7	15.3	54.2	28.1	1253	17.7
Normal	48	1.6	15.4	55	28.5	1276.9	18.2
SE		0.06	0.17	0.64	0.29	13.06	0.14
P value		0.566	0.805	0.372	0.281	0.198	0.011
Type*Form							
High Fat Mash	24	2.0 [c]	14.6	66.6	22.7 [a]	1150.8 [a]	17.9[b]
High Fat Pellet	24	2.1 [c]	16.6	81.5	26.3 [b]	1362.2 [b]	17.2[a]
Low Fat Mash	24	1.5 [b]	14	29.2	28.4 [c]	1115.4 [a]	19.5[c]

Low Fat Pellet	24	1.1 [a]	16.4	41.1	35.8 [d]	1431.4 [c]	17.3[a]
SE		0.08	0.24	0.91	0.41	18.47	0.2
P value		0.002	0.37	0.103	<.0001	0.006	0.001
Form*Temp							
Mash Elevated	24	1.7	14.3	47.3	25.6	1125.1	18.7[c]
Mash Normal	24	1.8	14.2	48.5	25.5	1141.1	18.8[c]
Pellet Elevated	24	1.6	16.3	61	30.6	1380.9	16.8[a]
Pellet Normal	24	1.5	16.6	61.5	31.5	1412.8	17.7[b]
SE		0.08	0.24	0.91	0.41	18.47	0.2
P value		0.327	0.422	0.696	0.236	0.668	0.048

[1] AMEdg AME required per unit gain for phase 1 of the growth period
Values with different superscripts [a, b, c, d] are significantly different from each other (LSD, P <0.05

Table 4.8 shows that the diet type, diet form, temperature as well as both type and form and form and temperature interactions had significant effects on ileal nutrient intakes and AMEdg at phase 2 (*P < 0.05*). The intakes of ash and fat were higher *(P <0.0001)* on the high fat diet measuring 3.3 g/b/d and 120.4 g/b/d for ash and fat respectively compared to low fat diets with ash and fat intakes measuring 2.1 g/b/d and 56.5 g/b/d respectively. The intake of starch on the low fat diet was 51.72 g/b/d which was higher (*P < 0.0001*) than the starch intake of 39.95 g/b/d on the high fat diets. AMEdg was lower (P = 0.008) on the high fat diet compared to the low fat diet (20.3 MJ/kg versus 20.8 MJ/kg). Altogether fat, starch, energy intakes and AMEdg were higher (*P <0.0001, P =0.005, P = 0.001 and P = 0.007*) on pellet diets measuring 92.3 g/b/d, 47 g/b/d, 2114 (KJ DM/b/d) and 20.8 MJ/kg respectively compared to mash diets where intakes measured 84.6 g/b/d, 44.7 g/b/d, 1985.5 KJ DM/b/d and 20.2 MJ/kg for fat, starch, energy and AMEdg respectively. Ash intake however was higher (*P < 0.0001*) on the mash diets measuring 3.1 g/b/d compared to pellet diets where the average intake was 2.4 g/b/d. Nitrogen and fat intakes under elevated temperature were 25.6 g/b/d and 90.6 g/b/d respectively which were higher (*P = 0.010, P = 0.025* for nitrogen and fat respectively) than intakes of 24.35 g/b/d and 86.33 g/b/d under normal temperature conditions for nitrogen and fat respectively. Ash intakes were highest (*P = 0.039*) for the high fat mash (3.5 g/b/d) followed by the high fat pellet diet (3.1 g/b/d) with intake being lowest (*P = 0.039*) for low fat pellet diet (1.6 g/b/d). For nitrogen, high fat mash had the highest (*P = 0.009*) intake of 26.1 g/b/d however this was comparable to nitrogen intake of 25.1 g/b/d on the low fat pellet diets. Nitrogen intake on low fat pellet diet was also comparable to nitrogen intakes on other interactions. The highest (*P = 0.007*) intake of fat was 121.7 g/b/d which was for the high fat pellet diet and the lowest (*P = 0.007*) intake was 50 g/b/d which was for the low fat mash. Starch intake for low fat pellet

of 54.8 g/b/d was the highest ($P < 0.0001$) and the lowest ($P < 0.0001$) intake of 48.7 g/b/d was on the low fat mash. With respect to energy intakes, the highest ($P < 0.0001$) intake was on the low fat pellet diet (2193 KJDM/b/d) followed by the comparable intakes of both the high fat mash (2058.7 KJDM/b/d) and high fat pellet (2034.9 KJDM/b/d) diets. The low fat mash diet had the lowest ($P < 0.0001$) energy intake of 1912.3 KJDM/b/d. Pellet diets under elevated temperature conditions had the highest ($P = 0.027$, $P = 0.018$ for fat and energy respectively) intake for both fat of 96.5 g/b/d and energy 2193.8 KJDM/b/d while intakes for the other treatments were similar.

Table 4.8 The influence of diet type, diet form and temperature and their interactions on the Ileal nutrient intakes of ash (g /b/d), nitrogen (g/b/d) fat (g /b/d) starch (g /b/d), (AME KJ /b/d) and the AMEdg (MJ/kg) for phase 2 of the broiler growth cycle (d 21 -d 34) (Least square means + Standard Error (LSMEANS + SE)).

		Intakes					
	n	**Ash**	**Nitrogen**	**Fat**	**Starch**	**AME**	**AMEdg[1]**
Type							
High Fat	48	3.3	25.4	120.4	40	2046.8	20.3
Low Fat	48	2.1	24.5	56.5	51.7	2052.7	20.8
SE		0.09	0.32	1.32	0.57	26.04	0.14
P value		<.0001	0.065	<.0001	<.0001	0.874	0.0076
Form							
Mash	48	3.1	25	84.6	44.7	1985.5	20.2
Pellet	48	2.4	24.9	92.3	47	2114	20.8
SE		0.09	0.32	1.32	0.57	26.04	0.14
P value		<.0001	0.841	<.0001	0.005	0.001	0.007
Temp							
Elevated	48	2.8	25.6	90.6	46.6	2085.2	20.4
Normal	48	2.6	24.4	86.3	45	2014.3	20.7
SE		0.09	0.32	1.32	0.57	26.04	0.14
P value		0.097	0.01	0.025	0.05	0.057	0.117
Type*Form							
High Fat Mash	24	3.5 [d]	26.1 [b]	119.2 [c]	40.7[a]	2058.7 [b]	19.8
High Fat Pellet	24	3.1 [c]	24.7 [a]	121.7 [c]	39.2[a]	2034.9 [b]	20.7
Low Fat Mash	24	2.6 [b]	24.0 [a]	50.0 [a]	48.7 [b]	1912.3 [a]	20.7
Low Fat Pellet	24	1.7[a]	25.1 [ab]	62.9 [b]	54.8 [c]	2193.0[c]	20.9
SE		0.12	0.46	1.86	0.81	36.82	0.2

P value		0.039	0.009	0.007	<.0001	<.0001	0.054
Form*Temp							
Mash Elevated	24	3	25.2	84.6 [a]	44.7	1976.6 [a]	20.2
Mash Normal	24	3.1	24.8	84.6[a]	44.7	1994.4 [a]	20.3
Pellet Elevated	24	2.6	25.9	96.5 [b]	48.6	2193.8 [b]	20.5
Pellet Normal	24	2.1	23.9	88.1 [a]	45.4	2034.2 [a]	21.1
SE		0.12	0.46	1.86	0.81	36.82	0.2
P value		0.067	0.076	0.027	0.058	0.018	0.177

[1] AMEdg AME required per unit gain for phase 2 of the growth period
Values with different superscripts [a, b, c, d] are significantly different from each other (LSD, P <0.05)

Base on Table 4.9, diet type, diet form as well as both the diet type and diet form and the diet form and temperature interactions had significant effects on the overall ileal nutrient intake and AMEdg ($P < 0.05$). High fat diets had a greater ($P < 0.0001$) intake of ash (2.7 g/b/d) and fat (97.9 g/b/d) than low fat diets where intakes were 1.7 g/b/d and 46.5 g/b/d for ash and fat respectively. Starch intake and AMEdg were higher ($P < 0.0001$*and* $P < 0.0001$ for starch and AMEdg respectively) on low fat diets (42.6 g/b/d and 19.8 respectively) than on high fat diets (32.4 g/b/d and 19.1 MJ/kg respectively). Overall pellet diets had higher ($P = 0.018$, $P < 0.0001$, $P < 0.0001$) for nitrogen, fat, starch and energy respectively) intakes for nitrogen (20.8 g/b/d), fat (76.9 g/b/d) starch (39.2 g/b/d) and energy (1763.2 KJDM/b/d). With respect to ash intake, there was a higher ($P < 0.0001$) intake of 2.4 g/b/d for mash compared to pellet diets where the intake was 2 g/b/d. For AMEdg, intake was higher ($P = 0.002$) on mash diets (19.7 MJ/kg) than on pellet diets (19.2 MJ/kg). Birds fed under elevated temperature conditions had a lower ($P = 0.030$) AMEdg of 19.3 MJ/kg compared to birds fed under normal temperature where the AMEdg was 19.6 MJ/kg. Ash intake of 2.8 g/b/d was highest ($P = 0.015$) on the high fat mash and the lowest ($P = 0.015$) intake of 1.4 g/b/d was on the low fat pellet diet. For nitrogen, the low fat pellet had the highest ($P = 0.012$) nitrogen intake of 21 g/b/d however the other diet type and diet form interactions had similar nitrogen intakes. Starch intakes were higher ($P < 0.0001$) for low fat pellets (45.8 g/b/d) than for low fat mash (39.3 g/b/d) and energy intake was highest ($P < 0.0001$) on low fat pellets (1831.8 KJDM/b/d) and the lowest ($P < 0.0001$) on low fat mash (1545.7 KJDM/b/d). AMEdg for high fat mash, high fat pellet and low fat pellet were comparable and measured 19.1, 19.1 and 19.4 (MJ/kg) respectively and low fat mash had the highest ($P = 0.003$) AMEdg value of 20.27. Pellet diets fed under elevated temperature conditions resulted in the highest ($P = 0.045$) intake of fat (79.2 g/b/d).

Table 4.9 The influence of diet type, diet form and temperature and their interactions on the ileal nutrient intakes of ash (g /b/d), nitrogen (g/b/d) fat (g /b/d) starch (g /b/d), (AME KJ /b/d) and the AMEdg (MJ/kg) for the overall growth period (d 10 -d 34) (Least square means + Standard Error (LSMEANS + SE)).

		Intakes					
	n	**Ash**	**Nitrogen**	**Fat**	**Starch**	**AME**	**AMEdg[1]**
Fat							
High Fat	48	2.7	20.6	97.9	32.4	1662.8	19.1
Low Fat	48	1.7	20.2	46.5	42.6	1688.7	19.8
SE		0.07	0.23	0.93	0.39	18.17	0.1
P value		<.0001	0.182	<.0001	<.0001	0.315	<.0001
Form							
Mash	48	2.4	20	67.4	35.8	1588.3	19.7
Pellet	48	2	20.8	76.9	39.2	1763.2	19.2
SE		0.07	0.23	0.93	0.39	18.17	0.1
P value		<.0001	0.018	<.0001	<.0001	<.0001	0.002
Temp							
Elevated	48	2.3	20.7	73.1	37.8	1689.9	19.3
Normal	48	2.1	20.1	71.3	37.2	1661.7	19.6
SE		0.07	0.23	0.93	0.39	18.17	0.1
P value		0.198	0.055	0.163	0.215	0.275	0.03
Type*Form							
High Fat Mash	24	2.8 [c]	20.6 [b]	94.4	32.2 [a]	1630.9 [b]	19.1[a]
High Fat Pellet	24	2.6 [c]	20.6 [b]	101.3	32.7 [a]	1694.7 [b]	19.1[a]
Low Fat Mash	24	2.1 [b]	19.4[a]	40.4	39.3 [b]	1545.7[a]	20.3[b]
Low Fat Pellet	24	1.4 [a]	21.0 [b]	52.5	45.8 [c]	1831.8 [c]	19.4[a]

SE		0.1	0.32	1.31	0.56	25.7	0.15
P value		0.015	0.012	0.053	<.0001	<.0001	0.0031
Form*Temp							
Mash Elevated	24	2.4	20.1	67.0 [a]	35.8	1577.5	19.7
Mash Normal	24	2.5	19.9	67.8 [a]	35.8	1599	19.7
Pellet Elevated	24	2.1	21.3	79.2 [c]	39.9	1802.2	18.9
Pellet Normal	24	1.8	20.3	74.7 [b]	38.5	1724.3	19.5
SE		0.1	0.32	1.31	0.56	25.7	0.15
P value		0.108	0.199	0.045	0.19	0.056	0.081

[1] AMEdg AME required per unit gain for the overall growth period

Values with different superscripts [a, b, c, d] are significantly different from each other (LSD, P <0.05)

5 Discussion

In this section, the impact of diet type (high or low fat), diet form (mash or pellet and temperature (normal or elevated) as well as the interactions diet type x diet form and diet form x temperature, on the nutrient digestibility, digestible nutrient intake, growth performance and growth efficiency will be discussed. Additionally the effectiveness of the Total collection method and indicator method for measuring AME digestibility will also be discussed.

5.1 Diet type (High fat or Low fat content)

The diet type had a significant effect on digestibility, ileal nutrient intake and mortality.

With respect to digestibility and intake, there was a higher coefficient of ileal apparent digestibility (CIAD) for ash, nitrogen and fat as well as a higher ileal nutrient intake of ash and fat for high fat compared to low fat diets. This higher digestibility and intake is consistent with previous literature (Bonnet et al., 1997; Dale & Fuller, 1979; Mujahid, 2011; Sinurat & Balnave, 1985) where a higher fat content in diets improves the absorption and overall digestibility of other nutrients by slowing the movement of nutrients through the intestines. This is important for heat stressed birds as different morphological and physiological changes to the gastrointestinal track result in a faster passage of feed and thus lower absorption and digestibility of nutrients (Ghazalah et al., 2008; Haitook, 2006). For the low fat compared to the high fat diet, there was a higher AME digestibility reported by both the indicator and total collection method as well as a higher AME content per kg dry matter according to the total collection method. Studies done by M. Abdollahi et al.

(2013), Abdollahi et al. (2014), Bonnet et al. (1997) and others, showed that starch has a very high digestibility and as a result may be a more digestible source of energy. This may explain the higher digestibility coefficient reported by both methods for the low fat diet where starch was used as the major source of energy. However in spite of the higher digestible AME content of the low fat diet, the high fat diet resulted in greater growth efficiency requiring less AME per kilogram gain. The longer passage time through the intestines coupled with the higher energy efficiency of fats compared to carbohydrates (starch) as a source of energy (Birkett & Lange, 2001; Emmans, 1994; Pirgozliev & Rose, 1999; Sakomura et al., 2005) may explain the higher performance of birds fed on the high fat versus low fat diet.

Diet type also affected mortality with the mortality rate on high fat being higher than the low fat diet. This outcome is parallel to that found in previous literature where high density diets lead to an increased growth rate of already rapidly growing broilers. This rapid growth rate associated with high fat diets was also supported by the current study as there was an overall higher growth efficiency of birds fed on high fat versus low fat diets. With growth rates accelerated beyond what the biological systems of birds can support, the chances of health complications and subsequent mortality are increased when birds are fed such diets (Brickett et al., 2007; Buyse et al., 1998; Garner et al., 2002; Scott, 2002).

5.2 Diet form (pellet or mash)

Overall, growth performance appeared to be significantly higher on pellet diets compared to mash diets. For pellet fed birds, live weight at 21 days was higher than that for mash fed birds. Though live weight at 34 days was not significantly affected by the diet form, higher

feed intake and a better FCR overall for pellet diets was a clear indication of the higher performance of pellet fed compared to mash fed birds. The higher performance of broilers on pellet versus mash diets has been reported by numerous studies (see Table 2.9). With respect to the CIAD of nutrients, the digestibility of ash and nitrogen were lower for pellet compared to mash diets. According to various studies done on the impact of feed processing on the digestibility of nutrients, the heat treatments of the pelleting process may decrease the digestibility of some ingredients which may explain the lower CIAD of ash and nitrogen in the pelleted diets (M. Abdollahi et al., 2013; Abdollahi et al., 2014; Abdollahi et al., 2011; M. R. Abdollahi et al., 2013). For other studies the digestibility may be enhanced like that of fats in studies conducted by M. Abdollahi et al. (2013). Though there was a lower digestibility of ash and nitrogen for pellet diets, the digestibility of AME and the overall intake were higher on pellet versus mash diets. The higher intake on pellet diets may be as a result of the higher density intake associated with the pelleted form of the diet which results in a higher intake at any given time for the pelleted versus the mash form of diets. Both the higher intake and digestibility of energy of pellet diets may have contributed to the overall higher performance of pellet versus mash fed birds (M. Abdollahi et al., 2013; Bolton, 1960).

Though the overall performance was higher on pellets versus mash diets, there was a higher mortality rate in pellet fed compared to mash fed birds. This may be as a result of the detrimental impact of accelerated growth rate of pelleted diets. Such may be supported by the findings of Brickett et al. (2007), Buyse et al. (1998), Garner et al. (2002) and Scott (2002) where accelerated growth rates associated with pelleted diets further exacerbated health complications and mortality in already rapidly growing broilers.

5.3 Temperature (normal versus elevated)

There was a higher feed intake, average daily gain as well as a better feed conversion ratio (FCR) overall for birds housed under elevated ambient temperatures. Maintaining high temperatures throughout the trial was a challenge. Apart from the evidence of heat stress in birds including panting and drooping feathers coupled with the strict animal welfare laws of New Zealand, elevated temperatures were gradually dropped from 30 °C at the start of the trial to 23 °C at the end of the trial, to ensure that heat stress was carefully managed throughout the study. This temperature range was lower than the average elevated temperature ranges used throughout previous literature (M. Abdollahi et al., 2013; Howlider & Rose, 1992; Sinurat, 1998; Sinurat & Balnave, 1986) and it may be argued that such explains the better performance reported by the current study. However the average and associated live weight of birds in the current study was higher than those reported by studies in Table 2.6, making them more sensitive to even minor temperature increases and thus more susceptible to heat stress than lighter weight birds. This is supported by Sinurat and Balnave (1985) as well as Bonnet et al. (1997) in their studies where heavier birds were seen as being more sensitive to heat stress. In spite of this, the performance of birds was higher under the elevated temperature versus normal temperature conditions.

5.4 Diet form x Diet type interaction

The overall performance was highest on the low fat pellet followed by the high fat pellet and high fat mash which were followed by the low fat mash.

Though performance appeared to be higher on high fat diets as a main effect, diet form and diet type interactions indicated higher performance on the low fat pellet versus high fat pellet diet. For most of the performance parameters examined, high fat pellet diets and mash diets (mainly high fat mash) were equivalent. For instance, the overall feed intake for high fat pellets and high fat mash were statistically equivalent ($P < 0.0001$). Based on the results of the pellet durability index (PDI), the PDI for the high fat diet was lower than that of the low fat pellet diet which may be an indication of a higher fine percentage on high fat diets. With a greater fine percentage both the pellet quality and the benefits associated with the pellet form of the diet is reduced which can impact negatively on performance (Behnke & Beyer, 2002). According to Briggs et al. (1999) and Steinke (1976) one factor that results in the degraded quality of pellets as a result of crumbling is the presence of a high fat content in diets which may have explained the lower PDI of the high fat pellets and thus the lower performance on the high fat compared to the low fat pellets. Further, simulation of the efficiency of energy utilisation for production between both diet types revealed only a small difference in energy efficiency (pNE/AME) between the high fat and low fat diet (0.63 vs 0.56 respectively). Such may be an indication that the higher performance associated with low fat pellets may be more a function of diet form than diet type. The birds fed on the low fat pellets had the highest feed intake of all the diet form x type combinations, however the pNE intake of those birds were similar to birds fed on the high fat pellet diet (1.07 MJ/d and 1.04 MJ/d respectively), resulting in similar FCR (1.45 vs 1.46 respectively).

Of all the diets, low fat mash had the lowest performance compared to other diets treatments. When both low and high fat mash were compared, the high fat mash had a

higher overall feed and energy intake as well as a higher growth efficieny than the low fat mash. The overall lower performance of low fat mash may be a combined effect of the lower efficiency of the mash form of the diet (see Table 2.9) as well as the lower efficiency of starch which was the main source of energy in this diet (see Table 2.8).

5.5 Diet form x Temperature interaction

Observations of diet and temperature interactions showed that birds fed pellet diets performed better than mash fed birds kept under elevated temperature which is similar to that found in previous literature (M. Abdollahi et al., 2013; Howlider & Rose, 1992; Jafarnejad et al., 2011; Jahan et al., 2006; Rosa et al., 2007; Svihus et al., 2004). The highest feed intake and ileal nutrient intake of fat and AME for phase 2 and ileal nutrient intake of fat for the overall period was in pellet fed birds housed under elevated ambient temperature. Pellet fed birds also had a higher growth efficiency requiring less AME per kilogram gain at phase 1 under elevated temperature. This may be an indication of the significance of diet form in allowing for high performance in spite of evident heat stress of birds housed under elevated temperature conditions.

5.6 Total collection method versus Indicator methods

There were significant differences in the results obtained from the indicator and total collection methods. The digestibility values for the indicator method had a coefficient of variation of 8.06% which almost doubled the 4.31 % coefficient of variation of the total collection method. Sales and Janssens (2003) concluded a 98% recovery for titanium, however in this study there was a recovery of 89% and an overall high coefficient of variation for titanium recovery of 18.68%. This may explain the high variability of

digestibility data from the indicator method. According to Marais and D'Mello (2000) and Sales and Janssens (2003), this variability is typical of the indicator method compared to the total collection method and as a result is seen as a less reliable method for estimating AME digestibility. As shown in Figure 4.1 the negative gradient of the digestibility data for AME was due to batch differences in the chemical analysis of the titanium. For instance there was a higher quantity of high digestibility samples from the elevated temperature treatments being processed first followed by higher quantities of the lower digestibility samples from the normal temperature treatment that were processed later. This may explain the downward sloping gradient of the digestibility data for the indicator method. This highlights the importance of having an equal number of samples from each treatment in each analytical batch.

6 Conclusion

Though there was no noticeable impact of diet type and diet form strategies on performance, diet form had a greater impact on performance under elevated temperature conditions. At different stages of their growth cycle, pellet fed birds housed under elevated temperature conditions had higher feed and ileal intakes as well as higher growth efficiencies than mash fed birds housed under elevated temperature conditions.

Based on the current study the diet form shows more potential over diet type, as a nutritional strategy for alleviating heat stress in broilers. The impact of diet form though negligible was present. This is consistent with the findings of Gous and Morris (2005) who underscored the negligible impact of various feed strategies in alleviating heat stress in broilers. Such may hold true, however there may be potential for improvements to nutritional studies which may elicit noticeable results. For instance the effects of high fat pellets were not recognised because of the compromised quality of the treatment. The true effects of high fat pellets may have been observed through improving the quality of pellets. One example of improving the quality of pellets include reconsidering the way high fat content is incorporated in pellet diets. Various studies indicate the benefit of applying fat on the surface of pellets immediately after processing rather than before pellets are processed, prevents the crumbling effect of high fat inclusion, on the pelleting process. With the high cost of imported sources of dietary fat, the use of locally available novelty materials including Linseed, Groundnut and Coconut meal that are rich in oils (see Table 2.7) may be potential alternatives that can reduce the high cost of imported fats. However

much work needs to be invested into such developments to ensure that challenges such as the many anti-nutritional factors characteristic of novelty feeds, is overcome.

7 References

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