INVESTIGATION OF ENERGY PARTITIONING IN MODERN BROILER CHICKENS

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ABSTRACT

Studies were conducted to estimate the energetic efficiencies for fat deposition from different energy sources (carbohydrate, protein, soybean oil and tallow) and to determine the maximum protein deposition (Pdmax) and minimum body lipid to protein ratio (minL/P) of the modern broiler chickens. Energetic efficiencies for fat deposition were assessed by feeding birds extra energy from different energy sources when protein was limiting in the diet. Comparison of birds slaughtered before and after the dietary treatments were applied allowed the determination of the energy retained as fat or protein. In the first experiment (Chapter 3), the energetic efficiencies of fat deposition from vegetable oil and starch were estimated to be 0.82 and 0.69, respectively. In the second experiment (Chapter 4), the energetic efficiencies of fat deposition were estimated to be 0.93 from soybean oil and 0.90 from tallow, but there was no significant difference between soybean oil and tallow. In the third experiment (Chapter 5), the efficiency of energy deposition as fat from non-essential amino acid intake was calculated to be 0.63.

In the fourth experiment (Chapter 6), the Pdmax and minL/P were determined by feeding diets not limited for protein with varying energy levels. The maximum daily protein deposition was predicted at 22 g/day. According to broken-line model, the rate of protein deposition increased when the apparent metabolisable energy intake above maintenance requirement (AMEIp) increased up to the break point of 1.2 MJ/day. Further increases of AMEIp did not lead to an increase in protein deposition rate whereas the fat deposition rate sharply increased. The body weight and energy intake affect the L/P ratio. Across all treatment groups, the minimum value of L/P ratio was observed at 0.31 for birds fed 1 MJ/day of AMEIp at 4 kg live body weight.

From the knowledge of net energy requirements and considering the efficiency of metabolisable energy for fat and protein deposition from all experiments, a simple mechanistic growth model was developed for modern broilers (Chapter 7). The model simulates the daily growth of broilers and it was able to predicting the broiler performance and carcass composition under a variety of nutritional conditions. Moreover, the model was evaluated with a range of experimental data (Chapter 8) and prediction values were in close agreement with observed values. The relative prediction errors were 3.8% and 7.3%, for prediction of slaughter live body weight for dependent and independent dataset, respectively.

In conclusion, the efficiencies of energy utilisation for fat deposition varied depending on energy sources with the highest values for soybean oil and tallow followed by starch and the lowest for protein. Modern broilers have an upper limit for protein deposition (22 g/day). The body weight and energy intake affect the L/P ratio and the minimal L/P ratio was observed at 0.31. The mechanistic growth model based on energy partitioning concepts can be a tool to predict the carcass composition and broiler performance and can deal adequately with the complexity of nutritional factors.

The finding of this thesis is that the broiler performance can be improved by formulating the diet to maximise the protein deposition with minimum fat deposition. The maximum protein deposition can be achieved when the birds consumed 1.2 MJ/day of AMEIp or 2.5 MJ/day of AME intake, further energy intake will deposited as lipid.

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Publications

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List of Abbreviations

AA	Amino acid
ADE	Apparent digestible energy
ADG	Average daily gain
AHP	Activity heat production
AME	Apparent metabolisable energy
AMEg	Apparent metabolisable energy for gain
AMEi	Apparent metabolisable energy intake
AMEIfpd	Apparent metabolisable energy intake free of energy for protein deposition
AMEIp	Apparent metabolisable energy intake for production
AMEn	Nitrogen corrected apparent metabolisable energy
ANOVA	Analysis of variance
CP	Dietary crude protein
CPi	Dietary crude protein intake
DCP	Digestible crude protein
DCPi	Digestible crude protein intake
DFCP	De-feathered carcass protein
DFCPf	De-feathered carcass protein final
DFCPi	De-feathered carcass protein initial
DFCW	De-feathered carcass weight
DFCWf	De-feathered carcass weight final
DFCWi	De-feathered carcass weight initial
DM	Dry matter
EAA	Essential amino acids
EE	Ether extract
EEP	Energy from excess protein intake
ER	Energy retention
ERF	Energy retention fat
ERP	Energy retention protein
EUBP	Energy from unbalanced protein
FA	Feed allowance
FI	Feed intake
FE	Feather energy retention
FHP	Fasting heat production
Fd pot	Potential body fat
Ff	Fat deposition from fat intake
FI	Feed intake
Fp	Fat deposition of unbalance/excess protein intake
Fs	Fat deposition from starch intake
g	Gram
GE	Gross energy
HI	Heat increment
HP	Heat production
IDBP	Ideal digestible balance protein
IDBP(AA)	Ideal digestible balance protein for each amino acid
IDBPI	Ideal digestible balance protein intake
k	Efficiency of metabolisable energy utilisation

kf	Efficiency of metabolisable energy utilisation for fat deposition
kf_f	Efficiency of metabolisable energy utilisation for fat deposition from digestible fat
kf_N	Efficiency of metabolisable energy utilisation for fat deposition from protein
kf_s	Efficiency of metabolisable energy utilisation for fat deposition from digestible starch
kf_{so}	Efficiency of metabolisable energy utilisation for fat deposition from soybean oil
Kf_t	Efficiency of metabolisable energy utilisation for fat deposition from tallow fat
k_g	Efficiency of metabolisable energy utilisation for weight gain
km	Efficiency of metabolisable energy utilisation for maintenance
kp	Efficiency of metabolisable energy utilisation for protein deposition
kJ	Kilo Joule
LBW	Live body weight
LBWf	Final live body weight
LBWi	Initial live body weight
L/P ratio	Lipid to protein ratio
ME	Metabolisable energy
MEm	Metabolisable energy for maintenance requirement
minL/P	Minimum lipid/protein ratio
MJ	Mega joule
N	Nitrogen
NEAA	Non-essential amino acids
NE	Net energy
NEp	Net energy for production
NRC	National research council
Pd	Protein deposition
PDI	Pellet durability index
Pdmax	Maximum protein deposition
Pg	Protein for body weight gain
Pm	Maintenance protein requirement
TME	True metabolisable energy

CHAPTER 1
General Introduction

World chicken meat production increased from 58.7 million tonnes in 2000 to 90.9 million tonnes in 2012 (FAO, 2012). In the same period, New Zealand chicken meat production increased from 108,929 to 168,336 tonnes; this is an increase of 4.5% per annum (Poultry Industry Association of New Zealand, 2013). In New Zealand, this increase was due to higher slaughter body weight (i.e. 1.6 kg in 2000 to 1.8 kg in 2012) and increased bird numbers (i.e. 67.067 million birds in 2000 to 93.941 million in 2012).

New Zealand leads the world in broiler chicken production efficiency with an average feed per gain ratio of 1.5 at 2.3 kg live body weight compared with a global average feed per gain of 1.62 at the same live body weight (Williams, 2012). This is partly due to New Zealand disease free status, high quality management and housing, as well as continuing improvements in genetic selection. The genetic selection has altered protein and fat deposition in broiler chickens (Sakomura *et al*. 2005).

It is necessary to understand the efficiency of energy utilisation for protein or fat deposition to establish the energy requirement that maximise the growth rate of high genetic merit broiler chickens. Lopez and Leeson (2005) partitioned the metabolisable energy intake (MEI) into energy requirement for maintenance (MEm) and energy retained in the body in the form of protein (ERP) and fat (ERF). They recognised that different energy efficiencies are associated with use of energy for protein (*kp*) and for fat (*kf*). Usually, *kp* and *kf* values are estimated using regression models for birds fed at different intake levels. However, the energy efficiencies of protein deposition ranged between 0.36 and 0.70 (Sakomura *et al*., 2005; Boekholt *et al*., 1994; De Groote, 1974) and the energy efficiencies of fat deposition between 0.55 and 0.92 (Sakomura *et al*., 2005; Scheimann *et al*., 1972; Emmans, 1994). These variations may have resulted from differences in the genetic merit, diet composition and environment. Energy efficiencies can be significantly affected by diet composition and the purpose for which the energy is used (Lopez and Leeson 2008; De Groote, 1974). For example, dietary energy is utilised more efficiently for body fat deposition than for body protein deposition. This variation prevents accurate prediction of broiler nutrient requirements. There is limited

information on how the nutrients (carbohydrate, fat and protein) are utilised for fat deposition with different relative efficiencies in the broiler chickens.

To optimise the feed efficiency and carcass quality in modern broiler chickens, updates on the maximum protein deposition with minimum fat deposition are needed. Usually, the potential protein depositions are described by Gompertz type equations in broiler chickens (Hancock *et al.*, 1995; Gous *et al.*, 1999; Marcato *et al.*, 2008). In pigs, the maximum daily protein deposition (Pdmax) and the minimum lipid to protein ratio (minL/P) are used to describe the partitioning of production energy between protein and lipid deposition (De Lange, 1995; De Greef and Verstegen 1995; Moughan *et al.*, 2006). Pdmax is important to establish the amino acid (AA) and energy requirements to maximise the growth rate. Pdmax and minL/P ratio could provide rules for energy partitioning in a broiler growth model. The validity of this theory has not tested in broiler chickens.

Knowledge about the potential for achieving maximum protein deposition and a better understanding of energy utilisation from various dietary components is important for a precise estimation of energy requirement of broiler chickens. Consequently, information about energy utilisation efficiency and maximum protein deposition can be used to develop a mechanistic growth model for increasing the accuracy of feeding formulation for broiler chickens. The objective of this thesis was to evaluate the efficiencies of energy utilisation for fat deposition from different energy sources and quantify the maximum protein deposition and minimum lipid to protein ratio in modern broiler chickens, this lead to development of a simple mechanistic growth model to predict the body composition.

A review of the literature in Chapter 2 looks at the energy system of broiler chickens, describes energy and amino acid partitioning, and provides a framework of a broiler growth model and finally touches on some limitations of the energy partitioning concept for broiler chickens. Some of the limiting parameters are examined in the experimental work. The objectives of the experiments conducted in this thesis were to investigate the efficiencies of energy utilisation for fat deposition from different energy sources such as carbohydrate, fat and protein (Chapter 3), saturated and unsaturated fatty acids (Chapter 4) and non-essential amino acids (Chapter 5). The energetic efficiencies were estimated from an extra energy intake under a protein limited deposition condition. The key assumption was that the extra energy intake (from different dietary sources) would be used for fat deposition only. The description of the

genetic upper limit of protein deposition and the minimum lipid to protein ratio was evaluated in Chapter 6. The maximum protein deposition was achieved by feeding the birds a diet with high digestible protein and high energy content. The results from Chapters 3 to 6 were compiled into growth model (Chapter 7), which was tested for validity in Chapter 8. Chapter 9 presents the general discussion of the experiments results, addressing the major findings and suggests further steps to develop the growth model.

CHAPTER 2

Review of literature

2.1. Energy system in the broilers chickens

2.1.1. Introduction

Feed is one of the most expensive input components in broiler chickens industry, and it represents approximately 70% of the total production cost. Therefore, the aim of modern broiler industry is to reduce the feed cost for optimal economic returns. Three strategies are involved to meet this purpose; firstly, to increase the efficiency of feed utilisation and the total amount of broiler meat production, secondly, manipulate the diet formulation to express the maximum growth rate for modern broiler strains, thirdly, to decrease the input cost without significantly reducing production. Intrinsically, the energy cost represents the greatest proportion of the feed cost (Noblet *et al*., 2010). Energy utilisation in poultry is usually expressed in terms of metabolisable energy (ME) which is determined by the difference between energy intake and energy excreted. Dietary carbohydrates, lipids and protein are the main sources of energy in poultry diet. The energy supplied from carbohydrate and lipid above the chicken maintenance and protein deposition requirements is stored in the body as fat (adipose tissue). Dietary protein can provide energy and unbalanced and excess of dietary protein can be converted into pyruvic acid and acetyl CoA and then into lipid by process of lipogenesis. However, the heat increment produced from utilisation of different chemical components is not constant (De Groote, 1974; Noblet *et al*., 2010). For instance, the heat increment is lower when the ME is derived from fat compared to when ME is derived from protein. The ME system is unable to account for the energy utilisation efficiency of the different energy sources (Emmans, 1994; Farrell, 1999). Therefore, understanding the efficiency of energy utilisation for fat deposition from different energy sources (carbohydrate, fat and protein) could improve nutrient utilisation and minimise the energy waste as heat increment. The aims of this section are firstly; present a brief discussion of the energy system used in broiler chickens; secondly, to provide a framework for a model of energy and protein partitioning; thirdly, to identify limitations of the energy partitioning concept and finally, to make suggestions for examining these limitations.

2.1.2. Energy requirement of the broiler chicken

The accurate estimation of energy requirements is important for maximising the broiler performance and making economic decisions. Energy is measured as either kilocalorie (kcal) or kilojoule (kJ), while 1 kcal is equal to 4.184 kJ (NRC, 1994). The energy value of a feed ingredient can be expressed in several ways: gross energy, digestible energy, metabolisable energy and net energy.

Gross energy (GE) is defined as the energy released as heat when a substance is completely oxidised to carbon dioxide and water. The GE depends exclusively on the chemical composition of the feed. The GE in feed ingredient is not necessarily all available to the bird (NRC, 1994).

Apparent digestible energy (ADE) is the GE of the feed consumed minus the GE of the faeces. Birds excrete faeces and urine together via a cloaca and it is difficult to separate the faeces and urine to measure the ADE. Consequently, ADE values are not generally employed in poultry feed formulation (NRC, 1994).

Metabolisable energy (ME) is the GE of the feed consumed minus the GE contained in the faeces, urine, and gaseous products of digestion (Farrell, 1974; NRC, 1994). For poultry, the gaseous products are usually small, so ME represents the GE of the feed minus the GE of the faeces and urine (Figure 2.1). Metabolisable energy is used to quantitatively describe energy requirements in commercial poultry nutrition, and is adapted to apparent metabolisable energy (AME). In poultry, AME is the GE of the feed consumed minus the GE content in the excreta (faeces, urine and endogenous losses). A correction for endogenous energy may be applied to give a true metabolisable energy (TME). True metabolisable energy is the difference between the GE of the feed composition and the GE of excreta of feed origin (NRC, 1994). A correction for nitrogen retained in the body is usually applied to yield a nitrogen-corrected AME (AME_n) value. The AME_n of the diet in kJ/kg was calculated using the following equation: $AME_n = AME - 34.39 \times$ nitrogen retained, where 34.39 is the nitrogen correction factor reported from previous research (Chuan *et al*., 2010).

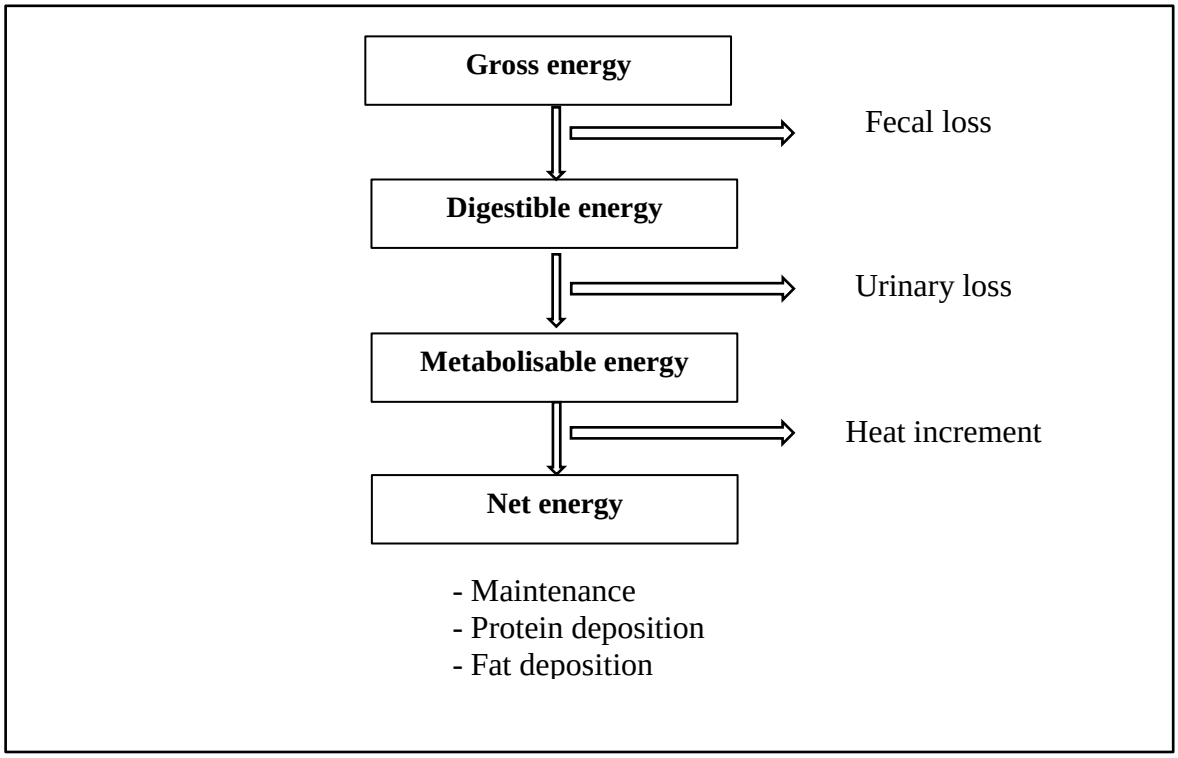

Figure 2.1. Energy partitioning in broiler chickens (adapted from Sibbald, 1982)

Net energy (NE) is the ME minus the energy lost as heat increment. Currently the AME system is preferred in poultry because it is simple and less expensive to determine AME contents than TME or NE. The composition of the diet may also affect the accuracy of measurement of ME (Nitsan *et al*., 1997; Noblet *et al*., 2010; Panja *et al*., 1995). De Groote (1974) showed that the metabolisable energy values are underestimated if the energy is utilised from fat rich feedstuffs, and overestimated if energy is utilised from protein rich feedstuffs.

The closest estimate of the true energy value of feedstuff is considered to be NE (Noblet *et al*., 2010). Net energy values are not available for poultry, but it is possible to measure the NE values by using two ways:

1- Indirect (respiration) calorimetry methods

The most common technique to measure the heat production uses respiration chambers, based on oxygen consumption and carbon dioxide production (Fuller *et al.*, 1983). However, this technique is expensive and difficult to be applied for long rearing period.

2- Comparative slaughter technique

This technique estimates the heat production (HP) as the difference between the ME intake and energy retention (ER) in the body tissues, the ER is measured by comparing the carcass energy composition of birds slaughtered at different body weights. This technique is laborious and time consuming and difficult when applied to large animals. However, it is suitable with small animal and poultry (Moon, 1967).

Many studies have presented equations for estimation the net energy values of feedstuffs for animals. These equations obtained based on the relationship between the chemical composition of diets (GE, crude protein, starch, fat and dry matter) and the carcass retention energy (Emmans, 1994; Noblet *et al*., 2010; 1994; Noblet and Van Milgen, 2004; Pirgozliev and Rose, 1999). However, these equations are based only on the chemical composition of the diet and do not consider the age, environment and genetic factors.

2.1.3. Validation of net energy system

Fraps (1946) reported the first data set on energy availability in 62 individual feedstuffs by determining their productive energy (NEp). A comparative slaughter technique was used to measure the energy retention in growing chickens. Unfortunately, no large data set has been published for broiler chickens since that of Fraps (1946). The relationship between AME and Nep for 28 ingredients commonly used in broiler nutrition is given in Figure 2.2. The proportion of starch or protein in the feed ingredients affects the efficiency of utilisation of the ME, because the results indicated that the NEp derived from high protein ingredients (animal sources) was lower than those derived from high starch ingredients (cereals sources). Feedstuffs with high fat content were not evaluated in this research. Also, the NEp was considered as the sum of fat and protein deposition, with no distinction made between fat and protein.

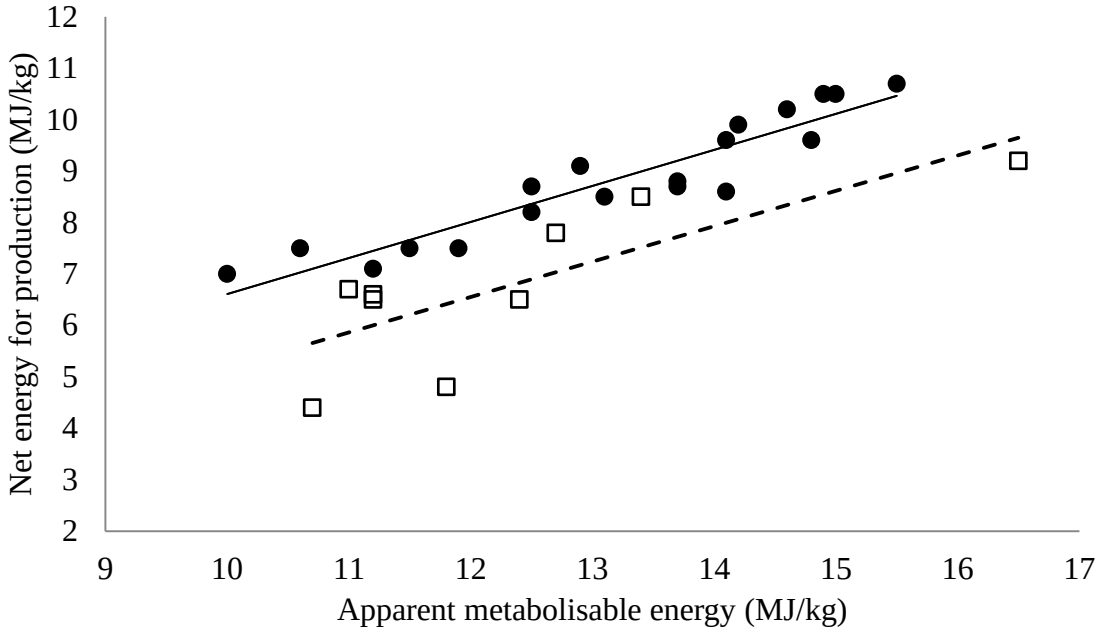

Figure 2.2. Relationship between determined apparent metabolisable energy and determined net energy for production for 28 ingredients (19 cereals ingredients (●); high in starch and 9 animal product ingredients (□); high in protein, (Source: Fraps, 1946 as cited by Pirgozliev and Rose, 1999)

De Groote (1974) compared the NE and ME systems. In the NE system, the utilisation efficiencies for using ME from protein, carbohydrate and fat were 0.60, 0.75 and 0.90, respectively. The NE concentration of feed was estimated by multiplying efficiency utilisation with the gross energy of individual digestible nutrients. The ME system was compared with the NE system, with a range of 12.55 (9.04) to 14.23 MJ ME/kg (10.71 MJ NE/kg) for starter and finisher diets. The least cost rations, based on the NE system, were cheaper than those based on the ME system. For equal NE content, the formula based on NE was 0.21 to 0.29 MJ /kg less ME/kg, than the one based on ME. For example, in a starter diet with a NE content of 9.04 MJ/kg, the formula based on NE contained 12.34 MJ/kg ME, whereas, in the formula based on ME the formula contained 12.55 ME/kg ME. The broiler performance was greater when using NE system, compared to the ME system.

Emmans, (1994) described a theoretical model (effective energy model) based on ME derived from different nutrient substance. In Emmans' (1994) model, the energy costs of five biological functions (nitrogen excretion, organic matter excretion, methane production, protein retention and lipid retention) were estimated. The minimum heat increment was achieved when dietary fat was directly used for lipid deposition. Variations in diet composition is a major factor affecting the relationship between an

effective energy system and ME, whereas, energy efficiencies can be significantly affected by diet composition and the purpose for which the energy is used (fat or protein deposition).

Recent studies have measured NE using respiratory chambers (Noblet *et al*., 2003; 2007; Van Milgen *et al*., 2001b). This method was developed to predict the energy retention (ER) and the total heat production. The heat production was partitioned into fasting heat production, physical activity and thermic effect of feed.

2.1.3.1. Energy retention

The metabolisable energy intake partitions into energy retained (ER) and energy lost for heat production (HP) (Close, 1990; Lawrence and Fowler, 2002). In the broiler, up to 52-64% of ME may go towards HP (Fuller *et al*., 1983; Noblet *et al*., 2003; Van Milgen *et al*., 2001b). Therefore, the retained energy is represented by the difference between ME and HP (approximately 36-48% of ME go towards production).

2.1.3.2. Heat production

The heat production is divided into the fasting heat production (FHP), heat production due to physical activity and the thermic effect of feeding. Van Milgen *et al*. (2001a) reported the FHP and physical activity represent 36-37% of total ME intake with physical activity being 8-10% of the total ME requirements. The total heat production per day was divided into 45%, 22%, 15% and 18% for fasting heat production, long term of thermic effect of feed, short term of thermic effect of feed and physical activity, respectively (Van Milgen *et al*., 1997).

Usually, heat production is determined by comparative slaughter techniques that estimate the difference between the ME intake and the energy retention in the body. Alternatively, respiration calorimetry is used to predict heat production, based on oxygen consumption and carbon dioxide production. The heat production (HP, in kilojoule) is calculated using the formula of Romijn and Lokhorst, (1961).

$$\mathrm{HP(kJ/day)} = 16.2 \times \mathrm{O_2(litre)} + 5.0 \times \mathrm{CO_2(litre)}$$

Heat production, as determined by energy balance versus gaseous exchange, was 1502 vs. 1456 kJ/bird/day in a group of 10-week-old chickens (Fuller *et al*., 1983). The HP in pigs was 9% higher when measured using the comparative slaughter method compared with the respiration chamber method (McCracken and Rao, 1989). Heat

production is higher with comparative slaughter method because activity is limited when the animal is confined to the chamber, reducing the maintenance energy required and HP. Also, the error from estimating the energy input and output becomes cumulative with the comparative slaughter technique (Reynolds, 2000). The HP is affected by many physical and biological factors. Birds fed a pelleted diet had a lower heat production than birds fed a mash diet and males have a higher HP than females (Latshaw and Moritz, 2009). Commercial breeds of broilers have a lower HP compared with the Leghorn breed (Lopez *et al.*, 2005; 2007).

The heat production increased when the environmental temperature changed from thermo-neutral zone (Sakomura *et al*., 2003; 2005). The calorimetry method partitioned the HP into three different component defined as;

2.1.3.2.1. Fasting heat production

Fasting heat production (FHP) is represented the energy expended in the fasting animal, which represented by ATP requirements at the cellular level, as well as heat produced by generating ATP from body nutrient stores (Birkett and de Lange, 2001a). Fasting heat production is either measured directly in fasting animal or calculated by extrapolating the HP measured at different feeding levels to zero ME intake (Noblet et al., 2010). There has been minimal investigation of FHP in broilers. The FHP is estimated to be 500 kJ/kg$^{0.60}$ per day in broiler chickens (Van Milgen *et al*., 2001b). In turkey, the average FHP is estimated to be 449 kJ/kg$^{0.75}$ per day (Rivera *et al*., 2010). The FHP is estimated from 701 to 801 kJ /kg$^{0.60}$ per day for growing pigs offered *ad-libitum* feed (Le Bellego *et al*., 2001; Van Milgen *et al*., 2001a). In experiments with pigs, obese strains have a lower FHP than lean strains and the feeding level before fasting also affects FHP due to the metabolic activity of visceral organs (Van Milgen *et al*., 1998). Fasting heat production is generally assumed to be directly related to ME for maintenance as MEm = FHP/*km*, where *km* is the efficiency of energy utilisation for maintenance (Van Milgen and Noblet, 2003).

2.1.3.2.2. Physical activity

There is a wide variation in heat production between different animals due to physical activity. Activity heat production (AHP) is estimated by force sensor detection, from statistical relations between the variations in heat production and variations in recorded physical activity (Schrama *et al*., 1996; Van Milgen *et al*., 1997). Van Milgen *et al*.

(1997) reported that the heat production from activity (AHP) in grower broilers is 18% of total heat production, environmental conditions, lighting programs and genetics affect this value. Van Kampen (1976) suggested that the AHP of eating represented about 3% of daily heat production in laying hens, however the broiler chicken normally eats more feed than those of laying and this value is possible to be higher for broiler chickens (Leeson *et al*.,1996a).

2.1.3.2.3. Thermic effect of feeding

The thermic effect of feeding (or heat increment) is defined as the heat production coming from feed utilisation. Thermic effect of feeding is calculated as the difference between the total heat production minus FHP and heat production due to physical activity (Van Milgen *et al*., 2000, Noblet *et al*, 2003). The thermic effect of feeding is divided into the short-term (heat production due to feed intake activity, energy losses from digestion and absorption processing) and long-term (heat production produced from processes such as fermentation and metabolism). In most situations, the thermic effect of feeding is affected by dietary composition. For example, the biochemical efficiency of the conversion of starch to lipid is 81% (Van Milgen, 2002). Therefore, the rest is lost as heat expenditure. The NE/ME ratio in pigs varies greatly with the chemical composition of the diet, with ratios 90%, 80% and 60% for fat, starch and dietary fibre, respectively. With broiler chickens, the variations on crude protein (CP) or fat content in the diet did not significantly change the NE/ME ratio (Noblet *et al*., 2003; Table 2.1).

Table 2.1. Effect of diet composition on heat production, energy retention and efficiency of ME for NE in broiler chickens

		kJ/kg $BW^{0.70}$/day				
Trial	Diet	MEI	HP	AHP	ER	NE/ME
1	18.0% CP	1609	853	146	756	75.1
	22.7% CP	1609	846	153	763	74.8
2	22.5% CP	1457	892	173	565	67.7
	27.3% CP	1457	872	168	585	68.6
3	2.8% EE	1873	904	141	969	75.0
	9.7% EE	1877	901	152	976	75.7

MEI is the metabolisable energy intake. HP is the heat production. AHP is the activity heat production. ER is the energy retention. NE is the net energy.
The variation in crude protein (CP) or ether extract (EE) in the diets is associated with an inverse variation in starch content. The data have been adjusted to a similar ME. Within trial, treatments gave the same results ($P > 0.05$), (Sources; Noblet *et al*., 2007; 2009; 2010).

The NE system has been applied successfully for pig nutrition (Noblet *et al.*, 2003; 2010) in countries such as France (Noblet *et al.*, 2003) and the Netherlands (CVB, 2003). In the case of poultry, recent data by Latshaw and Moritz, (2009), Noblet *et al*. (2010) and Noblet *et al*. (2007) did not show any significant superiority of the NE system over the ME system. This may be due to a smaller amount of variations between broiler diet composition, a smaller volume of research and a lower number of observations in each experiment (De Lange and Birkett, 2005; Noblet, 2010). De Lange and Birkett (2005) noted that broiler chicken NE system, as compared with ME system, only marginally improve the accuracy of broiler growth performance prediction. Furthermore, broiler chickens appear more susceptible to dietary factors, such as interactive effects among nutrients on digestive function and dynamics of nutrient digestion and utilisation, than growing pigs (De Lange and Birkett, 2005). For example, broiler chickens grew faster and more efficiently on a diet containing slowly digestible starch, than on a diet containing rapidly digestible starch (Weurding *et al*. 2003).

Currently, the ME partitioning system describes the energy availability for fat and protein deposition. However, there is little data available on energy partitioning in modern broiler chickens. Quantifying and partitioning the ME between protein and fat deposition can be used as basic step to establish a growth model. Such a model can be used to predict the NE retention and describe the carcass and growth performance of broiler chickens, taking into account the requirement of NE for optimising growth.

2.1.4. Metabolisable energy partitioning system in broiler chickens

Recently, there is interest in exploring ways to improve the ME partitioning model in modern broiler chickens by considering the total heat loss from protein and fat deposition (Latshaw and Moritz, 2009). Modelling the ME partitioning would be useful for predicting the net retention energy and describing the carcass and growth performance of broiler chickens. Information about how modern broiler chickens partition ME between maintenance and energy retention as protein or fat deposition is scarce.

Metabolisable energy intake (MEI) can be partitioned into retained energy (ER) and metabolisable energy for maintenance (MEm): MEI = MEm + $(1/k_g)$ ER, where the k_g is the ME efficiency for ER. This simple model failed, because did not account for the different efficiencies of protein and fat deposition (Birkett and De-Lange, 2001a). Boekholt *et al*. 1994 and Lopez *et al.* (2005, 2007) described the metabolisable energy

intake (MEI) using the Kielanowski (1965) equation as MEI = MEm + (1/*kf* × ERF) + (1/*kp* × ERP), Where ERF is the energy retention as fat (kJ/day), ERP is the energy retention as protein (kJ/day), *kf* and *kp* are efficiencies of utilisation of ME for fat and protein deposition, respectively. This latter approach allows the estimation of the energy efficiency for fat and protein deposition.

Estimated *k*-values using a multiple linear regression show a wide variation, and there are practical problems associated with precise measurement of ME. The utilisation of dietary protein influences the diet's ME content: i.e. partitioning of digestible protein between protein deposition and protein used as energy sources (De Groote, 1974; Birkett and de Lange, 2001a, c). Roux, (2013) reported two problems associated with multiple linear regression of MEI versus ERF and ERP, namely, the variation of protein deposition efficiency with degree of body protein maturity and possible inaccuracy in the estimates of *kf* and *kp*, due to statistically significant col-linearity between protein and fat deposition rates. In studies with pigs, the problems of co-linearity associated with multiple regression coefficients has been avoided by using theoretical efficiencies of protein and fat deposition (Roux, 2009). Theoretically, the protein deposition efficiency can be replaced by protein synthesis efficiency, in combination with the cost of protein turnover. Halas and Babinszky, (2010) estimated the efficiency of fat deposition from different energy sources under protein-limiting conditions, by using multivariate regression analysis. Birkett and de Lange (2001c) noted one possible procedure that minimised the variation of *k*-values, by creating a response between MEI and fat deposition at a fixed protein deposition for the estimation of *kf*, and creating a response between MEI and protein deposition at fixed fat deposition, in order to estimate the *kp*. Success in estimating the *k*-values requires a large number of observations and a special experimental design, in order to minimise the effect of the natural co-linearity between MEm, ERP and ERF (Birkett and de Lange 2001a; Roux, 2013).

2.1.4.1. Maintenance energy requirement

The maintenance energy requirement is the amount of energy and nutrients needed for basal metabolic processes and normal activity, when there is no influence of feed, environmental conditions and voluntary activity (Austic and Nesheim, 1990). The maintenance energy requirement has been estimated relative to the body surface area for growing animals. In poultry, Sakamora *et al*. (2005) estimated the MEm to be 469

kJ/LBW$^{0.75}$/day. Lopez and Leeson (2005) estimated the MEm to be 649 kJ/LBW$^{0.60}$/day or 143 kJ/LBW$^{0.75}$/day.

Boekholt *et al*. (1994) reported that, the estimation of MEm as a function of LBW$^{0.75}$ may not be the most suitable scale for broiler chickens, and the result may be not represent the most accurate estimates for MEm. Lopez and Leeson (2005) reported that maintenance requirements for broilers chickens based on LBW$^{0.60}$ showed the smallest residual variance and, therefore, appeared to be more precise than the values estimated using LBW$^{0.75}$. This supposition was implied in this thesis.

Differences in MEm are affected by changes in body composition. The ME are lower in fat animals than in lean animals (Close, 1990; Latshaw and Bishop, 2004). Macleod *et al*. (1988) found a higher fasting heat production and protein retention in broilers selected for leanness than those selected for fatness. This suggests an increased MEm requirement in lean birds. Increases in fat deposition in mature birds decreases MEm because the metabolic rate of fat tends to be lower than of other tissues (Sakomura, 2004; Roux, 2009). Latshaw and Bishop (2004) provided equations to estimate the MEm depending on body fat percentage and metabolic live body weight (LBW$^{0.75}$), and the result showed a chicken with 13% body fat was required 494 kJ /LBW$^{0.75}$/day for maintenance.

The MEm requirement of chickens raised on the ground was 20% higher than chickens raised in cages. This is due to more energy spent on locomotion activity. The hens raised on the ground also had a greater heat production (566 kJ/kg$^{0.75}$/day) than those raised in cages (273 kJ/kg$^{0.75}$/day) (Sakomura, 2004). Similarly, (Austic and Nesheim, 1990) found the energy requirement for activity of birds reared in uncaged conditions is 50% higher than birds in cages. Another factor affecting the MEm is the environmental temperature. For broilers housed on 13, 23 and 32°C, the MEm requirements were estimated to be 661, 469, and 531 kJ/LBW$^{0.75}$/day, respectively (Sakomura *et al*., 2005). The MEm requirement increases when birds have poor feathering and needed to consume more energy to maintain their body temperature (Pym *et al.*, 1984). In this context, it is necessary to know how the environmental temperature, body weight, body composition and breed affect MEm before estimating the total energy requirements for the broiler chickens.

2.1.4.2. Retained energy as protein and fat

In commercial broiler chickens fed *ad-libitum*, the energy deposited as body fat and protein represent 35% to 40% from the total of MEI with the rest was lost as heat (Lopez *et al*., 2007). The average proportion of energy retention between ERF and ERP is approximately 50% fat deposition and 50% to protein deposition (Lopez and Leeson, 2005). The description of energy requirements for maintenance protein and fat deposition is represented in Figure 2.3.

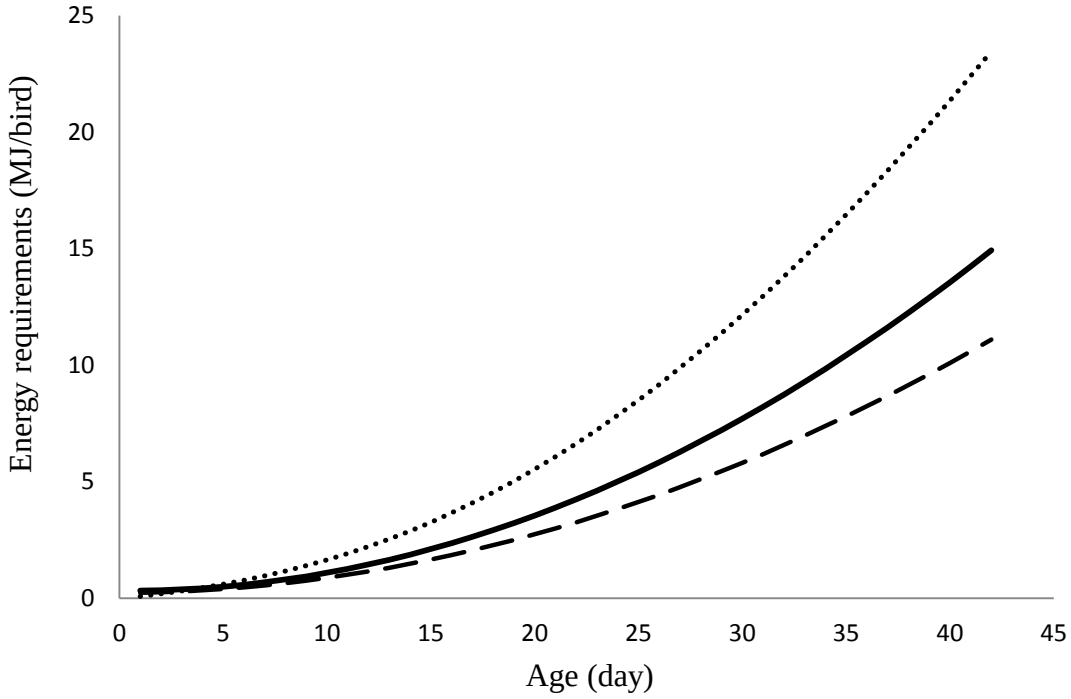

Figure 2.3. Metabolisable energy intake partitioning in broiler chicken from 1 to 42 days. ― is the energy required for protein deposition calculated as the estimated total gross energy deposited as protein divided by the energy efficiency for protein deposition (*kp* = 0.66, Boekholt *et al*., 1994). ---- is the energy required for fat deposition calculated as the estimated total gross energy deposited as fat divided by the energy efficiency for fat deposition (*kf* = 0.86, Boekholt *et al*., 1994). …. is the metabolisable energy for maintenance calculated as 649 kJ/LBW$^{0.60}$/day (Source: adapted from Lopez and Leeson, 2005)

2.1.4.3. Factors affecting the energy partitioning in broiler chickens

In order to maximise the energy efficiency in broiler chickens, factors that influence energy partitioning and energy efficiency should be known. The most common factors

that influence the energy partitioning are breed, sex, level of intake, maturity status and the form of feed. These factors are reviewed in the next sections.

2.1.4.3.1. Sex

Chung *et al*. (2007) reported that in Taiwanese native chickens, the males partitioned 64.1% of the ME to heat production and the remaining was partitioned to growth with 31.3% for body growth and 4.6% for feathers. The females partitioned 56.5% to HP and 39.1% for body growth and 4% in feathers growth. The same study found the daily ME for body growth was lower in females than males during early stages of growth. This difference became less after 7 weeks of age and at 10 weeks of age was greater in females than males. This phenomenon can be explained by the body energy deposition, mainly as fat, being higher for pullets approaching sexual maturity.

2.1.4.3.2. Intake level

The energy intake level is a very important factor affecting the energy retention in broiler chickens. An increase in the energy intake above the maintenance requirements will provide energy for fat and protein production. Many studies have focused on increasing voluntary feed/energy intake as a mechanism to improve production. Broilers fed limited amounts of feed gained less body weight per day and had a higher feed to gain ratio than those fed *ad-libitum* feed (Latshaw and Moritz, 2009). In the same report, as the daily ME intake increased, the proportion of each gram of feed needed for maintenance decreased, but the proportion for production and heat increment increased (Figure 2.4). Therefore, the regulation of energy intake is important for broiler performance and carcass composition. Increasing caloric intake above maintenance requirements may increases the fat deposition of broiler chickens. Each increase of 418.4 kJ of ME per day above maintenance increased body fat by 1.2% (Latshaw, 2008).

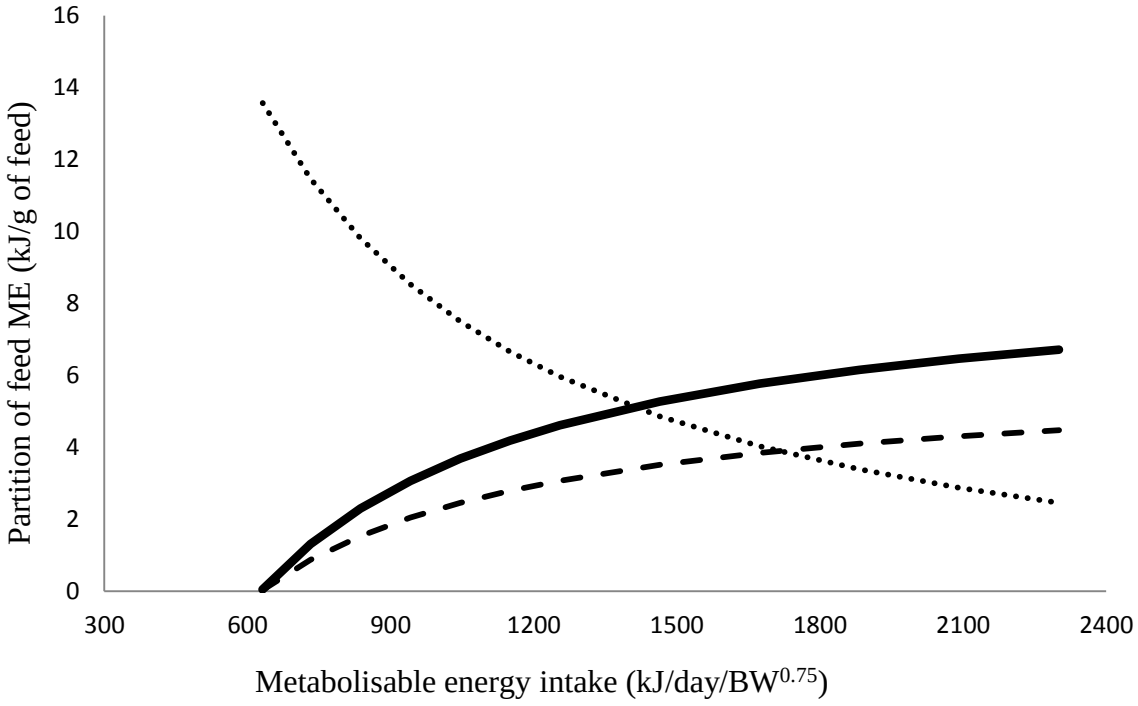

Figure 2.4. Effect of ME intake on partitioning of feed metabolisable energy (ME) for broilers chickens. ― is the energy for production. ----is the heat increment. ….. is the energy for maintenance (Source: Latshaw and Moritz, 2009)

2.1.4.3.3. Maturity statues

Broilers deposited a constant proportion (50%) of body energy as fat and protein deposition (Lopez and Leeson, 2005). However, in mature animals, in which skeletal and muscle growth is complete, may continue to consume excess energy which leads to deposition of fat. In broilers older than 35 days of age, energy is likely to be partitioned to fat rather than protein, which can affect meat quality and yield (Boekholt *et al*., 1994).

2.1.4.3.4. Form of feed

Broilers fed a pelleted diet had a decreased heat increment compared with broilers feed mash diet. In general, pelleting the diet does not increase digestibility and metabolisable energy content in the diet (Latshaw and Moritz, 2009). But, the birds fed pellet eat more feed with less physical activity than those fed mash diet. Consequently, the broilers fed pellet had higher energy available for production (Latshaw and Moritz, 2009). Jensen *et al*. (1962) observed that the pelleted feed for broilers reduced energy required for eating by 67% compared with mash feed. The number of meals consumed per day was similar

between the birds fed pelleted or mash diet, but the duration of meal was increased three-fold when mash fed was used.

2.1.4.4. Feather energy content

Feathers are complex branched skin appendages that contain 80-90% protein. Keratin represents about 85% of feather protein (Leeson and Walsh, 2004). There are two general kinds of feathers, the down feathers of newly hatched chicks; these consist of a large number of barbs attached to common base of main feather (Crawford, 1990). The down feathers are replaced by main feathers; and this usually takes 2 to 4 weeks in broiler chickens. Feathers develop from follicles, which are found in the dermal and epidermal layers of the skin. The feather is actively growing from the follicle. There is one artery and one vein that run through the feather to support the growth. Once the feather has reached the full size, the blood supply is no longer need and the vessels degenerate. Broiler chickens will have about 3-6% of body weight as feathers (Leeson and Walsh, 2004). Feathers are important for broilers body to provide insulation and reduce maintenance energy needs, and help prevent skin abrasions and infection.

There is limited information available on the energy requirement for feather growth. Most studies estimate the energy requirement for feather growth as part of the total energy requirement for protein retention (Sakomura *et al*., 2003; Harper, 2000; Lopez and Leeson, 2005). Chung *et al*. (2007) estimated the ME requirement for feather growth in Tawianese native chicken breeds and reported that the energy retained in feathers was similar in males and females, approximately 4% of the total ME requirement. Sakomura *et al*. (2005) found the growth rate for feathers was initially higher in female broilers but the males had a greater growth of feathers from 21 to 70 days of age. Sakomura *et al*. (2003) reported the energy content in the feather is 17.5 kJ/g. Dale (1992) estimated the energy content in the feather as 23.35 kJ/g.

2.1.4.5. The energy cost for uric acid synthesis in birds

The breakdown of amino acids (AA) and nucleic acids creates ammonia. In birds instead of converting this ammonia to urea, it is converted to uric acid ($C_5H_4N_4O_3$), which is excreted in the bird excreta. Uric acid production wastes less water than urea formation and allows birds to retain more moisture in their bodies but it is energy expensive biochemical process. The cost of uric acid synthesis in the birds is approximately 1406 kJ/*mol* compared with urea synthesis which uses only 432 kJ/*mol*

of urea formed (Harper, 2000). The molecular weight of one *mol* of uric acid is 168g, then the cost to produce one gram of uric acid is 8.37 kJ/g.

Commercial broilers had greater energy retention for fat and protein deposition compared with Plymouth Barred Rocks and Leghorns. That indicates higher protein syntheses and greater nitrogen excretion rate (300% more than other two breeds). The heat of combustion of uric acid was estimated to be 11.4 kJ/g (Lopez and Lesson, 2005).

2.1.4.6. Energetic efficiency of protein and fat deposition in poultry

The definition of energy utilisation efficiency is the total energy requirement to deposit 1 kJ energy in body mass. For example, the total energy requirement for protein deposition (*kp*) was 1.66 kJ/kJ and 1.25 kJ/kJ for fat deposition (*kf*) in pigs (Van Milgen and Noblet, 1999). For broilers it was 2.1 kJ/kJ for protein deposition and 1.4 kJ/kJ for fat deposition (Emmans, 1994; Emmans and Fisher, 1986). The energetic efficiencies can estimated experimentally or theoretically based on biochemical and biological transformations.

2.1.4.6.1 Experimental and theoretical energetic efficiency for protein and fat deposition

Observed efficiency of energy utilisation for protein and fat deposition varies, due to factors such as the nature of feed, dietary energy to protein ratio, strain, sex, age and environmental factors (Boekholt *et al*., 1994; Lopez *et al*., 2007; Sakomura *et al*., 2005).

Petersen (1970), using the White Plymouth Rock breed, estimated the efficiencies of energy utilisation to be 0.51 and 0.78 for protein and fat deposition, respectively. Sakomura *et al*. (2005), using the Ross breed, found the efficiency of energy utilisation for protein ranged from 0.36 to 0.58, and for fat it ranged from 0.55 to 0.92 (Table 2.2). The diet composition can have a major impact on energy efficiency (Lopez and Leeson, 2008a; De Groote, 1974). Experimental evidence of the energetic efficiency of nutrients (carbohydrate, fat and protein) in broilers is scarce.

Table 2.2. Experimental efficiencies of energy utilisation for protein (kp) and fat deposition (kf) in poultry

Reference	Breed	kp	kf
Petersen, (1970)	White Plymouth Rock	0.51	0.78
Scheimann et al. (1972)	Unknown	0.61	0.84
De Groote, (1974)	Hubbard	0.35- 0.70	0.70 -0.84
Boekholt et al. (1994)	Hybro strain	0.66	0.86
Nieto et al. (1995)	White Rock	0.40 -0.58	0.64 -0.9
Sakomura et al. (2005)	Ross broilers	0.36-0.58	0.55-0.92
Zoone et al. (1991)	Broiler	0.39	0.70
Emmans, (1994)	Broiler	0.41	0.70-0.90

The theoretical efficiency of fat and protein deposition can be estimated from biochemical considerations. The theoretical efficiency of fat and protein deposition in the animal body, from different energy sources, are summarised in Table 2.3

Table 2.3. The theoretical efficiency for fat and protein deposition from different energy sources

Energy sources	Product	Blaxter, (1989)	Baldwin, (1995)	Quiniou, (1996)	Van Milgen, (2006)
Carbohydrate	Body fat	0.80	0.78	0.81	0.81-0.84
Fat	Body fat	0.96	0.97	0.97	0.97
Protein	Body fat	0.66	0.67	0.81	0.52
	Body protein	0.86	0.83	-	0.85-0.90

The theoretical (biochemical) efficiency of using carbohydrate for fat deposition varies between 0.78 and 0.84, depending on the animal species and metabolic pathways, and whether the ATP yield from glucose is accounted for or not. When the ATP synthesis from glucose is not included in the efficiency calculation, the energy efficiency of triglyceride synthesis from glucose is 0.80: and when the ATP synthesis from glucose is included the efficiency is 0.84 (Baldwin, 1995).

The theoretical efficiency of fat deposition from dietary fat is very high and close to unity (Van Milgen, 2006). According to Baldwin (1995), after hydrolysis of dietary fat to monoglycerides and fatty acids, reformation of triacylglycerol and hydrolysis of blood triacylglycerol to glycerol and fatty acids, followed by re-esterification in adipose tissue, the net energy cost for fat synthesis is:

12 × 0.074 MJ/*mol* ATP)/ 31.74 MJ/ *mol* tripalmitin = 0.03. The energy expenditure of 0.074 per *mol* ATP calculated as (180.2 g/*mol* glucose × 0.0156 MJ/g glucose)/38 ATP/*mol* glucose. Then, the result of an efficiency of fat deposition from a dietary fat source is equal to 0.97.

Roux, (2009) estimated the theoretical efficiency of protein deposition from dietary protein as:

$kp = (22.6 \times PR)/[(22.6 \times PR) + (3.76 \times PS)]$

where

PR is the protein retention (kg/d),

PS corresponds to the given rate of PR, allowing for turnover, and

the coefficient 22.6 represents the energy content of protein (MJ/kg).

The cost of synthesis protein is 3.76 MJ/kg. A reasonable enough estimate can be derived, based on the assumption that 5 *mol* ATP is required to arrange one *mol* of peptide bonds (Van Milgen *et al*., 2001a), or 5/38 = 0.132 *mol* of glucose and then 0.132 *mol* of glucose × 2.82 MJ/*mol* glucose = 0.37 MJ of heat will be released per *mol* of peptide bond. Blaxter (1989) assumed that the average molecular weight of the constituent amino acids in protein is equal to 100g and then the energy cost per kg of protein deposition is 3.7 MJ, which is close to the value of Roux, (2009). If there is no protein turnover, then *kp* = 0.86. Similarly, Van Milgen (2001a) reported that the theoretical efficiency of deposition protein from amino acids is 0.87. However, the protein turnover, through repeated hydrolysis and synthesis of peptide bonds, contributed to a low efficiency of protein deposition. These terms are not easy to deal with and efficiency can change, depending on the number of turnover cycles. In pig, protein turnover typically occurs at a rate between two and three times (Reeds *et al*., 1980), which suggests an energy cost for turnover between 8 and 12 MJ/kg of protein deposition, and assuming that there is no other energy requirement, this implies an efficiency of protein deposition between 0.65 and 0.75. In chickens, based on 6.2 MJ/kg of synthesised protein, the efficiency of protein deposition would be 0.78 (Muramatsu *et al*., 1987).

Deposition of amino acid as fat deposition is implied with an additional energy cost for nitrogen excretion and energy for turnover cycle (assumed four times synthesis and three times breakdown). This situation would result in an efficiency of 0.63 for fat deposition (Van Milgen, 2001a).

There are differences when comparing theoretical efficiency with experimental efficiency. Van Milgen, (2006) indicated that the experimental efficiency for fat deposition from dietary fat is much lower than the theoretical efficiency, and this may be due to the oxidation of dietary fat for ATP synthesis combined with de-novo lipid synthesis from other nutrients. In addition, part of the energy coming from dietary fat may be used for maintenance requirements (Van Milgen *et al*., 2001a). Consequently, the available energy for fat deposition from dietary fat may be decreased.

Wongsuthavas *et al*. (2008) reported that, when polyunsaturated fatty acids were fed to birds, the birds preferred to oxidise the fatty acids and yield ATP. So the carbohydrates had shifted from oxidation to the lipogenic pathway and therefore the energetic efficiency for fat deposition decreased: i.e. the conversion of glucose to triglycerides is less efficient in energy deposition than conversion of fatty acids to triglycerides.

The maximum theoretical efficiencies for protein deposition from amino acids range between 0.85 and 0.90 (Van Milgen, 2006). However, experimental values range between 0.48 and 0.63, so it is clear that repeated hydrolysis and synthesis of peptide bonds contribute to a low efficiency. In addition, the estimated experimental efficiency of protein deposition is 0.52, when amino acids are used for support costs and 0.63 when glucose is used for support costs (Van Milgen, 2006).

2.1.5. Conclusion

Available literature clearly shows that, factors such as breed, sex, level of intake, maturity status and the form of feed affect the energy partitioning and metabolisable energy efficiency. A precise understanding of the partitioning of metabolisable energy is required to be able to assess the energy, which is available for fat and protein deposition. Therefore, understanding the efficiencies of metabolisable energy utilisation for fat deposition from variable energy sources is needed to improve the descriptions of the metabolisable energy partitioning system. A better description of energy flows could be developed via a mechanistic growth model. Such model would describe the amino acids and energy partitioning and the relationships between the energy intake and body composition.

2.2. The partitioning of dietary amino acids

2.2.1. Introduction

Dietary amino acid concentration in broiler diets influences protein deposition and growth rate (Hardy *et al*., 1999; Smith and Pesti, 1998). The cost of broiler feed is mainly associated with energy and protein content, and therefore, maximising lean meat production with minimum protein and amino acids intake is important for reducing the cost of feed. In addition, formulating diets that meet but do not exceed the birds requirements also results in less nitrogen excretion and increases the energy available for growth i.e. the excretion of excess AA nitrogen requires energy (Skaln and Plavink, 2002; Bartov, 1979). Amino acid requirements are based on ileal digestible AA and diets are supplemented with purified AA to balance the protein (Gorman and Balnave, 1995; Pesti, 2009). Ideal balanced protein is applied to maximise the efficiency of protein deposition and minimise the nitrogen excretion (Baker and Han, 1994; Smith and Pesti, 1998). Therefore, the fixed value for AA given as a percentage of the diet does not consider factors such as feed intake, genetics and the potential growth rate. An understanding of how the AA and protein intake is partitioned into maintenance and growth requirements is very important for optimising growth rate. The partitioning of total AA requirements changes with age, the body weight of the bird, genetics, and the AA profile in the diet.

2.2.2. Amino acids requirements in the broilers chickens

The body weight of commercial broiler chickens increases 55 to 60-fold by five weeks after hatching. Therefore, a high concentration of dietary AA is required to support the rapid growth of broiler chickens. A large part of the increase in weight is related to the protein accretion in the body tissues. Thus, adequate AA nutrition is required for a successful feeding program for broiler chickens.

Dramatic genetic changes have occurred in many commercial broiler lines during the past few decades with the objective to increase breast meat yield. The AA requirement for poultry has been published by the National Research Council (NRC, 1994). However, the NRC recommendation may no longer be sufficient to optimise the growth rate of modern broiler chickens. A feed specification is required for different classes of broilers based on maximum growth rate. The AA requirement for the Ross 308 broiler contains 20-30% higher AA than those given in NRC, 1994 (Table 2.4).

Table 2.4. The total amino acids requirement as percentage of diet composition for different growth period as presented in NRC 1994 and Ross 2007

	NRC (1994)			Ross (2007)		
	starter	grower	finisher	starter	grower	finisher
Amino acid	0-21	21-42	42-56	0-10	11-24	25- 42
Lysine (%)	1.10	1.00	0.85	1.43	1.24	1.09
Methionine (%)	0.50	0.38	0.32	0.51	0.45	0.41
Methionine-Cysteine (%)	0.90	0.72	0.60	1.07	0.95	0.86
Threonine (%)	0.80	0.74	0.68	0.94	0.83	0.74
Arginine (%)	1.25	1.10	1.00	1.45	1.27	1.13
Valine (%)	0.90	0.82	0.70	1.09	0.96	0.86
Tryptophan (%)	0.20	0.18	0.16	0.24	0.2	0.18
Isoleucine (%)	0.80	0.73	0.62	0.97	0.85	0.76
Leucine (%)	1.20	1.09	0.93	-	-	-
Phenylalanine (%)	0.72	0.65	0.56	-	-	-
Histidine (%)	0.35	0.32	0.27	-	-	-
Glycine-Serine (%)	1.25	1.14	0.97	-	-	-
Phenylalanine-Tyrosine (%)	1.34	1.22	1.04	-	-	-
Crude protein (%)	23.0	20.0	18.0	24.0	22.0	21.0

The digestibility of AA varies between dietary ingredients and the consideration of AA digestibility is important in diet formulation. Nowadays, the AA requirement is given based on ileal digestible AA (Table 2.5). The ileal digestibility of AA provide a good estimate for absorbed AA and ileal digestibility values of ingredients and well documented for broiler chickens (Ravindran *et al.,* 2005; Ravindran *et al.,* 2002; Bryden *et al*, 2009).

The diets are supplemented with purified AA to balance the protein in the diets. Ideal balanced protein is applied to describe the correct AA required to maximise the efficiency of protein deposition and minimise the nitrogen excretion (Baker and Han, 1994; Smith and Pesti, 1998). The ideal balanced protein concept uses lysine as a reference AA. Lysine is used as reference AA for several reasons. Firstly, in practical broiler diet (corn and soybean diet), lysine is the second limiting AA after methionine. Secondly, lysine analysis in the ingredients is straightforward. Finally, the lysine requirements in different circumstances have been well-documented (Emmert and Baker, 1997).

Table 2.5. The ileal digestible amino acids requirement as percentage of diet composition for male broilers (Ross, 2007)

	0-10 days	11-28 days	29-42 days	43-Slaughter
Amino acid				
Lysine (%)	1.27	1.06	0.88	0.84
Methionine (%)	0.47	0.40	0.34	0.33
Methionine-Cysteine (%)	0.94	0.81	0.69	0.66
Threonine (%)	0.80	0.68	0.58	0.55
Arginine (%)	1.33	1.13	0.96	0.92
Valine (%)	0.94	0.80	0.67	0.64
Tryptophan (%)	0.22	0.18	0.16	0.15
Isoleucine (%)	0.84	0.71	0.60	0.57
Leucine*(%)	1.02	0.92	0.79	0.79
Phenylalanine* (%)	0.61	0.55	0.48	0.48
Histidine* (%)	0.29	0.27	0.23	0.23
Glycine-Serine* (%)	1.06	0.97	0.82	0.82
Phenylalanine-Tyrosine* (%)	1.14	1.04	0.88	0.88
DCP (%)	20.4	18.7	17.0	15.3

*The values were adapted from NRC (1994) assumed that the ileal digestibility of AAs is 0.85.

2.2.3. Ideal balanced protein

The concept of ideal digestible balanced protein has been applied in feed formulations (Baker and Han, 1994; Smith and Pesti, 1998). The ideal digestible balanced protein (IDBP) is described as the perfect balance of AA required to maximise the efficiency of protein deposition per unit of absorbed protein. When a diet has IDBP, this means the protein in this diet should supply the exact proportion of required essential AA. This can be achieved if animals are fed protein with an appropriate amount of essential AA without energy limitation (Fuller and Chamberlain, 1983). Applying the IDBP in feed formulation makes it possible to adjust the AA intake to meet the broiler requirements with neither limitation nor excess of requirement. Few studies have been done with broilers that investigate the effect of ideal balanced protein on fat and protein deposition and profitability. Eits *et al*. (2005a, b) developed two models (growth simulation and economical model) that characterise the effect of balanced protein content in the broilers performance, carcass composition and feed costs. The first model was developed to estimate growth rate, feed per gain ratio, carcass yield and meat yield of broilers in response to dietary balanced protein content. The second model was developed to measure the effect of dietary balanced protein content on feed costs and gross margin. The results showed formulating diets with dietary balanced protein for maximum profit, instead of maximum broiler performance are able to increase the profitability of broiler

production. Moreover, Zuidhof (2009) created a growth model with different dietary energy and different dietary balanced protein. His results showed that each reduction in dietary balanced protein decreased the breast meat yield in males and females broilers, and increasing dietary balanced protein above the recommended concentration (up to 107.5% of recommended) levels increased the broilers breast yield in males, but did not improve the breast yield in females. This suggests that the requirement of ideal balanced protein may vary between sexes. The AA recommendation was specified for maximum growth rate, best feed per gain and maximum protein deposition (Rostagno *et al*., 2007). The digestible lysine requirement as a percentage of the diet ranged from 0.96 to 1.30 according to bird age, body weight gain, feed per gain lysine deposition and breast fillet (Table 2.6).

Table 2.6. Lysine requirements of broiler chickens for different performance categories

Response criteria	Value	Sex	Age(day)	Requirement	Reference
Age(day)	0-21	Mix	0-21	1.1 %[t]	NRC,1994
	22-42	Mix	22-42	1.0 %[t]	
	43-56	Mix	43-56	0.85%[t]	
Body weight gain(g)	277-410[1]	Male	0-14	1.28%[t]	Labadan *et al*., 2001
	684-801[2]	Male	15-28	1.13%[t]	
	1244-1483[3]	Male	35-56	0.81%[t]	
Body weight (g)	1504-1764	Male	14-28	1.09%[d]	Dozier *et al.*, 2009
	1329-1422	Female	14-28	0.98%[d]	
	1250	Male	-	1.37(g/d)	Rostagno *et al*., 2007
	1438	Male	-	1.55 (g/d)	
Feed per gain (g/g)	1.40	Male	14-28	1.15%[d]	Dozier *et al*., 2009
	1.60	Female	14-28	0.99%[d]	
	1.43	Male	10-21	1.16%[d]	Rostagno *et al.*, 2007
	1.69	Male	22-35	1.04%[d]	
	2.02	Male	36-49	0.96%[d]	
	1.47	Female	10-21	1.12%[d]	
	1.76	Female	22-35	1.10%[d]	
	2.18	Female	36-49	0.96%[d]	
Lysine deposition(g/d)	0.63	Male	10-21	1.30%[d]	Rostagno *et al.*, 2007
	0.46	Female	10-21	1.30%[d]	
Breast fillet (%)	23.6	Male	15-40	1.12%[t]	Rostagno *et al.*, 2007
	23.3	Female	15-40	1.12%[t]	

[t] is the total lysine % requirement in the diet; [d] is digestible lysine% requirement in the diet.
Labadan *et al.*, 2001 estimated the requirement based on broken-line regression analysis. [1]the mean of initial body weight was 33 g per chick, [2]the mean of initial body weight was 408 g per chick, [3] the mean of initial body weight was 870 g per chick. Dozier *et al*. (2009) estimated the requirement based on quadratic broken-line model analyses. Rostagno *et al*. (2007) estimated the digestible lysine as:
Digestible lysine (g/day) = $0.1\times BW^{0.75}$ + [(14.28+2.0439×BW) × DG], where $0.1\times BW^{0.75}$ is the lysine requirement for maintenance, BW is the live body weight (kg), DG is the daily gain (kg).

The requirement for AA has been traditionally determined by dose response trials (Rostagno *et al*., 2007; Rondon and Waldroup, 2002). It is assumed that all other nutrient requirements are supplied with sufficient amount. However, imbalanced diets may affect the production responses. The fixed value of AA given as a percentage of the diet does not consider factors such as genetic, feed intake, energy intake, body weight and the potential growth rate. Partitioning the digestible AA requirement into maintenance and retention would allow predicting of the accurate requirement of the ideal digestible AA (Emmert and Baker, 1997; Craig, 2004).

2.2.4. Partitioning the amino acid requirements

The total digestible AA requirements can be partitioned into maintenance requirement and retention requirement. The proportion of total AA required for maintenance increases as the bird grows (Emmert and Baker, 1997; Leeson and Summers, 2001). The partitioning of net methionine and cysteine requirements for body growth, feathers growth and maintenance requirements is represented in figure 2.5.

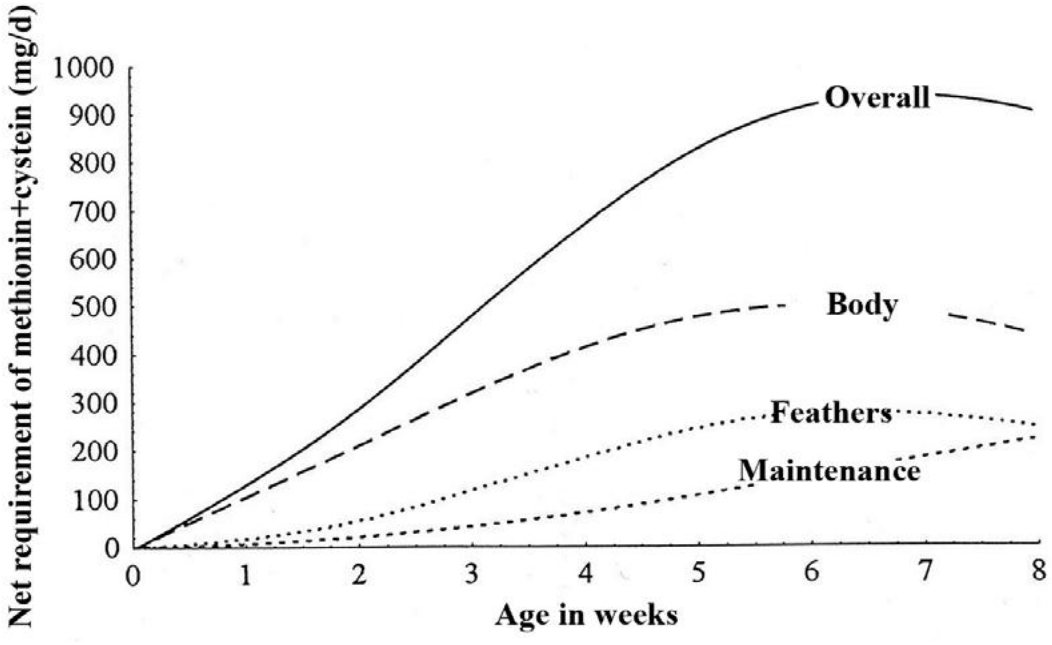

Figure 2.5. Net requirements of methionine and cysteine by male broilers in the growing period (Sundrum *et al*., 2005)

2.2.4.1. Amino acids requirement for maintenance

The maintenance AA requirement is the amount of AA needed for basal processes including protein turnover, integument AA loss, gut endogenous AA loss and urinary loss. The maintenance AA requirement is only a small proportion (about 10% or less) of

the total daily AA requirements for growing pigs (Moughan, 2003). Emmert and Baker (1997) suggested the minimal maintenance AA requirements are 3% to 6% of total AA requirements for young birds. Many studies have focused on calculating the protein and AA requirement to maximise the body growth rate. Some researchers calculated the maintenance requirements depend on body weight and protein body deposition. Zoon *et al*. (1991) presented a formula to calculate the protein requirement for maintenance and protein requirement for production as:

$$\text{total protein requirement(g/d)} = (0.0233 \times \text{LBW}^{0.66}) + \text{Pd} \times (\frac{1}{0.85})$$

Where:

LBW: is the live body weight and $0.0233\times \text{LBW}^{0.66}$ is the protein requirement for maintenance (g/day).

Pd: is the total protein deposition g/day.

0.85; is the efficiency of protein deposition.

The lysine needed for maintenance at zero lysine retention ranged from 8.3 to12.6 as a percentage of the total digestible lysine requirement. In contrast, the lysine requirement for maintenance at zero protein retention ranged between 0.65 to 0.99 as a percentage of the total digestible lysine requirement (Emmert and Baker, 1997, Table 2.7). The metabolic turnover of lysine may provide a positive protein deposition.

Table2.7. The partitioning of the digestible lysine requirement (mg/day/kg$^{0.75}$) into maintenance and retention in whole body

	0-21 days	21-42 days	42-56 days
Total lysine requirement(mg/day/kg$^{0.75}$)	1072	904	707
Maintenance[1]	89.0	89.0	89.0
Protein retention	983	815	618
Maintenance % of total	8.30	9.90	12.6
Maintenance[2]	7.00	7.00	7.00
Protein retention	1065	897	700
Maintenance % of total	0.65	0.77	0.99

[1] Based on zero lysine retention. [2] Based on zero protein retention. (Source; adapted from Emmert and Baker, 1997.

More recently, Samadi and Liebert (2006) reported that genetic selection has improved the potential for protein deposition, and estimated the requirement of AA according to the protein deposition. In the same report the nitrogen intake and total

nitrogen excretion was fitted into an exponential function for estimation of nitrogen for maintenance. The average daily nitrogen maintenance requirement was estimated to be 252 mg of nitrogen/$BW^{0.67}$ per day. In this study, they estimated the nitrogen for maintenance was based on zero nitrogen retention.

2.2.4.2. Amino acids requirement for growth

In broiler chickens, the crude protein ingested is almost completely broken down into peptides and AAs within the small intestine. These absorbed AAs are used for maintenance requirements and for tissue growth (muscle, viscera, feather, and ligaments). Excess AAs (above requirement for growth) are deaminated and used as a source of energy, and the breaking down of AAs produces nitrogenous excretions (Macleod, 1997). Amino acids cannot be stored as free molecules and they must follow catabolic or anabolic pathways (protein synthesis, lipogenesis, gluconeogenesis or uric acid formation).

The net retention of AA into body protein tissue explains a large part of dietary AA requirements ~90% (Moughan, 2003). In broiler chickens, 22 AA are needed to form body protein, some of which can be synthesised by the bird (non-essential AA), whereas others cannot be made at all and must be supplied by diet (essential AA). In order to prevent the conversion of essential AA into non-essential AA and to optimise the efficiency of AA utilisation, a sufficient amount of non-essential AA must also be supplied from the diet (Corzoa *et al*., 2005).

A specific AA profile is needed to support maximum growth rate with no limiting or excess AA and this profile has been reported as an ideal balanced ratio (NRC, 1994; Baker and Han, 1994; Emmert and Baker, 1997). In broiler, the lysine is the first limiting amino acid (NRC 1994, Dozier *et al*. 2009; Rostagno *et al*., 2007). Dozier *et al*. (2009) estimated the digestible lysine requirement for male Ross broilers to be between 1.07 and 1.09%, from 14 to 28 days of age. Berri *et al*. (2008) reported that, when the true digestible lysine increased from 0.83 to 0.93%, the growth rate increased from 91.8 to 95.5 g/day and the breast meat yield increased from 22.0 to 22.7% of body weight.

In order to determine an optimum dietary concentration of AA at different periods of broiler growth, the response of protein deposition to a range of lysine levels has been evaluated (Tavernari, 2009). When the digestible lysine level increased from 1.07 to 1.25% for male and 1.02 to 1.20% for female, the protein deposition slightly

increased, but this was not statistically significant for the period 1 to 21days of age for male and female. For the period 22 to 42 days of age, there was no significant effect of lysine levels on protein deposition in female broilers, but for male broilers the protein deposition was significantly increased from 20.4 (g/d) to 21.8 (g/d), as the digestible lysine level increased from 0.98 to 1.06 % of diet. A further increase of digestible lysine, up to 1.14%, did not improve the protein deposition.

Macleod, (1997) reported that, when the actual lysine intake increased from 0.17 to 0.55 g/day, the protein deposition rate significantly increased from 2.8 to 7.1 g/day. In many instances, the results of experiments have been used to define the requirement of AA for specific strains, sex, environmental conditions, feeding levels, and marketing objectives (Havenstein *et al*., 1994; Gous 2007). In order to estimate accurate requirements of AA, together with protein requirements, a better description of AA requirements for maintenance and growth is important for optimising growth rate.

The average digestible AA requirement for growth, as a percentage of the total digestible AA requirement, was decreased from 94% between 10 and 21 days of age into 79% between 32 and 43 days of age (Table 2.8, 2.9).

Table 2.8. The partitioning of total digestible amino acids requirements into maintenance and growth requirements (mg/day/kg $LBW^{0.75}$) for broiler chickens growing between 10 and 21 day of age (Craig, 2004)

Amino acid	Total[1]	Maintenance requirement[2]	Maintenance % of total[3]	Growth requirement[4]	Growth % of total[5]
Lysine	1003	-	-	1003	100
Methionine	319	19	6.0	300	94.0
Cysteine	345	28	8.1	317	91.9
Threonine	638	31	4.9	607	95.1
Tryptophan	147	6	4.1	141	95.9
Arginine	920	104	11.3	816	88.7
Valine	677	36	5.3	641	94.7
Leucine	1002	38	3.8	964	96.2
Isoleucine	635	56	8.8	579	91.2
Phenylalanine	529	27	5.1	502	94.9
Tyrosine	455	20	4.4	435	95.6
Glycine + Serine	927	86	9.3	841	90.7
Histidine	289	4	1.4	285	98.6

[1] is total amino acid requirement (mg/day/kg $LBW^{0.75}$). [2] is maintenance requirement (mg/day/kg $LBW^{0.75}$). [3] is the maintenance requirement as percentage of total requirement. [4] is growth requirement (mg/day/kg $LBW^{0.75}$). [5] is growth requirement as percentage of total requirement.

Table 2.9. The partitioning of total digestible amino acids requirements into maintenance and growth requirements (mg/day/kg $LBW^{0.75}$) for broiler chickens growing between 32 and 43 day of age (Craig, 2004)

Amino acid	Total[1]	Maintenance requirement[2]	Maintenance % of total[3]	Growth requirement[4]	Growth % of total[5]
Lysine	882	-	-	882	100
Methionine	292	50	17.1	242	82.9
Cysteine	213	62	29.1	151	70.9
Threonine	550	132	24.0	418	76.0
Tryptophan	157	25	15.9	132	84.1
Arginine	875	146	16.7	729	83.3
Valine	670	120	17.9	550	82.1
Leucine	967	187	19.3	780	80.7
Isoleucine	576	142	24.7	434	75.3
Phenylalanine	389	102	26.2	287	73.8
Tyrosine	294	-	-	294	100
Glycine + serine	541	-	-	541	100
Proline	504	-	-	504	100

[1] is total amino acid requirement (mg/day/kg $LBW^{0.75}$). [2] is maintenance requirement (mg/day/kg $LBW^{0.75}$). [3] is maintenance requirement as percentage of total requirement. [4] is growth requirement (mg/day/kg $LBW^{0.75}$). [5] is growth requirement as percentage of total requirement

Martine *et al*. (1994) described a factorial approach, in order to estimate the essential AA requirements for four body functions: body protein gain; body protein for maintenance; feather protein gain; and feather protein for maintenance. The utilisation efficiency of AA for body and feather protein was assumed to be 0.80. Baker (1991) reported the average utilisation efficiency of balanced protein as 76% and this value was variable among individual AAs. For example, the efficiency of lysine utilisation was 80%, whereas the efficiency of isoleucine utilisation was 61%.

Broiler chickens divert approximately 10% of their dietary protein needs in the first six weeks to feather formation (Hancock *et al*., 1995; Stilborn *et al*., 1994). Lean broiler strains have a higher requirement for sulphur AA, methionine and cysteine, especially during early growth period, due a lower feed intake and a greater relative requirement for feather protein synthesis (Leclerq *et al*., 1983). In the case of adult birds, the feather weight represents between 3% and 6% of body weight (Leeson and Walsh, 2004). Feather weight changes as birds get older and it is comprised as a percentage of body weight, rising from 0.90 to 5.1, between days 1 and 42 of age (Skland and Noy, 2004). The AA content of feathers, as a percentage of protein, was not influenced by strain or sex but instead it was influenced by age: especially between14 and 42 days of age (Stilborn *et al*., 1997). Nitsan *et al*. (1981) suggested that the AA content of feathers is quite consistent across most domesticated birds. Feathers have a

different AA composition compared to body protein (Table 2.10). Protein in feathers is low in lysine (18 g/kg protein) and high in cysteine (70 g/kg protein), whereas protein for the rest of the body is high in lysine (75 g/kg protein) and low in cysteine (11 g/kg protein) (Gous *et al.*, 1999). There is very little information about AA utilisation for feather growth. Cysteine utilisation is 94% for birds between 10 and 21 days of age and 65% for birds between 32 and 43 days of age. The greater utilisation of young birds is likely due to a greater gain of feathers between 10 and 21 days of age (Craig, 2004).

Table2.10: Amino acid composition as percentage of protein content in carcass and feathers for broiler chickens

	Feather composition			Carcass composition	
	Skland, 2004	Stilborn, 1997	Stilborn, 1997	Skland, 2004	Skland, 2004
Age (day)	21	28	42	21	42
Lysine (%)	1.67	1.91	1.90	7.32	7.45
Methionine (%)	-	0.61	0.65	-	-
Cysteine (%)	-	7.94	7.18	-	-
Threonine (%)	3.63	4.82	4.94	4.49	4.32
Arginine (%)	5.42	6.39	6.76	7.09	7.03
Valine (%)	5.67	6.45	6.49	4.83	4.95
Tryptophan (%)	-	0.76	0.73	-	-
Isoleucine (%)	3.72	4.47	4.64	3.89	3.45
Leucine (%)	7.14	7.68	7.93	7.59	7.84
Phenylalanine(%	3.48	4.65	4.76	4.02	4.10
Histidine (%)	0.61	0.67	0.61	2.86	3.06
Tyrosine (%)	2.19	2.80	2.62	3.03	3.01
Alanine (%)	4.12	4.03	4.12	6.87	6.99
Aspartic acid (%)	5.91	6.56	6.50	9.21	9.75
Glutamic acid (%)	8.43	10.42	10.62	15.16	14.63
Glycine (%)	5.94	6.95	7.08	8.67	8.88
Proline (%)	7.51	9.41	9.27	7.13	7.18
Serine (%)	8.89	10.85	11.2	4.65	4.32
Crude protein (%)	79.8	91.19	95.68	15.90	16.20

Further understanding of the partitioning of AA, for the expression of maximum genetic potential for lean growth of broiler chickens, would allow for an accurate estimation of AA requirement and diet formulation. To be able to estimate the AA requirement, information about the maximum rate of protein deposition (Pdmax), combined with information on the amino acid composition of whole body protein is required. The upper potential of protein deposition can be achieved by feeding the animal a highly digestible protein diet with high energy level under ideal husbandry

conditions (Gous, 1998; Moughan *et al*., 2006). The Pdmax needs to be known for each strain of broiler chicken, in order to accurately estimate the requirement of AA.

2.2.5. The upper limit for body protein deposition (Pdmax)

The potential protein deposition is not the maximum protein deposition that can be achieved under commercial husbandry conditions. However, the maximum rate of protein deposition can be achieved experimentally, when an animal is given perfect nutritional and husbandry conditions (Gous, 1998; Moughan *et al*., 2006). The maximum daily protein deposition has not been established for different broiler strains. Samadi and Liebert (2006) characterised the theoretical maximum daily protein deposition (PdmaxT = 26.8 g/day between 30 to 45 days of age) for the Cobb 500 breed, but that is not achievable due to dietary factors. The PdmaxT may not be achieved by optimising the feed strategies, but it is possible to define the protein deposition rate as a percentage of PdmaxT. Consequently, the AA requirements can be estimated depending on daily protein deposition. In the same report, between the ages of 10 to 25 days and 30 to 45 days of age, the calculated daily protein deposition corresponding to 80% of PdmaxT increased from 11.6 to 21.4 g/day for male and from 10.8 to 19.4 g/day for female broiler, respectively. According to Tavenari *et al.* (2009), the averages daily protein deposition were 6.6, 21.1 and 18.7 for male Avian farm broilers raised from 1 to 21, 22 to 42 and 43 to 56 days of age, respectively.

Broiler males have a genetically higher capacity for protein accretion than females (Chamruspollert *et al*., 2002; Marcato *et al*., 2008). Marcato *et al*. (2008) also reported different protein deposition rates between broiler breeds and sexes. Cobb broilers had earlier growth for protein and ash deposition, whereas, Ross broilers had earlier water deposition. The protein deposition potential rate for the Ross breed was 17.52 g/day at 42 days of age for males and 13.8 g/day at 35 days of age for females. Energy intake can limit the Pemex, for example, in pig experiments the daily protein deposition increased linearly when the daily digestible energy intake increased until the protein deposition approached a plateau (Weis *et al.*, 2004).

2.2.6. Effect of energy and protein supply on protein deposition

Many factors influence the protein deposition rate. Some of these factors are related to nutrients such as energy and balanced AA intakes, other factors are related to the animal's upper limit for protein deposition. The effect of energy intake on the rate of

protein deposition has been described comprehensively in pigs (Moughan, 1984; Moughan *et al*., 1987; Moughan and Verstegen, 1988; Moughan *et al*., 2006; Sandberg *et al*., 2005a, b; Whittemore and Fawcett, 1976). Whittemore and Fawcett (1976) proposed that the ideal protein intake is preferentially used for protein deposition unless energy availability, environmental, or genetic factors become limiting. The response of protein deposition to ideal protein supply is assumed to be linear (i.e. the efficiency of protein utilisation is constant) unless potential protein deposition or energy become limiting. This approach is called the linear plateau theory (see Figure 2.6; De Greef and Verstegen, 1995; Denis and Daniel, 1997).

De Greef and Verstegen (1995) reported that when the energy intake is limiting the protein deposition and the protein deposition is less than the Pdmax, an increase in energy intake is partitioned between protein and fat deposition according to some ratio. Van Milgen and Noblet (1999) proposed a model of energy intake partitioning between protein and fat deposition. When the animal growth was limited only by energy and not by protein supply, each extra MJ of ME above maintenance requirement is partitioned into protein and fat deposition by designated fractions; $Pd = kp \times Z \times (MEI - MEm)$, $Ld = kf \times (1 - Z) \times (MEI - MEm)$. Where Pd and Ld is the protein and lipid deposition, respectively; *kp* and *kf* are the energy efficiencies for protein and fat deposition respectively. Z is the proportion of energy that goes to protein or fat and this range from 0 to 1; MEI is the ME energy intake; MEm is the ME for maintenance. These assumptions provide rules for energy partitioning in growing animals.

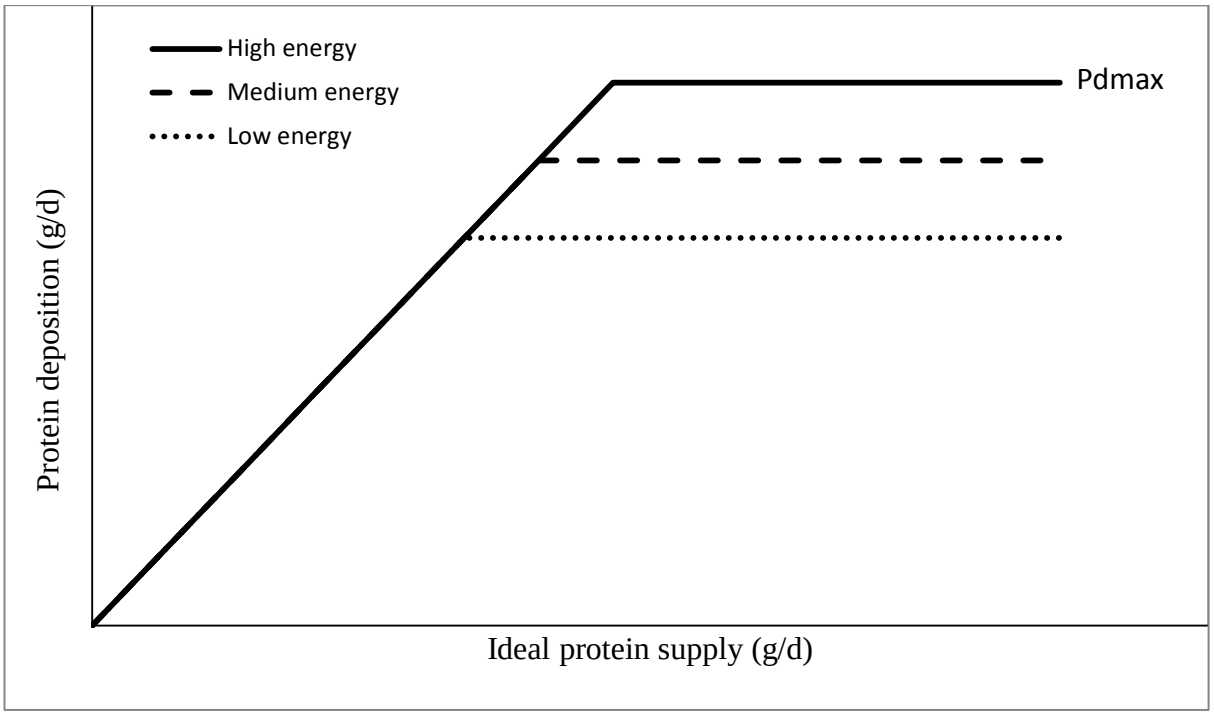

Figure 2.6. Response of protein deposition to protein and energy supply. Pdmax the maximum protein deposition for the animal (adapted from Black and Griffiths, 1975; as cited by Denis and Daniel, 1997)

Comparable evidence about the effect of energy intake on protein deposition for broiler chickens has been confirmed by Faulkner (1993) and Swatson (2003). The critical ratio of energy to protein is around 54 MJ ME per kg dietary protein, but below this ratio the efficiency of protein deposition decrease linearly. Assuming that the broiler starter diets contain 240 g of crude protein per kg feed, the ME content in this diet should be at least 13 MJ ME per kg feed. Any decrease in energy content or increase in protein content would result in a decrease in the efficiency of protein utilisation. Eits (2004) reported the protein deposition rate increased with additional AA intake ($P < 0.05$). Also, the protein deposition rate tended to increase with additional protein free energy ($P = 0.054$). Broilers fed a high energy diet had higher growth rate and better feed efficiency than those fed a low energy diet (Bartov, 1992; Lesson *et al.*, 1996 a, b). High energy intake may allow for more rapid protein gain.

2.2.7. Conclusion

Using a fixed requirement of amino acids in the diet is unlikely to maximise protein deposition and unlikely to be cost effective. However, providing evidence of how the amino acids and protein intake is partitioned into maintenance and growth is very important for optimising growth rate. The Pdmax is needed to be known for each strain

of broiler chickens in order to estimate the accurate requirements of ideal balanced protein and better description of protein deposition. Unfortunately, insufficient information is available on the impact of energy intake on protein deposition rate and therefore it is suggested that examining the effect of energy intake on the protein deposition is required. The methodology of mathematical growth modelling can be accepted as appropriate to determine the protein and other nutrients required to maximise growth of broiler chickens, as this will be deal adequately with the complexity of nutrient factors.

2.3. Growth models for broiler chickens

2.3.1. Introduction

A number of factors related to diet (protein, amino acids and energy concentration), animal (age, sex and genetic potential for growth) and environment (humidity, temperature and flock density) affect the growth rate of broiler chickens. Furthermore, the cost of ingredients is the key factor in determining profitability within the broiler industry. When making decisions for optimal production, such as cost per kg meat yield, all these interacting factors should be considered. Making the decisions needed to achieve maximum production can be assisted by combining all aspects and knowledge of animal growth within a computer growth model. Gous et al. (1999) mentioned that it is very important to simulate growth models for each genotype, as an essential step to predicting the effects of different diets, breeds and environmental conditions on broilers' performance and profitability. Another advantage of using a growth model is that it can enable manipulation of diet, in order to reduce carcass fat content and to increase protein deposition.

Models that can be used to predict growth performance in animals can be described as: empirical, mechanistic, deterministic, stochastic static or dynamic models (Zoons et al., 1991).

2.3.2 Model classification

2.3.2.1 Empirical and mechanistic models

Empirical models are models in which experimental data are used directly to quantify the relationship mathematically between two or more variables, without any explanation of biological processes (Zoons *et al*., 1991; France and Dijkstra, 2006). The empirical model can be associated with a curve fitting model for a specific data set. A mechanistic model describes the relationship between dependent and independent variables, by representing the biological process pathway, in order to predict growth (Zoons *et al*., 1991). A mechanistic model is constructed by looking at the overall structure of the system under investigation and dividing it into components, which come together to describe the behaviour of the whole system (France and Dijkstra, 2006).

2.3.2.2 Deterministic and stochastic models

Deterministic models describe the average of animal or flock outcome, without taking into account the probability distribution: e.g. the nutrition recommendations for feed formulation. In contrast, the stochastic model considers the probability distributions and it has a range of possible outcomes that represent natural variation (Zoons *et al*., 1991; Black, 1995).

2.3.2.3 Static and dynamic models

The static model represents the state of the system at only one fixed point of time, or for the mean of a fixed period, whereas the dynamic model takes time as a variable (Zoons *et al*., 1991; Black, 1995).

2.3.3 Application of empirical and mechanistic models

Empirical and mechanistic models are used to simulate the growth of broiler chickens. The majority of empirical models simulate growth, based on a nonlinear equation (Gous *et al*., 1999; Hancock *et al*., 1995; Roush *et al*., 2006; Zuidhof, 2005; 2009). Eits *et al*. (2005 a; b) developed empirical models to predict the feed composition required to maximise performance and profitability in broiler chickens. These models predict broiler responses of growth rate, feed conversion ratio, carcass yield and breast meat yield, to dietary balanced protein levels. These models make it possible to predict the response without actual experimentation under ad-libitum feed conditions. However, these models cannot deal with compensatory growth, nutrient digestibility and utilisation efficiency, or feed restriction.

Other empirical models describe growth as an increase in mass and as a function of time (Gompertz, Logistic, Richards, etc). In broiler, the Gompertz model has been used (Gous *et al*., 1999; Hancock *et al*., 1995; Roush *et al*., 2006; Zuidhof, 2005; 2009). In Gompertz model, the growth rate of a biological system is dependent on live body weight and time. The Gompertz equation requires knowledge of the mature live body weight, in order to estimate the parameters within the model (Zoons *et al*., 1991; Lopez and Lesson., 2005). The mature live body weight of the broiler chicken is unknown (Hancock *et al.,* 1995; Wang and Zuidhof, 2004). The method for evaluating the growth of live body and body component in broiler studies, using the Gompertz equation, has been based on data obtained for birds reared between 0 and 12 weeks and before maturity weight (Hancock *et al*., 1995; Wang and Zuidhof, 2004; Lopez *et al*.,

2007). Hancock *et al*. (1995) reported that, at 15 weeks of age, many broilers had stopped growing due to leg problems, and the growth curve was described up to 11 weeks of age only. Commercially, broiler chickens reach slaughter body weight at approximately five weeks and likely without achieving their maximum growth potential. Therefore, the asymptotic values for live body weight and body components are unlikely to represent commercial growth patterns.

Lopez *et al*. (2007) predicted empty body weight, protein and fat deposition as a function of time, by using the quadratic model with a good and reasonable accuracy (R^2 greater than 0.97). The disadvantage of the quadratic model is that there is no mathematical upper boundary for the response of body components growth with increased time, whereas in reality there is a biological limit for body component growth. Zuidhof, (2009) described an empirical approach (nonlinear mixed Gompertz model) to predict the growth responses for male and female broilers, under varied metabolisable energy and dietary balanced protein levels. This nonlinear mixed model could be extremely useful under a particular condition of the data obtained, but the response of the prediction might be limited under different arrays of data input. All empirical models, so far, have described growth as increasing with feed intake, nutrient intake, body weight or time. These empirical models create equations derived from observations and experiments, but they do not necessarily represent an understanding of biological theories. In contrast, mechanistic growth models prefer to describe the biological process and they are able to be applied and developed within a range of conditions.

A mechanistic growth model that has been widely used in animal science is referred to the work of Whittemore and Fawcett, (1976). This model describes the weight gain and carcass composition of a growing pig, as a function of feed intake, feed composition, breed and genetic parameters (maximum protein deposition and minimum lipid to protein ratio). The model is based on partitioning the amount of energy and protein taken up by the animal. The energy intake by the animal is first used to meet the maintenance requirement, followed by protein deposition with the physiologically necessary amount of fat, and any remaining energy is deposited as extra fat. If the protein intake is unbalanced, some of protein, which cannot be used for protein deposition, is also used for fat deposition. When the animal is given a diet that is adequate in protein, but limited by energy, the protein deposition increases as the energy intake increases, until it approaches the maximum protein deposition (Figure 2.7).

In broiler chickens, mechanistic growth models based on nutrient partitioning and utilisation can be used to predict growth performance, carcass composition and also to estimate an economic feeding strategy (Emmans, 1994; King., 2001; Zoons *et al*., 1991; EFG-Model; Emmans, 1981; Emmans and Fisher, 1986; Gous, 1998). The mechanistic growth model can be used for feed evaluation. In addition, research findings can be easily incorporated into a mechanistic growth model, which will improve the ability to predict the availability of nutrients (France *et al*., 2000). Recent research has suggested a merger between mechanistic and empirical models. The merging of these two models could improve the accuracy of the model prediction (Roush, 2006). An easy way to develop growth models for broilers is through an empirical approach but since this approach can only be used for a small range of circumstances, more mechanistic models based on nutrient partitioning concepts are needed (Zoons *et al*., 1991).

Implementation of the pig growth models of Whittemore and Fawcett (1976), Moughan (1984) and De Lange, (1995) can be re-stated for broiler chickens, by using a mechanistic based approach.

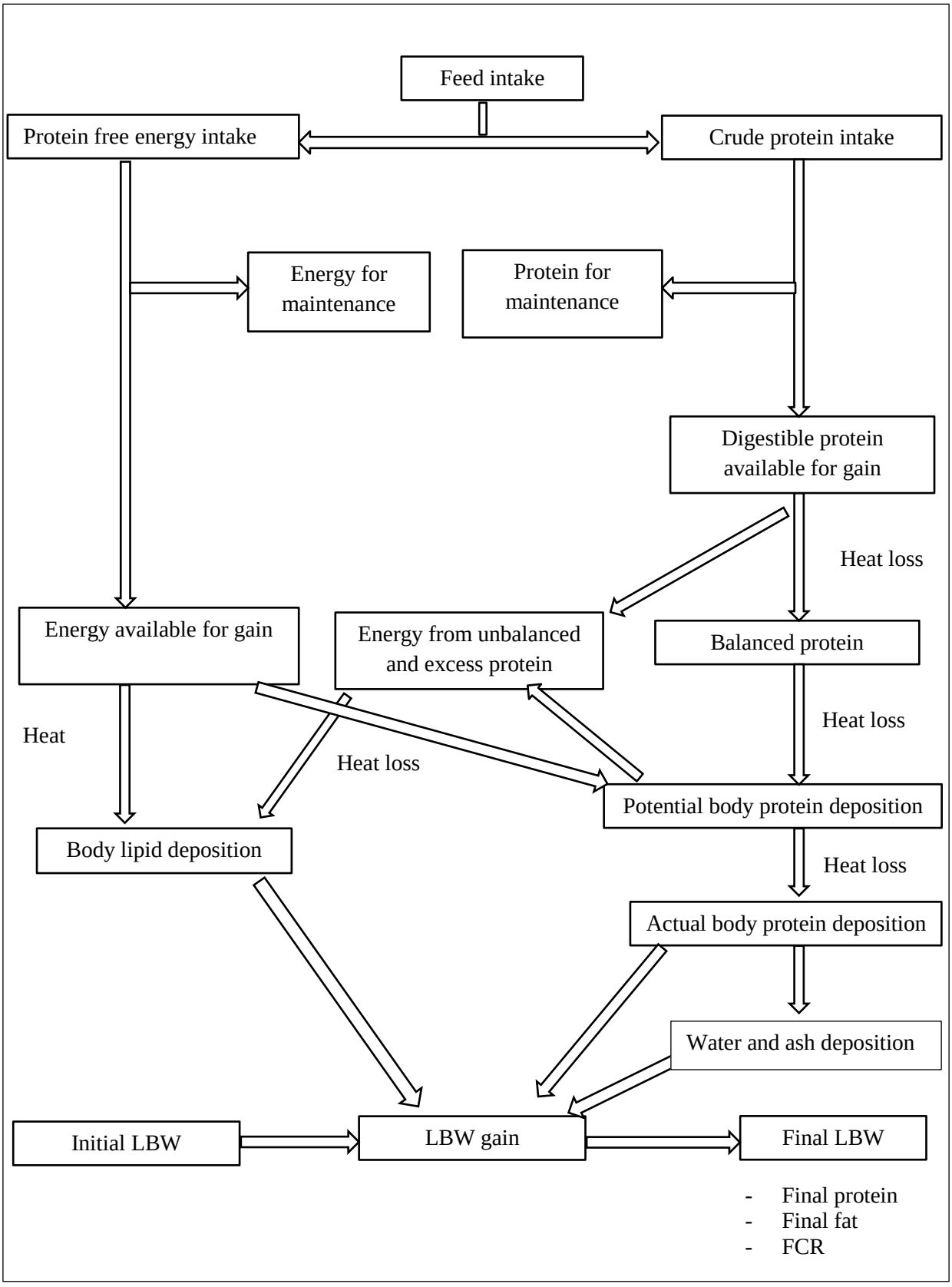

Figure 2.7. The pathway of protein and energy partitioning in broiler chickens (adapted from de-Lange, 1995)

2.3.4. Conclusion

The differences in nutrient requirements due to genetics, sex, environment and production objectives can be predicted through a mechanistic growth model. Therefore, a mechanistic growth model can be used to give a description of nutrient flows (energy and amino acids). In addition, a growth model can be used to predict the carcass composition, broiler growth performance and optimise their growth rate and feed efficiency.

2.4. Limitations of the previous research

- This review of the literature has presented the energy partitioning model for broiler chicken growth. Efficiency of metabolisable energy utilisation considers the efficiency at which dietary energy is used for protein (*kp*) deposition and for fat deposition (*kf*). The efficiency of metabolisable energy utilisation can be significantly impacted by diet composition and the purposes for which the energy is used. There is limited evidence of the efficiency at which metabolisable nutrients (carbohydrate, fat and protein) in different diet formulations are utilised by broilers. Further studies are warranted to estimate the efficiency of metabolisable energy utilisation from different energy sources.

- Updates of the maximum protein deposition and minimum fat to protein deposition ratio are needed to estimate the energy and amino acids requirements for maximum growth rate in modern broiler chickens. The maximum protein deposition and minimum fat to protein deposition ratio will provide rules for energy partitioning in the broiler growth model. Further studies are required to establish the maximum protein deposition and minimum fat to protein deposition ratio in different broiler strains.

- Incorporating the energy and amino acids partitioning concepts into a mechanistic broiler growth model will describe the nutrient utilisation, growth performance and carcass composition. Once developed and validated, the model will be able to predict nutrient requirements for maximising production and provide a mechanism to increase the accuracy of feeding formulation for broiler chickens.

CHAPTER 3

Efficiency of energy utilisation for fat deposition from different energy sources in broiler chickens

3.1. Abstract

An experiment was conducted to investigate the effect of additional energy intake from dietary fat (soybean oil), protein (casein) or carbohydrate (starch) on energetic efficiency of fat deposition, energy partitioning, carcass composition and growth performance of Ross 308 broilers between 15 and 36 days of age. Birds were allocated to a basal diet, or the basal diet supplemented with extra soybean oil (B+oil), casein (B+casein) or maize starch (B+starch) to provide 14% more daily energy intake than the basal diet. The birds were weighed weekly and feed intake was recorded daily. At the end of trial (36 days of age), chickens were weighed, slaughtered, de-feathered and weighed again to obtain the carcass weight. Protein, fat, GE and ash content of the carcass were compared to those of chickens slaughtered at the start of the trial. Additional energy intake increased ($P < 0.05$) the average daily gain and decreased ($P < 0.05$) feed intake per unit of gain. Birds fed B+oil or B+starch diets had a greater fat deposition than those fed the B+casein diet ($P < 0.05$). Birds fed B+casein had a greater protein deposition than those fed other diets ($P < 0.05$). The energetic efficiencies of fat deposition were 0.82 and 0.69 for soybean oil and starch, respectively. It is difficult to estimate the efficiency of energy utilisation for fat deposition from additional dietary casein due to the birds depositing protein rather than fat.

3.2. Introduction

Genetic selection of broiler chickens has caused changes in the rates of fat and protein retention in the broiler carcass (Sakomura *et al*., 2005). These changes are likely to have altered the partitioning of energy and protein intake and therefore the energy requirements should be established to express the target of protein and fat deposition. The deposition of body protein and fat in broilers is impacted by nutrition, the dietary energy-protein ratio, (Deschepper and De Groote, 1995; Leeson *et al*., 1996; Wiseman and Lewis, 1998), sex (Leeson and Summers, 1980), genotype (Edward and Denman, 1975; Havenstein *et al*., 1994a, b), degree of maturity (Carre *et al*., 1995), and environmental conditions (Sakomura *et al*., 2003; 2005). Successful prediction of nutrient requirements requires models that accurately describe the partitioning of

metabolisable energy (Sakomura *et al*., 2005). Boekholt *et al*. (1994) reported different ME efficiencies associated with the use of energy for protein (*kp* = 0.66) and for fat (*kf* = 0.86) deposition. However, efficiency of ME utilisation for protein deposition have ranged between 0.36 and 0.70 (Sakomura *et al*., 2005; De Groote, 1974) and the efficiency of energy utilisation for fat deposition between 0.55 and 0.92 (Sakomura *et al*., 2005; Scheimann *et al*., 1972; Emmans, 1994). The efficiencies of metabolisable energy can be impacted by diet composition and the purpose for which the energy is used (Lopez and Leeson 2008 a, b; De Groote, 1974). For example, ME is utilised more efficiently for body fat deposition than for body protein deposition. Therefore, information about how the nutrients (carbohydrate, fat and protein) are utilised with different relative efficiencies are important for the estimation the energy requirements and to understand the energy metabolism in broiler chickens.

The objective of this study was to investigate the effect of additional energy intake from dietary fat (soybean oil), protein (casein) or carbohydrate (starch) on ME efficiency utilisation for fat deposition, energy partitioning and growth performance of Ross 308 broilers between 15 and 36 days of age, under conditions that limited the protein deposition. The aim was to achieve a similar protein deposition rate but different fat deposition rate in the treatment groups. It was hypothesised that the efficiency of ME utilisation for fat deposition will be different among the energy sources.

3.3. Materials and Methods

All experimental procedures were in accordance with the New Zealand Revised Code of Ethical Conduct for the use of Live Animals for Research, Test and Teaching and approved by the Massey University Animal Ethics Committee (Anonymous, 2008, Ethics Code number of MUAEC 08/11).

3.3.1. Diets

Four treatment diets were prepared. The basal diet, based on maize and soybean meal was formulated to provide 200 g crude protein and 12.1 MJ ME per kg of diet (Table 3.1). For the other three diets, the basal diet was supplemented with soybean oil (B+oil), casein (B+casein) or starch (B+starch) to provide an extra 14% ME. The basal, B+oil, or B+starch diets were supplemented with crystalline amino acids so that the content of the first three limiting amino acids (methionine, lysine and threonine) were similar in all diets.

Table 3.1 Composition of the experimental diets based on equal nutrient intake except metabolisable energy intake

Ingredient (g)	Basal (B)	B+oil	B+casein	B+starch
Maize	641	641	641	641
Soybean meal, 48%	300	300	300	300
Soybean oil	0.0	45.5	0.0	0.0
Maize starch	0.0	0.0	0.0	100
Casein	0.0	0.0	97	0.0
Limestone	19	19	20	19
Dicalcium phosphate	15.2	15.2	11	15.2
Salt	3.0	3.0	3.0	3.0
DL-Methionine	2.6	2.6	0	2.6
Lysine. HCl	10	10	0	10
L-threonine	4.2	4.2	0	4.2
Trace mineral-vitamin premix [1]	2.0	2.1	2.2	2.2
Titanium dioxide	3.0	3.0	3.0	3.0
Total diet weight after mixing (g)[2]	1000	1045.6	1077.2	1100.2
Calculated analysis (g/kg as fed)				
Metabolisable energy (MJ/kg)	12.09	13.16	12.78	12.51
Crude protein	199.0	190.3	262.7	180.9
Lysine	18.35	17.55	16.99	16.68
Methionine	5.70	5.45	5.33	5.18
Methionine + Cysteine	9.06	8.66	8.75	8.23
Threonine	11.6	11.1	10.8	10.5
Calcium	11.6	11.1	10.8	10.5
Available phosphorus	4.84	4.63	4.49	4.40

[1]Supplied per kilogram of diet; Co, 03mg; Cu, 3.0 mg; Fe, 25mg; I, 1 mg; Mn, 125mg; Mo, 0.5 mg; Se, 200 μg; Zn,60 mg; antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; cholecalciferol, 60 μg; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4 mg; niacin,35 mg; pyridoxine,10 mg; trans-retinol, 3.33 mg; riboflavin, 12 mg; thiamine, 3.0 mg; dl-α-tocopheryl acetate, 60 mg; chlorine chloride, 638 mg.

[2]Intake of basal diet was identical across treatments. The total in this row reflects relative differences in the daily feed intake between the treatment groups to achieve an extra 14% energy intake in the B+oil, B+casein or B+starch treatment diets.

3.3.2. Birds and housing

One hundred and fifty, one day-old male broilers (Ross 308) were reared in floor pens and fed a commercial starter diet (230 g CP, 12.6 MJ ME per kg as fed) from day 1 to 14 days of age. On day 15, ninety birds were selected on the basis of weight uniformity. Eighty birds with a mean of weight 464 ± 27g were allocated to four experimental treatments (20 birds per group, in individual cages) and fed experimental diets between 15 and 36 days of age. Each bird was randomly assigned to a wire cage (60 × 60 cm) equipped with individual feeder and self-drinking system. All cages were in the same room as one layer and distributed in parallel rows with 6 cages each. Within each row, the treatments were assigned randomly. The 10 remaining birds (454 ± 47g) were

slaughtered at 15 days of age to measure initial carcass and feathers weights. Four birds out of the initial ten slaughtered were used to measure initial body composition. The experimental birds were housed in an environmentally controlled room with 20 h of fluorescent light per day. The temperature was 31 ºC on day 1, and this was gradually reduced to 22 ºC at 21 days of age. Live body weight was recorded weekly and feed intake and feed refusals were recorded daily for each bird.

3.3.3. Establishing feed allowance

To establish the daily feed allowance during the experiment, the *ad-libitum* feed intake records from the Poultry Research Unit (Massey University, Palmerston North, New Zealand) and the Ross broiler guide was consulted. The daily feed allowance (FA) for the basal group was 80% of the recorded *ad-libitum* feed intake. The daily feed allowances for B+oil, B+casein or B+starch diets were proportionately different to achieve an extra 14% energy intake from oil, casein or starch (Figure 3.1). The intake of the basal diet was constant cross all treatment groups. The daily feed allowances were:

FA_{Basal} = 80% of recorded *ad-libitum* intake,

$FA_{B+oil} = FA_{Basal} \times 1.0456$,

$FA_{B+casein} = FA_{Basal} \times 1.0772$,

$FA_{B+starch} = FA_{Basal} \times 1.1002$.

The feed allowance was provided in two mealtimes (8.00 am and 6.00 pm).

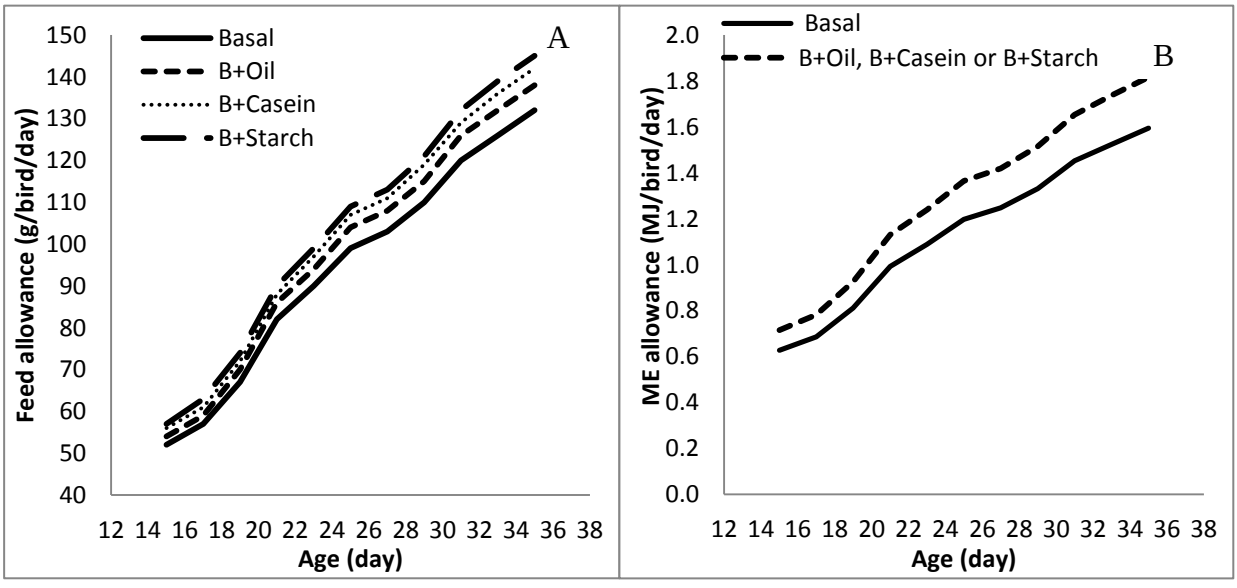

Figure3.1. The daily feed allowance (g as feed/bird/day) was adjusted (A) to achieve two levels of metabolisable energy intake (B); basal level and high level from B+oil, B+casein and B+starch diets for Ross broiler chickens for the treatment period of 15 to 36 days of age

3.3.4. Apparent metabolisable energy measurement

Apparent metabolisable energy (AME) was measured twice, from 21 to 24 and from 31 to 34 days of age. During each of four consecutive day periods, the feed intake was measured and total excreta was collected and weighed for each bird. Daily excreta collections were stored at −4 °C until mixed for subsamples. Excreta from the four collection days were pooled for each bird, mixed in a blender and subsampled. Each subsample was freeze-dried and ground, to pass through a 0.5 mm sieve and then stored at −4 °C in airtight plastic containers for further chemical analysis.

3.3.5. Measurement of ileal digestibility

Titanium dioxide was included as an indigestible marker in all the diets (3.0 g/kg). On day 35 and after 8 hours of ad-libitum feed, four birds per treatment were euthanised by intravenous injection of sodium pentobarbitone (1ml per 2 kg live body weight, Provet NZ Pty. Ltd., Auckland, New Zealand). Immediately after the birds were euthanised, ileal digesta was collected from the lower half of the ileum by gently flushing with distilled water into a plastic bag, as described by Ravindran *et al*. (2005). The start of the ileum was identified by the position of Merkel's diverticulum. These samples were freeze-dried and ground, to pass through a 0.5 mm sieve and then stored at −4 °C in airtight plastic containers until laboratory analysis.

3.3.6. Body composition

At the end of the trial (36 days of age), all remaining birds were fasted overnight, weighed and killed by cervical dislocation with minimum blood loss. Feathers were removed and the carcass weight was recorded (Note: In this thesis, the term 'carcass' refers to the whole body without feathers). The carcasses of eight birds from each group were randomly selected and stored at −20°C.

Each frozen carcass was cut into small pieces and minced twice in a meat grinder to obtain a homogenous sample. Subsamples of each minced carcass were analysed for dry matter, ash, protein, fat and energy. Samples of feathers were analysed for dry matter, protein, amino acids (AA) and energy content. The same mincing and sampling procedure was used to measure the initial body composition of birds that were slaughtered at the beginning of the experiment.

3.3.7. Laboratory analysis

Dry matter content of the diet, ileal digesta, excreta, carcass and feather samples was determined by drying a constant weight in a convection oven (AOAC 930.15, 2005). Ash content of diets, ileal digesta, excreta and carcass samples was determined by furnace at 550 °C (AOAC, 942.05, 2005).

Gross energy of the diet, ileal digesta, excreta, carcass and feathers samples was determined by adiabatic bomb calorimetry (Gallenkamp Autobomb, London, UK) standardised with benzoic acid.

Nitrogen content of the diet, ileal digesta and feathers samples was determined by total combustion (AOAC, 968.06, 2005) using a CNS-2000 auto analyser (LECO Corporation, St. Joseph, MI). For the carcass samples, the nitrogen content was determined using the Kjeldahl method (AOAC, 928.08, 2005). Amino acid content of the diets and feathers was determined by acid hydrolysis followed by HPLC separation (AOAC, 994.12).

Fat content of the diet and ileal digesta samples was determined by Soxhlet extraction (AOAC, 991.36, 2005). For the carcass samples, the fat content was determined by Mojonnier extraction (AOAC, 922.06, 2005).

Starch content of the diets and ileal digesta was measured using an assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland) based on thermostable alpha-amylase and amyloglucosidase (McCleary *et al*., 1997).

3.3.8. Calculations

The AME of the diets was calculated using the following formula:

AME (MJ/g of diet) = [(Feed intake × GE diet) - (Excreta output × GE excreta)]/ Feed intake.

Where: GE diet is the gross energy content per gram of the diet and GE excreta is the gross energy content per gram of the excreta. The daily AME intake (AMEI) was calculated as the AME (MJ/g of diet) multiplied by the feed eaten per day (g/day). The accumulated AMEI for all experiment period was calculated as AME (MJ/g) multiplied by the feed eaten per bird.

Ileal nutrient digestibility for all diets were calculated using the following formula:

$$\text{Ileal nutrient digestibility (\%)} = \frac{\left(\frac{\text{nutrient diet \%}}{\text{titanium diet \%}} - \frac{\text{nutrient ileal \%}}{\text{titanium ileal\%}}\right)}{\frac{\text{nutrient diet \%}}{\text{titanium diet \%}}}$$

The digestible AA% was calculated as AA% in the diet multiplied by crude protein ileal digestibility coefficient.

The ideal digestible balanced protein (IDBP) for each AA was calculated as:

The digestible AA% in the diet divided by the AA% requirements (Ross, 2007, NRC, 1994) multiplied by the digestible crude protein requirement.

Energy retention and maintenance requirement calculations

The initial body composition and energy content was estimated using the birds slaughtered at the start of the experiment. At the end of the experiment the body energy retention, fat and protein retention for each bird was estimated against the initial body composition. The energy in fat gained was assumed to be 39.0 kJ per gram and for protein gained as 23.7 kJ per gram (Leeson and Summers, 2001). Heat production was calculated as the difference between AMEI and energy retention.

Apparent metabolisable energy for maintenance (AMEm) per day was assumed to be 649 kJ per kg metabolic body weight (649 × $LBW^{0.6}$; Lopez and Leeson, 2005). The accumulative AMEm for the experiment period was calculated using integral equation as following:

$$\text{accumulative AMEm} = 649 \times \int_{t=0}^{t=21} (\text{LBWI} + t \times \text{ADG})^{0.6}$$

LBWI = initial live body weight.

t = day from 0 to 21,

ADG = average daily gain,

The integration by substitution was used to simplify this equation as following

$$\int (LBWI + t \times ADG)^{0.6}\, dt$$

Let $U = (\text{LBWI} + t \times \text{ADG})$

Then $du = ADG\, dt$

$\text{dt} = \frac{du}{ADG}$

Substitute U in for $(LBWI + t \times ADG)$ *and* $\frac{du}{ADG}$ *in for (dt)*

$$\int U^{0.6} \frac{du}{ADG}$$

Integrate the new integral

$$\int \frac{U^{1.6}}{ADG \times 1.6} \ du$$

Expression the original variable of U= (LBWI + t× ADG) given

$$\int \frac{(LBWI + t \times DG)^{1.6}}{ADG \times 1.6}$$

Then

$$\text{AMEm} = 649 \times \int_{t=0}^{t=21} \frac{(LBWI + t \times ADG)^{1.6}}{ADG \times 1.6}$$

$$= 649 \times \left[\frac{(\text{LBWI}+(21\times\text{ADG}))^{1.6}}{\text{ADG}\times 1.6} - \frac{(\text{LBWI}+(0\times\text{ADG}))^{1.6}}{\text{ADG}\times 1.6}\right] = 649\times\left[\frac{\text{LBWf}^{1.6} - \text{LBWI}^{1.6}}{\text{ADG} \times 1.6}\right]$$

Where, LBWf is the final live body weight.

3.3.9. Statistics analysis and determination of the efficiency of AME utilisation for fat deposition

Due to health problems that were not related to the treatment diets, two birds in B+starch group were excluded. The experiment data were analysed by one-way ANOVA (SAS, 2004) using individual bird as the experimental unit. Significance was given when $P < 0.05$, and significant differences between means were separated by the Least Significant Difference (LSD). The AME was analysed by using a linear model PROC GLM that included the fixed effects of age, diet and their interaction, a random effect of bird nested within diet, which was used as the error term to test for the diet effect. The effects of age and age × diet were tested against the means square error (SAS, 2004).

The following models were used to determine the efficiency of energy utilisation for fat and protein deposition.

Model 1: the overall efficiency of AME utilisation for fat and protein deposition

The efficiency of AME utilisation for fat and protein deposition were obtained from multiple linear regression of AMEI as a function of energy retention as protein (ERP) and energy retention as fat (ERF).

The data for AMEI and the energy retained as fat and protein of each bird was fitted into this model:

AMEI = intercept + (1/*kf*) ERF + (1/*kp*) ERP

Where:

kf is the efficiency of energy utilisation for fat deposition,

kp is the efficiency of energy utilisation for protein deposition.

Model 2: Predicting the efficiency of AME utilisation for fat deposition from extra oil (kf_o), casein (kf_p) or starch (kf_s) intake.

Under conditions where protein deposition is limited, the efficiency AME utilisation for fat deposition from different energy sources is obtained by linear regression of AMEI as a function of ERF. The efficiency of AME utilisation for fat deposition for each dietary source of energy was estimated relative to basal treatment group by:

AMEI = intercept + (1/*kf*) ERF

Model 3: Correcting AME intake for a protein free deposition (AMEIfpd)

To avoid the error that comes from protein deposition when predicting *kf*, the AME requirement for protein deposition was subtracted from the total AMEI.

Rearranging the equation given in model 1 as:

AMEI− (1/*kp*) ERP = intercept + (1/*kf*) ERF, then

AMEIfpd = intercept + (1/*kf*) ERF

Where:

AMEIfpd is the AMEI free protein deposition,

It was assumed that *kp* = 0.66 (Boekholt *et al*., 1994), the value of Boekholt *et al*. (1994) was used because agreed with commonly used data (Lopez and Leeson 2005; Lopez *et al*., 2007).

Data from the basal and B+oil treatments was fitted to above models to estimate the efficiency of AME utilisation for fat deposition from oil (kf_o). Data from basal and B+starch treatments was fitted to the models to estimate the efficiency of AME utilisation for fat deposition from starch (kf_s). Data from basal and B+casein treatments

was fitted to the models to estimate the efficiency of AME utilisation for fat deposition from casein *(kf_p).*

In order to test if the slopes (*kf*-values) from different sources of energy were statistically different from each other, a Student's t-test was used and a probability value less than 0.05 indicated that the two slopes were significantly different from each other.

3.4. Results

3.4.1. Diets

The AA analyses of the experimental diets (Table 3.2) were in close agreement with the calculated values. The concentrations of AA and protein in the diet decreased when the basal diet was supplemented with oil and starch. However, the dietary AA and crude protein concentration increased when the basal diet was supplemented with casein.

Table 3.2. Analysed amino acid and crude protein composition of experiment diets

	Basal (B)	B+oil	B+casein	B+starch
composition (g/kg as fed)				
Aspartic acid	20.2	19.5	25.7	18.0
Threonine	11.0	10.7	10.5	9.50
Serine	10.0	9.50	13.8	8.60
Glutamic acid	35.2	34.1	52.7	31.7
Glycine	8.50	8.50	10.3	8.00
Alanine	9.50	9.30	11.6	8.80
Valine	9.10	8.70	14.4	8.10
Methionine	5.20	6.00	5.00	5.20
Isoleucine	8.10	7.50	12.3	7.20
Leucine	16.9	15.4	23.6	14.9
Tyrosine	6.90	6.70	11.6	6.60
Phenylalanine	9.60	9.20	13.5	8.50
Histidine	4.90	4.30	6.40	4.00
Lysine	20.2	20.1	18.9	18.7
Arginine	13.7	12.7	16.3	12.7
Crude protein	201	197	264	183

3.4.2. Birds performance

Due to the feed allowance restrictions imposed, the feed intake was different between the groups with birds on the basal diet having the lowest followed by B+oil, B+casein and B+starch, respectively. Birds fed the B+casein diet had higher crude protein intake compared with those fed other diets. The birds fed the B+oil had higher crude protein intake than those fed B+starch or basal diet. The AME intakes for the B+oil, B+casein or B+starch groups were 13-15% higher than the basal group ($P < 0.05$; Table 3.3).

Broilers fed the B+casein diet grew faster (64 g/day; $P < 0.05$) than birds fed the other diets (54-59 g/day). The birds fed the B+casein diet had a lower feed per gain than birds on the other diets ($P < 0.05$).

Table 3.3. Influence of different sources of energy on broiler performance (15-36 days of age)

	Basal	B+oil	B+casein	B+starch	Pooled SE	P-value
n	20	20	20	18		
FI(g/bird/day)	94.1^{d}	98.8^{c}	100.8^{b}	102.7^{a}	0.33	0.0001
CPI (g/bird/day)	18.95^{c}	19.47^{b}	26.62^{a}	18.81^{c}	0.76	0.0001
AMEI (MJ/bird/day)	1.13^{c}	1.27^{b}	1.29^{a}	1.29^{a}	0.004	0.0001
Initial LBW (g)	470	465	459	461	6.30	0.630
Final LBW(g)	1604^{c}	1702^{b}	1805^{a}	1640^{c}	14.2	0.0001
Feed/gain(g/g)	1.748^{b}	1.678^{c}	1.575^{d}	1.833^{a}	0.018	0.0001
ADG (g/day)	53.9^{d}	58.9^{b}	64.0^{a}	56.1^{c}	0.64	0.0001

FI is average daily feed intake per bird calculated as the total feed intake per bird divided by 21 days, CPI is crude protein intake (g/bird/day) calculated as CP % in the diet multiplied by daily feed intake (g/bird/day). AMEI is apparent metabolisable energy intake per day, initial LBW is initial live body weight, final BW is final live body weight, ADG; average daily gain. ^{a,b,c,d} means in the same row with different superscripts are significantly different ($P< 0.05$).

Although, the first three limited AA were balanced by supplementing crystalline methionine, lysine and threonine into the basal, B+oil, or B+starch diet (Table 3.1) the birds fed B+casein diet still had a higher ideal digestible balanced protein intake (g/d, Table 3.4).

Table 3.4. Digestible and ideal balanced protein % of diet for broilers Ross chickens fed experimental diets from 15 to 36 day of age

	Digestible AA%[1]					Ideal digestible balanced protein% (IDBP)[2]				
AA	Basal	B+oil	B+casein	B+starch	NRC	Basal	B+oil	B+casein	B+starch	NRC
Aspartic acid	1.72	1.69	2.3	1.52	-	-	-	-	-	-
Threonine	0.93	0.92	0.94	0.80	0.74	25.2	24.9	25.3	21.7	20
Serine	0.85	0.82	1.24	0.73	-	-	-	-	-	-
Glutamic acid	2.99	2.96	4.72	2.68	-	-	-	-	-	-
Glycine +Serine	1.57	1.56	2.00	1.45	1.14	27.6	27.4	35.1	25.4	20
Alanine	0.8	0.8	1.04	0.74	-	-	-	-	-	-
Valine	0.78	0.75	1.29	0.68	0.82	18.9	18.4	31.5	16.7	20
Methionine	0.44	0.52	0.44	0.44	0.38	23.1	27.2	23.3	23.2	20
Isoleucine	0.69	0.65	1.10	0.61	0.73	18.9	17.9	30.1	16.6	20
Leucine	1.44	1.34	2.11	1.26	1.09	26.3	24.5	38.7	23.1	20
Phenylalanine	0.82	0.83	0.86	0.81	0.65	25.2	25.6	26.5	25	20
Phen+tyrosine	1.4	1.43	1.48	1.39	1.22	22.9	23.4	24.2	22.8	20
Histidine	0.42	0.37	0.57	0.34	0.32	26.3	23.1	35.6	21.4	20
Lysine	1.72	1.74	1.69	1.58	1.00	34.4	34.8	33.8	31.6	20
Arginine	1.17	1.1	1.46	1.07	1.10	21.2	20.0	26.5	19.5	20
Crude protein	17.1	17.1	23.6	15.5	20.0	17.1	17.1	23.6	15.5	20
Feed intake (g/day)						94.0	99.0	101	103	-
IBP intake (g/day)[3]						16.1	16.8	23.8	15.9	-

[1]Digestible AA% calculated as AA% in the diet multiplied by crude protein digestibility coefficient in the same diet. [2]Ideal digestible balanced protein (IDBP) for each AA calculated as [(the ratio of digestible AA in the diet to the digestible AA requirement) × recommended digestible crude protein]. [3]IDBP intake g/d calculated as the smallest amount of ideal digestible balanced protein % multiplied by the average feed intake (g/day).

3.4.3. Apparent metabolisable energy and ileal digestibility

Apparent metabolisable energy content differed between diets ($P < 0.05$). The AME in the basal diet was lower than that in the other three diets ($P < 0.05$). An age effect was observed ($P < 0.05$; Table 3.5) and the AME decreased as the experiment proceeded and the birds got older.

Table 3.5. Apparent metabolisable energy (MJ/kg) as fed of the experimental diets, determined between 21-24 and 31-34days of age

Diet	n	age	AME (MJ/kg)
Basal	4	21-24 days	12.03[b]
	4	31-34 days	11.88[b]
B+oil	4	21-24 days	13.02[a]
	4	31-34 days	12.66[a]
B+casein	4	21-24 days	12.91[a]
	4	31-34 days	12.64[a]
B+starch	4	21-24 days	12.69[a]
	4	31-34 days	12.47[a]
SEM			0.155
Main effects			
Diet			
Basal	8		11.95[b]
B+oil	8		12.84[a]
B+casein	8		12.77[a]
B+starch	8		12.58[a]
SEM			0.075
Age			
21-24 days	16		12.66[a]
31-34 days	16		12.41[b]
SEM			0.077
P-value	Diet		0.0001
	Age		0.040
	Diet × Age		0.910

[a,b] means in the same column with different superscripts are significantly different ($P < 0.05$).

Ileal digestibility of protein, starch and energy were similar between the four diets ($P > 0.05$). The B+oil diet group had a higher coefficient of digestibility for fat than the B+starch diet group ($P < 0.05$) but similar with B+casein and Basal diets. The basal diet group had a lower coefficient of digestibility for ash than the other diet groups ($P < 0.05$; Table 3.6).

As expected for this trial design the birds fed B+oil diet had higher digestible fat intake than those fed other diets ($P < 0.05$), and birds fed B+casein had higher digestible protein intake than those fed other diets ($P < 0.05$). Also, the birds fed B+starch diet had

higher digestible starch intake than those fed other diet (Table 3.6). The birds fed the B+oil, B+casein and B+starch had higher ileal digestible energy intake than those fed basal diet ($P < 0.05$).

Table 3.6. Influence of diet type on the apparent ileal digestibility of nutrients and energy

	Basal	B+oil	B+casein	B+starch	SEM	P-value
Ileal digestibility coefficient						
n	4	4	4	4		
Fat	0.71^{ab}	0.90^{a}	0.71^{ab}	0.58^{b}	0.06	0.02
Protein	0.85	0.87	0.89	0.85	0.019	0.31
Starch	0.96	0.97	0.96	0.96	0.007	0.51
Energy	0.71	0.79	0.76	0.71	0.024	0.103
Ash	0.40^{b}	0.54^{a}	0.49^{a}	0.47^{ab}	0.03	0.041
Ileal digestible nutrient intake(g/b/d)[1]						
n	8	8	8	8		
Feed intake	94.6^{d}	99.1^{c}	101.7^{b}	103.4^{a}	0.163	0.0001
Digestible fat intake	1.75^{b}	4.60^{a}	1.66^{c}	1.26^{d}	0.0028	0.0001
Digestible protein intake	16.2^{c}	17.0^{b}	23.9^{a}	16.1^{c}	0.028	0.0001
Digestible starch intake	36.3^{d}	37.9^{b}	37.0^{c}	44.0^{a}	0.067	0.0001
Digestible energy intake	1.07^{d}	1.30^{a}	1.27^{b}	1.17^{c}	0.002	0.0001

[a,b,c,d] Means in the same row with different superscripts are significantly different ($P < 0.05$). [1]The data presented are for the birds selected for carcass composition (8 birds per group). The digestible nutrients intake (g/bird/day) was calculated as nutrient intake per day multiplied by the nutrient digestibility coefficient.

3.4.4. Carcass composition

The birds fed the B+casein diet had higher percentage of carcass protein ($P < 0.05$; Table 3.7) than those fed B+oil or B+starch diets. The birds fed B+starch or B+oil diets had higher percentage of fat in the carcass than those fed basal or B+casein diets ($P < 0.05$). The effect of energy source on water and ash content were closely related to protein content. Birds fed the basal or B+casein diets had a similar gross energy concentration in the carcass (6.4 kJ/g) but lower than those fed B+oil and B+starch (6.9 and 7.1 kJ/g) respectively. The birds fed B+casein diet had greater feather weight compared to other diet groups ($P < 0.05$). The average GE of the dry feathers was 20.4 kJ/g (Table 3.7).

Table 3.7. Carcass composition of broilers fed diets with extra oil, casein or starch from 15-36 days of age[1]

	Body composition at 15 days Starter diet(1-14days)	Body composition at 36 day Treatment diets (15-36days)					
		Basal	B+oil	B+casein	B+starch	SEM	P-value
n	10	8	8	8	8		
Live body weight (g)	453	1589^{c}	1714^{b}	1819^{a}	1669^{b}	15.8	0.0001
Carcass(g)	436	1512^{c}	1630^{b}	1732^{a}	1588^{b}	16.8	0.0001
Protein (%)	16.8	18.0^{a}	17.3^{b}	18.2^{a}	17.4^{b}	0.19	0.010
Fat (%)	6.60	5.73^{b}	7.08^{a}	5.73^{b}	7.69^{a}	0.31	0.0001
Water (%)	73.9	73.7^{a}	72.7^{bc}	73.4^{ab}	72.2^{c}	0.28	0.001
Ash (%)	2.11	2.29^{bc}	2.11^{c}	2.55^{a}	2.35^{b}	0.06	0.001
Gross energy (kJ/g)	6.57	6.44^{b}	6.86^{a}	6.44^{b}	7.11^{a}	0.13	0.001
Dry feather weight (g)	4.46	28.8^{d}	31.3^{c}	39.8^{a}	34.0^{b}	0.31	0.0001
Feather protein (%)	83.6	90.7	90.9	92.2	92.1		
Gross energy (kJ/g)	20.3	20.2	20.7	20.1	20.4		

[1] Carcass is the whole body with no feather.

[a,,b,c,d] Means in the same row with different superscripts are significantly different ($P < 0.05$).

The crude protein and AA content of feathers is quite consistent across treatment groups (Table 3.8). The crude protein in the dry feather at 15 days of age was 83.5%, whereas the crude protein in the dry feather at 36 days of age was on average 91.5%. Feathers are low in methionine and histidine (less than 1%) and high in glutamic acid (8.7-10.2%), serine (7.1-9.1%) glycine (6.89-7.5%) and arginine (6.3-7.1%).

Table 3.8. Amino acids, crude protein and gross energy composition of dry feather from Ross broilers at 15 and 36 days of age

	15 day of age	36 day of age			
		Basal	B+oil	B+casein	B+starch
Amino acids (g/100g)					
Aspartic acids	5.94	6.34	6.41	7.01	6.37
Threonine	3.71	4.29	4.03	4.00	4.35
Serine	7.07	8.87	7.85	7.41	9.08
Glutamic acid	8.77	9.64	9.95	10.20	9.60
Glycine	6.89	7.35	6.89	6.94	7.49
Alanine	3.65	4.04	4.06	4.06	4.14
Valine	5.41	6.80	5.94	6.54	6.78
Methionine	0.83	0.66	0.85	0.92	0.61
Isoleucine	3.67	4.52	3.95	4.30	4.38
Leucine	6.30	7.09	6.92	7.11	7.18
Tyrosine	3.12	2.94	3.31	3.22	2.87
Phenylalanine	3.82	4.33	4.17	4.15	4.49
Histidine	0.98	0.80	0.93	1.14	0.63
Lysine	3.00	2.36	3.12	3.26	2.17
Arginine	6.29	7.13	6.73	6.66	6.93
Crude Protein (%)	83.5	90.7	90.9	92.1	92.1
Gross energy (kJ/g)	20.3	20.2	20.7	20.1	20.4

3.4.5. Deposition rate of chemical components

The birds fed B+casein diet had a greater rate of protein deposition (12.6 g/day) than the other diets groups ($P < 0.05$). Birds fed the B+oil and B+starch diet had a greater fat deposition per day, 4.1 and 4.4 g/day, respectively than the 3.3 g/day for the birds fed B+casein ($P < 0.05$, Table 3.9). The extra energy provided from oil or starch affected fat deposition, but extra energy provided from casein in the B+casein diet was utilised to deposit protein.

Table 3.9. Influence of different types of diet on protein, fat, water, ash and feather gain (g/day/bird) in Ross broiler carcass between 15 and 36 days of age

	Basal	B+oil	B+casein	B+starch	SEM	P-value
n	8	8	8	8		
Protein (g/d)	9.3^{c}	9.8^{b}	11.4^{a}	9.5^{cb}	0.15	0.0001
Fat (g/d)	2.70^{b}	4.05^{a}	3.29^{b}	4.37^{a}	0.24	0.0001
Water (g/d)	37.1^{c}	40.4^{b}	44.7^{a}	38.5^{c}	0.63	0.0001
Ash (g/d)	1.18^{b}	1.19^{b}	1.67^{a}	1.30^{b}	0.05	0.0001
Feather(g/d/bird)	1.29^{d}	1.41^{b}	1.52^{a}	1.37^{c}	0.013	0.0001

^{a,b,c,d} means in the same row with different superscripts are significantly different ($P < 0.05$).

3.4.6. Energy retention and body composition

The total heat production for broilers fed B+oil, B+casein or B+starch diet was higher than those fed basal diets ($P < 0.05$). The energy retention was similar between the three high energy intake groups (B+oil, B+starch or B+casein), and this was higher than the basal group ($P < 0.05$). The birds fed B+oil and B+starch diets had greater energy retention as fat than those fed basal or B+casein diets (Table 3.10). The heat production to AME intake ratio was lower for the broilers fed B+oil, B+casein and B+starch diet (0.67) compared with those fed basal diet (0.69). The efficiency of AME utilisation for weight gain (k_g) was calculated to be 0.73, 0.70, 0.70 and 0.67 for basal, B+oil, B+casein and B+starch diets, respectively.

Table 3.10. Utilisation of apparent metabolisable energy intake for growing birds fed diets with extra oil, casein or starch from15 to 36 days of age

Diet	Basal		B+oil		B+casein		B+starch		SEM[1]	P-value[1]
N	8	%	8	%	8	%	8	%		
AMEI (MJ/bird)	23.8^{c}	100.0	26.7 ^{b}	100.0	27.3^{a}	100.0	27.3^{c}	100.0	0.04	0.0001
Heat production	16.4^{b}	68.9	17.9^{a}	67.1	18.3^{a}	67.0	18.5^{a}	67.5	0.21	0.0001
Heat increment	2.7^{b}	11.2	3.7^{a}	14.0	3.8^{a}	13.8	4.4^{a}	16.3	0.25	0.0003
AMEm	13.7^{c}	57.7	14.2^{b}	53.1	14.5^{a}	53.3	14.0^{b}	51.3	0.09	0.0001
ER	7.4^{b}	31.1	8.8^{a}	32.9	9.0^{a}	33.0	8.9^{a}	32.5	0.20	0.0001
ERP	4.6^{c}	19.4	4.9^{b}	18.2	5.7^{a}	20.7	4.7^{bc}	17.2	0.08	0.0001
ERF	2.2^{b}	9.30	3.3^{a}	12.4	2.7^{b}	9.90	3.6^{a}	13.1	0.20	0.0001
FE	0.55^{c}	2.33	0.60^{b}	2.26	0.65^{a}	2.38	0.59^{b}	2.14	0.01	0.0001
k_g	0.73		0.70		0.71		0.67		0.02	0.117

[a,b,c] Means in the same row with different superscripts are significantly different ($P < 0.05$). AMEI is the apparent metabolisable energy intake, HP is the heat production, AMEm is the accumulative apparent metabolisable energy for maintenance requirement calculated as described in section 3.3.8. HI is the heat increment, ER is the total energy retention, ERP is the energy retention as protein, ERF is the energy retention as fat, FE is the energy retention as feather and k_g is the efficiency of energy utilisation for weight gain was calculated as ER/(AMEI-AMEm). [1] SEM and P-values is related to the absolute energy partitioning values

3.4.7. Efficiency of AME utilisation for fat and protein deposition

Birds fed extra casein had a higher protein deposition response. Therefore, it was difficult to obtain the efficiency of AME utilisation for fat deposition from extra casein intake.

Model 1: the overall efficiency of AME utilisation for fat (*kf*) and protein (*kp*) deposition

The efficiency of AME utilisation for fat (*kf*) and protein (*kp*) deposition was 0.82 and 0.58, respectively. This result was based on the multiple linear regressions using the

data from all treatments (Table 3.11, Model 1). However, the efficiencies of AME utilisation for fat deposition from additional oil or starch intake were 0.93 and 0.69, respectively. Also using the same model, the efficiencies of AME utilisation for protein deposition were 0.35 and 0.60 from oil and starch respectively (Table 3.11, Model 1). The intercept represent the AMEm, which ranged between 6.9-13.1 MJ/bird.

Table 3.11. Regression equations of AMEI as function of energy retention as fat (ERF) and as protein (ERP)

Diet	Model	Regression equations	*kf*	*kp*	R^2
All diets[1]					
	1	AMEI = 13.08+ 1.72ERP+1.22ERF (2.1) (0.35) (0.23)	0.82	0.58	0.62
	2	AMEI= 23.1+1.07ERF (0.93) (0.30)	0.93	-	0.29
	3	AMEIfpd= 14.33 +1.20ERF (0.68) (0.23)	0.83	-	0.49
Basal, B+oil					
	1	AMEI = 6.88+ 2.89 ERP+1.08ERF (5.04) (0.99) (0.30)	0.93	0.35	0.71
	2	AMEI = 21.43 + 1.37ERF (1.02) (0.36)	0.73	-	0.52
	3	AMEIfpd= 13.83+1.22ERF (0.85) (0.30)	0.82	-	0.55
Basal, B+starch					
	1	AMEI =12.58+1.68ERP+1.44ERF (7.08) (1.35) (0.35)	0.69	0.60	0.59
	2	AMEI = 21.3+1.46ERF (1.09) (0.36)	0.68	-	0.55
	3	AMEIfpd= 13.46+1.44ERF (1.03) (0.34)	0.69	-	0.57

[1]All diets from Basal, B+oil, B+ starch and B+ casein was included in the model.
AMEI is the apparent metabolisable energy intake. ERP is the energy retention as protein. ERP is the energy retention as fat. *kf* is the energy efficiency for fat deposition. *kp* is the energy efficiency for protein deposition. Numbers in the parentheses are the standard errors of above coefficient.
Model 1 multiple linear regression cross all treatment groups, AMEI= intercept + (1/*kf*) ERF + (1/*kp*) ERP.
Model 2 linear regression AMEI= intercept + (1/*kf*) ERF.
Model 3 linear regression AMEIfpd= intercept + (1/*kf*) ERF. AMEIfpd is the AME after ignoring the energy requirement for protein retention (see section 3.3.9).

Model 2: Predicting the efficiency of AME utilisation for fat deposition

When assuming that the extra AMEI from different energy sources is not used for protein deposition, the overall efficiency of AME utilisation for fat deposition from extra energy was 0.93. The AME utilisation efficiency of fat deposition from oil was 0.73 and higher than from starch 0.68 (Model 2, Table 3.11). The intercept constitute

the energy requirement for maintenance and for protein deposition with values range between 21.3 and 23.1 (MJ/bird).

Model 3: using AMEIfpd to estimate the efficiencies of AME utilisation for fat deposition from extra oil or extra starch intake

The protein deposition was 9.3, 9.8 and 9.5 g/day for basal, B+oil and B+starch, respectively. Further analyses were undertaken to avoid the response of protein deposition from extra energy, the AME intake was corrected to zero protein deposition (*kp* was assumed to be 0.66, as reported by Boekholt *et al*., (1994).

The overall efficiency of AME utilisation for fat deposition from extra energy was 0.83. However, the efficiency AME utilisation for fat deposition from oil (kf_o) was 0.82 and higher than from starch (kf_s) 0.69 (Table 3.11, Model 3). There were no statistically significant differences between kf_s and kf_o.

3.5. Discussion

The energy sources had an effect on body composition and energy partitioning in broiler chickens. In this study, additional energy from oil or starch increased fat deposition, but additional casein increased protein deposition.

3.5.1. Description the rate of gain body components

Chickens fed the B+casein diet had higher protein, water and ash deposition than the other groups, but less fat deposition. Water and ash deposition rates were closely related to protein deposition rate. The water:protein ratio in this study ranged from 4.0 to 4.2 (feather protein not included) at 36 days of age. Lopez *et al*. (2007) reported the ratio was 3.7 (feather protein included) at 42 days of age. The differences may be due to only carcass protein content being used in our calculation of water:protein ratio.

The dietary source of energy had an effect on the fat deposition, as the birds fed diet with extra AME from oil or starch had a greater fat deposition than those fed the diet with extra AME from casein. Under protein limiting conditions, extra AME consumed from oil or starch was utilised to form fat in the broiler chickens. In our study, from 15 to 36 days the fat deposition rates ranged between 2.7 to 4.4 g/bird/day, these results are lower than those of Lopez *et al*. (2007) who reported that in commercial broiler chickens fed *ad-libitum*, the fat deposition rate was 6.4 g/bird/day from 15 to 37 days. Tavernari *et al*. (2009) reported fat deposition rate of 9.3 g/bird/day

between 22 and 42 day of age. The reason for the differences between our experiment and previous studies is that the feed intake was restricted in our experiment.

3.5.2. Partitioning of metabolisable energy

The birds fed B+oil, B+casein and B+starch diets had similar energy retention, but the partitioning of energy retention between fat and protein deposition was different for these treatments. The source of AME did not affect the total heat production but did affect the carcass composition and the cost of protein and fat deposition separately. The heat production to AME intake ratio was higher for the broilers fed the basal diet compared with those fed other diets. Latshaw and Moritz (2009) indicated that the proportion of heat production decreases with increasing daily energy intake, and this improved daily body weight gain. Therefore, to improve the feed efficiency it is very important to approach the maximum feed intake to achieve high AME utilisation in the broiler industry.

3.5.3. Efficiency of AME utilisation for fat and protein deposition (Model 1)

The efficiencies of AME utilisation for fat *kf* and protein *kp* above the maintenance requirements were 0.82 and 0.58, respectively. Based on the values of the *kf* and *kp* and the GE content of body fat (39 kJ/g) and protein (23.7 kJ/g), the AME required to deposit one gram of fat and protein were 47.6 and 40.9 kJ, respectively. These values are similar to those of Chuan, *et al.* (2010) who estimated values of 46.4 and 43.1 kJ/g for fat and protein, respectively. Also the *kf* estimated in our study is in agreement to previous study of Boekholt *et al*. (1994) and Scheinmann *et al*. (1972) where reported values of the efficiency of AME utilisation for fat deposition of 0.86 and 0.84 respectively. In the present study the efficiency of AME utilisation for protein deposition was mid-range of values reported by previous studies of 0.36 to 0.66 (e.g., Boekholt *et al*., 1994; Nieto *et al.,* 1995; Sakomura *et al*., 2005).

3.5.4. Efficiencies of AME utilisation for fat deposition from extra oil or extra starch intake (Model 2 and 3)

Multiple linear regression has been used to estimate the efficiency of ME utilisation (*k*-value) in broiler chickens (Boekholt *et al.,* 1994; Lopez and Leeson 2005; Sakomura *et al*., 2005; 2004), they assumed that the ME intake partitioned into maintenance and protein and fat deposition with different efficiency. In practice, it is difficult to prevent

the correlation between fat and protein deposition during the growth, which introduces a wide range of *kf* and *kp* values (Leland, 2005). Hall (2010) reported the high degree of correlation between fat and protein deposition makes it difficult to separate the contribution from fat or protein on *k*-values. Some *kf* values have been found to be greater than 1, which makes it difficult to explain the results physiologically (Hall, 2010; Macleod, 1990; Sakomura *et al*. 2003), and these results limits the reliability of the multiple linear regression technique for estimating the *k*-value. In addition, the *kp* and *kf* values were estimated from the total chemical composition of the diet (carbohydrate, fat and protein). Therefore, in our case, it has been hypothesised, under limited protein deposition conditions the efficiencies of AME utilisation for fat deposition from extra oil, casein or starch would be obtained by linear regression of AMEI as a function of ERF. In case of casein, the birds deposited protein rather than fat therefore it was difficult to estimate the efficiency of AME utilisation for fat deposition from additional dietary casein. The efficiencies of AME utilisation for fat deposition from oil and starch were 0.73 and 0.68 respectively (Table 3.11; Model 2). The efficiency of AME utilisation for fat deposition from each source of energy was estimated relative to basal treatment group. The protein deposition was 9.3, 9.8 and 9.5 (g/day) for basal, B+oil and B+starch, respectively. Consequently, further analysis was undertaken to adjust for AMEIfpd (Table 3.11; Model 3). When the AMEI was corrected to zero protein deposition, (*kp* was assumed to be 0.66, Boekholt *et al*., 1994) the kf_o and kf_s was 0.82 and 0.69, respectively (Table 3.11; Model 3).

Applications of model 2 and model 3 should give similar answers when the protein deposition remained constant. There were no differences in protein deposition for birds fed basal diet or B+starch (Table 3.9) then the kf_s obtained by using model 2 was similar to value of model 3. In contrast, the protein deposition was different for the birds fed basal versus B+oil diet, and the kf_o was estimated to be 0.73 in model 2 and 0.82 in model 3. It appears that the energy requirement for protein deposition was not accounted for using model 2 (assuming that extra AME was used for fat deposition only), whereas the energy requirement for protein deposition was accounted for in model 3.

In the present study, the kf_s value was 0.69 and appears to be somewhat lower than that achieved by De Groote, (1974) and Carre *et al*, (2002) who reported values of 0.75, and 0.78 respectively. In previous studies, the efficiency of energy utilisation from starch was estimated from the total energy deposited as protein and fat. However, in our

case only the efficiency of AME for fat deposition was considered. Also, the levels of feed intake may have had an effect on the energetic efficiency of fat deposition from starch. Halas *et al.* (2010), in experiment with pigs, reported the marginal energetic efficiency of fat deposition from a starch source increased from 0.39 to 0.65 when the feeding level increased from 2.4 to 3.4 times the energy requirement of maintenance. In our study the AME intake was increased from 1.7 to 1.9 times the energy requirement for maintenance, and this may be partially resulted low efficiency of AME utilisation for fat deposition from starch intake.

In the current study, the kf_o value was 0.82, similarly Carre *et al.* (2002) reported that the efficiency of AME from fat for NE is 0.84 in poultry. The theoretical efficiency of energy utilisation for fat deposition from fat source is 0.97 (Quiniou, 1996; Van Milgen, 2002) and higher than the value obtained by Carre *et al.* (2002) and this current study. The digestible fat not only can be deposited as fat but also can be used for ATP synthesis (Van Milgen *et al*., 2001a) and this was not accounted for in this experiment. Further factors which could result in an underestimate of the *kf* value from oil may be the feed intake levels. The feed intake was limited, which may have affected the utilisation efficiency as Sakomura *et al*. (2005) reported that the energy retention efficiency decreased with increasing feed restriction.

3.5.5. Estimation of the maintenance requirement

In our study, the regression model was used and the intercept constitute AMEm requirement in Model 1 and 3. However, the intercept on model 2 presents the summation of protein deposition and maintenance energy requirement. Therefore, according to our results, from 15 to 36 day of age, the application of models 1 and 3 showed that 13.1and 14.3 MJ/ bird were used for the maintenance requirement (Table 3.11, Model 1 and 3), respectively. When the maintenance requirement was calculated using the value of Lopez and Leeson (2005), the result was 13.7 to 14.5 MJ/ bird (Table 3.10). Therefore, the value estimated by Lopez and Leeson (2005) is an applicable value and can be used for modelling the ME partitioning in modern broiler chickens. The estimation of AMEm can be applied for Ross 308 strain and under experimental condition. However, birds with different condition may have different AMEm.

3.5.6. An explanation of the response of protein deposition to extra casein intake

All diets except the B+casein diet were supplemented with crystalline lysine, methionine and threonine; in order to balance the first three limiting amino acids. Consequently, it was expected that the energy supplement from oil, casein or starch would affect only fat deposition, with similar protein deposition rates, but this was not the case in the B+casein diet group. B+casein diet group had a higher protein deposition rate (Table 3.9). Because all birds, including those fed the B+casein diet, consumed less protein and AA between 15 and 36 days than the recommended requirements of Ross 308 broiler (~575g protein/bird), the digestible protein and AA intake became limiting. The amount of digestible protein consumed by the birds was determined by feed intake and ileal protein digestibility coefficient, the digestible protein intakes were 16.1, 16.8, 15.9 and 23.8 g/day for basal, B+oil, B+starch and B+casein, respectively (Table 3.4). The B+casein diet contained 9% high quality protein in the form of casein, resulting in a diet with a higher digestible protein (23.6% from the diet). Also, the birds fed B+casein diet had better balanced protein compared with other groups and this improved the protein deposition rate. Figure 3.2 represents the ideal balanced protein intake compared with protein deposition rate.

Furthermore, the birds fed B+casein diet had better protein and nonessential amino acids intake compared to those fed other diets. This result confirms with that of Moran, (2011), who reported the deficiency of nonessential amino acids (glycine-serine and proline) with a low crude protein concentration in the diet could reduce the growth performance.

Several studies suggest that the energy-protein ratio affects carcass composition (Eits, 2004; Bartove and Plavnik, 1998; Macleod, 1997; Leeson *et al*., 1996a,b; Wiseman and Lewis, 1998). Leeson *et al*. (1996b) reported the body protein increased by 95 g/bird when the energy-protein ratio decreased from 15.7 to 12.7. This result is congruent with the results of our study, the energy-protein ratio was 14.2, 15.5, 11.6 and 16.4 for basal, B+oil, B+casein and B+starch, respectively, and the protein deposition rate was higher (11.4 g/day; Table 3.9) in B+casein diet compared with other dietary groups.

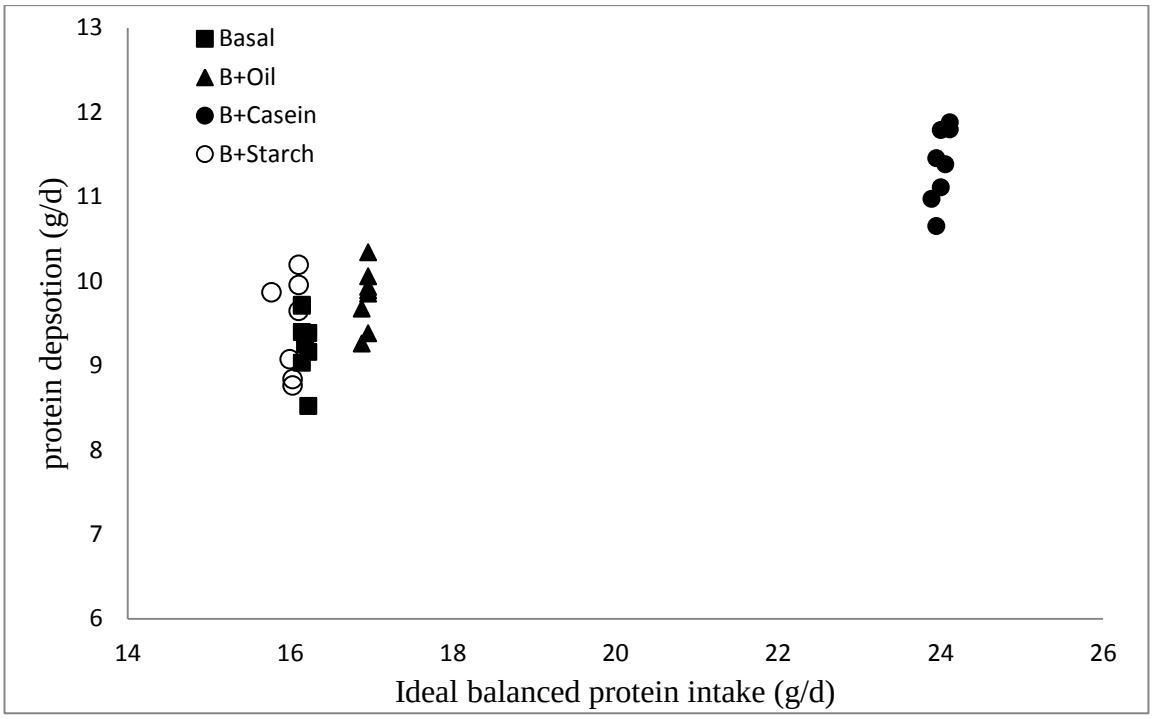

Figure 3.2. Effect of ideal balanced protein intake on the protein deposition rate with different dietary treatments. Ideal balanced protein calculation as described below Table 3.4

3.6. Conclusion

Under conditions of limiting protein deposition, the efficiencies of metabolisable energy utilisation for fat deposition from oil and starch were estimated to be 0.82 and 0.69 respectively. These values can be incorporated and use in a broiler growth model. Given the amino acids limitations in this study, it is difficult to obtain a definitive conclusion about the effect of additional dietary protein on the fat deposition. Therefore, efficiency of metabolisable energy for fat deposition from dietary protein was re-investigated using non-essential amino acids as protein source in Chapter 5. Although, the energetic efficiencies of fat deposition from different types of fat were investigated in Chapter 4.

CHAPTER 4

Efficiency of energy utilisation for fat deposition from soybean oil or tallow in broiler chickens

4.1. Abstract

This experiment was conducted to investigate the effect of additional energy intake from soybean oil (as source of unsaturated fatty acids) or tallow (as source of saturated fatty acids) on the energetic efficiency of fat deposition, energy partitioning, carcass composition and growth performance of Ross 308 broilers between 21and 42 days of age. Birds were allocated to one of five dietary treatments; basal diet or the basal diet supplemented with soybean oil or tallow to provide 12% or 24% extra metabolisable energy intake. The birds were weighed weekly and feed intake was recorded daily. At the end of the experiment, the chickens were slaughtered, defeathered and the carcass weighed. Protein, fat, gross energy and ash composition of the carcass was compared to that of chickens slaughtered at the start of the experiment. Additional metabolisable energy intake increased the carcass fat gain, abdominal fat gain and increased energy in the gain ($P < 0.05$). The energetic efficiencies of fat deposition were 0.93 and 0.90 for extra dietary soybean oil and tallow, respectively.

4.2. Introduction

Modern, high performing broilers require diets that are high in energy to allow them to express their genetic potential for growth. The energy concentration in maize and soybean meal is not enough to meet this high energy requirement in a balanced ration (Sadeghi and Tabiedian, 2005). Since the energy concentration of fat is three times higher than that of feed grains there is an advantage in using fat or oil supplements to increase the energy content of the diet.

Previous research has investigated the effects of including different types of fat in the diet on broiler growth (Alao and Balnave, 1984; Leeson and Atteh, 1995; Newman *et al*., 2002; Sanz *et al*., 1999; Sklan, 1979), diet digestibility (Carew *et al*., 1972; Freeman, 1984; Mossab *et al*., 2000), carcass characteristics (Laurin *et al*., 1984; Monfaredi *et al*., 2011; Sanz *et al*., 2000), and quantity of fat deposited in carcass and non-carcass tissues (Crespo and Esteve, 2001; 2002 a,b; Villaverde *et al*., 2005), but there is limited information on the energetic efficiencies of fat deposition from different dietary fat.

Excess energy ingested and absorbed above that required of maintenance and protein deposition will either be deposited as fat or dissipated as heat. Energy can also come from protein and carbohydrate and broiler growth responds to protein and carbohydrate intake. Assessment of the the energetic efficiency of fat deposition for broilers that have additional fat intake need to be considered relative to constant carbohydrate and protein intake. The objective of this study was to investigate the effect of additional metabolisable energy intake from soybean oil or tallow sources on energetic efficiency of fat deposition, metabolisable energy partitioning, carcass composition and growth performance in broiler chickens. The aim was to have a limited protein intake across all experimental groups to prevent a protein response and the metabolisable energy intake would only differ due to the additional fat intake. It was hypothesised that the efficiency of ME utilisation for fat deposition will be different between the fat types.

4.3. Materials and Methods

The procedure for this experiment was approved by Massey University Animal Ethics Committee as described in Chapter 3, Section 3.3. The Ethics Code number was MUAEC 11/04.

4.3.1. Diets

Five treatment diets were prepared. The basal diet, based on maize and soybean meal was formulated to provide 185 g crude protein and 12.7 MJ ME per kg of diet and designated as the low energy level. For the other diets the basal diet was supplemented with 12% extra ME from soybean oil (MO) or tallow (MT) and this designated as diets with a medium energy level, and the basal diet supplemented with 24% extra ME from soybean oil (HO) or tallow (HT) and this designated as diets with a high energy level. The aim was to have a similar daily intake of the basal diet across all treatments and the extra ME intake being a consequence of supplementary soybean oil or tallow intake (Table 4.1).

Table 4.1. Ingredient and composition of the experimental diets based on equal nutrient intake except metabolisable energy intake

	Diet				
Ingredient (g)	Basal	Medium oil	Medium tallow	High oil	High tallow
Maize	707	707	707	707	707
Soybean meal, 48%	260	260	260	260	260
Soybean oil	0.0	40	0.0	80	0.0
Tallow	0.0	0.0	46	0.0	92
Limestone	12	12	12	12	12
Dicalcium phosphate	12	12	12	12	12
Salt	3.0	3.0	3.0	3.0	3.0
Trace mineral-vitamin premix [1]	3.0	3.0	3.0	3.0	3.0
Titanium oxide	3.0	3.0	3.0	3.0	3.0
Total diet weight (g)[2]	1000	1040	1046	1080	1092
Calculated analysis (g/kg as fed)					
Metabolisable energy (MJ/kg)	12.7	13.7	13.6	14.6	14.4
Crude protein	185	177.9	176.9	171.3	169.4
Crude fat	28.3	65.3	70.6	99.5	109.3
Lysine	9.8	9.4	9.4	9.1	9.0
Methionine	3.3	3.2	3.2	3.1	3.0
Methionine + Cysteine	6.1	5.9	5.8	5.6	5.6
Threonine	8.0	7.7	7.6	7.4	7.3
Calcium	8.1	7.8	7.7	7.5	7.4
Available phosphorus	4.2	4.0	4.0	3.9	3.8

[1]Supplied per kilogram of diet; Co, 03mg; Cu, 3.0 mg; Fe, 25mg; I, 1 mg; Mn, 125mg; Mo, 0.5 mg; Se, 200 µg; Zn,60 mg; antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; cholecalciferol, 60 µg; cyanocobalamin, 0.017 mg; foilc acid, 5.2 mg; menadione, 4 mg; niacin,35 mg; pyridoxine,10 mg; trans-retinol, 3.33 mg; riboflavin, 12 mg; thiamine, 3.0 mg; dl-α-tocopheryl acetate, 60 mg; choline chloride, 638 mg.

[2]Intake of basal diet was maintained identical across treatments within different feeding level. The total in this row reflects relative differences in the daily feed intake between the treatment groups.

4.3.2. Birds and housing

Two hundred male broilers (Ross 308) were reared in floor pens and fed a commercial starter diet (230 g CP and 12.6 MJ ME per kg) from days 1 to 20 of age. At day 21, one hundred and fourteen birds were selected on basis of the weight uniformity. Eighty birds with a mean of weight of 566 ± 40g were allocated to five experimental treatments (16 birds per group, in individual cages) and fed the experiment diets between 21 and 42 days of age. Each bird was randomly assigned to a wire cage (60 × 60 cm), equipped with individual feeder and self-drinking system. All cages were in one layer and placed in the same room. Twenty-four birds (581 ± 18g, 4 birds per cage) were fed *ad-libitum* and used

as monitor birds to establish the daily feed allowance for the experimental birds. The 10 remaining birds (568 ± 44g) were slaughtered at 21 days of age to measure the initial carcass weight and feather weight, four birds out of the initial ten slaughtered were used to measure the initial body composition. Live weight was recorded weekly and feed intake and feed refusals were recorded daily for each bird. Housing conditions have been described in Chapter 3, Section 3.3.2.

4.3.3. Establishing feed allowance

High energy diets are known to reduce feed intakes in broiler chickens (Leeson and Summers, 2001; Mehdi *et al*., 2007). To ensure that additional ME from soybean oil and tallow will be consumed, the monitor birds were fed the experimental diet with the high tallow content and daily feed intake was measured. From the intakes achieved by the monitor birds, the feed allowances of the experimental birds for the following days were calculated to achieve the planned energy intake levels (Figure 4.1). The daily feed allowances (FA) for the treatments birds were calculated as:

FA_{HT} = Feed intake from monitor birds (FI monitor)

FA_{Basal} = FI monitor ×(1/1.092),

FA_{MO} = FI monitor ×(1.04/1.092),

FA_{MT} = FI monitor × (1.046/1.092),

FA_{HO} = FI monitor ×(1.08 /1.092).

The daily feed allowances for each treatment were proportional to diet formulation given in Table 4.1. The equation coefficients represented the relative differences in soybean oil or tallow daily intake.

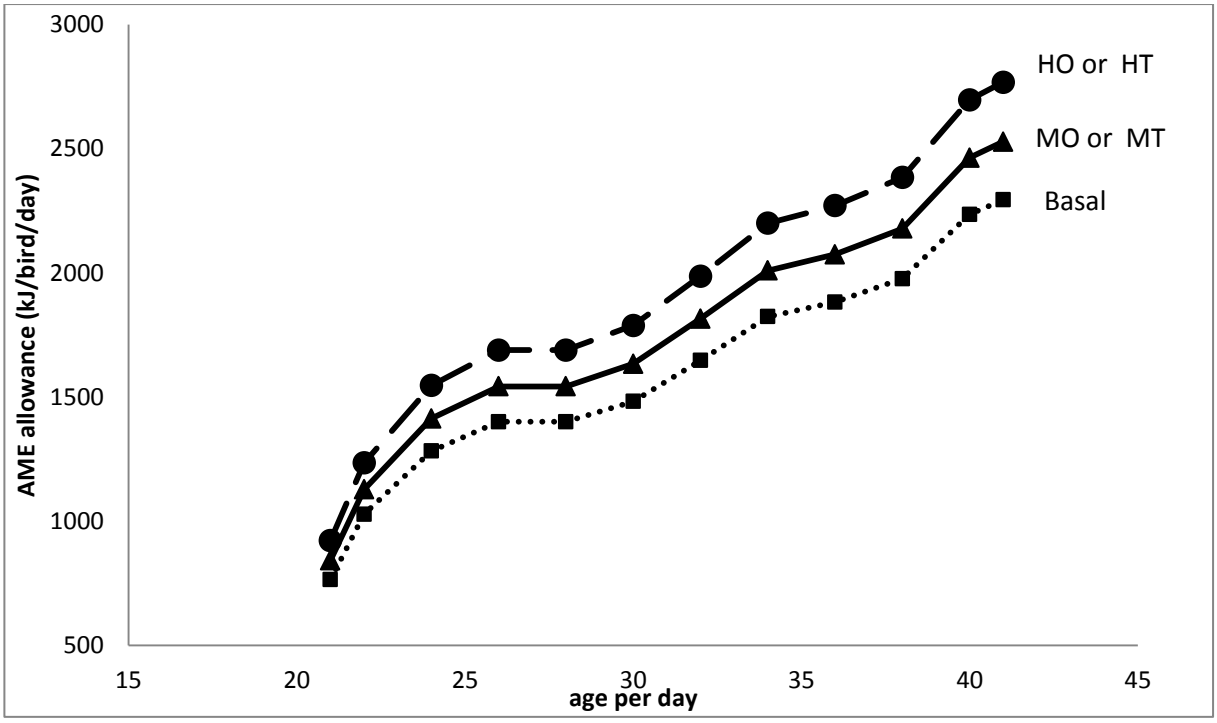

Figure 4.1. The daily apparent metabolisable energy (AME) allowances for the three levels of energy (basal level, medium level from MO or MT diet and high level from HO or HT diet

4.3.4 Pellet durability index

The pellet durability index (PDI) was measured in this experiment in order to explain the differences in the residual feed. The 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), with no fines, were rapidly circulated in an air stream around a perforated test chamber for 30 s. Fines were continuously removed through the perforations during the test cycle. After the test cycle, the subject pellets were ejected and weighed manually. The PDI was calculated as the ratio of the pellets not passing through the perforations after the test to whole pellets at the start.

4.3.5 Apparent metabolisable energy measurement

Apparent metabolisable energy was measured twice, when the birds were between 28 and 31 days of age and 38 and 41days of age. The AME procedure as described in Chapter 3, Section 3.3.4.

4.3.6. Measurement of ileal digestibility

Titanium dioxide was included as an indigestible marker in all diets (0.3% of diet). At 42 days of age, six birds per treatments were euthanised and ileal digesta was collected and processed as described in Chapter 3, Section 3.3.5.

4.3.7. Body composition and energy retention

At the end of the experimental period (at 42 day of age), the birds were fasted overnight, weighed and killed by cervical dislocation, de-feathered and weighed again. To measure the abdominal fat weight, the abdominal fat was dissected out of the carcasses, weighed and then returned back to the carcasses. The carcasses of eight birds were randomly selected from each treatment and stored at −20 °C prior to analysis for carcass composition and efficiency of energy utilisation. The carcasses were sampled and processed as described in Chapter 3, Section 3.3.6.

4.3.8. Laboratory analysis

The laboratory analysis was carried as described in Chapter 3, Section 3.3.7. The fatty acid composition was determined by the procedure reported by Sukhija and Palmquist (1988). Fatty acids were extracted by using methanolic hydrochloric acid (mixture of methanol and acetyl chloride) in culture tubes. Samples were vortexed and heated at 70 °C for methylation. After extraction and methylation, potassium carbonate and chloroform were added. Samples were vortexed and centrifuged to separate the methyl esters. Methyl esters were then transferred to the vials for fatty acid determination by using gas chromatography (Shimadzu GC-17A, Shimadzu Corporation, Tokyo, Japan), equipped with a flame ionisation detector.

4.3.9. Calculation

All calculations were made as described in Chapter 3, Section 3.3.8.

4.3.10. Statistics and determination efficiency of AME utilisation for fat deposition

The data of seven outlier birds were excluded from the performance analysis. Outliers were established by using a Boxplots quartile method (Minitab 15, 2006). These outliers were identified using inter-quartile range (IQR). Extreme values (within 1.5 times the inter-quartile range from the upper or lower quartile) were identified as outliers. The experiment data were analysed by one-way ANOVA (SAS, 2004) with a factorial orthogonal contrasts arrangement of treatments (2 types of fat × 2 levels of fat plus a basal treatment). These contrasts were formed, in order to compare the main effects of added fat (basal diet vs. other diets), types of fat (oil vs. tallow) and levels of fat (medium vs. high). Orthogonal polynomial contrasts (GLM, SAS 2004) were used to examine whether responses to increasing levels of energy were a linear or quadratic effect. The AME was analysed as described in Chapter 3, Section 3.3.9.

Multiple and linear regression models were used to estimate the efficiency of AME utilisation for fat and protein deposition.

Model 1: multiple linear regression of AMEI as a function of energy retention as protein (ERP) and energy retention as fat (ERF),

Model 2: linear regression of AMEI as a function of energy retention as fat (ERF),

Model 3: linear regression of AMEIfpd as a function of energy retention as fat (ERF),

The full description for each model is presented in Section 3.3.9.

Data from the basal, MO or HO treatments was fitted into the models to estimate the efficiency of AME utilisation for fat deposition from soybean oil (kf_{so}). Data from basal, MT or HT treatments was fitted to estimate the efficiency of AME utilisation for fat deposition from tallow (kf_t).

In order to test of the slopes (*kf*-values) from different types of fat were statistically different, Student's t-test was used and a probability value less than 0.05 indicates that the two slopes are significantly different from each other.

4.4. Results

4.4.1. Diet

The chemical composition and nutrient analysis of the diets are given in Table 4.2. The calculated crude protein was over estimated by approximately 1% compared to that measured in the diets. As expected, the fat percentage was increased when the basal diet was supplemented with extra fat from soybean oil or tallow.

Table 4.2. Analysed chemical composition of the experiment diets

	Diet				
	Basal	Medium oil	Medium tallow	High oil	High tallow
Composition (g/kg as fed)					
Crude protein	172.0	166.3	164.4	161.1	159.3
Essential amino acids					
Lysine	9.32	10.0	8.29	8.83	8.07
Methionine	2.58	2.63	2.75	2.46	2.53
Cysteine	2.52	2.59	2.65	2.40	2.37
Arginine	11.3	12.2	10.4	10.7	10.2
Threonine	6.72	7.12	6.24	6.36	6.22
Valine	8.66	9.24	8.03	8.32	7.95
Isoleucine	7.23	7.85	6.67	6.99	6.73
Leucine	15.4	16.3	14.6	14.7	14.2
Tyrosine	6.67	7.12	6.24	6.21	6.2
Phenylalanine	8.75	9.30	8.01	8.33	8.01
Histidine	5.51	5.77	5.10	5.31	5.23
Starch	530	533	524	515	525
Crude fat	30.3	64.2	74.1	101	117
Saturated fatty acids	5.60	10.9	21.5	15.9	36.1
Monounsaturated fatty acids	6.50	14.1	24.2	21.1	40.4
Polyunsaturated fatty acids	12.3	34.0	14.9	54.1	17.2
Total fatty acids	24.4	59.0	60.6	91.1	93.7

4.4.2. Feed intake and performance

The feed refused increased with increased level of fat supplement (Table 4.3) and was greater for the birds fed high fat diets compared to those fed low and medium fat diets ($P < 0.05$). This response in feed intake was unaffected by the type of fat, also there was no interaction effect between types and levels observed of fat in the feed intake ($P > 0.05$).

The pellet durability index (PDI) was measured in this experiment in order to explain the differences on the residual feed. A decrease in the pellet durability was associated with an increased fat concentration in the diet. In this experiment, the PDI was significantly different between diet groups, the PDI were 82.8, 85.4, 73.3, 60.4 and 16.4 for basal, MO, MT, HO and HT group respectively.

The final body weight was similar among all treatment groups ($P > 0.05$). The birds fed HO had similar average daily gain (ADG) with birds fed HT and however, it was lower than the ADG for birds fed the MO or MT diet ($P < 0.05$). There was no interaction effect observed on the ADG. The birds fed HO diet had greater feed conversion ratio than birds fed the other diets ($P < 0.05$; Table 4.3). The birds fed medium fat level had higher feed conversion ratio than those fed high fat level ($P < 0.05$). No significant interaction ($P > 0.05$) effect between types and levels of fat were observed for the feed conversion ratio.

Table 4.3. Influence of supplementing a basal feed with two levels of soybean oil or tallow on broiler chickens performance between 21-42 days of age

Diet	n	Feed intake (g/bird/d)	Feed refused (g/bird/d)	LBWi (g)	LBWf (g)	ADG (g)	FCR (g/g)
Basal	15	122.2	2.2^{b}	571	1868	61.8^{abc}	1.99^{b}
Medium oil	13	126.5	2.9^{b}	572	1915	64^{ab}	1.98^{b}
Medium tallow	15	126.3	3.8^{b}	565	1936	65.3^{a}	1.95^{b}
High oil	15	122.8	11.6^{a}	572	1792	58.1^{c}	2.15^{a}
High tallow	15	120.6	15.2^{a}	570	1844	60.7^{bc}	1.99^{b}
Pooled SE		2.04	2.04	10.68	38.71	1.63	0.046
P-value		0.18	0.0001	0.992	0.074	0.022	0.024
Contrast P-value							
Basal vs. Added fat diets		0.42	0.008	0.97	0.92	0.9	0.49
Fat type (oil vs. tallow)		0.57	0.27	0.699	0.347	0.236	0.034
Fat level (medium vs. high)		0.024	0.0001	0.82	0.007	0.002	0.015
Fat type × fat level		0.62	0.50	0.82	0.68	0.70	0.22

^{a,b,c,d} Means in the same column with different superscripts are significantly different ($P < 0.05$). LBWi is the initial live body weight, LBWf is the final live body weight, ADG is the average daily gain, FCR is the feed conversion ratio.

4.4.3. Apparent metabolisable energy and ileal digestibility

As designed, the additional fat increased the AME of the diets ($P < 0.05$) and the AME of the HO and HT diets were higher than those of MO and MT diets ($P < 0.05$), a tendency of age effect ($P = 0.06$) on AME was observed (Table 4.4)

Table 4.4. Apparent metabolisable energy (AME; MJ/kg) of the diets that differ in the concentration and types of fat, determined between 28 and 31 and 38 and 41 days of age

Diet	n	Age	AME(MJ/kg)
Basal	4	28-31days	12.97[c]
	4	38-41days	12.74[c]
Medium oil	4	28-31days	13.57[b]
	4	38-41days	13.66[b]
Medium tallow	4	28-31days	13.96[b]
	4	38-41days	13.68[b]
High oil	4	28-31days	14.49[a]
	4	38-41days	14.27[a]
High tallow	4	28-31days	14.27[a]
	4	38-41days	14.11[a]
SEM			0.117
Main effects			
Diet			
Basal	8		12.85[c]
Medium oil	8		13.62[b]
Medium tallow	8		13.82[b]
High oil	8		14.38[a]
High tallow	8		14.20[a]
SEM			0.138
Age			
28-31days	20		13.85
38-41days	20		13.68
SEM			0.052
P-value	Diet		0.0001
	Age		0.0598
	Diet × Age		0.5520

[a,b,c,] Means in the same column with different superscripts are significantly different ($P < 0.05$).

The ileal digestibility of protein, starch, ash and organic matter were similar among all treatment groups ($P < 0.05$). When birds were fed the diet with the higher inclusion of fat (HO and HT), the digestibility of fat was greater than that measured in birds fed basal or

MT diets (P > 0.05, Table 4.5). No significant (P > 0.05) effects of types, levels or interaction on protein starch and organic matter digestibility coefficients were observed.

Table 4.5. Apparent ileal digestibility coefficients for nutrients in the experimental diets that differ in the concentration and types of fat

Diet	n	Protein	Fat	Starch	Organic matter
Basal	6	0.73	0.86[c]	0.94	0.69
Medium oil	6	0.75	0.94[ab]	0.95	0.70
Medium tallow	6	0.75	0.90[b]	0.92	0.68
High oil	6	0.80	0.97 [a]	0.95	0.73
High tallow	6	0.80	0.97[a]	0.94	0.72
SEM		0.02	0.01	0.01	0.02
P-value		0.18	0.0001	0.31	0.24
Contrast P-value					
Basal vs. added fat diets		0.09	0.0001	0.81	0.31
Fat type (oil vs. tallow)		0.97	0.210	0.11	0.35
Fat level (medium vs. high)		0.07	0.005	0.26	0.07
Fat type × fat level		0.97	0.21	0.32	0.61

[a,b,c,] Means in the same column with different superscripts are significantly different (P < 0.05).

4.4.4. Digestible Nutrients and AME intake

As expected for this trial design, there were no differences in the digestible protein and starch intake (P < 0.05, Table 4.6). The daily digestible fat intake was lowest for birds fed the basal diet (P > 0.05), intermediate for birds fed the medium fat level diets (MO or MT) and highest for birds fed the high fat level (HO and HT).

Table 4.6. The ileal digestible nutrients and energy intake for broiler chicken fed a basal diet or basal supplemented with two levels of soybean oil or tallow between 21 and 42days of age[1]

Diet	n	Feed intake (g/b/d)	iDPI (g/bird/d)	iDSI (g/bird/d)	iDFI (g/bird/d)	iDEI from fat (MJ/bird/d)	AMEI (MJ/bird/d)
Basal	8	121.7	15.3	53.7	3.19^{e}	0.124^{e}	1.56^{b}
Medium oil	8	126.5	15.9	56.7	7.67^{d}	0.299^{d}	1.72^{a}
Medium tallow	8	127.2	15.7	54.6	8.57^{c}	0.334^{c}	1.76^{a}
High oil	8	121.4	15.7	52.9	11.90^{b}	0.464^{b}	1.75^{a}
High tallow	8	124.6	15.9	54.9	14.10^{a}	0.550^{a}	1.77^{a}
SEM		2.75	0.35	1.21	0.284	0.011	0.039
P-value		0.45	0.78	0.26	0.0001	0.0001	0.004
Contrast P-value							
Basal vs. added fat diets		0.30	0.23	0.45	0.0001	0.0001	0.0002
Fat type (oil vs. tallow)		0.49	0.95	0.94	0.0001	0.0001	0.48
Fat level (medium vs. high)		0.17	0.98	0.16	0.0001	0.0001	0.69
Fat type × fat level		0.67	0.65	0.11	0.027	0.027	0.84

[1]The data presented in this table are for the birds selected for carcass composition (8 birds per group).

[a,b,c,d,e] Means in the same column with different superscripts are significantly different ($P < 0.05$). The ileal digestible nutrients intake (g/birds/day) was calculated as the daily nutrient intake multiplied by nutrient coefficient (Table 4.5). iDPI is the ileal digestible protein intake, iDSI is the ileal digestible starch intake, iDFI is the ileal digestible fat intake, iDEI from fat is the ileal digestible energy intake derived from fat. AMEI is apparent metabolisable energy intake.

The ileal digestible energy intake derived from fat was higher for those birds fed high fat level compared with medium fat level diets. The AMEI from birds in medium and high fat levels was 10-13% higher than the birds on basal diet ($P < 0.05$). The fat types and levels were unaffected on the AMEI ($P > 0.05$).

4.4.5. Carcass composition

There was no difference in carcass weight between the treatment groups ($P > 0.05$, Table 4.7). The birds fed diet without additional fat had less abdominal fat than those birds fed diets with additional fat ($P < 0.05$). The birds fed diets with additional fat had a lower carcass protein concentration than the birds fed basal diet ($P < 0.05$). No significant effects of level, type and interaction were observed on the carcass protein concentration. The birds fed the diet supplemented with high fat level had a higher carcass fat and energy content compared with those fed diets supplemented with medium fat level ($P < 0.05$). The carcass fat and energy content was lower for the birds fed basal diet compared to the birds fed diet with medium fat level ($P < 0.05$, Table 4.7). No interaction effect was observed for the carcass composition ($P > 0.05$).

Table 4.7. De-feathered carcass composition for broiler chicken fed a basal diet or basal diet supplemented with two levels of soybean oil or tallow between 21 and 42 days of age

Diet	n	Carcass (g/bird)[1]	Abdominal fat (g/bird)	Abdominal fat (%)	Protein (%)	Fat (%)	Water (%)	Ash (%)	Energy (kJ/g)
Basal	8	1785	29.7[b]	1.65[b]	15.92[a]	13.59[c]	67.36[a]	2.59	8.94[c]
Medium oil	8	1841	47.1[a]	2.55[a]	14.76[b]	17.08[b]	65.08[b]	2.50	9.95[b]
Medium tallow	8	1839	48.4[a]	2.64[a]	14.87[b]	17.50[b]	64.38[b]	2.43	10.30[b]
High oil	8	1773	50.1[a]	2.81[a]	14.50[b]	19.99[a]	62.97[c]	2.4	10.92[a]
High tallow	8	1825	48.9[a]	2.66[a]	14.78[b]	18.91[a]	62.82[c]	2.46	11.04[a]
SEM		42.02	3.60	0.183	0.163	0.452	0.40	0.073	0.188
P-value		0.69	0.001	0.001	0.0001	0.0001	0.0001	0.44	0.0001
Contrast P -value									
Basal vs. added fat		0.46	0.0001	0.0001	0.0001	0.0001	0.0001	0.09	0.0001
Fat type (oil vs. tallow)		0.55	0.98	0.87	0.25	0.540	0.290	0.97	0.210
Fat level (medium vs. high)		0.33	0.62	0.48	0.28	0.0001	0.0001	0.65	0.0001
Fat type × fat level		0.53	0.73	0.55	0.62	0.130	0.490	0.41	0.520

[1]Carcass is the de-feather carcass weight (gram/bird). [a,b,c] Means in the same column with different superscripts are significantly different ($P < 0.05$).

4.4.6. Deposition rates of chemical components

The protein deposition rate was lower in the birds fed HO diet compared with the birds fed other diets. The rate of fat deposition increased as the energy intake derived from fat increased ($P < 0.05$). The broilers fed the high and medium fat diets had higher fat deposition rates than those fed the basal diet ($P < 0.05$, Table 4.8). The water deposition rate was closely related to protein deposition rate. There was no difference in ash growth rate among the groups. Linear ($P < 0.05$) increase in fat deposition rates were observed with increasing of oil or tallow levels. A tendency for a quadratic ($P = 0.07$) response was seen for fat deposition rate with increasing the level of tallow.

Table 4.8. Deposition rate (g/d) of fat, protein, ash and water for broiler chicken fed a basal diet or basal diet supplemented with two levels of soybean oil or tallow between 21 and 42 days of age

Diet	n	Protein (g/bird/day)	Fat (g/bird/day)	Water (g/bird/day)	Ash (g/bird/day)
Basal	8	9.44[a]	8.43[c]	38.77[a]	1.54
Medium oil	8	8.92[a]	11.87[b]	38.93[a]	1.54
Medium tallow	8	8.91[a]	12.20[ab]	37.87[ab]	1.47
High oil	8	8.09[b]	13.69[a]	34.47[c]	1.35
High tallow	8	8.69[ab]	13.32[ab]	35.82[bc]	1.47
SEM		0.271	0.595	1.012	0.0651
P-value		0.024	0.0001	0.013	0.244
Contrast P-value					
Basal vs. added fat		0.014	0.0001	0.09	0.28
Fat type (oil vs. tallow)		0.28	0.97	0.88	0.67
Fat level (medium vs. high)		0.061	0.02	0.003	0.14
Fat type × fat level		0.27	0.56	0.24	0.16
Linear effect (oil)		0.002	0.0001	0.007	0.001
Quadratics effect (oil)		0.64	0.24	0.08	0.103
Linear effect (tallow)		0.05	0.0001	0.03	0.52
Quadratics effect (tallow)		0.63	0.07	0.06	0.75

[a,b,c,] Means in the same column with different superscripts are significantly different ($P< 0.05$).

[1] linear and quadratics effect were used to examine whether responses to increasing levels of oil (basal, medium oil and high oil) or tallow (basal, medium tallow and high tallow) were of a linear or quadratic nature.

4.4.7. Energy partitioning

The total HP was similar among all treatment groups and the types, levels or interaction were not effect ($P > 0.05$) on HP. The energy retention in birds fed diets with additional fat from tallow or soybean oil was higher than the birds fed the basal diet ($P < 0.05$). The total energy retention was similar between the medium and high fat levels ($P > 0.05$). The fat types were not effect on the energy retention ($P > 0.05$). The birds fed high fat diets had higher energy retention as fat than those fed the basal or medium fat diets ($P < 0.05$, Table 4.9).

Table 4.9. Utilisation of apparent metabolisable energy (MJ/bird) for broiler chickens fed a basal diet or basal diet supplemented with two levels of soybean oil or tallow from 21to 42 day of age

Diet	n	AMEI	HP	AMEm	HI	ER	ERP	ERF	FE	k_g
Basal	8	32.84[b]	20.56	15.13	5.42	12.28[b]	4.70[a]	6.91[c]	0.68	0.69
%		100	62.61	46.07	16.5	37.39	14.31	21.04	2.07	
Medium oil	8	36.19[a]	21.33	15.32	6.00	14.86[a]	4.44[a]	9.73[b]	0.69	0.71
%		100	58.94	42.33	16.58	41.06	12.27	26.89	1.91	
Medium tallow	8	36.94[a]	21.85	15.33	6.52	15.09[a]	4.43[a]	10.00[ab]	0.66	0.7
%		100	59.15	41.5	17.65	40.85	11.99	27.07	1.79	
High oil	8	36.68[a]	20.8	15.11	5.68	15.89[a]	4.03[b]	11.22[a]	0.64	0.74
%		100	56.71	41.19	15.49	43.32	10.99	30.59	1.74	
High tallow	8	37.13[a]	21.21	15.31	5.89	15.92[a]	4.32[ab]	10.92[ab]	0.68	0.73
%		100	57.12	41.23	15.86	42.88	11.63	29.41	1.83	
SEM[1]		0.826	0.554	0.199	0.506	0.597	0.135	0.488	0.022	0.02
P-value[1]		0.004	0.524	0.874	0.631	0.0007	0.024	0.0001	0.473	0.41
Contrast P-value										
Basal vs. added fat diets		0.0002	0.24	0.54	0.30	0.0001	0.01	0.0001	0.57	0.23
Fat type(oil vs. tallow)		0.47	0.40	0.60	0.48	0.82	0.28	0.97	0.85	0.58
Fat level (medium vs. high)		0.68	0.29	0.58	0.35	0.13	0.06	0.02	0.50	0.14
Fat type x fat level		0.86	0.92	0.64	0.77	0.87	0.27	0.56	0.83	0.85

[a,b,c] Means in the same column with different superscripts are significantly different ($P < 0.05$). AMEI is the apparent metabolisable energy intake, HP is the heat production, AMEm is the metabolisable energy for maintenance requirement, HI is the heat increment, ER is the total energy retention, ERP is the energy retention as protein, ERF is the energy retention as fat, FE is the energy retention as feather and k_g is the efficiency of energy utilisation for weight gain was calculated as ER/ (AMEI–AMEm). [1] SEM and P-values is related to the absolute energy partitioning values.

4.4.9. Efficiency of AME utilisation for fat and protein deposition

The overall *k*-values for fat deposition using different models ranged between 0.93 to 0.99 for soybean oil and 0.89 to 0.90 for tallow (Table 4.10).

Model 1: the overall efficiency of AME utilisation for fat and protein deposition

The overall efficiency of AME for fat deposition (*kf*) was 0.94 and protein deposition (*kp*) was 0.67 (Table 4.10, Model 1). Using the same model, the efficiency of AME utilisation for fat deposition from additional AME from soybean oil was 0.93 and from additional AME from tallow it was 0.89.

Model 2: Predicting the efficiency of AME utilisation for fat deposition

The efficiencies of AME utilisation using a linear regression model assuming constant protein deposition were 0.93 across all diets and 0.99 and 0.89 for soybean oil and tallow intake, respectively (Table 4.10, Model 2).

Model 3: Subtracting the protein deposition energy requirement (protein deposition free AMEI; AMEIfpd) to estimate the efficiencies of AME utilisation for fat deposition from extra soybean oil or extra tallow intake

When correcting the differences of protein deposition across all diets, the kf_{so} value was 0.94 when feeding additional AME in the form of soybean oil and kf_t value was 0.90 when the additional AME was from tallow (Table 4.10, Model 3), but the kf_{so} and kf_t were not different ($P > 0.05$).

Table 4.10. Regression equations of AMEI as function of energy retention as fat (ERF) and protein (ERP) to estimates the efficiencies of energy utilisation for fat deposition from different types of fat in broiler chickens between 21 and 42 days of age

Diet	Model	Regression equations	*kf*	*kp*	R^2
All diets					
	1	AMEI = 18.14+ 1.49ERP+1.06ERF (3.0) (0.55) (0.124)	0.94	0.67	0.69
	2	AMEI = 25.53+1.07ERF (1.34) (0.134)	0.93	-	0.63
	3	AMEI fpd = 18.02+1.06ERF (1.22) (0.123)	0.94	-	0.66
Basal, MO, HO					
	1	AMEI = 18.72+ 1.3ERP+1.07ERF (3.85) (0.65) (0.150)	0.93	0.77	0.71
	2	AMEI = 25.86+1.01ERF (1.48) (0.156)	0.99	-	0.66
	3	AMEI fpd = 17.54+1.08ERF (1.36) (0.143)	0.93	-	0.72
Basal, MT, HT					
	1	AMEI=20.761+0.868ERP+1.12ERF (4.18) (0.785) (0.147)	0.89	1.12	0.74
	2	AMEI = 25.12+1.13ERF (1.40) (0.147)	0.89	-	0.73
	3	AMEI fpd =17.53+1.11ERF (1.38) (0.145)	0.90	-	0.73

Numbers in the parentheses are the standard errors of above coefficient. No significantly different between the two slopes; soybean oil and tallow groups at (P = 0.05).
Model 1 multiple linear regression cross all treatment groups, AMEI= intercept + (1/*kf*) ERF+ (1/*kp*) ERP.
Model 2 linear regression, AMEI= intercept + (1/*kf*) ERF, The efficiency of energy utilisation for fat deposition from each type of fat was expressed relative to basal treatment group, assumed extra energy intake would not affect protein retention and the intercept presented the protein deposition and maintenance energy requirement.
Model 3 linear regression, AMEIfpd= intercept + (1/*kf*) ERF, AMEIfpd; is the AME intake after ignoring the energy requirement for protein retention.

4.5. Discussion

4.5.1. Feed intake and performance

In this trial, the feed allowance (g/bird/day) was controlled to create three levels of energy intake: basal, medium and high. Greater refusal was observed for birds fed the high fat diets, which suggests that the energy concentration was controlling the feed intake and as the energy density increased the feed intake decreased. Several studies have reported that the feed intake decreases with increased dietary ME concentration because birds adjust the feed intake to reach their ME requirements (Hulan *et al*., 1984; Leeson and Summers,

2001; Mehdi *et al*., 2007; Nahashon *et al*., 2005; Perrault and Leeson, 1992; Pinchasov and Nir, 1992). Leeson *et al*. (1996b) showed that broilers fed up 49 days of age were able to adjust the feed intake to constant energy intake with variable ME concentration levels from 11.3 to 13.8 MJ/kg diet.

Another factor possibly affecting feed and energy intake was the PDI. When the fat percentage in the diet increased the PDI decreased. A decrease in the pellet durability and quality is commonly associated with increase dietary fat concentration (Benhke, 1996; Catala, 2009; Plavnik *et al*., 1997). Feed intake decreases when the pellet quality and durability reduced (Corzo *et al*., 2011; Cutlip *et al*., 2008). Factors such as ME concentration and PDI were likely both influences the bird feed intake in this experiment.

In the present study, the final body weight and feed intake were similar in all treatments. Leeson *et al*. (1996b) found no difference in the body weight gain between 25 and 49 days of age when the broilers were fed fixed quantities of feed with variable energy content but similar nutrient profile. Also, Hulan *et al*. (1984) and Pinchasov and Nir, (1992) reported that no effect of energy concentration on the body weight gain but an effect on the carcass composition was observed. Therefore, the additional energy intake was associated with no change in body weight, but the energy intake influenced on carcass composition and carcass energy content.

4.5.2. Description of body components growth rates

In this experiment, the additional AME intake increased fat deposition rate by 4.6 g/day for birds fed high fat diets when comparing with the birds in basal diet. The rate of protein deposition was constant across all treatments at approximately 9.0 g/day. Therefore, extra AME consumed from birds in in high and medium fat diets had an effect on the rate of fat growth. Body growth is determined mainly by protein gain and to a smaller extent by fat gain (Boekholt *et al*., 1994) and each gram of protein deposited is accompanied by 4 grams of water compared with the 0.25 grams of water that accompanies with each gram of fat deposited (Soller and Eitan, 1984). This suggests that the additional AME intake in our experiments, increased the fat gain with less water gain and the significance of fat gain were not reflected to the total live body weight gain. As a result, it is very important to approach a minimum AME intake per gram of gain in broiler industry to improve the AME

efficiency. The overall efficiency of AME utilisation could be improved by manipulating the diet based on AME per gram live body weight gain rather than the feed per live body weight gain.

4.5.3. Energy partitioning

The energy retention to AME intake ratio increased when the AME derived from fat intake increased (Table 4.9). A better AME utilisation after fat feeding implies a decreased heat production and increased energy retention. Therefore, the birds fed the basal diet produced more heat per unit of AME intake compared with birds fed diets with additional fat. The decreased heat production is the result of decreased maintenance requirements, decreased heat released from AME utilisation or both. It was assumed the AME for maintenance constant in all cases and this is valid because there were no differences in the live body weight between the treatment groups ($P > 0.05$). Therefore, the decreased heat production is likely to have resulted from increased efficiency of AME utilisation. Because the protein deposition was constant, the decrease in heat production was related to the efficiency of fat deposition. These results agree with other studies, such as Danicke *et al*. (2001) who found that the ER:AMEI ratio was 0.33 for the birds fed basal diet compared with 0.36 for birds fed diet with 12% soybean oil. Crespo and Esteve (2002b) observed an ER: AMEI ratio of 0.38 for birds fed a basal diet and the ratio was approximately 0.42 for birds fed diets with 10% tallow, olive oil, sunflower oil or linseed oil.

4.5.4. Efficiency of AME utilisation for fat and protein deposition (Model 1)

In the present study, increasing the levels of fat is resulted a linear response on fat deposition rate. These finding suggest the linear regression model is valid model to estimate the *kf*-values.

In the model 1, the *kf* was estimated to be 0.93 from soybean oil and 0.89 from tallow fat. This result is in agreement of those of Danicke *et al*., (2001) who estimated the *kf* of 0.89 from diet supplemented with soybean oil.

4.5.5. Efficiencies of AME utilisation for fat deposition from extra soybean oil or tallow intake (Model 2 and 3)

The efficiency of AME utilisation for fat deposition from soybean oil (kf_{so}) was estimated in model 2 to be higher than the value estimated in model 3 (kf_{so} = 0.99 model 2, kf_{so} = 0.93 in model 3). Whereas the efficiency of AME utilisation for fat deposition from tallow (kf_t) was similar using model 3 or model 2; approximately 0.90.

Applications of model 2 and model 3 should give similar answers when the protein deposition remained constant, but this was not the case for the birds fed HO diet, the birds fed the HO diet had lower protein deposition than the birds fed other diets. The differences between kf_{so} estimated in model 2 and model 3 is probably related to the changes in the rate of protein deposition. The higher estimation of AME requirement for maintenance and protein deposition may also have resulted in a high kf_{so} value for soybean oil on model 2.

In general, the estimation of efficiency of ME utilisation for fat deposition using the classical multiple linear regression is very variable. For growing broilers the *kf* ranges from 0.55 to 0.92 (Boekholt *et al*., 1994; Nieto *et al.,* 1995; Petersen 1970; Sakomura *et al*., 2005) and in some case *kf* values were found to be greater than 1 (Lin and Chiang, 2004; Macleod, 1990; Sakomura 2003). The multiple linear regression is sensitive to the value of maintenance requirement (Halas and Babinszky, 2010; Roux, 2009) and the *kp* and *kf* were assumed to be independent of genotype, but this may be not accurate if there are differences in metabolic pathways involved in protein and fat turnover rate related to ME intake and this may have changed the *k*-values (Azevedo *et al*., 2005). Other limitations of the multiple linear regression model was that the model is based on the assumption that the ME utilisation for protein and fat deposition are identical (Hall, 2010). This means that the ratio between extra fat and extra protein deposition is constant from an extra unit of ME intake. Unfortunately, this assumption was not valid in all cases. Boekholt *et al*. (1994) reported that for the broilers with low ME intakes the fat was mobilised, whereas the protein was deposited. In contrast, in the mature birds which have completed skeletal and muscle growth the extra ME consumed can be deposited only as fat.

In the present study the total protein deposition was similar in all treatment groups, suggesting that extra AME intake did not improve the protein deposition and the relative differences in the protein deposition was ignored by correcting to protein deposition free

AME intake (Table 4.10, Model 3). Therefore, the results obtained by this approach showed realistic values for *kf* from the additional soybean oil or tallow consumed. The linear regression model introduced here avoided the correlation between fat and protein deposition by fixing the protein deposition during the growth, which is difficult to do it in multiple linear regression.

It has been reported that the most efficient pathway for fat deposition is from dietary fat with *kf* value of 0.90 (E1mmans, 1994; Noblet *et al.,* 2010). It is likely due to the lower heat increment produced from fat utilisation as observed from our experiment.

Our results are consistent with previous studies in growing pigs, the efficiencies of energy utilisation for fat deposition from fat were 0.90 and 0.88 as obtained by Noblet *et al.* (1994) and Van Milgen *et al.* (2001a), respectively. In our experiment, the coefficients *(1/kf)* of energy retention as fat deposition ranged from 1.01 to 1.13 with an average 1.07 and the largest SE was 0.150 (Table 4.10) therefore, the 95% confidence interval is 1.07 ±0.31 (0.76-1.38). These confidence intervals are smaller than those reported by of Chuan *et al.* (2010), who estimated a 95% confidence interval of 1.2 ±0.71 (0.49-1.91) for the *(1/kf)* value of 1.20 (SE = 0.34, n = 20). Therefore, our study provides a *kf* value with more confidence than those in the literature.

4.6. Conclusion

In conclusion, under protein limiting conditions, the efficiency of AME utilisation for fat deposition from extra fat intake was 0.94 across all diets and 0.93 for extra soybean oil intake and 0.90 for extra tallow intakes. The results from this experiment provide important data about the efficiency of AME utilisation for fat deposition which will be used to improve the net energy system in broilers.

CHAPTER 5

Efficiency of energy utilisation for fat deposition from non-essential amino acids in broiler chickens

5.1. Abstract

This experiment was conducted to investigate the effect of additional energy intake from non-essential amino acids (NEAA), as source of protein on energetic efficiency of fat deposition, energy partitioning, carcass composition and growth performance of Ross 308 broilers between 21and 42 days of age. Birds were allocated to one of three dietary treatments; basal diet or the basal diet supplemented with extra NEAA to provide 7% (B+NEAA1) or 14 % (B+NEAA2) extra energy from NEAA intake. The birds were weighed weekly and feed intake was recorded daily. At the end of the experiment, the chickens were slaughtered, de-feathered and the carcasses were weighed. Protein, fat, gross energy and ash composition of the carcass was compared to that of chickens slaughtered at the start of the experiment. The carcass protein gain increased ($P < 0.05$) for birds fed B+NEAA1, however the carcass fat gain tended to increase ($P = 0.06$) for bird fed B+NEAA2. The energetic efficiency of fat deposition from NEAA intake was calculated as 0.63.

5.2. Introduction

The cost of feed in the broiler industry is critical as it contributes to 65 to 75% of the total production cost, and the supply of energy represents the greatest proportion of this cost (Noblet *et al.*, 2010). Energy utilisation in poultry is usually expressed in terms of metabolisable energy (ME), which is determined by the difference between energy intake and energy excreted. Net energy (NE) is another possible measure of energy utilisation in poultry. NE is the ME minus the energy lost as heat increment of feeding, and represents the energy used for maintenance and production of protein and fat in broiler chickens. Net energy values are not readily available for broiler chickens, but it is possible to predict the NE from dietary composition by using the ME partitioning model (Gous, 1999, Birkett and de Lange, 2001a). The ME partitioning model can be used to explore ways to improve growth efficiency. Data on the contribution of different energy sources to growth

performance and the efficiency of ME utilisation for protein and fat deposition are limited in broilers. The efficiency of ME utilisation for fat deposition from different energy sources such as starch, soybean oil and tallow have been investigated in Chapter 3 and 4. The objective of the present study was to investigate the effect of additional ME intake from NEAA as a protein source, on broiler growth performance, deposition of fat and protein, ME partitioning and on the energetic efficiency of fat deposition. It was hypothesised that the extra NEAA will be utilised for fat deposition.

5.3. Materials and Methods

The procedure for this experiment was approved by Massey University Animal Ethics Committee as described in Chapter 3, Section 3.3. The Ethics Code number was MUAEC 11/04.

5.3.1. Diets

Three experimental diets were prepared. The basal diet and the basal diet supplemented with NEAA (Glycine, Glutamic acid, Serine, Alanine, Aspartic acid and Proline) were calculated to provide either 7 or 14% extra ME intake. The basal diet was formulated to provide 212 g crude protein and 13.42 MJ ME per kg of diet as recommended for Ross308 broilers (Ross, 2007). The feed allowance of each treatment group was set to have a similar intake to that of the birds in the basal diet group, with the ME intake differing between treatments reflecting an additional NEAA intake (Table 5.1).

Table 5.1. Ingredients and composition of diets for growing broilers, based on equal nutrient intake except non-essential amino acids intake

Ingredients(g)	Basal	B+ NEAA1	B+NEAA2
Maize	671	671	671
Soybean meal, 48%	210	210	210
Casein	60	60	60
Soybean oil	20	20	20
Glycine	0.0	10	20
Glutamic acid	0.0	10	20
Serine	0.0	10	20
Alanine	0.0	10	20
Aspartic acid	0.0	10	20
Proline	0.0	10	20
Limestone	14	14	14
Dicalcium phosphate	15	15	15
Salt	2.0	2.0	2.0
DL-Methionine	2.0	2.0	2.0
Premix[1]	3.0	3.0	3.0
Titanium dioxide	3.0	3.0	3.0
Total diet weight after mixing (g)[2]	1000	1060	1120
Calculated analysis (g/kg as fed)			
Metabolisable energy (MJ/kg)	13.42	13.55	13.67
Crude protein	211	246	278
Lysine	13.0	12.2	11.6
Methionine	6.00	5.70	5.40
Methionine + Cysteine	9.00	8.49	8.04
Glycine	16.3	24.7	32.1
Serine	11.9	20.5	28.1
Glutamic acid	41.8	48.7	54.9
Alanine	10.7	19.4	27.1
Aspartic acid	20.1	28.2	35.4
Calcium	9.77	9.22	8.73
Available phosphorus	4.99	4.71	4.46

[1]Supplied per kilogram of diet; Co, 03mg; Cu, 3.0 mg; Fe, 25mg; I, 1 mg; Mn, 125mg; Mo, 0.5 mg; Se, 200 µg; Zn,60 mg; antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; cholecalciferol, 60 µg; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4 mg; niacin,35 mg; pyridoxine,10 mg; trans-retinol, 3.33 mg; riboflavin, 12 mg; thiamine, 3.0 mg; dl-α-tocopheryl acetate, 60 mg; choline chloride, 638 mg.

[2]Intake of basal diet was maintained identical across treatments within different daily feeding level. The total in this row reflects relative differences in the daily feed intake between the treatment groups.

5.3.2. Birds and housing

Two hundred male broilers (Ross 308) were reared in floor pens and fed a commercial starter diet (230 g crude protein and 12.6 MJ ME per kg) from 1 to 20 days of age. At 21 days of age, seventy four birds were selected on the basis of weight uniformity, forty eight

birds were allocated to three treatments (956 ± 35g, 16 birds per treatment, in individual cages) and fed the experimental diets between 21 and 42 days of age (Table 5.2), sixteen birds (958 ± 47g, in individual cages) were used as monitor birds to establish the daily feed allowances for the experimental birds, the remaining ten birds (937 ± 41g) were slaughtered at 21 days of age to measure the initial carcass and feather weight, and four birds out of the initial ten slaughtered were used to measure the initial body composition. Live body weight was recorded weekly and feed intake and feed refusals were recorded daily for each bird. Housing conditions have been described in Chapter 3, Section 3.3.2.

Table 5.2. Description of the treatments.

Treatment	Diet/offered	Number of birds
Monitor [1]	*ad-libitum* (basal diet)	16
Basal	~85% of monitor group intake (basal diet)	16
B+NEAA1	Basal + Non-essential AA (level1)	16
B+NEAA2	Basal + Non-essential AA (level2)	16

[1] Monitor birds were used to establish the *ad-libitum* feed intake.

5.3.3. Establishing feed allowance

Broilers in the monitor treatment were provided free access to feed to establish the *ad-libitum* feed intake. The daily feed allowances (FA) for the three treatments were established based on the previous day feed intake of the monitor group (Figure 5.1). The feed allowance was restricted to allow the birds to consume the extra NEAA with minimum refusal. The calculations of feed allowances were:

FA_{Basal} = Feed intake from monitor × 0.85

$FA_{B+NEAA1} = FA_{Basal} \times 1.06$

$FA_{B+NEAA2} = FA_{Basal} \times 1.12$

The daily feed allowances for each treatment were relative to diet formulation given in Table 5.1. The coefficients in the equations (above) represented the relative differences in daily intake.

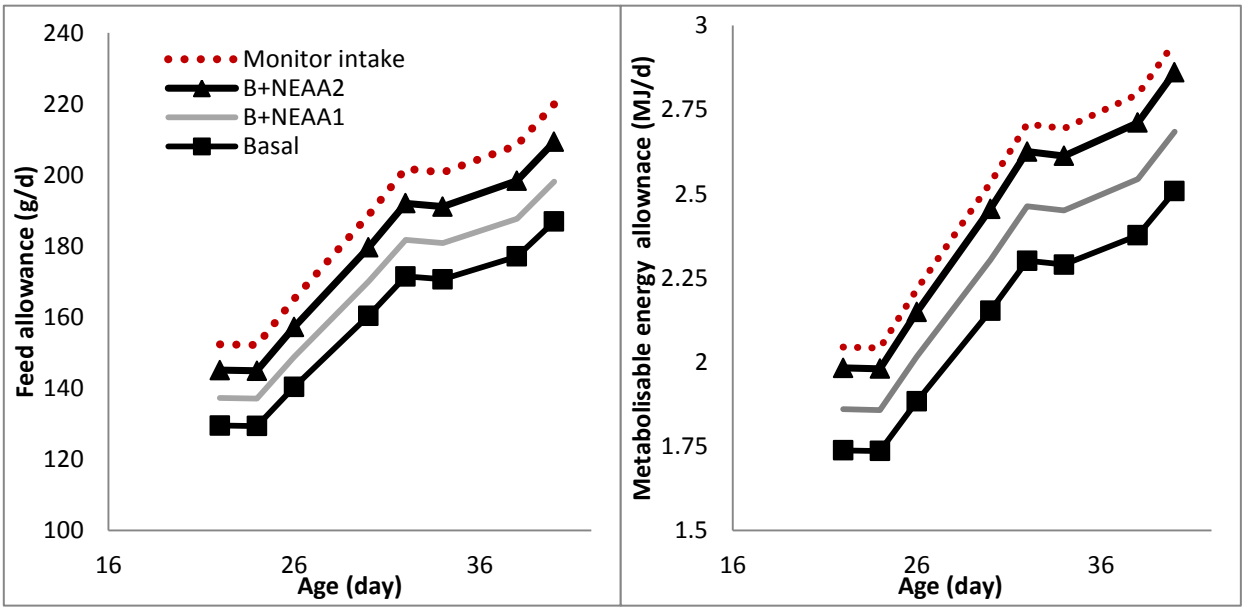

Figure 5.1. The daily feed allowance (g//bird/day) was obtained regarded to the monitor group daily intake to achieve the different levels of metabolisable energy intake

5.3.4. Apparent metabolisable energy determination

Apparent metabolisable energy was measured twice, when the birds were 28-31 and 38-41 days of age. The AME procedure as described in Chapter 3, Section 3.3.4.

5.3.5. Ileal digestibility

Titanium dioxide was included as an indigestible marker in all diets (0.3% of diet). At 42 day of age, six birds per treatments were euthanised and ileal digesta was collected and processed as described in Chapter 3, Section 3.3.5.

5.3.6. Body composition

At the end of the experimental period (42 day of age), the birds were fasted overnight, weighed and killed by cervical dislocation, defeathered and weighed again. De-feathered carcass (whole bird with no feathers) of eight birds from each group were randomly selected and stored at –20°C. The carcasses were sampled and processed as described in Chapter 3, Section3.3.6.

5.3.7. Laboratory analysis

The laboratory analysis was carried as described in Chapter 3, Section 3.3.7.

5.3.8. Calculations

The calculations were made as described in Chapter 3, Section 3.3.8. Moreover, the NEAA digestibility was calculated using formula of Adeola (2000) as follows:

$$\text{Digestibility of the NEAA (\%)} = \frac{(T \times t) - (B \times b)}{a}$$

where:

T is the digestibility of the NEAA in the treatment diet.

t is the amount of the NEAA in the treatment diet.

B is the digestibility of the NEAA in the basal diet.

b is the amount of the NEAA in the basal diet.

a is the amount of the NEAA added to the basal diet. The treatment diets are those with additional NEAA.

Calculation for the theoretical efficiency to produce carbon skeleton from digestible amino acids

The theoretical energetic efficiency to produce the carbon skeleton from each AA was calculated based on the molecular formula, molecular weight, nitrogen content and gross energy of AA.

Efficiency of AA to produce carbon skeleton = $[(\text{GEAA} - \text{EUE})/\text{GEAA}]$

Where:

GEAA is the gross energy of AA (adapted from Van Milgen, 2002)

EUE is the energy required to excrete uric acid,

$\text{EUE(kJ)} = \text{gram uric acid} \times 8.37\ \text{kJ}$,

Where 8.37 kJ is the cost to produce one gram of uric acid (Harper, 2000)

The amount of uric acid produced from AA calculated as:

$$\text{gram uric acid from AA} = (\text{gram nitrogen per gram AA}) \times 3$$

Note: each gram of nitrogen is equivalent to 3 gram uric acid (calculated from uric acid molecular formula and the molecular weight).

The gram nitrogen per gram AA (g/g) = molecular weight of nitrogen in AA divided by the molecular weight of the same AA. Similar logic was used for all AA.

5.3.9. Statistics Analysis and determination of the efficiency of NEAA utilisation for fat deposition

The data of four outlier birds were excluded from the performance analysis. Outliers were established by using Boxplots quartile method (Minitab 15, 2006). The outliers were identified using inter-quartile range (IQR). The extreme values (within 1.5 times the inter-quartile range from the upper or lower quartile) were identified as outlier. The experiment data were analysed by orthogonal polynomial contrasts (GLM, SAS 2004) to examine whether responses to increasing levels of energy were a linear or quadratic effect. The AME was analysed as described in Chapter 3, Section 3.3.9.

The following models were used to estimate the efficiency of NEAA utilisation for fat deposition.

Model 1: Overall efficiency of NEAA utilisation for fat and protein deposition

The efficiency of NEAA utilisation for fat (*kf*) and protein (*kp*) deposition were obtained from multiple linear regression of ileal digestible energy intake (iDEI) as a function of energy retention as protein (ERP) and energy retention as fat (ERF), using this model:

$$\text{iDEI} = \text{intercept} + \left(\frac{1}{k_f}\right)\text{ERF} + \left(\frac{1}{k_p}\right)\text{ERP}$$

Model 2: Predicting the efficiency of digestible energy utilisation for fat deposition from extra NEAA (kf_N)

The efficiency of digestible energy utilisation for fat deposition from NEAA was estimate relative to basal treatment group, using a linear regression of iDEI as a function of ERF.

$$\text{iDEI} = \text{intercept} + \left(\frac{1}{k_f}\right)\text{ERF}$$

Model 3: Correcting to protein deposition free ileal digestible energy intake (iDEIfpd)

To avoid the error in model 1 and 2 that came from protein deposition, the digestible energy for protein deposition requirement was subtracted from the total iDEI. Rearrange of the equation given in model 1 as:

$$\text{iDEI} - \left(\frac{1}{k_p}\right)\text{ERP} = \text{intercept} + \left(\frac{1}{k_f}\right)\text{ERF}$$

To correct a protein deposition free iDEI (iDEIfpd) it is Assumed that *kp* = 0.66 (Boekholt *et al*., 1994).

Then

$$\text{iDEIfpd} = \text{intercept} + \left(\frac{1}{k_f}\right)\text{ERF}$$

5.4. Results

5.4.1. Diets

The chemical analysis of the AA and crude protein of the three diets are shown in Table 5.3. The AA analyses of the experimental diets were in close agreement with the calculated values. The dietary individual and total essential amino acid (EAA) were similar in all the experimental diets. However, the dietary Glycine, Glutamic acid, Serine, Alanine, Aspartic acid and Proline increased as the synthetic NEAA supplementation increased. The crude protein value increased when the diet was supplemented with NEAA.

5.4.2. Feed intake and performance

Linear and quadratic ($P < 0.05$) responses were observed for final live body weight, feed intake and the daily body gain with increasing intake of NEAA. The birds fed a diet with additional NEAA had a greater final live body weight, feed intake and daily body weight gain than those fed the basal diet ($P < 0.05$).

The feed per gain was decreased linearly ($P < 0.05$) in response to increasing NEAA intake (Table 5.4). The feed per gain was higher for birds fed basal diet compared with birds fed the B+NEAA1 or B+NEAA2 diet.

Table 5.3. Analysed amino acid and crude protein composition (g/kg) of treatment diets

Diet	Basal	B+NEAA1	B+NEAA2
EAA(g/kg as fed)			
Lysine	11.7	12.1	11.6
Methionine	5.75	6.05	5.74
Cysteine	3.03	2.83	2.74
Arginine	10.4	10.5	10.4
Threonine	7.34	7.29	7.07
Valine	11.3	11.5	11.1
Isoleucine	8.93	9.06	8.69
Leucine	17.7	17.5	16.9
Tyrosine	7.81	7.89	7.71
Phenylalanine	9.79	9.98	9.39
Histidine	5.80	5.91	5.69
Total EAA	**99.55**	**100.61**	**97.03**
NEAA			
Glycine	6.75	16.7	23.6
Serine	8.42	15.7	21.0
Glutamic acid	35.1	46.5	51.7
Alanine	8.35	17.3	22.3
Aspartic acid	17.3	27.7	35.1
Proline	14.4	22.9	31.8
Total NEAA	**90.32**	**146.8**	**185.5**
Crude protein	204	231	255

Table 5.4. Influence of extra non-essential amino acids intake on broiler performance between 21and 42 days of age

Diet	n	LBWi	LBWf	FI(g/d)	Extra NEAA intake	ADG (g)	FCR (g/g)
basal	15	952	2791^{b}	153.0^{b}	-	92^{b}	1.67^{a}
B+NEAA1	14	961	2979^{a}	162.0^{a}	9.0	101^{a}	1.61^{b}
B+NEAA2	15	952	3008^{a}	164.0^{a}	15.6	103^{a}	1.60^{b}
Pooled SE		9.22	18.2	1.05	-	0.80	0.014
P-value		0.723	0.0001	0.0001	-	0.0001	0.002
Linear effect		0.98	0.0001	0.0001	-	0.0001	0.001
Quadratic effect		0.42	0.001	0.0110	-	0.0008	0.154

[a,b] Means in the same column with different superscripts are significantly different ($P < 0.05$). LBWi is the initial live body weight, LBWf is the final live body weight, ADG is the average daily gain, FCR is the feed conversion ratio.

5.4.3. Apparent metabolisable energy and ileal digestibility

The measured AME value of the basal diet was similar to that of B+NEAA2 diet but greater than that of the B+NEAA1 diet for the period between 27 and 31 or between 37 and 41 day of age ($P < 0.05$). There was no effect of age on AME values ($P > 0.05$, Table 5. 5).

Table 5.5. Apparent metabolisable energy (AME; MJ/kg) of the diets, determined between 28 to 31 and 38 to 41 days of age

Diet	n	Age	AME (MJ/kg)
Basal	6	28-31 days	13.71[a]
	6	38-41days	13.61[a]
B+NEAA1	6	28-31 days	13.46[b]
	6	38-41days	13.48[b]
B+NEAA2	6	28-31 days	13.68[ab]
	6	38-41days	13.55[ab]
SEM			0.068
Main effects			
Diet			
Basal	12		13.66
B+NEAA1	12		13.47
B+NEAA2	12		13.61
SEM	12		0.091
Age	24		13.61
28-31 day	24		13.54
38-41 day			0.040
SEM			
P-value			
	Diet		0.32
	Age		0.21
	Diet × age		0.54

[a,b] Means in the same column with different superscripts are significantly different ($P < 0.05$).

A linear tendency ($P = 0.06$ to 0.09) occurred with increasing the NEAA intake for the ileal digestibility of organic matter, protein, fat, starch and energy. No effects were observed ($P > 0.05$) for the ileal ash digestibility with increasing the NEAA intake (Table 5.6).

Table 5.6. Influence of extra non-essential amino acids intake on the ileal digestibility of nutrients

Diet	n	OM[1]	Protein	Fat	Starch	Energy	Ash	NEAA
Basal	6	0.74	0.77	0.91	0.91	0.74	0.39	-
B+NEAA1	6	0.77	0.80	0.93	0.95	0.77	0.39	0.85
B+NEAA2	6	0.80	0.83	0.95	0.97	0.79	0.39	0.89
SEM		0.02	0.024	0.017	0.024	0.02	0.025	-
P-value		0.170	0.162	0.224	0.204	0.225	0.980	-
linear effect		0.065	0.061	0.09	0.093	0.089	0.930	-
Quadratic effect		0.910	0.940	0.910	0.570	0.970	0.860	-

[1]OM; is the organic matter digestibility.

5.4.4. Energy and digestible nutrients intake

The ileal digestible protein intake increased linearly ($P < 0.05$) in response to increase NEAA intake. No effects were observed ($P > 0.05$) for the digestibility fat intake with increasing the NEAA intake (Table 5.7). The digestible starch intake increased linearly ($P < 0.05$) in response to increase NEAA intake.

A linear ($P < 0.05$) response was observed for the iDEI and AMEI with increasing the NEAA intake. The AMEI was lowest in the basal treatment group, moderate in the B+NEAA1 treatments and greatest with the B+NEAA2 treatment group ($P < 0.05$). The same patterns of ileal digestible energy intake were observed across all treatments. The differences between the AME intake and ileal digestible energy intake decreased as the birds consumed more NEAA.

Table 5.7. Ileal digestible nutrient intake (g/bird/day) and energy intake (MJ/bird/day) for broiler chickens fed extra non-essential amino acids between 21 and 42days of age[1]

	n	FI	Protein	EAA[2]	NEAA[3]	Fat	Starch	iDEI[4]	AMEI[5]	AMEI-iDEI
Basal	8	153.8[c]	24.2[c]	11.79[c]	10.71[c]	6.4	58.0[c]	1.91[c]	2.10[c]	0.19[a]
B+NEAA1	8	162.0[b]	29.9[b]	13.03[b]	19.46[b]	6.4	61.6[b]	2.07[b]	2.18[b]	0.11[b]
B+NEAA2	8	168.5[a]	35.7[a]	13.57[a]	26.87[a]	6.4	63.7[a]	2.23[a]	2.29[a]	0.06[c]
SEM		0.81	0.18	0.07	0.12	0.31	0.31	0.01	0.01	0.0001
P-value		0.0001	0.0001	0.0001	0.0001	0.259	0.0001	0.0001	0.0001	0.0001
linear effect		0.0001	0.0001	0.0001	0.0001	0.405	0.0001	0.0001	0.0001	0.0001
Quadratic effect		0.375	0.930	0.0005	0.0002	0.156	0.0500	0.960	0.300	0.0001

[a,b,c] Means in the same column with different superscripts are significantly different ($P < 0.05$).

[1]The data presented in this table are for the birds selected for carcass composition (8 birds per group), ileal digestible nutrients intake (g/birds/day) was calculated as nutrient intake multiplied by nutrients ileal digestible coefficient (Table 5.6). [2]EAA is ileal digestible essential AA intake, was calculated as essential AA intake multiplied by protein digestibility coefficient. [3]NEAA is ileal digestible of non-essential AA intake, was calculated as the sum of the digestible non-essential AA intake from basal and from additional NEAA. [4]iDEI is the ileal digestible energy intake. [5]AMEI is apparent metabolisable energy intake.

5.4.5. Body composition

A quadratic ($P < 0.05$) response was observed for the carcass protein concentration with increase the NEAA intake. The birds fed B+NEAA2 diet had a lower carcass protein concentration than those fed other diets ($P < 0.05$).

A linear ($P < 0.05$) response was observed for the carcass fat concentration with increase the NEAA intake. There was no difference in the water ash and energy content between the treatment groups ($P>0.05$, Table 5.8).

Table 5.8 Influence of extra non-essential amino acids intake on broiler de-feathered carcass[1] composition between 21 and 42 days of age

Diet	n	Protein (%)	Fat (%)	Water (%)	Ash (%)	Energy (kJ/g)[2]
Basal	8	17.1[a]	11.34	67.3	2.47	8.80
B+NEAA1	8	17.0[a]	10.58	68.0	2.62	8.63
B+NEAA2	8	16.56[b]	11.21	67.6	2.60	8.75
SEM		0.106	0.23	0.233	0.071	0.098
P-value		0.005	0.07	0.16	0.31	0.46
linear effect		0.430	0.03	0.06	0.16	0.23
Quadratic effect		0.002	0.40	0.77	0.56	0.77

[1]De-feathered carcass; is the whole body with no feather. [a,b] Means in the same column with different superscripts are significantly different ($P < 0.05$). [2]The measured energy content in kJ/gram.

5.4.6. Deposition rates of chemical components

A linear ($P < 0.05$) response was observed for the protein deposition rate with increase the NEAA intake. The birds fed basal diet had a slower rate of protein deposition than those fed the diet with additional NEAA. The birds fed the B+NEAA1 or B+NEAA2 diets had similar protein deposition rates. A quadratic ($P < 0.05$) response was observed for the fat deposition rate with increase the NEAA intake (Table 5.9). The change on water deposition was most likely related to protein changes. The ash deposition was linearly ($P < 0.05$) increased as the birds fed extra NEAA intake.

Table 5.9. Influence of extra non-essential amino acids intake on protein, fat, water and ash gain (g/day/bird) in de-feathered carcass between 21 and 42days of age

	n	Protein	Fat	Water	Ash
Basal	8	14.8[b]	11.06	55.2[b]	2.10[b]
B+NEAA1	8	16.2[a]	11.09	62.3[a]	2.53[a]
B+NEAA2	8	15.88[a]	12.13	62.7[a]	2.54[a]
SEM		0.189	0.332	0.873	0.10
P-value		0.0001	0.055	0.0001	0.007
linear effect		0.0001	0.990	0.0001	0.006
Quadratic effect		0.1090	0.017	0.0010	0.083

[a,b] Means in the same column with different superscripts are significantly different ($P < 0.05$).

5.4.7. Energy partitioning

Linear and quadratic increased ($P < 0.05$) were observed for the heat production as the NEAA intake increased. Whereas, a quadratic increased ($P < 0.05$) was observed for energy retention with increasing the NEAA intake. The energy retention was higher for the birds fed B+NEAA2 than those fed the basal diet (Table 5.10; $P < 0.05$). Broilers fed the B+NEAA2 diet had a numerically higher total energy retention than the birds fed B+NEAA1 diet, but this difference was not significant ($P > 0.05$). In this study, the heat production was approximately 60% of the AME intake. Therefore, the retained energy represents the remaining 40% of AME intake. In addition, the efficiency of AME utilisation for weight gain (k_g) was calculated to be 0.72 for the basal diet, 0.72 for B+NEAA1diet and 0.69, for B+NEAA2 diet (Table 5.10).

Table 5.10. Utilisation of apparent metabolisable energy (MJ/bird) for growing broilers fed extra non-essential amino from 21 and 42days of age

	n	AMEI	HP	AMEm	HI	ER	ERP	ERF	EF	k_g
Basal	8	44.14[c]	26.67[c]	19.83[b]	6.83[b]	17.47[b]	7.36[b]	9.08	1.04[b]	0.72
%		100	60.4	44.9	15.5	39.6	16.7	20.6	2.36	
B+NEAA1	8	45.84[b]	27.58[b]	20.48[a]	7.10[b]	18.26[ab]	8.06[a]	9.08	1.13[a]	0.72
%		100	60.2	44.7	15.5	39.9	17.6	19.8	2.47	
B+NEAA2	8	48.15[a]	29.16[a]	20.67[a]	8.48[a]	18.99[a]	7.90[a]	9.94	1.15[a]	0.69
%		100	60.6	42.9	17.6	39.4	16.4	20.6	2.39	
SEM[1]		0.232	0.298	0.095	0.32	0.291	0.0941	0.272	0.01	0.011
P-value[1]		0.0001	0.0001	0.0001	0.0028	0.0052	0.0001	0.055	0.0001	0.159
linear effect		0.0001	0.043	0.0001	0.560	0.067	0.0001	0.989	0.0001	0.940
Quadratic effect		0.0001	0.0001	0.0002	0.0008	0.005	0.107	0.017	0.0001	0.052

[a,b,c] Means in the same row with different superscripts are significantly different ($P < 0.05$). AMEI is the total apparent metabolisable energy intake, HP is the heat production, calculated from the different between AMEI and energy retained (ER) (kJ/bird), AMEm is the metabolisable energy for maintenance requirement. HI is the heat increment was calculated from the different between the HP and AMEm. ER is the total energy retention, ERP is the energy retention as protein, ERF is the energy retention as fat, EF is the energy retention as feather and k_g is the efficiency of energy utilisation for weight gain was calculated as ER/(AMEI-AMEm). [1] SEM and P-values is related to the absolute energy partitioning values.

5.4.8. Energetic efficiency of fat and protein deposition

Model 1: The overall efficiency of digestible energy utilisation for fat and protein deposition

The efficiency of digestible energy utilisation for fat and protein was 0.75 and 0.28 respectively. This result was based on the multiple linear regressions using the data from all treatment groups (Table 11, Model 1).

Table 5.11. Regression equations to predict the efficiency of digestible energy utilisation for protein (*kp*) and fat (*kf*) deposition

diet		Model regression equation	*kf*	*kp*	R^2
Basal, NEAA1, NEAA2	1	iDEI= -3.56 +3.9 ERP+1.33ERF (9.24) (0.97) (0.51)	0.75	0.28	0.56
Basal, NEAA1, NEAA2	2	iDEI= 28.3 +1.62 ERF (6.19) (0.66)	0.62	-	0.22
Basal, NEAA1, NEAA2	3	iDEIpfd= 16.0 + 1.51 ERF (5.28) (0.56)	0.66	-	0.25

Numbers in the parentheses are the standard errors of above coefficient.
Model 1 multiple linear regression cross all treatment groups, iDEI= intercept + (1/*kf*) ERF+ (1/*kp*) ERP.
Model 2 linear regression iDEI= intercept + (1/*kf*) ERF, data from Basal, NEAA1 and NEAA2 was fitted, assumed extra energy intake would not affect protein retention and the intercept presented the protein deposition and maintenance energy requirement.
Model 3 linear regression iDEIpdf= intercept + (1/*kf*) ERF, iDEIpdf is the net energy after subtracting the energy requirement for protein retention.

Model 2: The efficiency of NEAA utilisation for fat deposition

The kf_N value was estimated as 0.62 using linear regression model with the data from basal, NEAA1 and NEAA2 treatment groups (see Table 11, Model 2). This estimation is based on the assumption that the extra digestible energy from NEAA intake would not increase the protein deposition but increase the fat deposition.

Model 3: Corrected to protein deposition free ileal digestible energy

The results showed that both the protein and fat deposition systematically increased when the birds consumed more NEAA. Further analysis was taken to predict the efficiency of digestible energy utilisation for fat deposition from extra NEAA intake. The efficiency of digestible energy utilisation for fat deposition from extra iDEIfpd was 0.66; this result was based on the linear regression using the data from Basal, NEAA1 and NEAA2 treatment groups (Table 11, Model 3). The iDEIfpd was provided not only from extra NEAA intake,

but also from digestible starch intake (see Table 5.7). Thus, the efficiency of NEAA utilisation for fat deposition calculated using this formula:

$$kf_{N+starch} = 0.66 = (\ kf_N)X + (kf_s\ )Y$$

Rewriting the equation

$$kf_N = \frac{0.66 - (kf_s)Y}{X}$$

Where:

X is a fraction of additional digestible energy from NEAA intake toward fat deposition.

Y is a fraction of additional digestible energy from starch intake toward fat deposition.

kf_s is the efficiency of digestible energy utilisation for fat deposition from starch (used value 0.75 from De Groote, 1974).

kf_N is the efficiency of digestible energy utilisation for fat deposition from NEAA.

This assumes that the maintenance energy requirements coming from digestible starch and NEAA were of similar proportion. From this calculation it is clear that the kf_N would be 0.63.

5.4.9. The theoretical efficiency to produce carbon skeleton from amino acids

The energetic efficiency for essential amino acids to produce carbon skeleton ranged from 0.66 to 0.93 with an average of 0.85. For non-essential amino acids the energetic efficiency to produce carbon skeleton ranged from 0.72 to 0.89 with an average of 0.80 (Table 5.12).

Table 5.12. Energetic efficiency of amino acids to produce carbon skeleton, based on the energy balanced description

AA	Formula	MW(g/*mol*)[1]	Nitrogen (g/*mol*)	GE (kJ/g AA)[2]	N% (g/g AA)[3]	g uric acid[4]	kJ/uric acid[5]	Efficiency[6]
NEAA								
Glycine	C2H5NO2	75.0	14	17	0.186	0.559	4.70	0.72
Serine	C3H7NO3	105	14	16.6	0.133	0.399	3.36	0.80
Glutamine	C5H10N2O3	146	28	20.1	0.190	0.571	4.83	0.76
Alanine	C3H7NO2	89.0	14	22.8	0.157	0.471	3.96	0.83
Aspartic acid	C4H7NO4	133	14	14	0.105	0.316	2.65	0.81
Proline	C5H9NO2	115	14	28.1	0.122	0.365	3.06	0.89
Average of NEAA								**0.80**
Essential amino acids (EAA)								
Arginine	C6H14N4O2	174	56	23.9	0.322	0.966	8.11	0.66
Cysteine	C3H7NO2S	121	14	21.6	0.116	0.347	2.92	0.86
Histidine	C6H9N3O2	155	42	24.6	0.271	0.813	6.83	0.72
Isoleucine	C6H13NO2	131	14	31.6	0.107	0.321	2.69	0.91
Methionine	C5H11NO2S	149	14	21.2	0.094	0.282	2.37	0.88
Phenylalanine	C9H11NO2	165	14	31.6	0.085	0.255	2.14	0.93
Threonine	C4H9NO3	119	14	20.3	0.118	0.353	2.97	0.85
Tyrosine	C9H11NO3	181	14	27.2	0.077	0.232	1.95	0.93
Valine	C5H11NO2	117	14	29.5	0.120	0.359	3.02	0.90
Tryptophan	C11H12N2O2	204	28	30.2	0.137	0.412	3.46	0.88
Average of EAA								**0.85**
Overall average								**0.83**

[1] MW(g/*mol*) is the molecular weight (g/*mol* AA). [2]GE; is the GE per gram AA (adapted from Van Milgen 2002). [3] N% is the nitrogen percentages calculated as molecular weight of nitrogen content in AA divided by the molecular weight of the AA. [4]g uric acid produced from AA calculated as N content multiplied by 3. [5]kJ/uric acid the energy required to produce the uric acids calculated as gram uric acid multiplied by 8.37 kJ. [6]Efficiency of the energy retained in carbon chain pivot.

5.5. Discussion

The experiment was designed to assure that the intake of the basal diet was identical among the treatment groups. Therefore, it was anticipated that the excess supply of NEAA would have an effect on fat deposition. Indeed, the additional energy from NEAA resulted in increase in both the fat and protein deposition. This finding suggests that the levels of extra NEAA intake was either insufficient or was not completely utilised for fat growth. Also, the birds fed the diet with additional NEAA had higher essential and NEAA intake compared with those fed basal diet.

5.5.1. Feed intake and performance

The growth performance parameters for live body weight, daily gain and feed per gain showed the superiority of the birds fed extra energy in form of NEAA compared to those fed the basal diet. It is known that bird performance improves with the increase in energy intake (Shyam *et al*., 2007; Boekholt *et al*., 1994; Leeson, 1996 b; Arafa *et al*., 1983). Broiler body weight gain and the feed per gain improved when the birds were fed a diet supplemented with 2% NEAA between 5- 21 day of age (Corzoa *et al*., (2005). Aletor *et al*., (2000) also reported the feed per gain improved when broiler diets were supplemented with NEAA. Moran, (2011) reported that the deficiency of NEAA (glycine-serine and proline) with low crude protein diet could reduce the growth performance.

5.5.2. Description of body components growth rates

In our experiment, the protein deposition was increased when the birds were fed the B+NEAA1 diet, but no further increases were observed when the birds consumed more NEAA with the B+NEAA2 diet. For fat deposition, the birds fed the B+NEAA1 diet had similar fat deposition to the basal diet, but the fat deposition increased when the birds consumed more NEAA with the B+NEAA2 diet. Extra NEAA intake appeared to be supporting protein deposition and once the requirement for protein deposition was met, the excess of NEAA was used for fat synthesis. This finding suggests that a deficient supply NEAA could cause a breakdown of EAA for formation of NEAA, which means a limitation of EAA for protein synthesis and then a reduction in protein deposition. Moran and Stillborn, (1996) showed that added glutamic acid to low crude protein diet improved live weight gain in broiler chickens. The fat deposition was increased when the birds were fed the B+NEAA2 diet compared to those fed basal diet,

this is because of the additional AA were being converted into lipids by lipogenesis process, as a mechanism of store nutrients in the body. The broiler carcass fat content increases when the glycine and glutamic AA were added to low protein diet balanced with EAA (Deschepper and de-Groote, 1995).

It appears that the provision of NEAA in broiler diets is important to prevent AA deficiencies. The NEAA should be considered in broiler industry to improve the growth performance, particularly when boilers have restricted feed intake.

5.5.3. Partitioning of energy intake

Energy is retained in fat, protein and feathers and lost as heat and maintenance energy requirements. Compared to the birds fed the basal diet, feeding additional NEAA was associated with greater energy retention and heat production. The energy retention in broilers is commonly between 35 and 41.4% of the ME intake (Sakomura *et al*., 2005; Latshaw and Moritz, 2009; Lopez and Leeson, 2005), in this study the energy retention was between 39.4 and 39.9% of AME intake. Birds fed extra energy intake from B+NEAA2 diet had greater total energy retention which suggests that the efficiency of energy utilisation increased with an increased energy intake.

5.5.4. Efficiency of digestible energy utilisation for fat and protein deposition (Model 1)

This experiment was designed to estimate the efficiency of additional NEAA utilising for energy. Uric acid is assumed the end product of NEAA utilisation. The uric acid might be degraded by caeca microflora and can be recycled as nitrogen or converted to volatile fatty acids (Svihus *et al.,* 2013; Barnes *et al.,* 1972). The volatile fatty acids cannot be accounted for in the AME measurement. Consequently, the measured AME was higher than ileal digestible energy (i.e. AME was 13.7, 13.5 and 13.6 MJ/kg when the ileal digestible energy was 12.4, 12.8 and 13.2 MJ/kg for basal, B+NEAA1 and B+NEAA2 diets, respectively). Therefore, caeca microflora metabolism can be providing differences between digestibility measured from the ileum or fecal excreta (Scott *et al*., 1998; Ravindran *et al.,* 1999). To avoid the effect of caeca microflora functions on energetic efficiency, the ileal digestible energy was considered to be the best estimate the energetic efficiency.

Fat deposition is a more efficient process than protein deposition as indicated by a higher *kf* value in contrast to a lower *kp* value estimated with the broilers chicken in this study. Boekholt *et al.* (1994), Nieto *et al*. (1995) and Chuan *et al*, (2010) reported *kf*- values of 0.86, 0.65 and 0.84 and *kp*-values of 0.66, 0.58, and 0.55, respectively. In the present study, the *kf* and *kp* were appears to be lower than those reported in the previous studies. In our case we are considering only the effect of additional NEAA on protein and fat deposition which is likely to have resulted in low values for *kf* and *kp* compared with previous studies.

5.5.5. Efficiency of digestible energy utilisation for fat deposition from extra NEAA (Model 2 and 3)

Efficiency of digestible energy utilisation for fat deposition from extra NEAA intake (kf_N) was estimated to be 0.62, using a linear regression model (Table 11, Model 2). The diet composition and the daily feed intake schemes were designed to limit the protein deposition in broiler bodies. However, due to response of protein deposition to extra NEAA intake, further analysis was taken to correct for the residual protein response. The digestible energy intake was corrected to a zero protein deposition (Table 11, Model 3). In addition, the effect of starch intake was also corrected for and the kf_N calculated to be 0.63. In this regression equation the coefficient of determination was low ($R^2 = 0.25$). The poor coefficient of determination was principally caused by large variation of fat deposition in same treatments, small number of observations per treatment group and the NEAA intake was not totally utilised for fat deposition purposes.

Information on the efficiency of digestible energy utilisation for fat deposition (over the maintenance and protein retention requirement) from NEAA intake in the broilers is limited. Noblet *et al.* (2010) indicated that the NE/ME ratio varies greatly with the nutrient composition of the diet with efficiency from fat (0.90) and starch (0.80) and protein (0.60), the energy efficiencies were estimated from the total energy retention. However, in our case only the energy efficiency of fat deposition was considered. In an experiment using pigs, the energy efficiency of using crude protein intake for fat deposition was 0.52-0.53 (Black and De-lange, 1995; Van Milgen, 2001a), while Kielanowski (1971) give a somewhat higher efficiency of 0.60-0.65. Roux, (2009) and Quiniou *et al*., (1996) reported that the theoretical efficiency from digestible crude protein to body fat was 0.67. These results are congruent with those of our study.

5.5.6. Using a theoretical aspect to calculate the energy efficiency for fat deposition from amino acids

When AAs are absorbed and in excess of the requirement for protein deposition, the AAs will be deaminated (removal of the amino group) and the carbon skeleton converted to acetyl-CoA, pyruvate, α-ketoglutarate, succinyl-CoA, fumarate and oxaloacetate, which are intermediate components of the citric acid cycle. Part of amino group can be used to synthesis NEAA and the remaining is converted to uric acid through the urea cycle.

No energy input is required for the synthesis and catabolism of glutamate, aspartate, alanine and glycine. Also, minimal energy is required for the synthesis and breakdown of the other NEAA (asparagine and glutamine) (Van Milgen, 2002). Based in this information, the average energetic efficiency to produce a carbon skeleton from NEAA was calculated to be 0.80 and 0.85 from EAA (Table 5.12). Furthermore, the average energetic efficiency of the intermediate components is 0.81 (Van Milgen, 2002), then the maximum efficiency of NEAA utilisation for fat deposition is approximately 0.80 × 0.81 = 0.65 for NEAA, and 0.85 × 0.81 = 0.69 for EAA. The average of all AA will be 0.67.

The above calculation is the maximum efficiency and this efficiency may be decreased when associated with other metabolic processes, such as protein and fat turnover that may be involved with lipid gain.

In the present study, the theoretical values for the efficiency of NEAA utilisation were calculated, based on input/output energy and they do not include the energy (ATP) cost for other metabolic process. Schulz (1978) reported that, in a gluconeogenesis process, the AAs (except lysine and leucine) can be converted to urea, CO_2, H_2O, ATPs and glucose. The glucose produced ranges from 0.15 to 0.5 *mol*/*mol* AA. In addition this glucose can be completely oxidised and produce ATPs. Some ATP (obtained from glucose) can be used for other metabolic process (maintenance) instead of using the glucose for fat deposition.

It is important to mention that, when protein deposition is limited by energy intake, part of the AA will catabolism to generate ATP and therefore, the catabolism of AA to generate ATP could decrease the efficiency of AA utilisation to form fat. In addition, the NEAA is required for basal processes (maintenance requirements) and this is directly associated with the amount of body mass. As the rate of body protein

increases, the requirement of AA also increases for turnover of body protein and then NEAA can be used for maintenance requirements, instead of being converted from NEAA into fat (Moughan and Fuller, 2003).

Furthermore, nutrient intake can influence partitioning of NEAA. It has been suggested that NEAA may become essential when essential amino acids from dietary sources is limited. In this respect, NEAA is directly involved in protein synthesis and it is not converted through the gluconeogenesis process (Corzoa *et al*., 2005; Waldroup *et al*., 2005). In a study by Makoto *et al*. (1998), carcass fat decreased with an increase in EAA/NEAA ratio in dietary protein, for male broiler chickens reared between 7 and 21 days of age. They mentioned that the EAA/NEAA ratio might shift the energy intake to be used for protein deposition, instead of fat deposition.

The efficiency of utilisation of AA may be vary among the individual AA. The cost for excretion of N from an AA as uric acid requires from 2 to 4 kJ for most AA. However, the cost for the excretion of N from histidine and arginine were calculated to be 6.8 and 8.1 kJ, respectively (Table 5.12), indicating the histidine and arginine are used less efficiently. Macleod (1997) indicated that excretion of each N from an AA as uric acid requires 6 moles ATP/g atom N and a molecule such as histidine that has 3 N will require 18 ATP, which may decrease the energy available for production. Sklan and Plavnik (2002) reported that the chicken diets should be formulated to provide all AA sufficiently for protein synthesis and that excess AA should be avoided as this decreases the efficiency of energy utilisation.

The experimental design used here provides a method for predicting the efficiency of NEAA utilisation for fat deposition. The energetic efficiency of fat deposition from dietary crude protein is considered to be low compared with the efficiency of fat deposition from dietary fat or starch (Noblet *et al.*, 1994; 2004; 2010; Van Milgen *et al.*, 2001a). This is because of the excess of AAs is converted into one of pyruvic acid, acetyl-CoA, α-ketoglutarate, succinyl-CoA, fumarate or oxaloacetate and then converted into lipids or glucose by lipogenesis and gluconeogenesis processes respectively. The lipogenesis process from AA is a more costly process than from starch or fat, since there is additional energy cost of 8.37 kJ for each gram of uric acid synthesis (Harper, 2000).

5.6. Conclusion

The live body weight, daily gain and feed per gain were improved when the birds consumed extra NEAA. The efficiency of digestible energy utilisation for fat deposition from NEAA was 0.63. Similar to this finding the theoretical value was calculated to be 0.65 from NEAA. The results from this experiment provide important data about efficiency of NEAA utilisation for fat deposition, which will be used to improve a broilers growth model.

CHAPTER 6

A method to predict the upper limit for body protein deposition and the minimum lipid to protein ratio in broiler chickens

6.1. Abstract

An experiment was conducted to determine the upper limit for daily body protein deposition (Pdmax) and the minimum lipid to protein ratio (L/P ratio) for male Ross 308 broiler chickens. Birds were allocated to four energy intake levels; low energy (L-Energy), medium energy (M-Energy), medium-high energy (MH-Energy) or high energy (H-Energy). The daily protein, amino acids, vitamins and minerals intake were calculated to be non-limiting for growth. The protein and lipid deposition rate were measured by serial of comparative slaughter technique; between approximately 0.60 to 1.0, 1.0 to 1.75, 1.75 to 2.50, 2.50 to 3.25, and 3.25 to 4.0 kg of live body weight.

The results indicate that the daily body weight gain increased linearly with age and metabolisable energy intake. The predicted Pdmax was 22 g/day. According to a broken line relationship model, the protein deposition rate was increased with AME intake above maintenance requirement (AMEIp) up to the break point of 1.2 (MJ/d), but further increases of AMEIp did not lead to increase in protein deposition. When the birds achieved the Pdmax the lipid deposition increased sharply as AMEIp increased. The body weight and energy intake affect the L/P ratio and the minimum value of L/P ratio was observed at 0.31.

6.2. Introduction

Genetic selection, over the past few decades, has resulted in improvements in the growth rate and feed per gain of broilers. The rate of protein deposition has a considerable impact on the growth rate, feed efficiency and body composition of broiler chickens (Sakomura *et al.*, 2005; Gous, 1998). Information about the upper limit of protein deposition (Pdmax) for modern broiler strains is scarce. Therefore, knowledge about the Pdmax of broilers is important for the formulation of diets that maximise growth performance and profitability. Under practical growing condition, the genetic potential of maximum protein deposition may be hard to achieve, because of effects due to factors such as nutrition, level of disease and environmental condition (Whittemore et al., 2001; Moughan and Fuller, 2003). For this reason the term of "operational Pdmax"

has been considered with operational Pdmax being determined under typical production conditions (Morel *et al*., 1993).

A high energy diet is used to maximise the growth rate of modern broiler chickens, but may cause excess fat deposition with unrestricted feeding. This can affect the total edible carcass yield since the abdominal fat is removed during the evisceration process (Sadeghi and Tabiedian, 2005). Broiler growth rate is improved when birds are fed high energy and protein in starter and finisher diets (14.2 MJ ME and 250 crude protein per kg for starter diet and 14.3MJ ME and 220 crude protein per kg for finisher diet), but the carcass fat is also increased (Wiseman and Lewis, 1998). Establishing the ME requirement for maximum lean tissue growth rate with minimum fat deposition is important for increasing the accuracy of feed formulation for modern broiler chickens.
The effect of dietary energy to protein ratios on growth rate and body composition has been investigated previously (Jones and Smith, 1986; Bartov, 1992; Jones and Wiesman, 1985). Variable in the ME concentration in the diet with constant dietary energy to protein ratio has different effect on broiler performance and energy efficiency (Kamran *et al*., 2008). In pig experiments, the effect of energy and protein intake on the efficiency of protein deposition is often used instead of considering the dietary energy to protein ratio (de-Greef and Verstegen, 1993; Quiniou *et al.*, 1995; Weis *et al*., 2004). The relationship between energy intake and protein deposition is reflected in the ratio of whole body lipid to whole body protein (minL/P, Whittemore, 1995; Moughan *et al.*, 1987; Weis *et al*., 2004). The minL/P is important for model development and it represents the effect of energy intake, or body weight, on the partitioning of retained energy between fat and protein, during the energy-dependent phase of protein deposition (de Lange *et al*., 2008). During this phase, some amino acids are preferentially catabolised, in order to provide energy to support lipid deposition (Moughan and Fuller, 2003). During the energy-dependent phase, the protein deposition is not limited by the animal upper limit of protein but by the energy intake.

Information about Pdmax and minL/P ratio at different body weights in growing broilers could be used in broiler growth simulation models. The Pdmax will represent the maximum protein deposition, where the minL/P ratio will used to describe the partitioning of production energy between protein and lipid deposition when ME intake, but not protein intake, is limiting. Thus, Pdmax and minL/P ratio would provide rules for ME partitioning in a broiler growth simulation model.

The objectives of the present study were to determine the genetic upper limit of daily body protein deposition (Pdmax), the minimum lipid to protein ratio (minL/P ratio) and to establish the effects of ME intake on the deposition rate of body chemical components in male Ross broiler chickens. The hypotheses proposed were, that (1) for broiler chickens between 0.60 and 4.0 kg of LBW, protein deposition rate would increase linearly with increasing the ME intake, until approached the maximum protein deposition rate. (2) When the protein deposition limited by ME intake, the ratio between protein and lipid depositions would represent the minL/P ratio.

6.3. Materials and Methods

The procedures for this experiment were approved by Massey University Animal Ethics Committee as described in Chapter 3, Section 3.3. The Ethics Code number was MUAEC 12/116.

6.3.1. Diet

The diets were formulated to ensure that dietary intake of vitamins, minerals, and ideal balanced protein exceeded the Ross 308 requirements for growing-finishing broiler chickens (Ross 308 recommendation is 220 g crude protein and 13.4 MJ ME per kg), but with four different ME intakes manipulated by feed restriction (Table 6.1). This was done to ensure that ME intake and live body weights (LBW) were the only factors affecting broilers performance and carcass composition.

Table 6.1. Ingredients and composition of diets fed to growing broilers, based on intensive nutrients with variable metabolisable energy intake

Ingredient (g)	L-Energy	M-Energy	MH-Energy	H-Energy
Maize	510	510	510	510
Soybean meal, 48%	332	332	332	332
Casein	80	80	80	80
Vegetable oil	0.0	30	60	90
DL-Methionine	4.0	4.0	4.0	4.0
Lysine. HCI	3.0	3.0	3.0	3.0
Threonine	2.0	2.0	2.0	2.0
Limestone	13	13	13	13
DCP	17	17	17	17
Salt	3.0	3.0	3.0	3.0
Premix [1]	3.0	3.0	3.0	3.0
Titanium	3.0	3.0	3.0	3.0
Total diet weight after mixing (g)[2]	970	1000	1030	1060
Calculated analysis (g/kg as fed)				
ME (MJ/kg)	12.55	13.33	14.06	14.75
Total crude protein	286	278	270	262
Digestible crude protein	246	238	232	225
Lysine	21.4	20.8	20.2	19.6
Methionine	9.50	9.20	8.90	8.70
Methionine + Cysteine	13.4	13.0	12.6	12.3
Threonine	14.3	13.9	13.5	13.1
Calcium	10.5	10.2	9.90	9.60
Available phosphorus	6.00	5.80	5.60	5.50
Sodium	2.50	2.40	2.30	2.20

[1]Supplied per kilogram of diet; Co, 03mg; Cu, 3.0 mg; Fe, 25mg; I, 1 mg; Mn, 125mg; Mo, 0.5 mg; Se, 200 μg; Zn,60 mg; antioxidant, 100 mg; biotin, 0.2 mg; calcium pantothenate, 12.8 mg; cholecalciferol, 60 μg; cyanocobalamin, 0.017 mg; folic acid, 5.2 mg; menadione, 4 mg; niacin,35 mg; pyridoxine,10 mg; trans-retinol, 3.33 mg; riboflavin, 12 mg; thiamine, 3.0 mg; dl-α-tocopheryl acetate, 60 mg; choline chloride, 638 mg. [2] The total in this row reflects relative differences in the daily feed intake among the treatment groups.

6.3.2. Birds and housing

Two hundred male broilers (Ross 308) were reared in floor pens and fed a commercial starter diet (230 g crude protein and 12.6 MJ ME per kg) from 1 to 13 days of age. At 14 days of age, a hundred birds were selected on weight basis (593 ± 16 g; mean ± SD). Ninety-six birds were allocated to four treatment groups and placed in individual cages (24 birds per treatment). The four remaining birds were slaughtered at 14 days of age to measure the initial carcass weight, initial feather weight, initial body composition and used as comparison to birds slaughtered as the trial proceeded. For each dietary treatment group the 24 birds were divided into 20 birds for carcass composition and four birds for AME and ileal measurements. The birds were housed in an

environmentally controlled room with 20h of fluorescent illumination per day. The temperature was maintained at 31 ºC on day one, and gradually reduced to 22 ºC by 21 days of age. The birds were fed the four treatment diets between approximately 0.60 kg to 4.0 kg LBW. For each dietary treatment, the birds were slaughtered once they achieved one of five slaughter body weights which were 1.0, 1.75, 2.50, 3.25 and 4.0 kg LBW. At each target slaughter body weight, four birds were randomly selected and slaughtered for each treatment. Individual LBW and feed intakes were recorded daily.

6.3.3. Feed allowance procedure

The experimental diets were formulated to ensure that the bird's daily intake of digestible balanced protein, amino acids, vitamins and minerals exceeded requirements, and only ME intake was variable. As a consequence of this design, AME intakes across treatments were changed by adding extra soybean oil to the diets (see Table 6.1).

In order to establish the daily feed allowance (g/d), the birds in the H-Energy diet were provided free access to feed to establish the *ad-libitum* feed intake. Then, the daily feed allowances for the other three treatments were established based on the feed intake achieved from the birds fed H-Energy diet on the previous day. The daily feed allowances were calculated as:

$FA_{\text{H-Energy at day (i)}} =$ *ad-libitum*

$FA_{\text{MH-Energy at day (i+1)}} = FI_{\text{H-Energy at day (i)}} \times (1.03/1.06)$

$FA_{\text{M-Energy at day (i+1)}} = FI_{\text{H-Energy at day (i)}} \times (1/1.06)$

$FA_{\text{L-Energy at day (i+1)}} = FI_{\text{H-Energy at day (i)}} \times (0.97/1.06)$

where:

i is the age of birds.

The equation coefficients (1.03/1.06), (1/1.06) and (0.97/1.06) was taking into account the relative additional oil as described on formulated diets (Table 6.1).

Any spillage or feed refusals were collected daily and weighted for each individual bird to determine the daily feed intake.

6.3.4 Body composition

The birds were weighed daily and the average LBW for each treatment group was calculated immediately. When the treatment groups reached approximately 1.0, 1.75, 2.5, 3.25, or 4.0 kg LBW, four birds were randomly selected from each group, weighed,

killed by cervical dislocation, de-feathered and weighed again. De-feathered carcasses (whole bird with no feathers) were stored at −20°C. The carcasses were minced, subsampled and processed as described in Chapter 3, Section 3.3.6.

6.3.5. Apparent metabolisable energy and ileal digestibility

Four birds from each treatment group were used to measure the AME and ileal digestibility. The AME was measured at two periods. The first AME was conducted at a live body weight of 1.86 ± 0.21 kg (mean ± SD) and the second AME was conducted at a live body weight of 3.98 ± 0.27 kg over four days for each AME. The AME procedure as described in Chapter 3, section 3.3.4.

Titanium oxide was included (0.3%) as an indigestible marker in all diets. When the excreta collection for the second AME was finished, the same birds were euthanised and ileal digesta was collected and processed as described in Chapter 3, Section 3.3.5.

6.3.6. Laboratory analysis

The laboratory analysis was carried out as described in Chapter 3, Section 3.3.7.

6.3.7. Calculations

The average daily gain (ADG) for each bird was calculated as the final observed LBW minus the initial observed LBW divided by time (number of days). The feed conversion ratio was calculated as the total feed intake divided by the LBW gain.

The daily protein gain per bird was calculated as the final observed protein content minus the initial estimated protein content divided by the number of days. The initial estimated protein content was derived by multiplying the LBW and the mean of protein percentage measured from previously slaughtered birds. Similar calculations were used to calculate the fat, water, ash daily gain.

The calculations of AME, ileal digestibility and AME for maintenance (AMEm) were made as described in Chapter 3, Section 3.3.8.

The daily metabolisable energy intake above the maintenance requirement (AMEIp) was calculated as;

AMEIp = (AMEI-AMEm). AMEIp was considered because only energy above the maintenance requirements is available for protein and lipid deposition. The AMEm was assumed to be 649 kJ per kg metabolic $LBW^{0.6}$ (Lopez and Leeson, 2005).

6.3.8. Statistics

Growth performance and carcass composition for each specific growth period was analysed by ANOVA (SAS, 2004) using individual bird as the experimental unit. Differences were considered to be significant at $P < 0.05$ and significant differences between means were separated by the Least Significant Difference Test.

The AME was measured at two LBW range (at LBW of 1.86 ± 0.21 kg and at LBW of 3.98 ± 0.27). The AME was analysed by using a linear model (PROC GLM) that included the fixed effects of LBW, diet and their interaction, a random effect of bird nested within diet, which was used as the error term to test for the diet effect. The effects of LBW and LBW × diet were tested against the means square error (SAS, 2004).

The protein and fat deposition rates (y, g/d) was regressed against AMEIp (x, MJ/d) using a broken line linear $y= \alpha + \beta1\ x$, if $(x > c)$ else, $y = \alpha + c\ (\beta1 - \beta2) + \beta2\ x$ and quadratic $y = \alpha + \beta1\ x + \beta2\ x^2 + e$ regression models. These broken-line and quadratic models were conducted using PROC NLIN (SAS, 2004).

The relationships between carcass water and carcass ash (y, g/d) with carcass protein (x, g/d) were determined, by fitting a power regression function as $y = \alpha\ x^b$. Where:

a is the extrapolation of y for x = 1, b = allometric coefficient, the ratio of percentage change in y to the corresponding percentage change in x.

In order to examine the effect of LBW and AMEIp on the L/P ratio (y),

a parallel-slopes analysis of covariance with PROC GLM was used as:

$y = \beta0 + LBW + \beta1AMEIp + \beta2\ LBW*AMEIp + e$.

where β0 is the intercept, β1is the overall slope, β2 is the deviation of overall slope for LBW, LBW is the fixed effect of live body weight, AMEIp(x) is the covariate, LBW*AMEIp, is the interaction between LBW and AMEIp to test for the homogeneity of the slope, e is the random residual error.

6.4. Results

The data of four birds were excluded from the carcass composition analysis due to leg problems, illness or death. During excreta collections for AME measurements one bird died and the data of this bird was excluded. The data for three birds from ileal

measurements were excluded because one died and two birds had outlier results for titanium ileal content.

6.4.1. Diets

The amino acids and protein content of the experimental diets (Table 6.2) were in close agreement with the calculated values (Table 6.1). The analysed nutrient composition of the experimental diet was higher in comparison with that given for the Ross requirement, indicating that the nutrient intake did not limit the protein deposition.

Table 6.2. Analysed amino acid and crude protein composition of experiment diets

Diet	L-Energy	M-Energy	MH-Energy	H-Energy
Composition (g/kg as fed)				
Lysine	19.1	18.5	18.4	17.2
Methionine	9.00	9.40	8.90	8.30
Cysteine	3.50	3.40	3.50	3.20
Arginine	16.2	15.1	16.0	14.5
Threonine	12.1	12.0	11.4	11.3
Valine	16.0	15.3	15.4	14.2
Isoleucine	13.1	12.5	12.7	11.7
Leucine	24.0	23.0	23.1	21.0
Tyrosine	10.8	10.7	10.6	9.60
Phenylalanine	13.9	13.2	13.5	12.5
Histidine	6.90	6.70	6.70	6.30
Serine	12.3	12.0	12.4	11.3
Proline	18.7	18.2	18.0	16.6
Glycine	9.90	9.30	9.80	9.00
Alanine	11.0	10.4	10.8	9.80
Aspartic Acid	26.4	24.6	25.8	24.1
Glutamic Acid	52.4	49.6	50.2	45.8
Crude protein	288.1	279.8	270.9	258.9

6.4.2. Apparent metabolisable energy

The AME was higher ($P < 0.05$) for the birds fed the H-Energy than those fed L-Energy, M-Energy and MH-Energy. The AME for birds fed M-Energy and MH-Energy was the same ($P > 0.05$), but higher than those fed L-Energy ($P < 0.05$, Table 6.3). The difference in AME observed for each diet was not dependent on the body weight of the birds.

The ileal digestibility coefficients of protein, energy, ash and organic matter were the same among all treatments (Table 6.4, $P > 0.05$).

Table 6.3. Apparent metabolisable energy (MJ/kg) as fed for the experimental diets, determined at a live body weight (LBW) range from 1.6 to 2.0 kg and 3.8 to 4.2 kg

diet	n	LBW range	AME(MJ/kg)
L-Energy	4	1.6-2.0 kg	12.77
	4	3.8-4.2 kg	12.61
M-Energy	4	1.6-2.0 kg	13.51
	4	3.8-4.2 kg	13.77
MH-Energy	4	1.6-2.0 kg	14.10
	4	3.8-4.2 kg	13.97
H-Energy	4	1.6-2.0 kg	14.73
	3	3.8-4.2 kg	14.56
Pooled SE			0.341
Main effects			
Diet			
L-Energy	8		12.69[c]
M-Energy	8		13.64[b]
MH-Energy	8		14.03[b]
H-Energy	7		14.83[a]
Pooled SE			0.276
LBW			
1.6-2.0 kg	16		13.79
3.8-4.2 kg	15		13.75
Pooled SE			0.172
P-value	Diet		0.003
	LBW		0.845
	Diet*LBW		0.902

[1] The number of observations that used for AME determination. [a,b,c] Means in the same column with different superscripts are significantly different ($P < 0.05$).

Table 6.4. Influence of diet type on the apparent ileal digestibility coefficient of nutrients

Diet	L-Energy	M-Energy	MH-Energy	H-Energy	Pooled SE	P value
n	4	4	2	3		
Protein	0.88	0.84	0.81	0.85	0.018	0.136
Energy	0.79	0.78	0.77	0.81	0.011	0.213
Ash	0.38	0.34	0.34	0.38	0.024	0.517
Organic matter	0.77	0.75	0.74	0.78	0.018	0.273

6.4.3. Growth performance

The feed allowances for each treatment were calculated on a daily basis from the intake achieved by H-Energy group. The actual feed intakes were similar to the feed allowance due to minimal refusal for all birds, and the AME intake increased with LBW. The broilers fed more energy per day reached the target slaughter body weight faster than

those fed less energy. Whilst the birds fed H-Energy reached 4.0 kg at 44 days, the birds fed L-Energy reached the same LBW at 51 days (Table 6.5). The maximum daily gains between 1.75 and 2.5kg were 126, 116 and 114 g/day for birds fed H-Energy, MH-Energy and M-Energy, respectively. The birds fed L-Energy reached their maximum growth rate 110 g/day at LBW range between 2.5 and 3.25 kg (Table 6.5). The daily gain increased as the ME intake increased up to 2.5 MJ/d, then further increases on AME intake did not increased the daily gain.

The feed per gain decreased as the energy intakes increased. The lowest feed per gain was observed from 0.6 to 2.5 kg LBW for the birds fed H-Energy diet compared with other three dietary groups ($P < 0.05$). Above 2.5 kg LBW the feed per gain was increased as the energy intake increased but this difference was not statistically significant ($P > 0.05$, Table 6.5).

Table 6.5. Growth performance of broiler chickens slaughtered at different LBW and fed various levels of energy intake

LBW range	L-Energy	M-Energy	MH-Energy	H-Energy	Pooled SE	P-value
0.6-1 kg						
Age (day)[1]	21	20	20	19		
n	20	20	20	20		
Initial LBW(g)	592	594	591	601	3.58	0.201
FI (g/b/d)	86.6^{c}	86.5^{c}	89.1^{b}	95.7^{a}	0.77	0.0001
AMEI (MJ/b/d)	1.10^{d}	1.18^{c}	1.25^{b}	1.42^{a}	0.011	0.0001
CPI (g/b/d)	25.0^{a}	24.2^{b}	24.1^{b}	24.8^{a}	0.2	0.0075
Feed per gain (g/g)	1.46^{a}	1.32^{b}	1.28^{b}	1.14^{c}	0.021	0.0001
Final LBW (g)	1011^{ab}	991^{b}	1012^{ab}	1020^{a}	7.39	0.046
ADG (g)	59.8^{d}	66.1^{c}	70.1^{b}	83.9^{a}	1.17	0.0001
1-1.75kg						
Age (day)	29	28	27	26		
n	16	16	16	16		
Initial LBW (g)	1007	995	1008	1018	8.24	0.286
FI (g/b/d)	132.6^{b}	130.3^{b}	133.12^{b}	137.05^{a}	1.38	0.0097
AMEI (MJ/b/d)	1.68^{d}	1.78^{c}	1.87^{b}	2.03^{a}	0.020	0.0001
CPI (g/b/d)	38.2^{a}	36.5^{b}	36.1^{b}	35.5^{b}	0.36	0.0001
Feed per gain (g/g)	1.46^{a}	1.41^{b}	1.33^{c}	1.24^{d}	0.017	0.0001
Final LBW (g)	1736^{b}	1739^{b}	1710^{b}	1790^{a}	16.3	0.0097
ADG (g)	91.2^{c}	93.1^{c}	100.3^{b}	110.3^{a}	1.62	0.0001
1.75-2.5kg						
Age (day)	37	34	34	32		
n	11	12	11	12		
Initial LBW (g)	1729.5	1755	1707	1787	21.15	0.063
FI (g/b/d)	163.8	161.1	166.5	168.6	2.55	0.184
AMEI (MJ/b/d)	2.08^{d}	2.20^{c}	2.34^{b}	2.50^{a}	0.037	0.0001
CPI (g/b/d)	47.2^{a}	45.1^{b}	45.1^{b}	43.6^{b}	0.675	0.0069
Feed per gain (g/g)	1.65^{a}	1.42^{b}	1.44^{b}	1.34^{c}	0.025	0.0001
Final LBW (g)	2525	2438	2521	2540	33.9	0.136
ADG (g)	99.5^{c}	113.7^{b}	116.2^{b}	125.7^{a}	2..41	0.0001
2.5- 3.25kg						
Age (day)	43	41	40	38		
n	7	8	7	7		
Initial LBW (g)	2543	2476	2551	2513	39.13	0.516
FI (g/b/d)	184.6	179.7	182.9	179.5	2.03	0.237
AMEI (MJ/b/d)	2.34^{d}	2.45^{c}	2.57^{b}	2.66^{a}	0.029	0.0001
CPI (g/b/d)	53.2^{a}	50.3^{b}	49.6^{b}	46.5^{c}	0.53	0.0001
Feed per gain (g/g)	1.688	1.6	1.6	1.54	0.0436	0.159
Final LBW (g)	3201	3266	3238	3217	49.07	0.797
ADG (g)	109.6	112.7	114.5	117.4	3.45	0.477
3.25-4kg						
Age (day)	51	48	46	44		
n	3	3	3	3		
Initial LBW (g)	3230	3240	3295	3292	90.6	0.933
FI (g/b/d)	188.3^{a}	187.0^{a}	193.2^{a}	206.7^{b}	5.43	0.1117
AMEI (MJ/b/d)	2.39^{c}	2.55^{bc}	2.71^{b}	3.06^{a}	0.077	0.0016
CPI (g/b/d)	54.2	52.3	52.3	53.5	1.45	0.7424
Feed per gain (g/g)	1.93	1.83	1.73	1.65	0.091	0.246
Final LBW (g)	4015	3965	3997	4010	105.9	0.986
ADG (g)	98.1	103.6	116.9	119.7	5.89	0.088

[1] The age of birds when they reached the target slaughter body weight. The initial age at 0.60 kg was 14 days. ^{a,b,c,d} Means in the same row with different superscripts are significantly different ($P < 0.05$).

6.4.4. Body composition

The changes on water percentage and water mass were most likely related to protein changes. There were no differences on body ash percentage and mass at all LBW.

The carcass water and ash mass were highly related to protein mass. From the observed carcass component data, fitting allometric equations gave high correlation coefficient between carcass water mass and carcass protein mass (P); water = $5.997 \times P^{0.940}$ ($R^2 = 0.99$) and between carcass ash mass and carcass protein mass; ash = $0.222 \times P^{0.924}$ ($R^2 = 0.95$).

At 1.0, 2.5 and 3.25, kg LBW the birds fed L-Energy had higher protein percentage than those fed the other three levels of energy ($P < 0.05$). At 1.75 kg LBW the energy intake tended to increase the protein percentage of the L-Energy group ($P = 0.06$). At 4 kg LBW, the birds fed L-Energy and M-Energy had similar protein percentage but was higher than those fed the MH-Energy and H-Energy diets (Table 6.6).

The body lipid concentration was lower for the birds fed L-Energy compared with those fed the other three levels of energy at 1.0 and 1.75 kg LBW. At 2.5 kg LBW, the lipid percentage was 2% higher for the birds fed MH-Energy and H-Energy compared with those fed L-Energy and M-Energy. At 3.25 kg, the birds fed L-Energy and M-Energy had a similar carcass lipid percentage but lower lipid percentage than those fed the MH-Energy and H-Energy diets. At 4 kg, the birds fed H-Energy had a higher carcass lipid percentage compared with the other three levels of energy, the birds fed L-Energy and M-Energy and MH-Energy had similar carcass lipid percentages.

The energy intake tended to influence the final body protein mass at LBW of 1.0 ($P = 0.06$), 1.75 ($P = 0.05$) and 2.5 ($P = 0.09$) kg, whereas no differences were observed at other LBW. The energy intake influenced the final body lipid mass at all LBW. At 1 kg LBW, the birds fed H-Energy had higher lipid content than those fed the other three levels of energy ($P < 0.05$). The birds fed M-Energy and MH-Energy had similar lipid concentration but the birds fed M-Energy had higher lipid content than those fed L-Energy ($P < 0.05$). At 1.75 kg the lipid mass was low for the birds fed L-Energy compared with those fed the other three levels of energy ($P < 0.05$), the bird fed M-Energy, MH-Energy and H-Energy had similar lipid mass. At 2.5 kg LBW, the birds fed MH-Energy and H-Energy had similar final body lipid but higher than those fed L-Energy and M-Energy ($P < 0.05$). At 3.25 kg LBW, the birds fed MH-Energy and H-Energy had similar final body lipid but higher than those fed L-Energy diet ($P < 0.05$).

At 4.0 kg LBW, the birds H-Energy had higher lipid content than those fed the other three levels of energy ($P < 0.05$), the birds fed L-Energy, M-Energy and MH-Energy had similar lipid mass content.

Table 6.6. Influence of different types of diet on broiler de-feathered carcass composition, on a percentage (%) or on mass content (g/bird)

body weight	L-Energy	M-Energy	MH-Energy	H-Energy	SE	P-value
At 1 kg						
Water (%)	73.19	72.2	72.82	71.88	0.35	0.0841
Ash (%)	2.48	2.45	2.65	2.43	0.17	0.771
Protein (%)	17.01^{a}	16.28^{b}	16.40^{b}	16.23^{b}	0.13	0.0035
Lipid (%)	5.96^{b}	7.44^{a}	6.57^{ab}	7.52^{a}	0.37	0.0309
Water (g)	719.7	677.1	719	718.4	15.1	0.1801
Ash (g)	24.4	23.0	26.3	24.3	1.77	0.633
Protein (g)	167.2	152.6	161.9	162.2	3.41	0.063
Lipid (g)	58.3^{c}	69.8^{ab}	64.7^{bc}	75.2^{a}	3.39	0.0243
lipid/protein ratio	0.35^{b}	0.46^{a}	0.40^{ab}	0.46^{a}	0.02	0.0211
At 1.75 kg						
Water (%)	73.40^{a}	71.05^{b}	71.15^{b}	70.64^{b}	0.29	0.0001
Ash (%)	2.57	2.33	2.38	2.52	0.19	0.7871
Protein (%)	17.17	16.68	16.42	16.2	0.23	0.0633
Lipid (%)	5.60^{b}	8.38^{a}	8.46^{a}	8.83^{a}	0.42	0.0005
Water (g)	1238.1^{a}	1145.9^{c}	1175.6^{bc}	1222.4^{ab}	17.7	0.0112
Ash (g)	43.3	37.6	39.3	43.7	3.48	0.546
Protein (g)	289.7	269.1	271.2	280.3	5.09	0.0518
Lipid (g)	94.4^{b}	135.0^{a}	139.6^{a}	153.2^{a}	7.12	0.0005
lipid/protein ratio	0.33^{b}	0.50^{a}	0.52^{a}	0.55^{a}	0.03	0.0008
At 2.5 kg						
Water (%)	72.15^{a}	72.00^{a}	70.34^{b}	70.38^{b}	0.47	0.0253
Ash (%)	2.48	2.23	2.43	2.24	0.09	0.1935
Protein (%)	17.62^{a}	16.94^{b}	17.00^{b}	16.52^{b}	0.15	0.0023
Lipid (%)	6.81^{b}	7.19^{b}	9.08^{a}	9.30^{a}	0.47	0.0044
Water (g)	1727.1	1620.3	1666.2	1714	44.1	0.3448
Ash (g)	59.4	50.2	57.5	54.6	2.68	0.1382
Protein (g)	421.8	381.0	402.0	402.5	10.1	0.0915
Lipid (g)	162.7^{b}	162.3^{b}	213.6^{a}	227.0^{a}	11.0	0.0018
lipid/protein ratio	0.39^{b}	0.43^{b}	0.54^{a}	0.56^{a}	0.03	0.0018
At 3.25 kg						
Water (%)	72.34^{a}	71.68^{ab}	70.26^{bc}	70.01^{c}	0.52	0.0212
Ash (%)	2.46	2.61	2.35	2.22	0.14	0.303
Protein (%)	17.75^{a}	17.14^{b}	17.06^{b}	17.16^{b}	0.15	0.0263
Lipid (%)	6.44^{c}	7.32^{bc}	9.15^{ab}	9.39^{a}	0.60	0.0122
Water (g)	2201.2	2210.7	2139.8	2124.7	49.7	0.5405
Ash (g)	74.9	80.5	71.4	67.3	4.22	0.2073
Protein (g)	539.9	528.7	519.6	520.7	12.3	0.6408
Lipid (g)	195.7^{c}	225.2^{bc}	277.6^{ab}	284.7^{a}	17.1	0.0086
lipid/protein ratio	0.36^{c}	0.43^{bc}	0.54^{ab}	0.55^{a}	0.04	0.0118
At 4 kg						
Water (%)	72.20^{a}	72.15^{a}	71.02^{a}	68.23^{b}	0.85	0.0334
Ash (%)	2.5	2.42	2.37	2.20	0.07	0.0803
Protein (%)	18.15^{a}	17.75^{ab}	17.13^{bc}	16.90^{c}	0.24	0.0245
Lipid (%)	5.67^{b}	6.41^{b}	8.00^{b}	11.69^{a}	0.83	0.0038
Water (g)	2790.2	2714.7	2702.8	2609.5	99.5	0.659
Ash (g)	96.6	91.5	90.3	84.2	5.25	0.4625
Protein (g)	701.4	666.4	652	646.3	18.9	0.2421
Lipid (g)	218.6^{b}	238.0^{b}	302.8^{b}	448.9^{a}	31.9	0.0036
lipid/protein ratio	0.31^{b}	0.36^{b}	0.47^{b}	0.69^{a}	0.05	0.0033

[a,b,c] Means in the same row with different superscripts are significantly different ($P < 0.05$).The number of observations for slaughter live body weight 1.0, 1.75, 2.5, 3.25 and 4.0 kg were 4, 4, 4, 4, and 3 respectively.

6.4.5. Deposition rate of chemical components

The deposition rates were affected by the energy intake levels between 0.6 to 2.5 kg (Table 6.7). There was no effect of energy level on the gain of body components between 2.5 to 4 kg LBW.

At LBW ranging between 0.60 to 1.0 kg and 1 to 1.75 kg, the daily gain of water increased in the birds fed the H-Energy diet compared to those fed the other three levels of energy ($P < 0.05$). Between 1.75 to 2.5 kg, the birds fed M-Energy, MH-Energy and H-Energy had a similar daily water gain, which was higher than those fed L-Energy. There was no effect of energy level on the ash content at all of LBW.

The daily lipid gain was increased as the energy intake levels increased. At a LBW between 0.6 to1.0 kg, the daily lipid gain was lowest with L-Energy intake, intermediate with M-Energy and MH-Energy intake, and highest with the H-Energy intake ($P < 0.05$). At the LBW range between 1.0 to 1.75 kg the birds fed L-Energy had lower lipid gain than those fed other three levels of energy, the lipid gain was similar when the birds fed M-Energy, MH-Energy and H-Energy. At the LBW range between 1.75 to 2.5 kg the birds in L-Energy and M-Energy had similar daily lipid gain but, lower than those fed MH-Energy and H-Energy.

At the LBW ranging between 0.6 and 1.0 kg, the daily protein gain (g/b/d) was higher for birds fed H-Energy than those fed the other three levels of energy ($P < 0.05$). At LBW range between 1.0 and 1.75 kg, the daily protein gain was similar in all treatment groups ($P > 0.05$). Between 1.75 and 2.5 kg LBW, the birds fed L-Energy had lower daily protein gain than those fed the other levels of energy, the birds fed M-Energy and MH-Energy had similar daily protein gain. At LBW range 2.5 to 4.0 kg, the daily protein gain was similar in all treatment groups ($P > 0.05$). The maximum daily protein depositions were 24.6, 22.4, 23.6 and 21.7 for the birds fed H-Energy, MH-Energy, M-Energy and L-Energy for period of 2.5 to 3.25, 1.75 to 2.5, 3.25 to 4.0 and 2.5 to 3.25 kg, respectively.

Table 6.7. Chemical body component gain (g/day) of broiler chicken slaughtered at different live body weight and fed diet with various levels of energy intake

LBW range	L-Energy	M-Energy	MH-Energy	H-Energy	SEM	P-value
0.6-1.0kg						
n	4	4	4	4		
Water (g/d)	42.54^{b}	43.7 ^{b}	50.5 ^{b}	59.29 ^{a}	2.66	0.0028
Ash (g/d)	1.64	1.72	2.26	2.28	0.31	0.3314
Lipid (g/d)	3.87^{c}	6.50^{b}	5.65^{cb}	8.79^{a}	0.59	0.0006
Total protein (g/d)	12.31^{b}	11.49^{b}	12.99^{b}	15.3^{a}	0.50	0.0008
Carcass protein (g/d)	10.88^{b}	10.52^{b}	12.04^{b}	14.24^{a}	0.59	0.0033
Feather protein (g/d)	1.43	0.97	0.95	1.06	0.15	0.1211
1.0-1.75kg						
n	4	4	4	4		
Water(g/d)	65.2^{b}	59.91 ^{b}	65.3 ^{b}	72.4 ^{a}	1.81	0.0034
Ash(g/d)	2.38	1.88	1.87	2.78	0.47	0.4910
Lipid (g/d)	4.50^{b}	8.28^{a}	10.67^{a}	11.185^{a}	0.97	0.0014
Total protein (g/d)	17.45	17.35	17.54	18.94	0.50	0.1350
Carcass protein (g/d)	15.39	14.85	15.62	16.95	0.50	0.0620
Feather protein (g/d)	2.06	2.50	1.92	1.99	0.17	0.1140
1.75-2.5kg						
n	4	4	4	4		
Water(g/d)	64.67^{b}	78.1 ^{a}	74.16^{ab}	82.17 ^{a}	3.40	0.0190
Ash(g/d)	2.13	2.07	2.73	1.84	0.33	0.3044
Lipid (g/d)	8.79^{ab}	4.39^{b}	11.04^{a}	12.40^{a}	1.58	0.0185
Total protein (g/d)	19.85^{c}	20.88^{bc}	22.35^{ab}	24.5^{a}	0.80	0.0080
Carcass protein (g/d)	17.35^{b}	18.45^{ab}	19.60^{ab}	20.40^{a}	0.75	0.0064
Feather protein (g/d)	2.50^{b}	2.43^{b}	2.75^{b}	4.10^{a}	0.24	0.0012
2.5-3.25kg						
n	4	4	4	4		
Water(g/d)	73.8	73.5	72.1	72.2	4.16	0.987
Ash(g/d)	2.43	3.99	2.06	2.23	0.67	0.209
Lipid (g/d)	4.98	7.96	9.56	10.19	2.97	0.620
Total protein (g/d)	21.71	20.36	21.06	24.61	1.11	0.086
Carcass protein (g/d)	18.42	18.54	17.91	20.61	0.93	0.232
Feather protein (g/d)	3.29^{a}	1.82^{b}	3.15^{a}	4.00^{a}	0.39	0.015
3.25-4kg						
n	3	3	3	3		
Water(g/d)	69.2	74.1	82.8	66.3	8.19	0.538
Ash(g/d)	2.57	1.63	2.78	2.33	0.51	0.458
Lipid (g/d)	2.44^{b}	1.98^{b}	2.57^{b}	25.39^{a}	4.73	0.019
Total protein (g/d)	20.37	23.58	21.36	18.18	1.85	0.296
Carcass protein (g/d)	19.10	20.18	19.37	17.38	1.91	0.771
Feather protein (g/d)	1.27	3.40	1.99	0.80	0.56	0.051

^{a,b,c} Means in the same row with different superscripts are significantly different ($P < 0.05$).

6.4.6. Effect of body weight on protein deposition

The protein deposition rates were affected by LBW between 0.6 to 2.5 kg, but further increases on LBW did not influence the protein deposition rate ($P > 0.05$). The measured protein deposition rates were 13.0, 17.8, 21.9, 21.9 and 20.9 (g/day) for LBW ranged from 0.6 to 1.0 kg, 1.0 to 1.75 kg, 1.75 to 2.5 kg, 2.5 to 3.25 kg and 3.25 to 4.0

kg, respectively. Fitting a quadratic relationship established that the protein deposition reached a maximum of 23.2 g/day at 3.28 kg LBW and then decreased to 22g/day at 4.0 kg (Figure 6.1).

The quadratic relationship of LBW and protein deposition rate was described as;

$Pd(g/d)=1.735+0.0131\times LBW - 0.000002\times LBW^2$ ($R^2 = 0.73$)

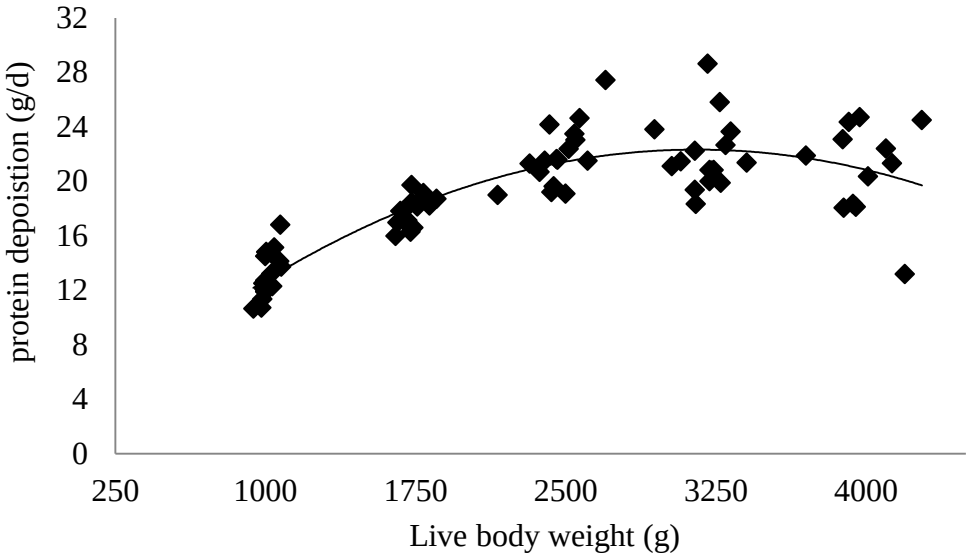

Figure 6.1. The regression line between live body weight and the body protein deposition

6.4.7. Effect of energy intake on protein deposition

The relationship between the AME intake for production (AMEIp) and the body protein and lipid deposition was described using quadratic and broken line regression models (Table 6.8, Figure 6.2).

Table 6.8. The regression equation to predict the daily protein and lipid deposition from the apparent metabolisable energy intake above maintenance requirement (AMEp, MJ/d)

Model	Prediction equation for protein and lipid deposition(g/d)	R^2
Quadratic regression	$Pd= - 4.57 + 34.69 \times AMEIp - 11.2 \times AMEIp^2$	0.64
	$Ld= - 16.6 - 29.4 \times AMEIp + 18.9 \times AMEIp^2$	0.49
Broken line	$Pd=2.53 + 16.15 \times AMEIp$. If $AMEIp \leq 1.19$ (MJ/b)	0.65
	$Pd=22.1 - 0.317 \times AMEIp$, If $AMEIp > 1.19$ (MJ/b)	
	$Ld =3.31 + 3.58 \times AMEIp$, If $AMEIp \leq 1.28$ (MJ/b)	0.56
	$Ld= - 39.3 + 36.9 \times AMEIp$, If $AMEIp > 1.28$ (MJ/b)	

When the AMEIp increased from 0.5 to 1.2 MJ/day protein deposition increased from 11 to 22 g/day, but further increases of AMEIp did not lead to increases in protein

deposition. Therefore, the broken line model was the best model to describe the protein deposition rate data (Figure 6.2). The broken line model indicated a Pdmax value of 22 g/day at AMEIp breakpoint of 1.2 MJ/day. Also, lipid deposition rate was sharply increased after the break point of 1.28 MJ/day.

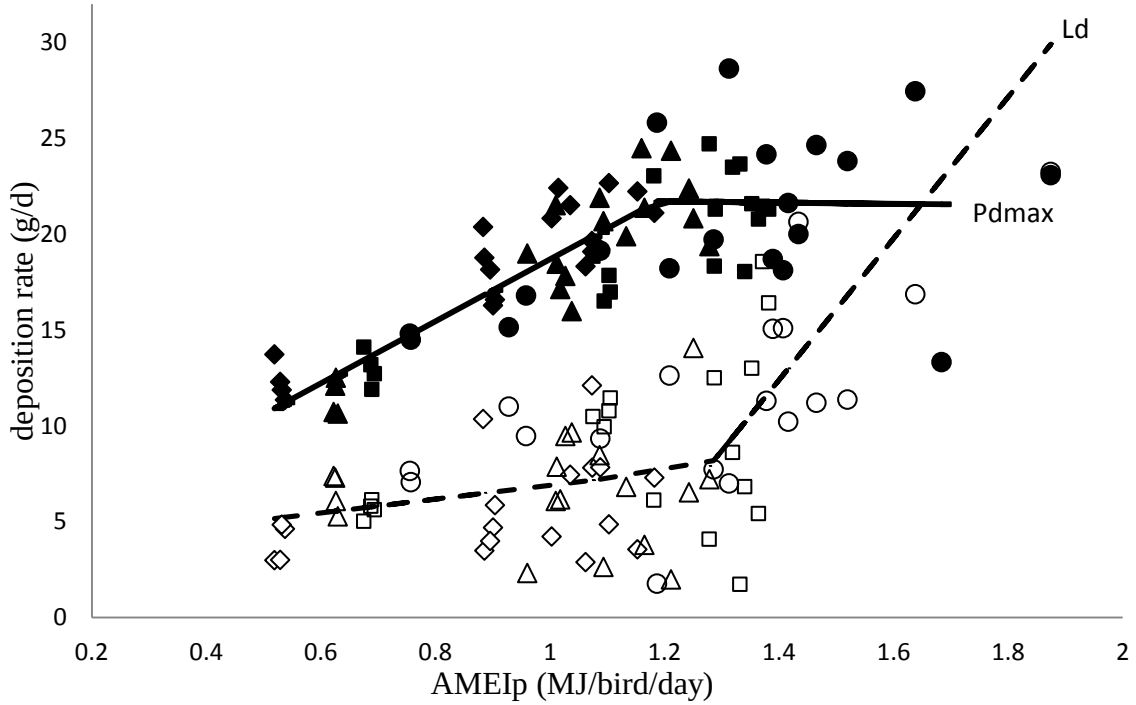

Figure 6.2. The regression lines between the apparent metabolisable energy intake for production (AMEIp) and the body protein (sold fill symbols) and lipid deposition (no fill symbols), ♦ Low energy, ▲ Medium energy, ■ Medium high energy, ● High energy diet. Models of the responses of protein deposition (—) and lipid deposition (- - - -) on AMEIp supply

6.4.7. Ratio of body lipid to protein

The result from covariance analysis indicated that the LBW and AMEIp had an effect on the L/P ratio ($P < 0.05$), but no interaction between AMEIp and LBW was observed. The L/P ratio ranged between 0.31 to 0.39 for the birds fed L-Energy and increased to 0.46-0.69 for the birds fed the H-Energy diet. At a LBW of 1.0 kg, the L/P ratio was 0.35 to 0.46 where the L/P ratio at 4.0 kg was 0.31 to 0.69. A heavier body weight was associated with a greater increase in the L/P ratio as the AMEI above maintenance increased (Figure 6.3).

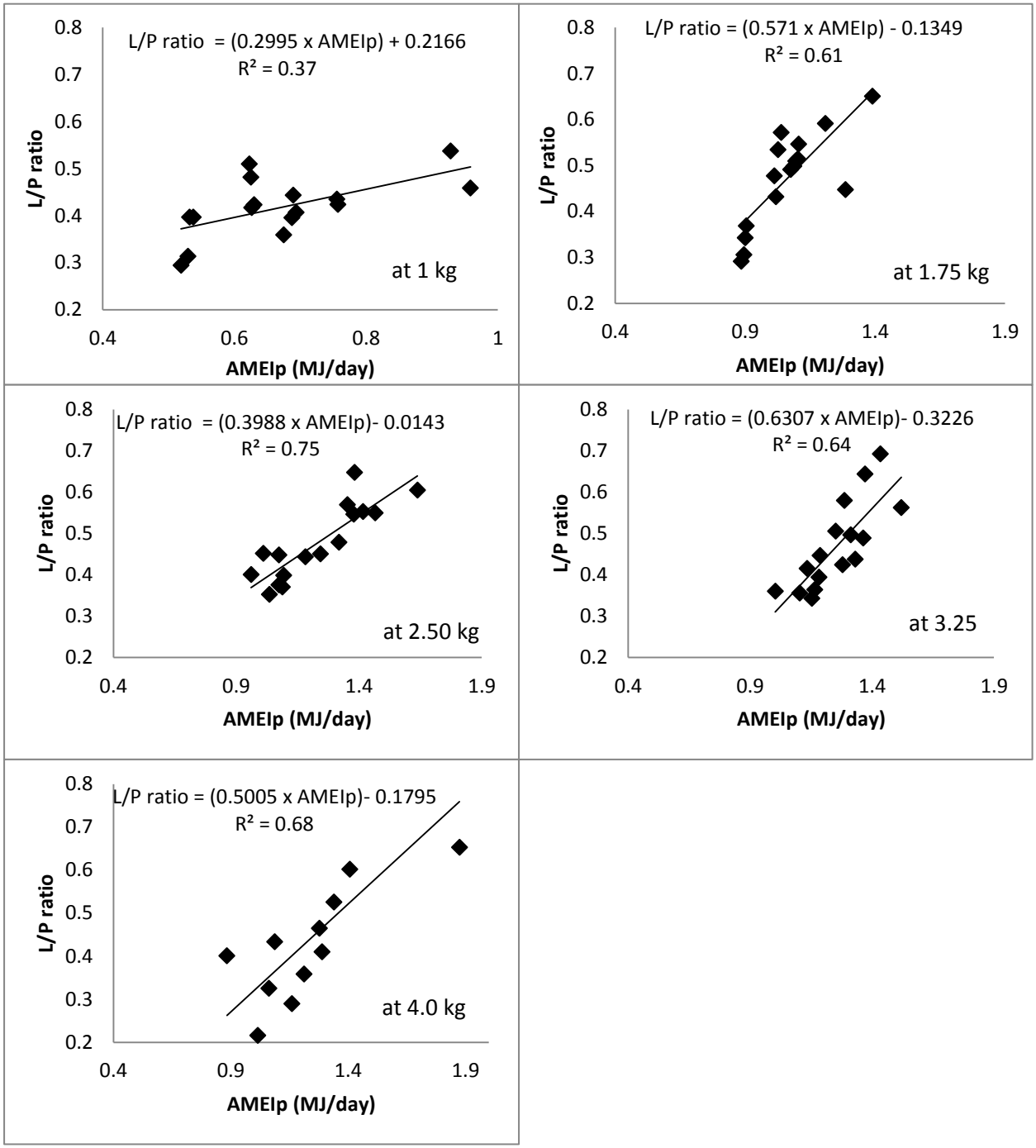

Figure 6.3. The linear regression lines between apparent metabolisable energy intake for production (AMEIp) and body lipid/protein ratio (L/P ratio) at different live body. The slopes for each of the LBW were not significantly different ($P > 0.05$).

6.5. Discussion

6.5.1. Effects of body weight and energy intake on daily gain

Results from this experiment clearly showed that the average daily gain increased as the body weight increased from 0.6 kg to 4 kg. The maximum growth rate of 126 g/day was achieved for birds fed the H-Energy diet at 26 to 32 days of age. Marcato *et al.,* (2008) reported that the maximum daily gain was 111 g/day at 42 days in male Ross broilers. Body weight gain is dependent on feed intake and particularly, energy and protein intakes. The reasons for superlative daily gain in our experiment was due to the achievement of Pdmax by feeding the birds nutrient-rich diets and the birds were reared in cages with perfect conditions to express their maximum genetic potential.

The daily body weight gain was increased when the ME intake increased and the maximum daily gain was achieved when the birds consumed 2.2 to 2.5 MJ/day in all treatment groups. As a result of improving the daily gain there was an improvement in the feed per gain ratio.

The results from this experiment indicate that, the male Ross broiler can be slaughtered between 26-32 days of age with a reasonable marketable body weight of 1.75-2.50 kg. Body weight gain, market age, feed conversion ratio and caloric conversion ratio may be improved by increasing the levels of energy and balanced protein in the diet.

6.5.2. The upper limit for protein deposition

The present experiment was performed to measure the upper limit protein deposition. The highest average daily protein deposition was 24.6 (g/day) achieved at LBW range between 1.75 to 3.25 kg for the birds fed H-Energy diet. Usually, the potential protein deposition is described by Gompertz type equations in broiler chickens (Hancock *et al.*, 1995; Gous *et al.*, 1999; Marcato *et al*., 2008). However, potential growth rate is not the same as maximum growth rate that can be achieved by broilers under commercial condition (Gous, 1998). The Gompertz equations described a specific data set mathematically rather than described the overall biological processes. In Gompertz equation, the protein deposition (Pd) expressed as a function of mature protein weigh (Pm), a rate of maturing parameter (b) and time (t; days), and time (t*) when the growth rate is maximum as following:

$$Pd \text{ at } (t) = Pm \times \exp(-\exp(-b(t - t^*)))$$

The mature protein weight (Pm) and the rate of maturing (b) are considered to be genotype and sex dependent. Previous studies have provided values to describe the protein deposition in broiler chickens by using Gompertz model but the values for the parameters have differed across experiments (Table 6.9). The nutrient intake and the conditions of the experiment used to obtain data for fitting the growth curve might be the factors that affect the b and Pm parameters. The maximum growth rate of protein has ranged between 11 and 18 g/day and this is achieved anywhere between 37 to 48 days of age (Table 6.9, Marcato *et al.*, 2008; Hancock *et al.*, 1995; Gous *et al.*, 1999). This age range is substantially different when comparing with present experiment where the maximum protein deposition rate was achieved at 26 to 38 days of age when BW ranged between 1.75 and 3.25.

Table 6.9. The Gompertz parameters of protein deposition for male broilers chickens

Strain	b	Pm (g)	t*	Pd	Age (day)	BWm (g)	Reference
Ross 308	0.037	1309	44	17.8	1-56	-	Marcato *et al*., (2008)
Cobb 500	0.047	1042	37.2	18.0	1-56	-	
Ross	0.0483	616	31.0	10.9	1-112	6050	Sakomura *et al*.,(2005)
Ross x Arbor Acres	0.0356	1010	46.5	13.2	1-112	6087	Gous *et al*.,(1999)
Steggles x Arbor Acres	0.0354	1003	47.6	13.0	1-112	5888	
Broiler strains[1]	0.0350	889	45.6	11.4	1-77	5629	Hancock *et al*., (1995)

b; is the rate of maturing parameter. Pm is the mature protein weight. t* is the time (days) when growth rate is maximal. Pd is the protein deposition (g/day) at maximum growth rate. Age (day) is the experiment period. BWm (g) is the mature body weight. [1] The values described the average of six strains of male broilers (Ross 708, Ross 788, Ross 608, Ross 688, Hubbard and Hybro breed).

In our study it was possible to measure the upper limit for protein deposition (Pdmax) by feeding the birds a diet that had a high nutrient and energy concentration (H-Energy group) and decreasing the energy intake for the other groups to create the relationship between energy intake and protein deposition. The average ideal digestible balanced protein (IDBP) intakes between 1.75 and 2.5 kg BW were 41.4, 36.2, 36.5 and 36.8 g/day, for the L-Energy, M-Energy, MH-Energy and H-Energy diets, respectively. The amount of protein for production was adequate to reach the Pdmax (assumed the protein efficiency = 0.85, Talpaz *et al.*, 1986). Likewise the AME intake in H-Energy treatment was sufficient to support the Pdmax with additional energy deposited as lipid, while the AME intake in L-Energy treatment did not allow for maximum protein deposition to be achieved.

The maximum protein deposition estimated in this study 24.6 g/day at 26-38 days and this is greater than that observed by Marcato *et al*., (2008) who reported the protein deposition potential rate for the Ross breed to be 17.52 g/day at 42 days old. Samadi and Liebert (2006) also characterised the theoretical maximum daily protein deposition (PdmaxT) for the Cobb 500 breed. The PdmaxT was 26.8 g/day between 30 to 45 days old. The PdmaxT was 26.8 g/day between 30 to 45 days old. The PdmaxT is not attainable in practical conditions, but it is possible to define the protein deposition rate as a percentage of PdmaxT. In the same report, between age period of 10 to 25 day and 30 to 45 days of age, the calculated daily protein deposition corresponding to 80% of PdmaxT increased from 11.6 to 21.4 g/day for male and from 10.8 to 19.4 g/day for female broiler, respectively. According to Tavenari *et al*. (2009), the daily protein deposition was 6.6, 21.1 and 18.7 for male Avian farm broiler for periods 1 to 21, 22 to 42 and 43 to 56 days of age, respectively.

Values for the Pdmax have been provided in pig experiments. The effect of energy intake on the daily protein deposition has been described with the linear plateau theory (Van Moughan *et al*., 2006; Denis and Daniel, 1997). When an animal is given a diet adequate in protein but limited by energy intake the protein deposition increases as the energy intake increases (independent of protein intake) until it approaches the Pdmax (De Greef and Verstegen, 1995). Similar evidence was provided in our study, the protein deposition was increased linearly as the energy intake increased and Pdmax was achieved at 2.2 and 2.5MJ/day AME intake or 1.2 MJ/day AMEIp, and further energy intake was deposited as lipid.

6.5.3. Lipid to protein ratio

It has been proposed that animals have an inherent ratio between body protein and body lipid deposition and animal will always attempt to maintain this ratio (Ferguson and Theeruth, 2002; Gous *et al*., 2012). In the current experiment, the energy intake and LBW were affecting the L/P ratios. The L/P ratios ranged between 0.31-0.69 in all treatment groups and the birds fed L-Energy diets had lowest L/P ratio. The L/P ratio increased as the LBW increased. Gous *et al.,* (2012) reported that the L/P ratio increased from 0.36 at 1.0 kg to 0.64 at 1.80 kg LBW for a lean broiler genotype fed on high protein diet. Comparable results have been observed with growing pigs (Ferguson and Theeruth, 2002), where the L/P ratio was 0.67 and 0.86 for pigs fed high protein

and low protein diet, respectively at 30 kg LBW and the ratios increased to 0.86 and 1.04 at 60 kg LBW.

The protein deposition to the digestible protein intake ratios were 0.49, 0.57, 0.60 and 0.62 for birds fed the L-Energy, M-Energy, MH-Energy and H-Energy, respectively. The influence of dietary energy to protein ratios on growth rate and body composition has been reported in many previous studies (Leeson *et al*., 1996; Swennen *et al*., 2004; Bartov, 1992). The broiler performance is improved with a high energy diet and it was shown that protein utilisation is improved with higher levels of dietary energy (Reginatto *et al*., 2000 and Leeson *et al.*, 1996). The minimum L/P ratio has been proposed as the inhibiting factor for protein deposition when pigs have energy restriction (de-Greef and Verstegen, 1995; de-Lange, 1995; de-Lange *et al*., 2008). When the energy intake is limiting the protein deposition, the production energy is partitioned according to some partitioning rule to have minimum amount of lipid per unit of protein deposition ((Whittemore, 1983; De-Greef and Verstegen, 1995 and Moughan *et al*., 2006). Green *et al*. (2006) reported that when the energy supply is insufficient to maximise protein growth and the protein deposition is less than the Pdmax, the pig needs to deposit some fat. The proposition can be framed in terms of the minL/P ratio and the daily ratio for lipid and protein gain may be range from 0.4 to 1.2 in the growing pig. Similarly, in our study the birds fed L-Energy diets had insufficient energy intake to achieve the Pdmax with necessary lipid deposition. Consequently, part of protein intake was utilised to deposit lipid instead of protein deposition. The low energy intake had a negative effect on protein deposition for the birds fed the L-Energy diet as the birds attempted to maintain the genetic minL/P ratio. The minL/P ratio value was observed when the bird consumed AMEIp less than 1.16 (MJ/d) and the possible minL/P ratio ranged between 0.31 and 0.55.

6.6. Conclusion

Under conditions of protein non-limiting diets, the modern broiler chicken has an upper limit for protein deposition of 24.6 g/day. Pdmax was constant over a body weight range 1.75 to 3.25 kg and above 3.25 kg Pdmax was no longer achievable. When the energy intake was insufficient to deposit a minimum amount of lipid per unit of protein deposition the birds have attempted to maintain the genetic L/P ratio by decreased the protein deposition efficiency. The possible minL/P ratio ranged between 0.31 and 0.55.

Body weight gain, market age, feed conversion ratio and carcass composition may be improved by manipulating the broiler diet formula to maximise the protein growth rate with minimum fat deposition.

CHAPTER 7

Mechanistic growth model of broiler chickens

7.1. Abstract

This chapter presents a broiler growth model, which is a mechanistic, deterministic and dynamic approach to evaluate the effect of diet on carcass composition and performance for modern broiler chickens and has been calibrated for Ross 308 broilers chickens. The model is based on the nutrient partitioning concept, information derived from literature and the data from previous experiments. A feed formulation program was also developed to calculate the nutrients and energy content in the diet. The initial live body weight, maximum protein deposition (Pdmax), minimum lipid to protein ratio (minL/P ratio), daily ME and ideal balanced protein intakes were used as data input. The output of the growth model provides information about protein, fat, water and ash deposition. Live body weight, carcass weight and meat yield (breast and leg meat). The results indicate that the growth model based on nutrients and energy partitioning concepts can predict the carcass composition and broiler performance.

7.2. Introduction

Over the past two decades, interest has been shown in the development of growth models for poultry (Hruby *et al*., 1994; Gous *et al*., 1999; King, 2001; Zuidhof, 2009; Rivera *et al*., 2011). Empirical and mechanistic models have been used to simulate the growth of broiler chickens (Gous *et al*., 1999; Eits *et al*. 2005a; Zuidhof, 2005; 2009; King, 2001; Zoons *et al*., 1991; EFG-model, Emmans, 1981; Emmans and Fisher, 1986).

Empirical models are based on direct observations and measurements and experiment data records, by using mathematical or statistical equations without an explanation of biological processes (Zoons *et al*., 1991; France and Dijkstra, 2006). The empirical model is a mathematical function resulting fitted to data, whereas, the mechanistic model describes the relationship between dependent and independent variables, by representing the biological process pathways (Zoons *et al*., 1991; France and Dijkstra, 2006). For example, an empirical model can be constructed to predict the feed intake from the diet composition, without understanding how the diet composition affect feed intake. A mechanistic model attempts to describe and understand how the diet composition affect feed intake.

In broiler chickens, mechanistic growth models, based on nutrient partitioning and utilisation, are preferred (Morris, 2006; France *et al*., 2000). Mechanistic models can provide more flexibility for evaluation and development of models under variable conditions. In addition, a mechanistic growth model can be used for feed evaluation: i.e. research findings can be easily incorporated into a mechanistic growth model, which will improve the ability to predict availability of nutrients (France *et al*., 2000). Recent research has suggested a merger between mechanistic and empirical models. The merging of these two models could improve the accuracy of model prediction (Roush, 2006).

In order to optimise growth rate and develop feed strategies for different stages of growth, an accurate prediction of responses in body growth and carcass yield, to nutrients and energy intake, is important. Therefore, the aim of this study was to develop a mechanistic growth model to predict broiler performance and carcass composition under varied nutritional conditions. Some parameters and equations, which are not achieved mechanistically, will be described by the empirical approach.

7.3. Materials and methods

7.3.1. Feed formulation program

The composition of AME, calcium, available phosphorus, fat, crude protein, ileal digestible protein and ileal digestible amino acids of 85 feedstuffs were obtained from Bryden et al. (2009) and Leeson and Summers (2005). A feed formulation programme was used to calculate the sum products of the nutrient profiles of specified ingredients. The digestible protein and amino acids requirements for male broilers (Ross, 2007) were used to calculate the limiting amino acids, and then to calculate the ideal digestible balanced protein. This information was used as input data into the main growth model.

7.3.2. Growth model

Several equations were obtained from experiments and literature. Data of 195 birds from our experiments were used to obtain the regression equations to estimate the de-feathered carcass weight, body water, body ash, feather protein, initial body protein and initial body fat. Data from 96 birds from the Massey Poultry Unit was used to estimate the yield of primary meat components of the carcass from the live body weight (i.e., edible carcass, breast and leg meat weight).

In addition, data were extracted from literature to establish the ME energy for maintenance requirement (649 kJ/kgLBW$^{0.6}$, Lopez and Leeson, 2005), the protein requirement for maintenance (1.575 g/kgLBW$^{0.67}$, Samadi and Liebert, 2006), the efficiency of protein utilisation for growth after protein requirement for maintenance (0.85, Scheele *et al.*, 1977; Talpaz *et al.*, 1986), the energetic efficiency for protein deposition (0.66, Boekholt *et al*., 1994) and the energy cost of uric acid excretion (8.4 kJ/g uric acid, Harper, 2000).

3.3. Basic principles for describing the growth model

The following are the key assumptions and principles of the proposed model.

A. The feed intake is characterised into digestible crude protein intake and AME intake.
    1. The ideal digestible balanced protein is calculated from the digestible dietary AAs profiles (Bryden *et al*, 2009; Leeson and Summers, 2005) and the digestible AA requirements for the 308 broiler.
    2. The ideal digestible balanced protein in excess of that needed for maintenance is available for growth. Daily protein deposition can be limited mainly by three factors; Pdmax, energy intake or balanced protein intake. The linear plateau concept can describe the protein deposition. When the animal given a diet deficient in ideal balanced protein, the protein deposition rate increases linearly as the ideal balanced protein intake increases (independent of energy intake) until it approaches the Pdmax. When the animal is given a diet adequate in protein but limited by energy intake (independent of protein intake), the protein deposition increases as the energy intake increases until it approaches the Pdmax. The Pdmax varies with sex, genetics and age. The Pdmax was established for male Ross 308 broilers as 22g/day (Chapter 6). It is assumed the efficiency of ideal balanced protein utilisation for protein deposition is constant at 0.85 (Scheele *et al*., 1977, Talpaz *et al*., 1986). In addition, the efficiency of protein deposition will be zero when the protein deposition reaches the Pdmax.
    3. Excess protein (AAs) is used as energy and converted into fat.

B. The AME intake is used for maintenance, protein and fat deposition requirements.
    1. Available metabolisable energy in excess of that needed for maintenance and required for protein deposition is used for the deposition of fat.

2. The energy needed for the uric acid production is subtracted from the available energy for fat deposition.
3. Fat deposition is calculated dependent on the different energy efficiencies of the different energy sources.
4. The minimum fat to protein ratio (this ratio also varies related to genetic, sex and body weight) provides rules for the energy partitioning. In our case the minimum fat to protein deposition was = 0.31.

C. The quantity of protein and fat deposited is added to the original body composition.

D. The feather protein, water and ash content of the final body weight is calculated from the de-feathered carcass protein content.

E. The body weight is calculated as the summation of all body contents.

7.4. Results

7.4.1. Broiler growth model component

The flow of the model components is described as follows:

- Data Input

Actual body weight at start (g)

Age at start (day)

Diet composition (from feed formulation program)

Actual feed intake per day or per period (g)

Daily potential protein deposition/Pdmax (g/day)

MinL/P (%)

Feed cost ($/kg feed) (optional)

Initial carcass composition (optional)

- Model components

Calculate the initial body composition (g/bird)

Calculate the energy requirement for maintenance (kJ/day/bird)

Calculate the protein requirement for maintenance (g/day/bird)

Calculate the protein deposition (g/day/bird)

Calculate the water and ash deposition related to protein deposition (g/day/bird)

Calculate the available energy for fat and then calculate the fat deposition (kJ /day)

Calculate the final body composition (g).

-Output from the model

Daily gain (g/day)

Final body weight (g)

Feed per gain (g/g)

Carcass composition (g/bird)

7.4.2. Simulation to predict of broiler performances

This model simulates the daily growth of male Ross 308 broiler chickens, by using information on initial age, initial live weight of bird, number of days over which diet is to be fed, feed intake and diet composition (output from feed formulation programme). The model runs with a daily time-step. The output provides information on a daily basis or, alternatively, for the end of a growth stage, with information on daily or accumulated deposition and current bird status for protein, fat, water, ash body content, live body weight, edible carcass yield, breast meat yield and feed per gain.This model can be used to predict growth response at any given live body weight and age over any period of growth.

The model was developed and run within Microsoft Excel, 2010 (Windows7). The overall description of the model presented in figure 7.1 and 7.2.

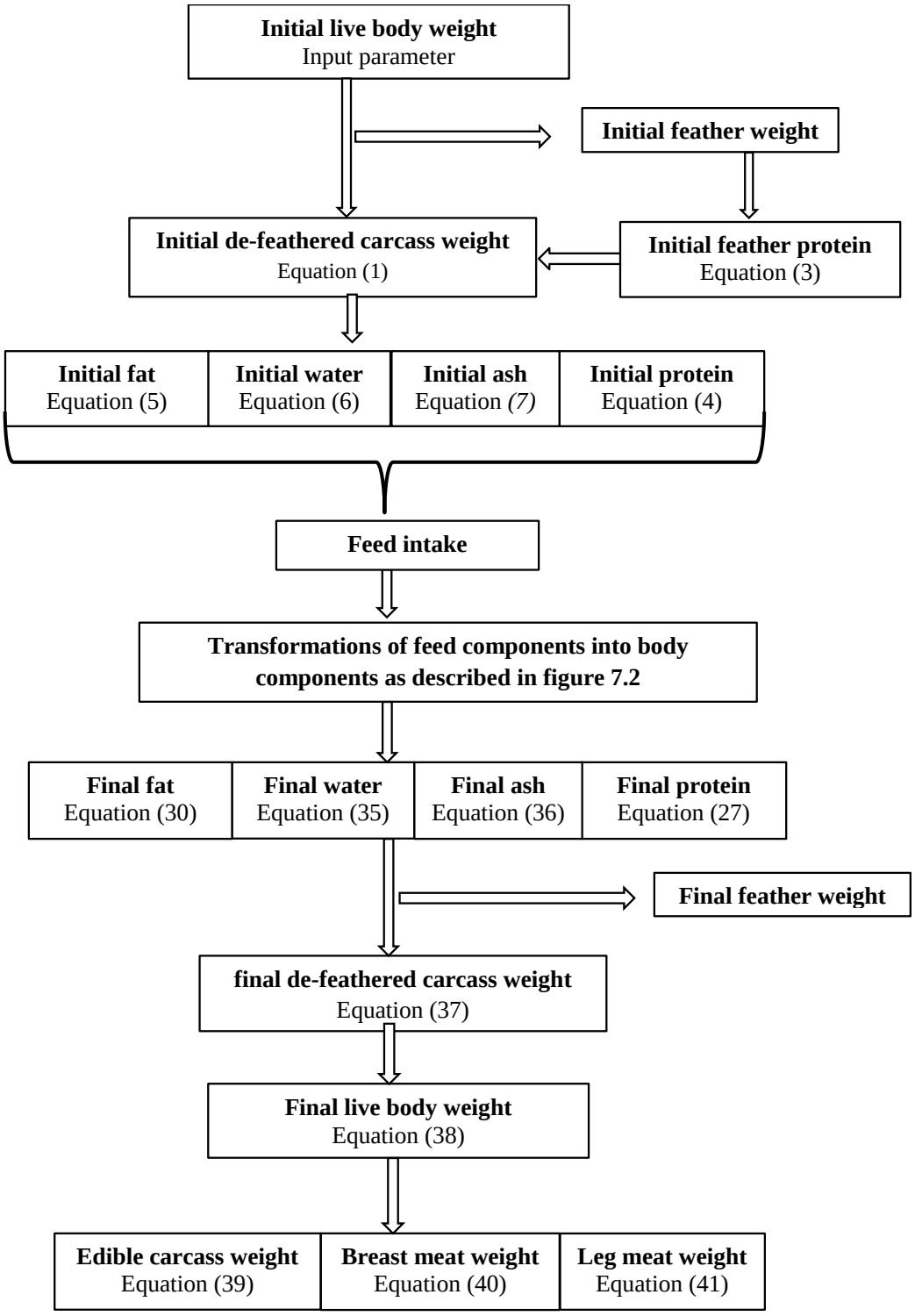

Figure 7.1. Flow diagram of the main steps for broiler chickens growth model

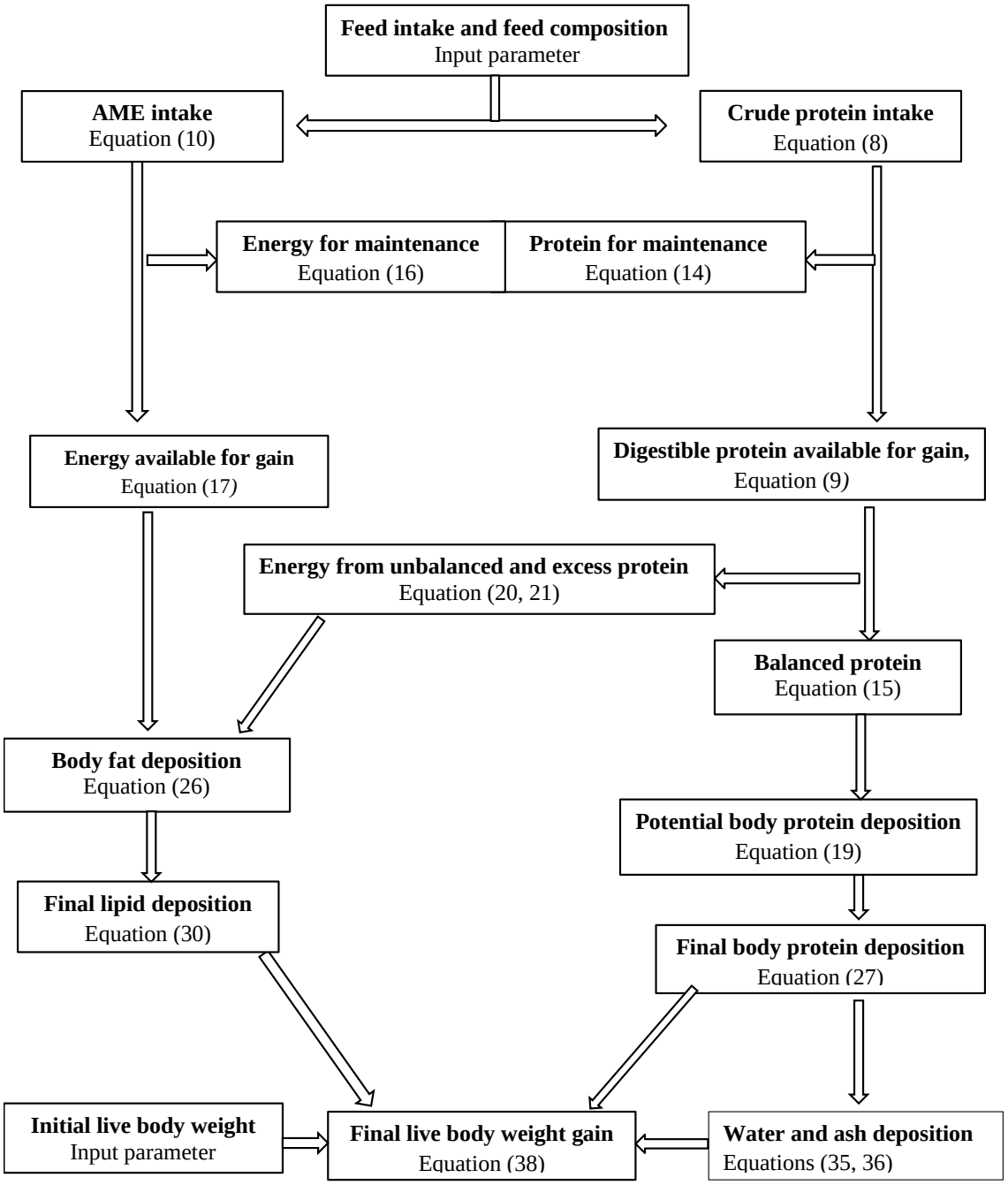

Figure 7.2. The pathway of nutrient transformations into body components in broiler chickens

Step 1- Calculations for initial body composition

The initial live body weight (LBWi) is used as input data, then the de-feathered carcass weight and compositions were calculated as follows:

Initial de-feathered carcass weight (DFCWi) (g) $= 0.9525 \times LBWi$

(n=195, R^2=0.99). (1)

Note; de-feathered carcass means whole bird without feather.

Initial de-feathered carcass protein (DFCPi) (g) $= (0.1686 \times \text{DFCWi})$ (n = 195, $R^2 = 0.98$). (2)

Feather protein (g) $= (0.117 \times DFCPi)$ (n = 114, $R^2 = 0.93$). (3)

Total initial body protein = DFCPi + feather protein (4)

Initial fat (g) $= (0.0281 \times DFCWi^{1.1593})$ (n = 195, $R^2 = 0.68$). (5)

Initial water (g) $= (6.082 \times DFCPi^{0.9355})$ (n = 195, $R^2 = 0.99$). (6)

Initial ash (g) $= (0.174 \times DFCPi^{0.968})$ (n = 195, $R^2 = 0.94$). (7)

The equation from 1-7 were created using data from Chapters 3, 4, 5 and 6.

Step 2- Calculations for daily nutrients intake

The daily feed intake (FI), dietary crude protein (CP), amino acids (AA) profile and the apparent metabolisable energy (AME) in the diet are required as data inputs to calculate the available protein and energy for maintenance and growth.

Crude protein intake (CPI, g) $= [\text{CP\% in the diet} \times \text{FI (g)}]/100.$ (8)

Digestible CPI (DCPI, g) $= [\text{CPI} \times \text{protein digestiblity coefficent (\%)}]/100.$ (9)

AME intake (AMEI, kJ) $= \text{AME (kJ/g) in the diet} \times \text{FI (g)}.$ (10)

Calculation of ideal digestible balanced protein intake

The requirements of all the essential AAs are necessary as data inputs for calculating the ideal digestible balanced protein intake (IDBPI, Table 7.1).

Table 7.1. The digestible amino acids requirement for male broilers grown up to 59 days (Ross, 2007)

	Age (day)			
	0-10	11-28	29-42	43-Slaughter
Composition (g/100g diet)				
Digestible crude protein	20.4	18.7	17.0	15.3
Threonine	0.80	0.68	0.58	0.55
Valine	0.94	0.8	0.67	0.64
Methionine	0.47	0.40	0.34	0.33
Isoleucine	0.84	0.71	0.60	0.57
Leucine*	1.02	0.92	0.79	0.79
Phenylalanine*	0.61	0.55	0.48	0.48
Histidine*	0.29	0.27	0.23	0.23
Lysine	1.27	1.06	0.88	0.84
Arginine	1.33	1.13	0.96	0.92
Methionine-Cysteine	0.94	0.81	0.69	0.66
Glycine-Serine*	1.06	0.97	0.82	0.82
Phenylalanine-Tyrosine*	1.14	1.04	0.88	0.88
Tryptophane	0.22	0.18	0.16	0.15

*The values were adapted from NRC (1994) assumed the digestible AAs is 0.85.

The ideal digestible balanced protein% (IDBP%) is calculated as:

$$\text{IDBP\%} = \text{minimum [IDBP\% (AA), digestible crude protein\% (DCP \%)].} \quad (11)$$

Where; IDBP% (AA) is the IDBP for each AA calculated as;

$$\text{IDBP \% (AA)} = \frac{\text{digestble AA\% in the diet}}{\text{recommended digestible AA\% in the diet}} \times \text{recominded DCP\%} \quad (12)$$

The ideal digestible balanced protein intake is calculated as

$$\text{IDBPI (g)} = [\text{IDBP\% in the diet} \times \text{FI (g)}]/100. \quad (13)$$

Step 3- Calculations of the protein requirement for maintenance and for gain

Protein requirements for maintenance (Pm) are given as a function of LBW

$$\text{Pm (g)} = 1.575 \times \text{LBW}^{0.67}\text{(kg)} \quad \text{Samadi and Liebert (2006).} \quad (14)$$

$$\text{Protein for gain (Pg) (g)} = \text{IDBPI} - \text{Pm.} \quad (15)$$

Step 4- Calculations for energy requirement for maintenance and for growth

Daily AME requirement for maintenance (AMEm) are given as a function of body weight

$$\text{AMEm (kJ)} = 649 \times \text{LBW}^{0.6}\text{(kg)} \quad \text{Lopez and Leeson (2005).} \quad (16)$$

$$\text{AME for gain (AMEg) (kJ)} = \text{AMEI} - \text{AMEm.} \quad (17)$$

Step 5- Calculations for protein deposition from ideal digestible balanced protein intake

The protein deposition rate (g/day) is calculated from the daily IDBPI, Pm and the post-absorptive efficiency for the utilisation protein for growth. The principal goal is to calculate the protein deposition from IDPBI because the IDPBI represents the actual balanced protein that can be used for protein deposition above maintenance.

Protein deposition (Pd) from IDBPI = 0.85 × (IDBPI-Pm) (18)

where, the value 0.85 is the efficiency of protein utilisation for growth after the protein requirement for maintenance (Scheele *et al.*, 1977; Talpaz *et al.*, 1986)

Pd (g/d) =minimum [Protein deposition from IDBPI, Pdmax]. (19)

Note: the protein deposition is calculated linearly related to IDBPI unless:

1) When the protein deposition exceeds the genetic potential for protein deposition, (Pdmax) then extra IDBPI will be converted to fat.
2) The energy intake limits protein deposition. When the energy intake is limiting the body protein depositions, further calculations can represent the protein reduction and fat increases based on minL/P.

MinL/P ratio and Pdmax are 0.31 and 22 (g/day), respectively (Chapter 6).

Step 6- Calculations for fat deposition

The fat in the body can be synthesised from unbalanced or excess protein, digestible starch or from digestible fat.

- **Fat deposition from unbalanced or excess protein intake (Fp)**

A) The energy available from unbalanced protein (EUBP) is calculated as

EUBP (kJ) = (DCPi – IDBPI) × 8.25 (20)

where, the value 8.25 represents the available energy derived from protein catabolism, i.e. the difference between the GE content of protein 23.7 kJ/g and the energy content of uric acid and the amount of energy required to synthesise the uric acid (assuming that the uric acid is the only nitrogenous excretory).

B) The energy from excess protein intake (EEP)

EEP (kJ) = (pd from IDBPI – Pd) × 8.25 (21)

EEP = 0 if the pd ≤ Pdmax.

Then Fp (g) =[(EUBP+EEP) × kf_N]*/39* (22)

kf_N= 0.63, is the energy efficiency of fat deposition from the extra or unbalanced protein, estimated from our experiments (Chapter 5). The value of 39 is the energy content of fat (kJ/g) (Leeson and Summers, 2001).

- **Fat deposition from AMEI**

Since the microbial fermentation and methane production has much smaller impact on the energetic value in broiler chickens compared with other species (Sibbald, 1980; de-Lange and Birkett, 2005; Emmans, 1995), and the conventional way of describing the energy supplied from feedstuffs is based on apparent digestibility rather than true digestibility, and so the AME values are used to calculate the available energy for fat gain

AMEI (kJ) for fat deposition ($AMEI_{fd}$) = AMEg-(Pd × 23.7/*kp*) – EUBP – EEP. (23)

Where AMEg is calculated from equation 17. *kp*= the energetic efficiency for protein deposition (0.66, Boekholt *et al*., 1994)

Fat deposition from available digestible starch

Fs (g) = ($AMEI_{fd}$ × Y × kf_s)*/39.* (24)

Where,

Y is the starch energy fraction which is the proportion of digestible energy of starch from the total of digestible energy from starch and fat.

kf_s=0.69, is the energy efficiency of fat deposition from digestible starch, estimated from our experiments (Chapter 3).

Fat deposition from digestible fat intake (Ff)

Ff (g) = ($AMEI_{fd}$ × (1–Y)× kf_f)*/39* (25)

kf_f*=0.*94 or 0.90 is the energy efficiency of fat deposition from digestible soybean oil or tallow fat, respectively, estimated from our experiments (Chapter 4).

Note: equation 24 and 25 are based on the assumption that the maintenance energy requirements coming from digestible starch and fat are in similar proportion. The

difference between ileal and faecal digestible energy values are likely to be due to microbial fermentation and have been ignored in the model.

Then the potential fat deposition (Fd) (g/d) = Fp (g) + Fs (g) +Ff (g) (26)

Step 7- Calculations for final body component

Final body protein (g) = initial protein (g) + Pd (g) (27)

Final feather protein (g) = 0.1047× final body protein (g) (n = 114, R^2 = 0.94) (28)

Final de-feathered carcass protein (DFCPf) (g)

= final body protein – final feather protein (29)

Final fat (g) = initial fat (g) + Fd (g) (30)

If the final de-feathered carcass protein divided by the final fat is less than the minL/P ratio then the final body protein deposition will be reduced as:

Final body protein (g) = initial protein (g) + Pd (g) – $Pd_{reduction}$ (31)

(i.e: the balanced digestible protein is preferred to catabolise to increase the amount of energy that is available for increasing the body fat deposition ($Fd_{increased}$).

Final fat (g) = initial fat (g)+ Fd (g) + $Fd_{increased}$ (32)

The equations for $Pd_{reduction}$ and $Fd_{increased}$ were derived from de-Lange, (1995) as follows:

$Pd_{reduction}$= [minL/P × (DFCPf) – final fat] ÷ [((23.7/*kp*)-$Ep_{excretion}$)/(39/kf_N)) + minL/P]

Where, 23.7 and 39 are the GE content of protein and fat, respectively.

$Ep_{excretion}$ is the energy cost of protein excretion in the form of uric acid (15.45 kJ/g)

Rearranging the equation gives:

$Pd_{reduction}$ = [(0.31 × DFCPf) – final fat] ÷ (0.64) (33)

$Fd_{increased}$ = $Pd_{reduction}$ × [((23.7/kp) – $Ep_{excretion}$)/(39/kf_N)]

Then $Fd_{increased}$ = $Pd_{reduction}$ × 0.33 (34)

Final water(g) = 6.082 × (DFCPf $^{0.9355}$) (n = 195, R^2=0.99) (35)

Final ash (g) = 0.174× (DFCPf $^{0.9968}$) (n = 195, R^2=0.94). (36)

Final de- feathered carcass weight (DFCWf) (g)

= DFCPf + final fat + final water + final ash. (37)

The final protein, fat, ash water and live weight at day x were used as data input for next day run (x+1).

Step 8- Calculations for final live body weight and production.

Final live body weight (LBWf) = DFCWf /0.9525. (n = 195, R^2 = 0.99) (38)

Edible carcass yield (g) = LBWf (g) × 0.724 (n = 96, R^2 = 0.97) (39)

Total breast meat yield (g) = LBWf (g) × 0.181 (n = 96, R^2 = 0.74) (40)

Total leg meat yield (g) = LBWf (g) × 0.193 (n = 96, R^2 = 0.86) (41)

The data for equations (39), (40) and (41) were obtained from study of Abdollahi and Ravindran, (2014), assumed that the relationship between the live body weight and body components is a linear relationship.

Feed per gain = feed intake/body weight gain (42)

7.4.3. Modelling the daily feed intake

The feed intake is the main factor effect on the growth performance and it is influenced by genetics, sex, breed, body weight, age, nutrient density and environmental conditions. Therefore, it is hard to predict (Emmans, 1995; Gous, 2007; Mbajiorgu *et al.*, 2011). In our model we preferred to use the daily feed intake as an input parameter. When an intake cannot be provided for input the model estimates the daily feed intake from live body weight (LBW) as follows:

$FI = 17.36 + 0.1105\ LBW - 0.000012 \times LBW^2$ (R^2 = 0.99) (43)

Adapted from recent data of Ross, (2012)

7.5. Discussion

Conventionally, the total AA composition of ingredients was used to formulate the broiler chicken diet (NRC, 1994). However, the digestibility of AAs varies between the ingredients and therefore, in our feed formulation program consideration of the digestible AA content in the ingredients was made (Bryden *et al*, 2009). The digestible AA requirement was used for calculations of the IDBP% in the diet.

The energy and protein balances measured in the previous four experiments and the serial slaughter analysis of 195 birds provided good datasets to estimate the parameters that were needed. King *et al.* (2001) developed a broiler growth model and estimated the water as a sum of water in lean tissue (4 gram per g protein), water in adipose tissue (0.25 g per gram of fat) and in ash (0.43 gram per gram of ash). The relationship between water and protein in the body of animal was not affected by genotype or nutrition (Emmans, 1988; De Greef *et al*., 1992; Eits, 2004). According to

Emmans and Kyriazakis (1995) and de Lange *et al*. (2003) in pigs and Hruby *et al*. (1994) and Eits *et al*. (2004) in broilers, whole body water and ash content are best expressed per kilogram of body protein based on an allometric relationship. Therefore, in our case the allometric relationships indicated that the water weight was 6.08 gram per gram of $(\text{protein})^{0.94}$ ($R^2 = 0.99$). Also, the ash weight was 0.17 gram per gram of $(\text{protein})^{0.97}$ ($R^2 = 0.94$). The allometric slope was below unity (0.94 for water and 0.97 for ash). This means that, with increasing body protein weights, the amount of water and ash per unit of body protein decreases continuously. Eits (2004) used a logarithmic scale to represent the relationships between water and ash weight with protein weight, the equation for water was ($\ln \text{water} = 1.22 + 0.945 \times \ln \text{protein}$, $R^2 = 0.99$) and for ash ($\ln \text{ash} = -1.90 + 0.999 \times \ln \text{protein}$, $R^2 = 0.99$). In the same report, the data used were for broilers slaughtered at fixed body weight of 200, 800 and 1600 g. In contrast, the data in our model was described a wide range of live body weight.

The current growth model predicts the effect of nutrient intake on carcass composition and live body weight. The model is based on the partitioning of the daily amount of energy and protein taken by the bird. The available energy for fat gain is defined by the protein free energy and the energy come from protein intake but not used for protein deposition due to either imbalanced protein intake or over the Pdmax requirements. Previously, the energy efficiency of fat deposition was described by one *k*-value (*kf* = 0.86, Boekholt *et al*., 1994; Lopez and Lesson, 2005; Lopez *et al*., 2007). King (2001) used the value of 0.69 for the efficiency of fat deposition, whereas, Zoon *et al*. (1991) used a value of 0.70. In our model the efficiency of fat deposition is differentiated by the source of energy namely: 0.93 with soybean oil, 0.90 with tallow fat, 0.69 with starch and 0.63 with imbalanced or excess protein.

The IDBP intake was used for maintenance and growth. The protein deposition then calculated as $Pd = b \times [\text{IDBP intake} - Pm]$, where supplying the IDBP intake is not in excess of Pdmax and independent of energy intake. The value of b is the efficiency of protein utilisation for growth after protein maintenance requirement. Whittemore, (1995), Whittemore (1983) and Fisher (1976) considered the efficiency of protein utilisation for growth to be between 0.80 and 1.0 for growing pigs. Under a non-limiting supply of dietary protein, the efficiency of protein deposition is 0.45 for pigs with a low energy intake and 0.81 with a high energy intake (Kyriazakis and Emmans 1992). In broiler chickens, the efficiency of utilisation of AA for growth is 0.75 to 0.80 (Gous, 2007). Hruby *et al*. (1994) reported that the ideal digestible protein requirement for

protein deposition is 1.15 per gram body protein deposition (i.e., an efficiency of 0.87) from 1 to 14 days of age. In our model, the efficiency of protein deposition over maintenance requirement was 0.85 as reported by Scheele *et al*. (1977) and Talpaz *et al*. (1986). However, the IDBP was calculated when the AA in the diet was limiting and assuming all essential AA had similar growth response. Baker (1991) reported that the utilisation efficiency from individual AA varies and the slow turnover AA such as lysine is more efficient than the fast turn over AA such as isoleucine. Some research is needed to estimate the efficiency of protein deposition depending on which AA is derived for the IDBP calculation.

The requirements of AA depend on the AA profile of the protein being gained. As the AA composition of carcass protein and feather protein are very different (Skland and Noy, 2004; Gous *et al*., 1999), a total ideal digestible balanced intake needs to be partitioned into that for carcass and feather growth to accurately calculate the AA requirement.

It has been proposed that broilers have an inherent ratio between body protein and lipid and birds will always attempt to maintain this ratio (Gous *et al*., 2012). However, no references available describe this relationship in broilers and therefore the mathematical functions of de-Lange, (1995) are referred to use when the lipid to protein ratio was less than the minL/P ratio (equations, 33 and 34).

In comparison to other growth models (Zoon *et al*., 1991; King, 2001; EFG Broiler Growth Model version 5.1) the model described here deals with the nutrients partitioning concept and could be modified for different genders and genetics, however, it does not account for the effect of environment (temperature, stock density and humidity) on the growth responses. Also, the growth response for a population may be different from that predict from individual bird (Pomar *et al*., 2003; Gous and Berhe, 2006). Information about effect of gender, variation of strain and environment may be used to validate this model under commercial condition.

7.6. Conclusion

The results indicate that the mechanistic broiler growth model that has been developed based on nutrient and energy partitioning concepts can be an acceptable tool to predict the carcass composition and broiler performance. Further research is needed in order to validate the model. Therefore, the model evaluation is described in Chapter 8.

Table 7.2. Mathematical model symbols and parameter values

Symbol	Description	Role	Value
AA	AA (%)	-	-
AME	Apparent metabolisable energy (kJ/g)	Output[1]	-
AMEg	Apparent metabolisable energy for gain(kJ)	Output[2]	-
AMEI	Apparent metabolisable energy intake (kJ)	Output[2]	-
AMEIfd	Apparent metabolisable energy intake for fat deposit(kJ)	Output[2]	-
CP	Dietary crude protein %	Output[1]	-
CPI	Dietary crude protein intake (g)	Output[2]	-
DCP	Digestible crude protein %	Output[1]	-
DCPI	Digestible crude protein intake(g)	Output[2]	-
DFCP	De-feather carcass protein(g)	-	-
DFCPf	De-feather carcass protein final(g)	Output[2]	-
DFCPi	De-feather carcass protein initial (g)	Output[2]	-
DFCW	De-feather carcass weight (g)	-	-
DFCWf	De-feather carcass weight final(g)	Output[2]	-
DFCWi	De-feather carcass weight initial (g)	Output[2]	-
EEP	Energy from excess protein intake (kJ)	Output[2]	-
EUBP	Energy from unbalanced protein (kJ)	Output[2]	-
Fd pot	Potential body fat (g/d)	Output[2]	-
Ff	Fat deposition from fat intake (g/d)	Output[2]	-
FI	Feed intake (g)	Input	-
Fp	Fat deposition of unbalance/excess protein intake (g/d)	Output[2]	-
Fs	Fat deposition from starch intake (g/d)	Output[2]	-
IDBP(AA)	Ideal digestible balance protein for AA	Output[1]	-
IDBP	Ideal digestible balance protein	Output[1]	-
IDBPI	Ideal digestible balance protein intake (g)	Output[2]	-
kf_f	Energy efficiency of fat deposition from fat	Parameter	0.90-0.93
kf_N	Energy efficiency of fat deposition from protein	Parameter	0.63
kf_s	Energy efficiency of fat deposition from starch	Parameter	0.69
kp	Energy efficiency of protein deposition	Parameter	0.66
LBW	Live body weight (g)	-	-
LBWf	Final live body weight (g)	Output[2]	-
LBWi	Initial live body weight (g)	Input	-
MEm	Metabolisable energy for maintenance requirement(kJ)	Parameter	$649 kJ/kg^{0.6}$
minL/P	Minimum lipid/protein ratio	Input	0.31
Pd	Protein deposition (g/d)	Output[2]	-
Pdmax	Maximum protein potential (g/d)	Input	22g/d
Pg	Protein for gain (g)	Output[2]	-
Pm	Protein for maintenance (g)	Parameter	$1.575/kg^{.67}$
Y	Starch fraction	Output[1]	0-1

[1] is the output from feed formulation program. [2] is the output from growth model.

CHAPTER 8

Model evaluation and further development

8.1 Abstract

A mechanistic growth model for modern broiler chickens has been developed in this thesis (Chapter 7). This model predicts live body weight and carcass composition, based on metabolisable energy and protein intake. Protein deposition is predicted using information on an upper limit for protein deposition and an ideal balanced protein intake. Fat deposition is predicted using information on metabolisable energy intake and utilisation efficiencies: with the constraint that lipid to protein ratio must be equal or greater than minL/P ratio (0.31). Water and ash contents are predicted from protein content using allometric equations. Live body weight is calculated as a summation of body components. This model was applied to three different datasets: (a) dataset obtained from the literature on experiments conducted in New Zealand (independent, NZ); (b) dataset obtained from the literature on experiments conducted outside New Zealand (independent, outside NZ); and (c) dataset from our experiments, that were used for model calibration (dependent). Then the model output was evaluated using different methods:

(i) A comparison between actual and predicted values using a paired t-test to accept or reject the differences between predicted and actual values.

(ii) Statistical properties were considered by linear regression.

(iii) Prediction errors were characterised by the relative prediction error (RPE) and the concordance correction coefficient (CCC).

(iv) Model parameters were evaluated by sensitivity analysis.

Results indicate that the predicted values were in close agreement with actual values. The linear regression coefficient between observed and predicted values was approximately 1, and the coefficient of determination was 99%, for the prediction of live body weight. The RPEs were 3.8, 7.5 and 7.3% for prediction of live body weight for dependent, independent NZ and independent out of NZ dataset, respectively. The predicted live weight always appears to be more sensitive to parameters related to protein deposition, than to lipid deposition.

The objectives in this study were to evaluate a broiler growth model, by comparing the means of treatment observation values with predicted values for dependent and independent datasets, and to find which parameters have the most impact on growth prediction.

8.2. Introduction

Model evaluation is important to prove the accuracy of the model. The evaluation of the model involves several steps including setting the boundaries of the model, evaluating the model structure, testing the model and making a comparison between model predicted and actual values and evaluating the model in practical applications (Fisher and Gous, 1998). There are many methods available to compare the predicted and actual values; the linear regression of actual versus predicted values may be provide a useful insights the R^2 values and evaluation of the slope and intercept values where good prediction has a slope = 1 and intercept = 0 (Vanclay and Skovsgaard, 1997).

The relative prediction error (RPE) is another statistical method used for evaluating the accuracy of the model prediction. The RPE is defined as the ratio between the root square of the mean square prediction error (MSPE) and the mean of observed actual values (Fuentes-Pila *et al*., 2003; Fuentes-Pila *et al*., 1996). A desirable reproducibility index such as the concordance correlation coefficient (CCC) evaluates the agreement between actual and predicted values (y and x) as described by Lin (1989) and Nickerson (1997).

The sensitivity analysis is used to evaluate the responses of model parameters. The sensitivity analysis can be help to determine which parameters are the key drivers of the model output (Majkowski and Bramall, 1980). The objectives in this study were to evaluate the broiler growth model, comparing the observed experimental treatment means against the predicted values for dependent and independent datasets, and to find which model parameters have the most impact on the growth prediction.

8.3. Materials and Methods

The model was tested using three different datasets:

- A dependent dataset was used for model calibration obtained from our experiments (4 experiments with 17 different dietary treatment means).
- An independent dataset was taken from recent experiments conducted in New Zealand (6 experiments with 22 dietary treatment means).
- An independent dataset collected from recent experiments conducted outside New Zealand (5 experiments with 10 different dietary treatment means). Details on source, bird characteristics, diet characteristics and study consideration are presented in Tables 8.1, 8.2 and 8.3.

Table 8.1. Dependent dataset profile

	bird characteristics				diet characteristics		
Treatment	growth phase	LBWi	LBWf	FI (g)	AME (MJ/kg)	CP (%)	Consideration
Basal(Ch3)	15-36	473	1589	1987	12	20.1	
B+Oil	15-36	473	1714	2080	12.8	19.7	efficiency of ME from oil
B+Casein	15-36	468	1819	2137	12.8	26.4	efficiency of ME from casein
B+Starch	15-36	475	1669	2173	12.6	18.3	efficiency of ME from starch
Basal(Ch4)	21-42	576	1856	2555	12.8	17.2	
MO	21-42	566	1921	2657	13.6	16.6	efficiency of ME from soybean oil
HO	21-42	583	1847	2550	14.4	16.1	efficiency of ME from soybean oil
MT	21-42	578	1910	2672	13.8	16.4	efficiency of ME from tallow
HT	21-42	583	1900	2616	14.2	15.9	efficiency of ME from tallow
Basal(Ch5)	21-42	955	3387	3863	13.4	20.4	
Basal-res	21-42	948	2861	3229	13.7	20.4	
B+NEAA1	21-42	961	3064	3399	13.5	23.1	efficiency of ME from NEAA
B+NEAA2	21-42	968	3120	3556	13.6	25.5	efficiency of ME from NEAA
L-Energy	14-51	592	4015	5605	12.7	28.8	Low ME intake-high protein intake
M-Energy	14-48	594	3965	5149	13.6	28	medium ME intake-high protein intake
MH-Energy	14-46	591	3997	4895	14	27.1	medium-high ME intake-high protein intake
H-Energy	14-44	601	4000	4796	14.8	25.9	high ME intake-high protein intake

Table 8.2. Dataset profiles for experiments conducted in New Zealand.

	Bird characteristics				Diet characteristics		
Treatment	growth phase	Bwi	LBWf	FI (g)	CP %	AME	consideration
Tancharoenrat (2012)							Different dietary fat content
Tallow 0%	1-21	40	795	1004	22.8	12.17	
Tallow 4%	1-21	40	847	1050	22.8	12.79	
Tallow 8%	1-21	40	955	1195	22.8	13.34	
Maize-Tallow	1-21	40	822	1054	23	12.74	
Abdollahi (2011)							
Sorghum based	1-21	40	991	1228	22.7	13.2	Sorghum and soybean based diet
Maize based	1-21	40	1089	1279	22.7	12.87	Maize and soybean based diet
Mash	1-21	40	915	1150	23.7	13.02	Mash diet
Pellet	1-21	40	1005	1308	23.7	13.02	Pellet diet
Maize based 90 ºC	1-21	40	1055	1279	22.7	12.87	Maize and soybean based diet
Wheat based 90 ºC	1-21	40	948	1255	23.7	13.02	Wheat and soybean based diet
Pellet binder	1-35	40	2724	4148	(24.1) (21.6)	(13.1)(13.4)	Different pelleting temperature
Pellet diameter/length 3-3	11-42	330	3374	5102	(22.9)(21.1)	(13.4)(13.8)	Different pelleting diameter
Singh (2012)							
Whole maize 0%	1-21	40	1045	1303	22.3	13.05	Whole maize inclusion
Pre pellet Whole wheat	11-35	330	2334	3371	20.4	12.87	Pre pellet Whole wheat inclusion
Post pellet Whole wheat	11-35	330	1955	2893	20.4	12.87	Post pellet Whole wheat inclusion
Ravindran, unpublished							Different energy-lysine ratios
Treatment 1	1-42	40	3580	5086	(26.6)(26.6)(24.6)	(13.5)(13.5)(13.9)	
Treatment 2	1-42	40	3325	4938	(21.5)(21.5)(19.5)	(13.5)(13.5)(13.9)	
Treatment 3	1-42	40	3557	5361	(24) (24)(22.3)	(12.2)(12.2)(12.8)	
Treatment 4	1-42	40	3209	5216	(19.5)(19.5)(17.4)	(12.2)12.2)(12.8)	
Zaefarian et al. (2013)							
Maize based	1-21	40	866	1121	23	12.73	Maize and soybean based diet
Wheat based	1-21	40	833	1110	23.8	12.8	Wheat and soybean based diet
Nalle et al. (2011)							
Maize soy diet	1-21	40	973	1240	24.45	11.92	Maize and soybean based diet

The initial body weight was assumed to be 40 g at 1 day of age and 330 g at10 days of age. The diet composition was estimated by our feed formulation program and the feed intake was used as a data input for growth model. Numbers in the parentheses represented the composition for starter, grower and finisher diets, respectively.

Table 8.3. Dataset profiles for experiments conducted outside of New Zealand.

	Bird characteristics				Diet characteristics		
	growth phase	LBWi	LBWf	FI (g)	CP(%)	ME (MJ/kg)	consideration
Selle et al. (2007)							
Lysine 10	7-28	200	1023	1476	20.9	12.79	Effects of dietary lysine on growth performance
Lysine 11.8	7-28	200	1099	1473	21.2	12.83	
Behrooz et al. (2011)							
Standard	1-21	40	489	727	(22.3)(20.4)	(12.3)(13)	Effects of dietary energy and protein dilution
Standard	1-42	40	1874	3181	(22.3)(20.4)(18.4)	(12.3) (13)(13.2)	
Diluted	1-21	40	518	783	(21.5)(19.3)	(11.7)(12.3)	
Diluted	1-42	40	1990	3367	(21.5)19.3)(17.4)	(11.7) (12.3) (12.5)	
Peter et al. (2010)							
Ross 308	1-35	40	1645	2621	(21.73)(21.7)(19.6)	(11.9)(12.5)	
Zand and Foroudi (2011)							
Control	1-42	40	2367	3763	(24.9)(22)(19.7)	(12.8)(13.9)(14)	Used of commercial produced feed mixtures
Scheurer et al. (2013)							
Control	1-22	40	871	1161	(22.4)(21.6)	(12.4)(13.2)	Different starter, grower and finisher diets
Control	1-39	40	2472	3278	(22.4)(21.6)(21.5)	(12.4)(13.2)(13.1)	

The initial body weight assumed to be 40 g at day 1 of age and 200 g at days 7 of age. The diet composition was estimated by our feed formulation program and the feed intake was used as a data input for growth model. Numbers in the parentheses represented the composition for starter, grower and finisher diets, respectively.

The data regarding dietary composition, feed intake, and initial live body weight were used as data input for the model and then the output from the model was evaluated by following methods:

1) *Paired t-test*

Comparison between actual and predicted values and using the paired t-test to accept or reject the differences between predicted and actual values.

The t-test compared the actual differences between the means of actual values ($M_{A)}$ and predicted values ($M_{P)}$ in relation to standard deviation of the actual values (S_A).

The null hypothesis is stated as H_0: $M_P = M_A$, and the alternative hypothesis is

H_A: $M_P \neq M_A$

$$t = \frac{(MA-Mp)}{SA/\sqrt{n}}$$

where:

n: is the number of actual values.

If the t-test results accept the H_0: good prediction (t calculated < t table).

If the t-test results reject the H_0: poor prediction (t calculated > t table).

2) Linear regression

Linear regression was used (with no intercept) to study the linear regression of the predicted on actual values. Y (prediction values) = X (actual values).

3) *The relative prediction error (RPE) and concordance correlation coefficient (CCC).*

$$RPE = (MPE/ \bar{A}) \times 100$$

$$MPE = \sqrt{MSPE}$$

$$MSPE = \frac{1}{n}\sum_{i=1}^{n} (Ai - Pi)^2$$

According to Bibby and Toutenberg, (1977) the MSPE can be considered as the sum of three components: mean bias, line bias and random variation around the regression line.

MSPE = error in central tendency (mean bias) + errors due to regression (line bias) + errors due to disturbances (random variation). Then the MSPE is calculated as follows:

$$MSPE = (\bar{A} - \bar{P})^2 + (S_P - RS_A)^2 + S_A^2 (1-R^2).$$

The concordance correlation coefficient was used to indicate how far the regression line deviates from slope of unity at 45° as following equation;

$CCC = 2S_{AP}/ (S_A^2+S_P^2+ (\overline{A}-\overline{P})^2)$

Where;

MSPE is the mean square prediction error,

Ai is the ith actual value,

Pi is the ith predicted value,

n is the number of pairs of A and P values being compared,

$\overline{A}$ and $\overline{P}$ are the means of A and P values,

S_A^2 and S_P^2 are the variances of A and P values, respectively,

b is the slope of the regression of A on P with intercept zero,

R is the correlation coefficient of A and P.

S_{AP} is the covariance between A and P values.

Fuentes-Pila *et al*. (1996) assumed that, when RPE was less than 10%, it indicated a good prediction; when RPE was between 10%-20% it indicated a relatively acceptable prediction; and higher than 20% indicated an unsatisfactory prediction. A model is considered to be robust if RPE for most of the datasets is less than 10%. Visser *et al*. (2012) suggested that a CCC value higher than 0.81 indicates an acceptable prediction.

4- Sensitivity analysis

Sensitivity analysis was also used to evaluate the model output response to the change of the input parameters. Sensitivity calculated as the ratio of the standard change on model response to the standard change on parameter value (Majkowski and Bramall, 1980);

$$S = \frac{(Ra-Rn)/Rn}{(Pa-Pn)/Pn}$$

where

S is the sensitivity

Ra and Rn are the model responses for altered and nominal parameters, respectively.

Pa and Pn are the altered and nominal parameters, respectively.

The sensitivity analysis was applied to five different feeding conditions; low energy intake and low protein intake (basal diet, chapter 3), low energy intake and high protein intake (L-Energy, chapter 6), high energy intake and low protein intake (HO, chapter 4), high energy intake and high protein intake (H-Energy, chapter 6) and standard diet (21 g CP, 13.4 MJ ME

per kg, *ad-libitum* feed intake, monitor group, chapter 5). The objective of using different feeding conditions was to determine the growth responses to changes of model parameters and to explain all combinations of nutrition factors.

8.4. Results

8.4.1. A comparison between actual and prediction values using the t-test

The predicted values were in close agreement with the actual values and the absolute prediction error for lipid was 3.9-37.7 g, for protein it was 0.2-46.3 g and for live body weight it was 1.4-192g. The t-test indicated that there were no statistical differences ($P > 0.05$) between the actual and predicted value for most of the dependent datasets (Table 8.4, 8.5 and 8.6). The absolute prediction errors for the feed conversion ratio were ranged between 0.0-0.35 (Table 8.7).

Table 8.4. A comparison between the actual and predicted lipid deposition (g/bird), using data of current thesis (dependent dataset)

Treatment	Actual Lipid (g/bird)	Predicted Lipid (g/bird)	Difference	significances
Basal(Ch3)	86.7	80.1	-6.6	NS
B+Oil	115.0	104.8	-10.3	NS
B+Casein	98.7	87.5	-11.2	*
B+Starch	122.0	110.4	-11.6	NS
Basal(Ch4)	242.7	272.7	30.0	**
MO	313.8	334.3	20.5	*
HO	353.9	364.4	10.5	NS
MT	322.0	332.4	10.4	NS
HT	346.2	378.2	32.0	NS
Basal-ad-lib	388.6	371.7	-16.9	NS
Basal-res	305.2	313.6	8.4	NS
B+NEAA1	306.3	310.2	3.9	NS
B+NEAA2	328.9	333.8	4.8	NS
L-Energy	218.6	212.7	-5.9	NS
M-Energy	238.0	253.9	15.9	NS
MH-Energy	302.8	296.0	-6.8	NS
H-Energy	448.9	411.2	-37.7	NS

NS is not significant, * $P < 0.05$, ** $P < 0.01$. The full definitions of treatment diets existed in experiment chapters.

When the model was applied to an independent dataset (experiments conducted in New Zealand), the differences between the actual and predicted values for live body weight ranged

between 2.0 to 287g, for FCR it ranged between 0.0 and 0.37 (Table 8.8, 8.9). When the model was applied to a dataset from experiments conducted out of New Zealand, the differences between actual and predicted for live body weight ranged between 7.0 to 265 g and for FCR were ranged between 0.01 to 0.25 (Table 8.10, 8.11).

Table 8.5. A comparison between the actual and predicted protein deposition (g/bird), using data of current thesis (dependent datasets)

Treatment	Actual protein	Predicted protein	Difference	significances
Basal(exp1)	298.3	297.4	-0.9	NS
B+Oil	311.5	356.8	45.3	***
B+Casein	345.5	329.6	-16.0	**
B+Starch	304	343.0	39.0	***
Basal(exp2)	317.2	270.9	-46.3	***
MO	305.9	286.4	-19.6	*
HO	289.7	278.2	-11.5	NS
MT	307.3	296.2	-11.1	*
HT	303.3	285.1	-18.1	NS
Basal-ad-libitum	575.9	595.3	19.3	*
Basal-restriction	513.3	510.6	-2.7	NS
B+NEAA1	548.6	548.4	-0.2	NS
B+NEAA2	544.4	573.9	29.5	***
L-Energy	783	756.9	-26.1	NS
M-Energy	749	787.5	38.5	NS
MH-Energy	728	762.4	34.4	NS
H-Energy	723	727.9	4.9	NS

NS is not significant, * $P < 0.05$, ** $P < 0.01$, ***$P < 0.001$.

Table 8.6. A comparison between the actual and predicted live body weight (g/bird), using data of current thesis (dependent datasets)

Treatment	Actual LBW	Predicted LBW	Difference	significances
Basal (exp1)	1589	1590.4	1.4	NS
B+Oil	1714	1900.4	186.4	***
B+Casein	1819	1752.2	-66.8	NS
B+Starch	1669	1840.6	171.6	***
Basal (exp2)	1856	1664.9	-191.1	***
MO	1921	1804.1	-116.9	**
HO	1847	1796.4	-50.6	NS
MT	1910	1849.3	-60.7	NS
HT	1900	1844.4	-55.6	NS
Basal-ad-libitum	3387	3299.5	-87.5	**
Basal-restriction	2861	2844.7	-16.3	NS
B+NEAA1	3064	3017.3	-46.7	NS
B+NEAA2	3120	3160.4	40.4	NS
L-Energy	4015	3877.6	-137.4	*
M-Energy	3965	4060.6	95.6	NS
MH-Energy	3997	3990.3	-6.7	NS
H-Energy	4000	3952.7	-47.3	NS

NS is not significant, * $P < 0.05$, ** $P<0.01$, *** $P < 0.001$.

Table 8.7. A comparison between the actual and predicted feed conversion ratio (FCR, g/g), using data of current thesis (dependent datasets)

Treatment	Actual FCR	Predicted FCR	Difference
Basal(exp1)	1.78	1.78	0.00
B+Oil	1.68	1.46	-0.22
B+Casein	1.58	1.66	0.08
B+Starch	1.82	1.59	-0.23
Basal(exp2)	2.00	2.35	0.35
MO	1.96	2.15	0.19
HO	2.02	2.10	0.08
MT	2.01	2.10	0.09
HT	1.99	2.07	0.08
Basal-ad-libitum	1.59	1.65	0.06
Basal-restriction	1.69	1.70	0.01
B+NEAA1	1.62	1.65	0.03
B+NEAA2	1.65	1.62	-0.03
L-Energy	1.64	1.71	0.07
M-Energy	1.53	1.49	-0.04
MH-Energy	1.44	1.44	0.00
H-Energy	1.41	1.43	0.02

Table8.8. A comparison between the actual and predicted live body weight (g/bird) for independent dataset (experiments conducted in New Zealand).

Treatment	Actual LBW	Predicted LBW	Difference
Tancharoenrat (2012)			
Tallow 0%	795	631	-164
Tallow 4%	847	736	-111
Tallow 8%	955	957	2.0
Maize-Tallow	822	739	-83
Abdollahi (2011)			
Sorghum based	991	891	-100
Maize based	1089	996	-93
Mash	915	859	-56
Pellet	1005	1030	25
Maize based 90 ºC	1055	1004	-51
Wheat based 90 ºC	948	972	24
Pellet binder	2724	2886	162
Pellet diameter/length 3-3	3374	3667	293
Singh (2012)			
Whole maize 0%	1045	1040	-5.0
Pre pellet Whole wheat	2334	2491	157
Post pellet Whole wheat	1955	2242	287
Ravindran, unpublished			
Treatment 1	3580	3535	-45
Treatment 2	3325	3396	71
Treatment 3	3557	3392	-165
Treatment 4	3209	3270	61
Zaefarian *et al*. (2013)			
Maize based	866	828	-38
Wheat based	833	808	-25
Nalle *et al*. (2011)			
Maize soy diet	973	823	-150

The feed intake and diets composition as described in Table 8.2.

Table 8.9. A comparison between the actual and predicted feed conversion ratio (FCR, g/g) for independent dataset (experiments conducted in New Zealand)

Treatment	Actual FCR	Predicted FCR	Difference
Tancharoenrat (2012)			
Tallow 0%	1.33	1.70	0.37
Tallow 4%	1.30	1.51	0.21
Tallow 8%	1.31	1.30	0.01
Maize-Tallow	1.35	1.51	0.16
Abdollahi (2011)			
Sorghum based	1.29	1.44	0.15
Maize based	1.22	1.34	0.12
Mash	1.31	1.40	0.09
Pellet	1.36	1.32	-0.04
Maize based 90 ºC	1.26	1.33	0.07
Wheat based 90 ºC	1.38	1.35	-0.03
Pellet binder	1.55	1.46	-0.09
Pellet diameter/length 3-3	1.68	1.53	-0.15
Singh (2012)			
Whole maize 0%	1.30	1.30	0.00
Pre pellet whole wheat	1.68	1.56	-0.12
Post pellet whole wheat	1.78	1.51	-0.27
Ravindran, unpublished			
Treatment 1	1.44	1.46	0.02
Treatment 2	1.50	1.47	-0.03
Treatment 3	1.52	1.60	0.08
Treatment 4	1.65	1.62	-0.03
Zaefarian *et al*. (2013)			
Maize based	1.36	1.42	0.06
Wheat based	1.40	1.45	0.05
Nalle *et al*. (2011)			
Maize-Soybean diet	1.33	1.58	0.25

Table 8.10. A comparison between the actual and predicted live body weight (LBW, g/bird) for independent dataset (experiments conducted outside of New Zealand)

	Actual LBW (g)	Predicted LBW (g)	Difference (g)
Selle *et al*. (2007)			
Lysine 10	1023	1064	41
Lysine 11.8	1099	1106	7.0
Behrooz *et al*. (2011)			
Standard	489	437	-52
Standard	1874	1986	112
Diluted	518	455	-63
Diluted	1990	1981	-9.0
Peter *et al*. (2010)			
Ross 308	1645	1625	-20
Zand and Foroudi (2011)			
Control	2367	2632	265
Scheurer *et al*. (2013)			
Control	871	857	-14
Control	2472	2595	123

Table 8. 11. A comparison between the actual and predicted feed conversion ratio (FCR, g/g) for independent dataset (experiments conducted outside of New Zealand)

Treatment	Actual FCR	Predicted FCR	Difference
Selle *et al*. (2007)			
Lysine 10	1.79	1.71	-0.08
Lysine 11.8	1.64	1.63	-0.01
Behrooz *et al*. (2011)			
Standard	1.62	1.83	0.21
Standard	1.73	1.63	-0.1
Diluted	1.64	1.89	0.25
Diluted	1.73	1.73	0.01
Peter *et al*. (2010)			
Ross 308	1.63	1.65	0.02
Zand and Foroudi (2011)			
Control	1.62	1.45	-0.17
Scheurer *et al*. (2013)			
Control	1.4	1.42	0.02
Control	1.35	1.28	-0.07

8.4.2. Overall accuracy of the model prediction

The results of the evaluation of the model accuracy are presented in Table 8.12. In our evaluation, the slope (b) of linear regression was close to one, indicated a good prediction accuracy. When the b is < 1, the model tends to be under-prediction, whereas, when the b is > 1, the model tends to be over-prediction. The coefficient of

determination (R^2) were 0.99 for prediction of LBW and between 0.24 and 0.82 for prediction of FCR.

The RPE for live body weight was 3.8 for the dependent dataset, whereas the RPE for independent datasets were approximately 7.3. Also, with independent datasets the RPE for feed conversion ratio ranged between 7.7-10.1.

For dependent data, the MSPE for LBW, lipid and protein deposition prediction were mostly due to random error bias (> 0.82). For FCR, the MSPE was mostly due to line and random error bias. For the New Zealand independent dataset, the contribution of random error to MSPE was 0.72 and 0.87 for LBW and FCR prediction, respectively.

Using data from out of New Zealand, for the prediction of LBW, the MSPE was mostly due to line and random error bias for prediction of LBW and FCR.

Table 8.12. Evolution the model prediction accuracy by the components of mean square prediction error (MSPE) and the concordance correction coefficient (CCC) by using different datasets.

		Mean (g)						MSPE					
	n	Actual	Predicted	SA^2	SP^2	R^2	b	Mean bias	Line bias	Random bias	MPE	RPE	CCC
Dependent data													
Lipid	17	267	269	10808	11345	0.97	1.007	3.04(0.01)[1]	15.4(0.05)[2]	283(0.94)[3]	17.4	6.5	0.99
protein	17	467.5	471	33283	36613	0.98	1.01	11.91(0.02)	109(0.16)	559(0.82)	26.1	5.57	0.99
LBW	17	2626	2603	868864	862225	0.99	0.99	520.9(0.05)	2.36(0.0)	9489(0.95)	100	3.81	0.99
FCR (g/g)	17	1.73	1.76	0.0387	0.0756	0.82	1.022	0.0011(0.06)	0.01(0.54)	0.007(0.40)	0.133	7.69	0.85
Independent data (NZ)													
LBW	22	1691	1691	1111516	1245294	0.99	1.015	0.02(0.0)	4507(0.28)	11545(0.72)	126.7	7.49	0.99
FCR (g/g)	22	1.42	1.46	0.0235	0.0117	0.24	1.02	0.002(0.08)	0.001(0.05)	0.018(0.87)	0.143	10.1	0.44
Independent out NZ													
LBW	10	1435	1474	482538	594076	0.99	1.04	1533(0.14)	6134(0.57)	3060(0.29)	103.6	7.22	0.99
FCR (g/g)	10	1.61	1.62	0.0179	0.0319	0.51	1.005	0.00007(0.005)	0.007(0.44)	0.009(0.56)	0.125	7.75	0.68

R^2 is the coefficient of determinationof actual and predicted, SA^2 is the variance of actual values, SP^2 is the variance of predicted value. [1] is the main bias proportion of MSPE, [2] is the line bias proportion of MSPE, [3] is the random variation proportion of MSPE. MPE is the mean prediction errors = √MSPE. RPE is the relative prediction error MPE/$\bar{A}$. CCC is the concordance correlation coefficient

Analysis of prediction error behaviour showed that the prediction error had a homogeneous distribution as indicated by the prediction errors plotted relative to the mean (Figure 8.1) However, some points were overestimated, this may be associated with the nature of the experiments (i.e. whole wheat inclusion in the diet, prediction error =~300g).

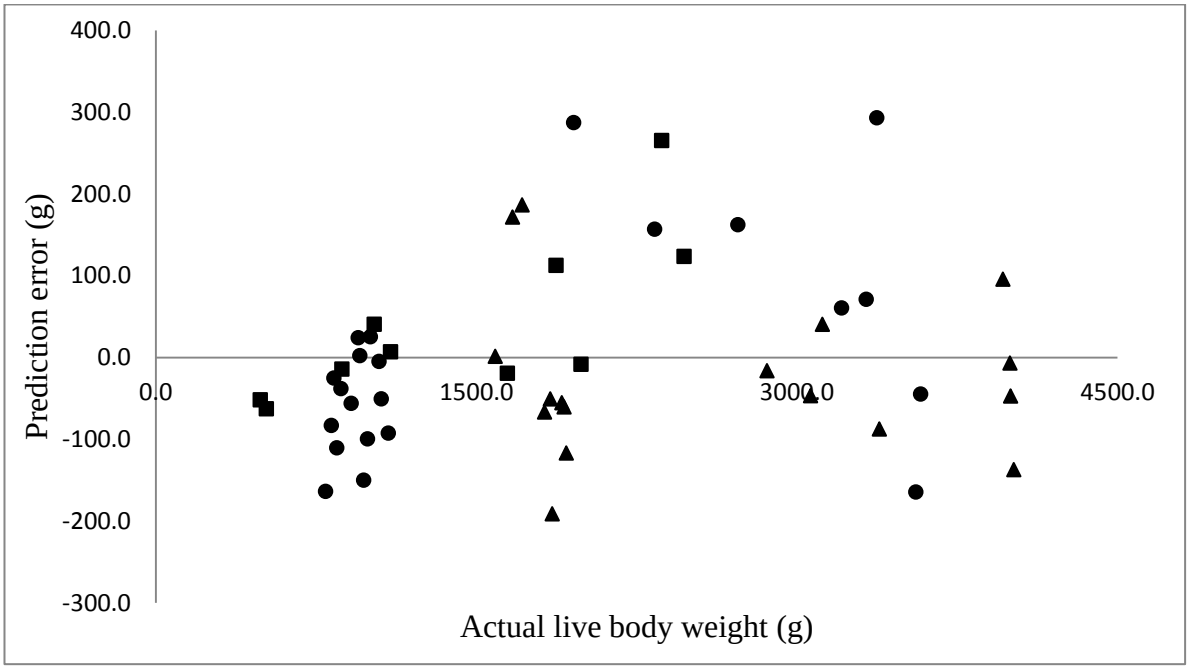

Figure 8.1. Distribution of the prediction error (differences between predicted and actual LBW) as function of the actual live body weight, ▲ dependent dataset, ● Independent dataset (inside New Zealand), ■ Independent dataset (outside of New Zealand)

8.4.4. Sensitivity analysis

Sensitivity analyses for the present broiler growth model parameters with various nutritional situations were determined. The model responses were tested with ± 10 % changes of the variable parameters (the maintenance energy requirement, protein utilisation efficiency, the energetic efficiency for protein deposition, the protein requirement for maintenance, the maximum protein deposition, and the minimum lipid to protein ratio).

8.4.4.1. Application to various nutrition situations

The data from our experiments was chosen for the sensitivity analysis because our data had a different nutrition conditions and included information about lipid, protein

deposition and live body weight. Also, the nutrients and ME intakes were recorded for variable periods.

Case1 Simulation for birds fed low energy low protein

For birds fed low energy low protein the model output for lipid deposition more sensitive to changes of MEm, minL/P and *kp* values than to those changes in other parameters. In regard to the prediction of protein deposition the model response was more sensitive to the changes on the MEm than the other parameters. For live body weight the model response was more sensitive to changes in MEm than the changes of other parameters (Table 8.13).

Table 8.13. Sensitivity analysis for model output to the changes (± 10%) in the model parameters for birds had low energy and low protein intake

	Lipid deposition		Protein deposition		Body weight	
Parameter[1]	S-10%	S+10%	S-10%	S+10%	S-10%	S+10%
MEm	-1.03	-0.93	-0.88	-0.80	-0.84	-0.76
Protein utilisation efficiency	-0.48	-0.47	-0.28	-0.28	-0.28	-0.27
kp	0.65	0.59	0.52	0.49	0.50	0.47
Pm	0.05	0.05	0.03	0.03	0.03	0.03
Pdmax	0.00	0.00	0.00	0.00	0.00	0.00
minL/P	0.72	0.68	-0.29	-0.27	-0.22	-0.21

[1]MEm is the maintenance energy requirement. Protein utilisation efficiency is the protein utilisation efficiency above maintenance requirement. *kp* is the energetic efficiency for protein deposition. Pm is the protein requirement for maintenance. Pdmax is the maximum protein deposition. minL/P is the minimum lipid to protein ratio. S is the sensitivity calculated as the ratio of the standard change on model response to the standard changes (±10%) on parameter value.

Case2 Simulation for birds fed high energy and low protein

For birds fed high energy and low protein the model responses for lipid deposition is more sensitive to changes of MEm than to changes in other parameters. In regard to the prediction of protein deposition, the model was more sensitive to the changes in the protein utilisation efficiency. Also, for live body weight prediction the model was more sensitive to changes to protein utilisation efficiency than to changes of the other parameters (Table 8.14).

Table 8.14. Sensitivity analysis for model output to the changes (± 10%) in the model parameters for birds had high energy and low protein intake

	Lipid deposition		Protein deposition		Body weight	
Parameter	S-10%	S+10%	S-10%	S+10%	S-10%	S+10%
Mem	-0.73	-0.72	0.01	0.01	-0.15	-0.15
Protein utilisation efficiency	-0.44	-0.43	0.61	0.60	0.36	0.36
kp	0.35	0.29	0.00	0.00	0.07	0.06
Pm	0.07	0.07	-0.10	-0.10	-0.06	-0.06
Pdmax	0.00	0.00	0.00	0.00	0.00	0.00
minL/P	0.00	0.00	0.00	0.00	0.00	0.00

Case 3 Simulation for birds fed low energy and high protein

For birds fed low energy and high protein and in regard to lipid deposition prediction the model was more sensitive to changes of MEm, *kp* and Pdmax than to those changes in other parameters. Changing the minL/P ratio had a positive effect on lipid deposition. For protein deposition the model was more sensitive to the changes on the MEm. Also, for live body weight the model was more sensitive to changes in MEm than to the changes to the other parameters (Table 8.15).

Table 8.15. Sensitivity analysis for model output to the changes (± 10%) in the model parameters for birds had low energy and high protein intake

	Lipid deposition		Protein deposition		Body weight	
Parameter	S-10%	S+10%	S-10%	S+10%	S-10%	S+10%
Mem	-1.22	-0.92	-0.76	-0.78	-0.75	-0.75
Protein utilisation efficiency	-0.05	-0.05	-0.05	-0.05	-0.05	-0.04
kp	0.65	0.60	0.53	0.48	0.51	0.47
Pm	0.00	0.00	0.00	0.00	0.00	0.00
Pdmax	-0.99	-0.39	0.07	-0.23	0.00	-0.22
minL/P	0.70	0.66	-0.26	-0.24	-0.19	-0.18

Case 4 Simulation for birds fed high energy and high protein

For birds fed high energy and high protein the prediction of the lipid deposition was more sensitive to changes of MEm, *kp* and Pdmax than to those changes in the other parameters. For protein deposition prediction the model was more sensitive to the changes on the Pdmax than to changes in the other parameters. Also, the model prediction for live body weight was more sensitive to changes in Pdmax than to changes in the other parameters (Table 8.16).

Table 8.16. Sensitivity analysis for model output to the changes (± 10%) in the model parameters for birds had high energy and high protein intake

	Lipid deposition		Protein deposition		Body weight	
Parameter	S-10%	S+10%	S-10%	S+10%	S-10%	S+10%
Mem	-1.35	-1.33	0.00	0.00	-0.15	-0.15
Protein utilisation efficiency	-0.53	-0.39	0.23	0.15	0.14	0.09
kp	1.10	0.90	0.00	0.00	0.12	0.10
Pm	0.03	0.03	-0.01	-0.01	-0.01	-0.01
Pdmax	-1.10	-0.96	0.70	0.63	0.47	0.42
minL/P	0.00	0.00	0.00	0.00	0.00	0.00

Case 5 Simulation for birds fed standard diet with ad-libitum feed intake condition

For birds fed a standard diet with *ad-libitum* feed intake. The parameters of MEm, *kp*, protein utilisation efficiency and Pdmax play a greater role for lipid deposition. The protein utilisation efficiency and Pdmax drove the protein deposition. Furthermore, the protein utilisation efficiency and Pdmax were the most important parameters driving the body growth (Table 8.17).

Table 8.17. Sensitivity analysis for model output to the changes (± 10%) in the model parameters for birds fed standard diet (21 g CP, 13.4 MJ ME per kg) *ad- libitum* feed intake

	Lipid deposition		Protein deposition		Body weight	
Parameter	S-10%	S+10%	S-10%	S+10%	S-10%	S+10%
Mem	-1.00	-1.00	0.00	0.00	-0.12	-0.12
Protein utilisation efficiency	-0.49	-0.43	0.31	0.27	0.20	0.17
kp	0.81	0.66	0.00	0.00	0.10	0.08
Pm	0.04	0.04	-0.03	-0.03	-0.02	-0.02
Pdmax	-0.55	-0.50	0.44	0.41	0.31	0.28
minL/P	0.00	0.00	0.00	0.00	0.00	0.00

8.5. Discussion

Comparison of model predictions with the results actual from the experiments provides methods to evaluate the present model. There was reasonable agreement between the model prediction and the actual values. In the t-test the alternative hypothesis was that the prediction value did not equal the actual value, if the t-test accepted the alternative hypothesis then the model prediction is wrong and there are differences between prediction and actual values. Otherwise, the model prediction can be accepted. Paired t-

test analysis can falsely reject the alternative hypothesis when the residual error is small. Using paired t-test, the simulation of our model appears more accurate for lipid deposition prediction than for protein and live body weight prediction. These appear to be because the rate of lipid deposition was predicted from the energy supply and energy efficiency from different energy sources and this was well documented in our work (Chapter 3, 4, and 5). However, the main factor affecting the growth rate is the protein deposition rate, and the protein deposition prediction is quite difficult as it depends on the calculation of IDBP, the coefficient of the utilisation of IDBP and the energy supply. Therefore, further study is needed in order to improve the prediction of the protein deposition.

The simplest but most efficient method for evaluating the model predictions is the linear regression of actual versus predicted data (Vanclay and Skovsgaard, 1997). The R^2, slope and intercept of the fitted line provide useful insights to the prediction accuracy with the best prediction giving $R^2 = 1$, slope = 1 and intercept = 0. In our evaluation the slopes were close to one and R^2 were 0.99 for dependent and independent datasets for prediction of LBW.

In this study, the MSPE is expressed as the sum of three components: first is the mean bias, i.e. the difference between the mean of actual and the mean of predicted value. The second component is line bias, which represents the errors due to regression, and the third component is the product of the variance of actual values and the deviation from unity of the coefficient of determination (R^2) of the regression of actual on predicted value. Examination of MPSE components provides some indication about the source of errors. For experiments conducted in New Zealand (dependent and independent data sets), the MPSE was mostly due to random bias for LBW prediction, whereas, for experiments conducted outside of New Zealand (independent dataset), the MPSE were mostly due to line and random errors bias.

Application of the MPSE decomposition indicated that the model had over-prediction for FCR for independent dataset, and this was associated with 0.54 as systemic bias.

In this study, the RPEs were less than 10%, indicating a good prediction accuracy (Fuentes-Pila *et al*., 1996), compared with a study by Rivera *et al*. (2011), where the RPEs were 25% and 10% for daily body weight gain predictions for male and female turkeys, respectively. Fuentes-Pila *et al*. (1996) assumed that when the RPE was less than 10% it indicated a satisfactory prediction, when the RPE was between 10%-

20% it indicated a relatively acceptable prediction and higher than 20% indicated an unsatisfactory prediction.

The model was tested using independent datasets for an experiment conducted in NZ and a dataset for an experiment conducted out of New Zealand. For the experiment conducted in New Zealand, the model predicted was similar to actual mean LBW, whereas, for experiments conducted outside New Zealand, the model over-predicted for LBW, proportionally, by 0.03. This indicates that the model had a better accuracy when using data that originated from New Zealand.

The CCC values can range between 1 to −1. A CCC = 1 corresponds to perfect positive agreement. A value of CCC = −1 corresponds to perfect negative agreement, and a value of CCC = 0 corresponds to no agreement between predicted and actual values. Visser *et al*. (2012) suggested a CCC value higher than 0.81 indicates an acceptable prediction. For our model, the application of CCC indicated that the model has good prediction accuracy for live body weight, whereas the CCC indicated poor prediction for FCR.

The sensitivity analysis is another analysis used to evaluate the model parameters. The sensitivity analysis provides information about the parameters which produce either strong or weak effect on the model output. Sensitivity analysis can help to determine which parameters are the key drivers of the model output (Hoch and Agabriel, 2004). The simulation of lipid deposition appears more sensitive to parameters such as MEm, energetic efficiency of protein deposition and Pdmax. The sensitivity coefficients were quite different when the energy and protein intakes changed. Furthermore, minL/P plays a greater role in the simulation of lipid deposition at low energy intake, but this is not the key parameter at high energy levels.

The simulation of protein deposition appears sensitive to Pdmax and protein utilisation efficiency at high protein intake level and sensitive to MEm when the energy intake was low. Simulation of body weight appears more sensitive to parameters such as MEm at low energy intake and to Pdmax and efficiency of protein utilisation at high protein intake level. The ME for maintenance represents 45-50% from the total ME intake (Lopez *et al*., 2007; Latshaw and Moritz., 2009). Therefore, simulation of live body weight always appears highly impacted by MEm.

The sensitivity analysis can lead to improvement in the model prediction, but because of the nature of our experiments (restricted feed, unbalanced diets, variable age and live body weight) it is difficult to consider which parameter would have the most

impact on model prediction under commercial conditions. However, it seems that parameters like MEm and the efficiency of protein utilisation have the most significant impact on model predictions and requires priority attention and re-investigation.

8.6. Conclusion

The model described in this thesis was evaluated and the results indicate that the model provides a reasonable prediction. The model validation indicates that the predicted values were in close agreement with actual values. Parameters like MEm and the efficiency of protein utilisation have the most significant impact on model predictions. Therefore, further studies to identify these parameters are required to improve the model prediction accuracy.

CHAPTER 9
General Discussion

9.1. Introduction

The cost of feed in the broiler industry is critical as it contributes 65 to 75% of the total production cost, and the supply of metabolisable energy represents approximately 70% of the feed cost (Golian and Murice, 1992; Moosavi *et al.*, 2011; Noblet *et al.*, 2010). Therefore, the cost of metabolisable energy comprises approximately 49% of the total production costs in the broiler industry. The challenge for broiler chicken nutrition is to improve energy efficiency and adjust the energy and nutrient supply as accurately as possible to the bird's requirements for maintenance and production. Researchers continually attempt to improve energy efficiency (Darmani *et al*., 2003; Saleh *et al.*, 2004), and energy partitioning models have been used in order to get a better understanding of the efficiency of energy utilisation (Boekholt *et al*., 1994; Lopez and Leeson, 2005; 2007; Sakomura *et al*., 2005). Usually, the metabolisable energy intake is partitioned into energy requirements for maintenance and energy retained in the body in the form of protein and fat. Different energy efficiencies are associated with the use of energy for protein (*kp*) and for fat (*kf*). In terms of fat deposition, published data on the energetic efficiencies of fat deposition (*kf*-values) from different energy sources are limited in modern broiler chickens. The results from our experiments (Chapter 3, 4 and 5) showed there are a differences in *kf*-values due to differences in efficiencies of AME utilisation between nutrients with the highest values for soybean oil (0.93) and tallow fat (0.90) followed by starch (0.69) and the lowest for protein (0.63).

The protein gain (lean tissue gain) is associated with body growth rate. Therefore, knowledge about the maximum protein deposition for modern broiler chickens is important for the formulation of diets that maximise growth performance and profitability (Rostagno *et al.,* 2007). Most previous studies described the potential protein deposition by Gompertz type equations (Hancock *et al.*, 1995; Gous *et al.*, 1999; Marcato *et al*., 2008). The Gompertz equations described a specific data set mathematically rather than described the overall biological processes.

An appropriate description for protein deposition has been proposed for pigs (Van Moughan *et al*., 2006; Denis and Daniel, 1997; Quiniou *et al.,* 1996; Van Milgen and Noblet, 1999). The protein deposition rate has been determined from the ideal digestible balanced protein intake, but the rate of protein deposition may be limited by the maximum protein deposition (Pdmax) and the energy intake. A similar theory has

been applied for broiler chickens by feeding the bird's high digestible protein and variable energy intake (Chapter 6), the maximum protein deposition has been predicted to be 22 g/d. The Pdmax was reached at 1.2 MJ/day with AME intakes above the maintenance requirements.

Broilers with *ad-libitum* feed access have the ability to store the excess energy as fat, and the carcass fat had a negative impact on the total edible carcass yield, reducing the feed efficiency and providing a less desirable meat composition for consumers (Sahraei, 2012). Therefore, establishing the energy requirements to maximise the lean growth rate with minimum fat deposition is important for increasing the accuracy of feed formulation for modern broiler chickens and producing a carcass with minimum fat. The minimum lipid to protein ratio (minL/P ratio) identifies the allowable ratio required to optimise the protein growth with minimum lipid deposition and the possible minL/P ratio ranged between 0.31- 0.55 (Chapter 6).

In order to combine all experimental results in one place, a broiler growth model was proposed in Chapter 7 and this semi-mechanistic broiler growth model based on energy partitioning concepts can be used to predict the carcass composition and broiler performance. The validation process (Chapter 8) indicated that the predicted values were in close agreement with observed actual values. Further studies are required to improve the model performance.

9.2. Theory and methods use to estimate the energy efficiency (*k*-value)

Existing metabolisable energy systems attribute a single energy value commonly used in broiler nutrition, the ME system is simple and relatively robust (Sibbald, 1980; Farrel 1999). The limitation of the ME system it cannot account for the energy losses as heat and the interaction between animal and the feed. This has led to the use of the energy partition model for feed evaluation and predicting the response of the animal to a changing nutrient intake. The simple approach to partitioning the ME intake (MEI) has been used in the following formula (Leland, 2005; Sakomura *et al*. 2003; 2004; 2005):

$MEI = MEm + (1/k_g)\ ER$

where

MEm is the metabolisable energy for maintenance,

RE is the energy retained in the body,

k_g is the efficiency of energy utilisation for body weight gain.

The efficiency of energy utilisation for body weight gain was determined by linear regressions of AMEI as a function of energy body retention. Sakomura *et al*. (2005) reported k_g values of 0.60, 0.57 and 0.64 for broilers housed on 13, 23 and 32°C.

However, this approach does not account for the different efficiencies that relate to energy retention as fat or as protein. In order to improve the model and estimate the energy utilisation efficiency for fat and protein deposition, the classical equation of Kielanowski (1965) has been widely used for estimating the energetic efficiency for fat and protein deposition:

MEI = MEm+ (1/*kf*) ERF + (1/*kp*) ERP

where

MEm is the ME requirement for maintenance,

ERF is the energy retention as fat (kJ/d)

ERP is the energy retention as protein (kJ/d),

kf and *kp* are efficiencies of utilisation of ME for fat and protein deposition, respectively.

Energy efficiencies can be significantly affected by diet composition and the purpose for which the energy is used (Lopez and Leeson 2008a; De Groote, 1974). The previous ME partitioning system does not account the differences of efficiencies from different energy sources.

Reported *k*-values obtained by multiple linear regression show wide variation, and there are practical problems associated with accurately of measuring ME, particularly if the increments of the ME intake involve crude protein sources (Sakomura *et al*., 2005; Boekholt *et al*., 1994; De Groote, 1974; Birkett and de Lange, 2001a, c). Multiple linear regression models have had poor precision and estimated energetic efficiency of fat deposition as greater than 1 (1.09, Sakomura *et al*. 2003) for which it is difficult to give biological explanation. Also, it has been admitted that the multiple linear regression model is sensitive to the value given for the maintenance requirement (Halas and Babinszky, 2010; Roux, 2009) and thus, inaccurate estimation of the energy requirement for maintenance may also impact on *k*-values (Azevedo *et al.*, 2005).

In practice the dependent variable (MEI) affects the independent variables (ERP and ERF). However, it is difficult to prevent the correlation between fat and protein deposition during growth, which introduces unexpected and unstable *kf* and *kp* values (Birkett and de Lange 2001a; Hall, 2010). Multicollinearity is a statistical phenomenon when two or more predictor variables in a multiple regression model are highly

correlated, meaning that one can be linearly predicted from the others with a good degree of accuracy. In this case the coefficient estimates from the multiple regression may unreliably change in response to small changes in the data (Dereny and Rashwan, 2011; Mason *et al*., 1975). Multicollinearity is not necessarily a problem if the aim of the model is to predict the independent variable. However, as the aim is to obtain *kp* and *kf* through the multiple regression model, predicting the parameter coefficients becomes difficult (Leahy, 2000).

The mathematical model introduced here has avoided the problems of the Kielanowski (1965) model by limiting the protein deposition. The assumption was that the MEI effect on the ERF provided a reasonable estimation of the *kf* -value. Birkett and de Lange (2001c) noted one possible procedure that minimised the variation of *k*-values by creating a response between MEI and fat deposition at fixed protein deposition for the estimation of *kf* -value, and creating a response between MEI and protein deposition at fixed fat deposition to estimate the *kp*-value. In our case, the *kf* -values were obtained at a fixed protein deposition rate. In addition, the efficiencies of energy utilisation for fat deposition were estimated from starch (kf_s) from fat (kf_f) and from protein (kf_N). The assumption was that the additional ME intake from different energy sources increased the fat deposition, whereas the protein deposition remained constant.

As a result, the protein deposition remained constant with the extra energy intake from starch, soybean oil or tallow fat (Figure 9.1) and the residual variation of protein deposition was accounted for by using AMEifpd, the efficiencies of energy utilisation for fat deposition were therefore, estimated from the energy remaining after the energy requirements for maintenance and protein deposition using a linear regression model.

In the present work, the energetic efficiency of fat deposition from starch (kf_s) was 0.69 and this appears to be somewhat lower than those of Carre *et al*., (2002) who reported a value of 0.78. Also, the theoretical biochemical efficiency of using glucose for fat deposition varies between 0.81-0.84 depending on ATP synthesis during lipogenesis from glucose is accounted for or not (Van Milgen, 2001a; 2002; Baldwin 1995). In our experiment, the efficiency of energy utilisation for fat deposition from starch was 15% less than the theoretical values. Van Milgen, (2006) indicated that the experimental efficiencies for fat are much lower than the theoretical efficiencies, and this may be due to the oxidation of dietary lipids for ATP synthesis combined with *de-novo* lipid synthesis from other nutrients. Also, the levels of feed intake may have had

an effect on the efficiency of energy utilisation for fat deposition from starch. Birekt and de Lange (2001a) mentioned it is essential to have observations with a wide range of ME intakes to increase the accuracy of the regression coefficients (*k*-value). In our case, only two levels of ME intakes were used and this may have contributed to the low efficiency of energy utilisation for fat deposition from starch intake that was estimated in this study. In addition, the MEm was assumed identical between treatment groups, The MEm is difficult to kept constant across all treatment groups, since the MEm changes in regard to body weight, environmental temperature and body composition (Latshaw and Bishop, 2004; Sakomura *et al*., 2005).

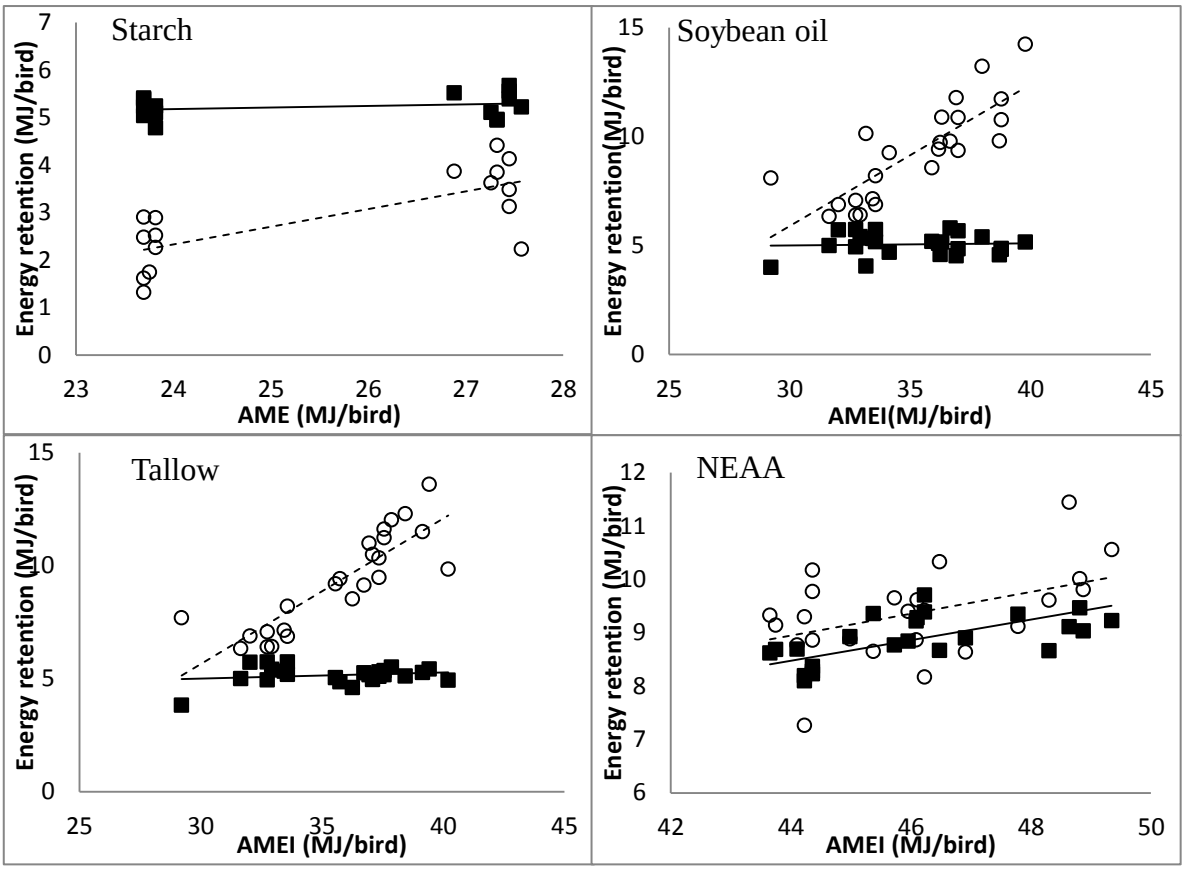

Figure 9.1. The partitioning of apparent metabolisable energy intake (AMEI) into energy retention for fat (...○.....) and protein (-■-) from different energy sources

De Groote (1974) presented the energetic efficiency for fat deposition from digestible fat intake as 0.90, and Carre *et al*. (2002) report a value of 0.84. The work reported here also showed that, under protein limiting conditions, the efficiencies of energy utilisation for fat deposition from extra soybean oil and tallow intakes were 0.93 and 0.90, respectively. Hydrolysis and re-esterification of fatty acids are the main

reason for energy loss involved in the synthesis the body fat from the digestible fat in animal. The theoretical energetic efficiency of fat deposition from dietary fat is 0.97 (Van Milgen, 2001a). In our experiment, the estimation of kf_{so} and kf_t were for fat deposition only whereas, the theoretical *kf* was calculated using dietary fat for both fat deposition and ATP synthesis. In addition, part of the energy coming from dietary fat may be used for maintenance requirement (Van Milgen *et al*., 2001a). Consequently, the available energy for growth from dietary fat may be decreased and therefore, the *kf* values decreased compared to theoretical values.

In case of the energetic efficiency of fat deposition from dietary protein sources, it was difficult to estimate the efficiency of energy utilisation for fat deposition from additional dietary casein (Chapter 3) due to the birds depositing protein rather than fat. There was a similar difficulty for obtaining the efficiency from dietary protein as reported in pig experiments because part of the casein intake was deposited as protein rather than deaminated and used for fat deposition (Van Milgen *et al.*, 2001a). To avoid the response of protein deposition from extra protein intake and to estimate the *kf* from protein sources, non-essential AA were used (Chapter 5). However, both fat and protein depositions were increased when the birds were fed extra NEAA (Figure 9.1). Further analysis was taken to ignore the residual response of protein deposition. The digestible energy intake was corrected to be zero protein deposition (see Chapter 5), and the kf_N was calculated to be 0.63. Furthermore, the theoretical efficiency of fat deposition from digestible NEAA was calculated to be 0.65. Similar results have been reported for efficiency of fat deposition from digestible protein with values ranging between 0.60-0.67 (Kielanowski, 1971; Roux, 2009; Quiniou *et al*., 1996). In pig experiments, the excess protein that was used for fat deposition was used with an efficiency of 0.52 and NE/ME ratio was 0.59 (Van Millgen, 2001). The different metabolic processes for obtaining energy from AA also utilise energy in the form of ATP. The synthesis of a peptide bond from AA requires at least 5 ATPs, hydrolysis of a peptide bond also requires 1 ATP, deamination of AA and uric acid synthesis also require ATP and lead to low energetic efficiency from AA.

9.3. The upper limit for protein deposition rate in broiler chickens

The traditional Gompertz function has been used to describe the protein growth curve in broilers (Gous *et al.*, 1999; Hancock *et al.*, 1995; Sakomura *et al*., 2005). Many of studies created under ideal conditions (balanced diet, thermo-neutral temperature, *ad*

libitum feed and genotype). The Gompertz equations described a specific data set mathematically rather than described the overall biological processes. Consequently, using this function may not give solid and precise predictions of protein deposition rates with different growth situations (Lopez *et al*., 2007). Therefore, describing the protein deposition sourced from ideal digestible balanced protein and as a function of ME intake may provide more precise information about the daily body protein deposition rate.

Pig growth models assumed that protein deposition increased as the ideal balanced protein intake increased (independent of energy intake) up to the genetic upper limit for protein deposition rate (Pdmax, Moughan *et al*, 2006; de-Longe 1995; Quiniiou *et al*., 1995; 1996). Also, the Pdmax changed with the live body weight for specific strains and with sex (Quiniiou *et al*., 1995). The study reported in Chapter 6, predicted the Pdmax as 22 g/day. However, in broilers it was difficult to identify Pdmax in the early growth stage as the feed intake capacity limits the protein deposition. The Pdmax was achieved at a body weight range of 1.75-3.25 kg. At this stage the broiler's had sufficient amounts of ideal balanced protein and AME intake to express their maximum protein deposition. Therefore, Pdmax cannot be found with the linear plateau model at early growth stage. It is suggested that it may be possible to identify the Pdmax as one single value for all periods of growth instead of the specific value for each range of body weight and this value could be identified for each broiler breed and sex of broiler chickens.

9.4. Effect of energy intake on Protein and lipid deposition

The study described in Chapter 6 was designed to validate the theory that is commonly used in animal growth simulation models (Moughan et al, 2006; Whittemore and Fawcett 1976; Whittemore, 1995; De Greef and Verstegen 1995) that at non-limiting protein intake the protein deposition is dependent on energy intake. The work reported here validated this theory for broiler growth (Chapter 6). The results showed that when the ME intake is less than the minimum energy required for maximum protein deposition (minAMEIp for Pdmax), the protein and lipid deposition increases as the AMEIp increases. When the protein deposition reached the Pdmax, all surplus energy goes toward the lipid deposition (Figure 9.2).

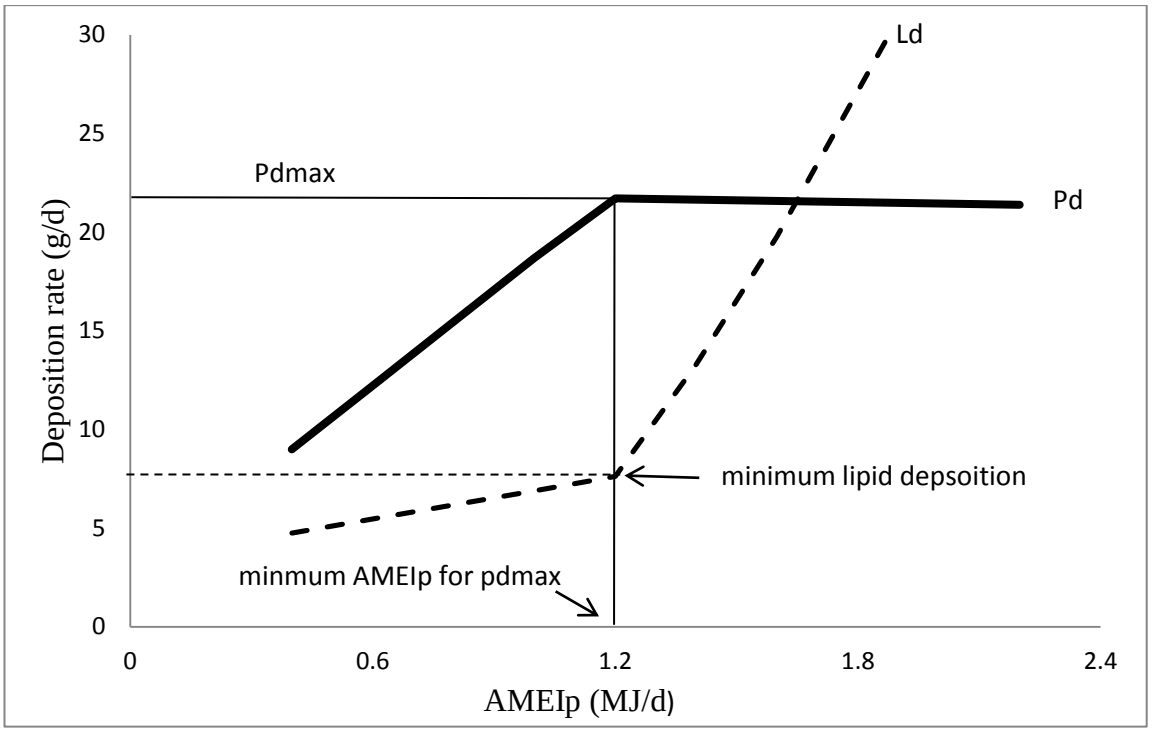

Figure 9.2. Modelling the responses of protein (Pd) and lipid (Ld) deposition in relation to AMEI above maintenance requirement (AMEIp)

Applying the mechanism of energy partitioning (Pdmax and minL/P) provides a flexible mechanism most likely change when the nutrition changes during growth. Moreover, these parameters can be identified for specific genotypes, sex and environmental conditions.

In pig growth models, the Pdmax and minimum lipid to protein ratio are considered as genetic input parameters. Knap *et al*. (2003) reported that the genetic parameters for a growth model can be estimated by either direct measurement from experiments or by fitting the experiment performance data into a mechanistic model (Model inversion). In our case the Pdmax was predicted at 22 g/day and the possible minL/P ratio ranged between 0.31 to 0.55. Applying these parameters in the growth model resulted a reasonable agreement between the predictions and the actual values.

Since the cost of feed is the major cost in broiler production, the feed conversion efficiency and the factors that affect feed conversion efficiency such as residual feed intake, residual daily gain and breast muscle weight have been used as selection criteria in the broiler breeding programs (Deeb and Lamont, 2002). A negative value for the residual feed intake is beneficial, whereas positive values for residual daily gain is favourable (Willems *et al*., 2013). However, the selection for gain has generally increased the lipid to protein ratio in the modern broiler chicken (Soller and Eitan 1984;

Gous, 1986). Therefore, the selection for maximum gain should be classified with minimum lipid to protein deposition ratio, and this should improve energy efficiency by changing the energy partitioning between protein and fat deposition. The parameters of Pdmax, minL/P ratio and minAMEIp for Pdmax could be considered in the genetic selection criteria for improvements of energy efficiency, with maximum growth and minimum fat deposition.

9.5. Predicting the protein and fat deposition

Protein deposition can be predicted from IDBP intake with the assumption that the only factor deriving the protein deposition is the IDBP intake as in the following equation.

$Pd = b \times [\text{IDBP intake} - Pm]$

Where b is the efficiency of protein utilisation which is 0.85 for growth after the protein maintenance requirement has been accounted for. Pm is the protein for maintenance = 1.575/kg LBW $^{0.67}$ (Samadi and Liebert, 2006).

The protein deposition linearly increased as the IDBP increased up to Pdmax (Figure 9.3). When the protein deposition achieved the Pdmax then the b value become zero and extra IDBP is converted into fat deposition.

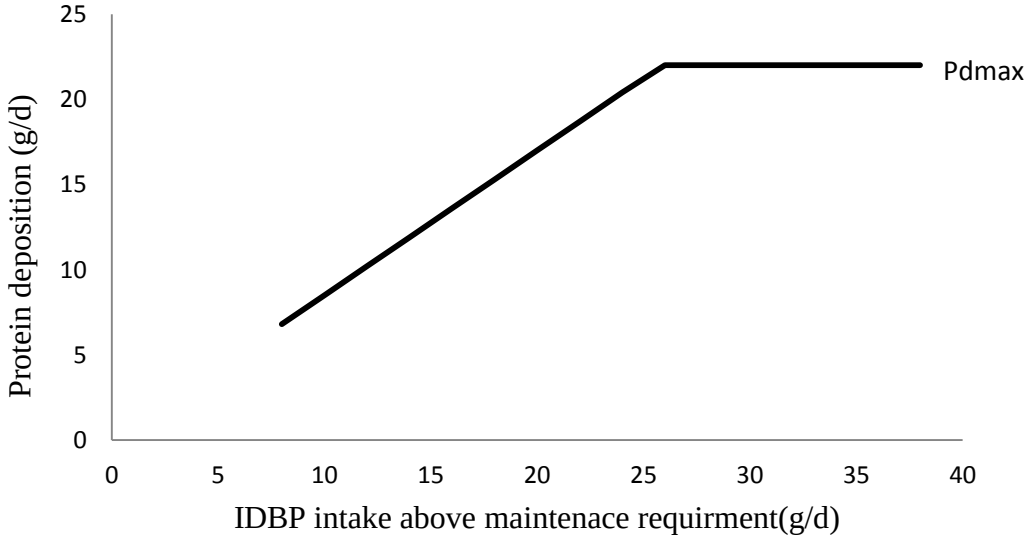

Figure 9.3. Protein deposition in relation to ideal digestible balanced protein intake above maintenance requirement under non-limit energy intake condition

Predictions of fat deposition can be calculated from AMEI, energy requirement for the protein deposition and the metabolic body weight using the following equation:

$AMEI = MEm + (1/kp) \times ERP + (1/kf) \times ERF$

When there is no excess protein or unbalanced protein intake, then the energy retained as fat is calculated as:

ERF = [AMEI – MEm – (ERP/*kp*)] × *kf*

Then the fat deposition = [AMEI – MEm – (ERP/*kp)*] × *(kf/39).*

The ERP = protein deposition × 23.7 (kJ/g).

The *kf* value can range from 0.69-0.93 depending on energy sources. The above calculations can be used when the L/P ratio is greater than the minL/P ratio or ERF/ERP ratio is greater than [(39/23.7) × minL/P]. When the lipid to protein ratio is less than the minL/P or ERF/ERP ratio is less than [(39/23.7) × minL/P], the lipid deposition competes with protein deposition. Consequently, protein deposition is reduced when the lipid deposition increases (Chapter 7, Equation (33) and (34)). The water and ash mass were calculated from final protein mass, using empirical equations. The body weight was calculated by summation of all four component i.e., protein, fat, water and ash.

9.6. Simulation of metabolisable energy and ideal digestible balanced protein requirement

The NRC (1994) presents the broiler requirements for AAs and ME as a percentage of the diet. However, the NRC recommendations have become insufficient to optimise the maximum growth rate for modern broiler chickens. The nutritional specifications of the diet should be variable and depend on the objectives of production and not given as fixed value in the diet. Using the model inversion presented in this thesis (Chapter 7), could allow for the estimation of ME requirements by the summation of energy requirements for protein deposition, fat deposition and maintenance energy. Also, the digestible balanced protein requirements can be estimated from the protein required for maximum protein deposition plus the protein required for maintenance.

The energy requirements for protein deposition are measured from the *kp* and target protein deposition. The target protein deposition presents the maximum daily protein deposition achieved when the birds are managed under perfect conditions (Table 9.1). In our experiment (Chapter 6), from 0.60-1.75 kg LBW the protein deposition was limited by the gut capacity and energy intake. From 1.75 to 4.0 kg of LBW the protein deposition was limited by the upper genetic limit potential. The ERP was calculated as protein deposition × 23.7 (kJ/g).

Our model assumed that the bird partitioned the energy intake to the preferred ratio of L/P or ERF/ERP, and then the ERF calculated as ERP × preferred ERF/ERP. The MEm was calculated as 649 kJ/kg LBW $^{0.6}$.

The AME intake requirements can then be estimated by the following equation.

AMEI (kJ/d) = MEm + (1/*kp*) × ERP + (1/*kf*) × ERF.

The minimum AMEI (kJ/d) is therefore calculated as:

minAMEI (kJ/d) = 649 × LBW$^{0.6}$ + (1/*kp*) × ERP + (1/*kf*) × (ERP × preferred ERF/ERP). When *kf* = 0.71 and this assumes that 10% of the digestible energy toward fat gain comes from dietary fat sources and 90% comes from dietary starch sources and there was no excess or unbalanced protein intake, the *kp* = 0.66 (Boekholt *et al.*, 1994). Therefore, for a given live body weight, target protein deposition and preferred ERF/ERP, the minimum MEI and IDBP intake requirements can be estimated as described in Table 9.1.

Table 9.1. Simulation of broiler requirements by applying the metabolisable energy and IDBP intake partitioning model

				Preferred		Requirement prediction			
BW range (kg)	Target Pd (g/d)	ERP (kJ/d)	MEm (kJ/d)	ERF/ERP Ratio[1]	ERF (kJ/d)	MEI (kJ/d)	IDBP[2] (g/d)	FI[3] (g/d)	IDBP[4] %
0.60-1.00	15.3	362.6	567.7	0.51	184.9	1376.2	19.4	103.5	18.7
1.001-1.75	18.9	448.9	785.6	0.51	228.9	1786.5	24.2	134.3	18.0
1.751-2.50	24.5	580.7	1020.1	0.51	296.1	2314.7	31.4	174.0	18.1
2.501-3.25	24.6	583.3	1223	0.51	297.5	2523.3	32.1	189.7	16.9
3.251-4.00	23.6	559.3	1405.5	0.51	285.3	2652.5	31.5	199.4	15.8

[1] is the preferred ERF/ERP calculated from minL/P, assumed minL/P = 0.31.
[2] is the average IDBP intake calculated as (Pd/0.85) + (1.575 kg LBW $^{0.67}$)
[3] is the average feed intake requirement when the energy concentration 13.3MJ/kg.
[4] is the IDBP concentration requirement in the diet.

The values given in Table 9.1 are generally the minimum levels of requirement that satisfy with minimum fat deposition. However, safety margins are a common practice to protect nutrients deficiencies (Alexandre, 2012). Therefore, a method of safety margins can be used to avoid the errors coming from growth conditions and individual variations. Further study needs to validate this requirement method with commercial conditions for broiler chickens.

9.7. Growth model

The New Zealand poultry industry leads the world in many areas of broiler production with the highest growth rates and best feed efficiency. One of the aims of our

investigation was to develop a simple broiler growth model, which could be easily used under New Zealand conditions. In the literature review, it was introduced that the mechanistic model has advantages and it is a more valid approach compared to the empirical model. The mechanistic model presented in this thesis includes a theory on how the nutrition and broiler genetic affects growth performance. In addition, the model was evaluated and tested with variable datasets and the results indicated that the model provides a reasonable prediction. However, further research needs to deal with the effects of environment (temperature, stock density and humidity) on the growth response.

Eits (2004) proposed that the mechanistic models generally predict the chemical body composition, but in practice, the physical body parts (carcass yield, breast and leg meat) are important. In our model data showed that, the physical body parts can be predicted directly from the live body weight with reasonable accuracy. The Edinburgh Growth Model (EFG-model; Emmans, 1981; Emmans and Fisher, 1986; Gous, 1999) has been commercially used. The EFG model is easy and clear to use, but at the same time there is a relative error when it is applied on New Zealand broiler farms, due to three major factors.

Firstly, the disease free New Zealand environment allows the broiler chickens to grow faster with low feed per gain.

Secondly, the EFG model was developed to predict the feed intake under *ad-libitum* condition and then used the predicted feed intake as an input parameter for the growth model. However, the feed intake is an extremely complex factor which can drastically affect growth performance prediction results (Gous, 2007; Mbajiorgu *et al.*, 2011). The feed intake is difficult to predict and it is not essential to predict because the feed intake data can be available in farm feeding systems (Stacey *et al*., 2004). When feed intake is given as data input, the model prediction is stronger (Fisher and Gous, 1998). Therefore, in our model the feed intake is preferred as a data input.

Thirdly, the EFG model does not deal with the effect of genetic potential and will probably become outdated quickly, especially under New Zealand conditions. Also, the interaction between the energy intake and protein deposition is not considered. The mathematical growth model described herein was able to predict the growth performance, carcass composition and estimate the energy and protein requirements.

Not all the parameters and equations adapted from the literature we believed to be correct. Some of the parameters, as indicated by the sensitivity test, had strong effect

on the model output. For example, the energy requirement for maintenance was assumed to be a fixed value in all situations (649 kJ/kg $LBW^{0.6}$, Lopez and Leeson, 2005). Sensitivity analysis indicated that the MEm had a greatest impact on model predictions. Therefore, further research is required on this matter. Also, the model should be calibrated with a large amount of data more relevant to commercial conditions. Once this is done and it validates the general concept and the model could then be linked with least formula program to maximise the profitability. The optimisation procedures would then identify the feed formula which maximises the profitability and the growth performance.

9.8. Summary and conclusions

At limited protein intake, additional energy intake had differing energetic efficiencies for fat deposition depending on the energy source. The efficiency of energy utilisation was the highest from soybean oil (0.93) and tallow fat (0.90) followed by starch (0.69) and lowest from protein (0.62).

Under unlimited protein intake, the modern broiler chicken has an upper limit for protein deposition (Pdmax) of 22 g/day. The response of protein deposition to ideal protein supply increased linearly, unless the genetic potential for protein deposition (Pdmax) is reached or energy intake becomes limiting. When birds are given a diet adequate in protein but limited by energy intake, the protein deposition increases as the energy intake increased. The minimum energy requirement for maximum protein deposition is 2.5 AME intakes per day or 1.2 MJ/day AMEIp, and further energy intake will deposited as lipid.

Factors such as diet composition, feed intake, Pdmax and minL/P ratio affect broiler performance and carcass composition, knowledge about the relationships and interactions can be simulated by the mechanistic broiler growth model. The mechanistic broiler growth model is a tool to predict the carcass composition and broiler performance. In our model, parameters like MEm, *kp* and the efficiency of protein utilisation were obtained from literature and were used as a constant. Sensitivity testing indicated the importance of these parameters and therefore, further research is needed to understand how these parameters could be incorporated into the model for better prediction of broiler performance.

The NRC energy system (NRC, 1994) has limitations to describe the modern broiler chickens requirements. The current mechanistic broiler growth model describes

the requirement of modern broiler chickens for IDBP and AME based on a target of live body weight and partitioning of nutrients based on Pdmax and minL/P. The proposed mathematical growth model could provide a roadmap for future nutrients and energy requirements for modern broiler chickens.

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