A MODEL OF CALCIUM AND PHOSPHORUS GROWTH IN BROILERS

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Details of contribution to publications that form part and/or include research presented in this thesis:

Publication

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ABSTRACT

A model is a simplified representation of a system. It can be used to organise knowledge and to develop theory in an academic setting, but also for practical applications. This research sought to incorporate as much as possible of the theory of calcium and phosphorus in broiler chickens into a model. This would allow researchers to see where there are gaps in the theory and to suggest ways in which experiments might be designed to fill these. It is hoped that this model will provide a guide for producers when they feed broilers, particularly under changing conditions. Current tables of requirements reflect empirical data on bird performance, collected at a certain point in time. As genetic progress, welfare considerations and environmental pressures change the constraints on an animal production system, a dynamic model allows the nutritionist to be more responsive to these.

The calcium/phosphorus model is located within an existing broiler model that simulates energy metabolism and protein growth. This model is linked to a feed formulation component and an optimiser that allows producers to manage their enterprise to meet production and profit targets. Because standardised digestibility values have proved elusive for minerals, a digestibility module assesses the complete feed and calculates mineral quantities available to the bird. These are then assessed against the requirements for soft tissue which is given priority and then bone growth. Excess mineral is excreted and this, and bone mineralisation are considered for the optimiser module of the main model.

The model was calibrated and validated using two body composition studies. It was demonstrated that reasonable predictions of performance could be made, but that modelling digestibility is a critical component. Perhaps most importantly, the model maps a way forward for research targeted at filling the gaps in the body of knowledge. These have been shown to be surprisingly large: very little whole carcass body composition work has been done and few studies of calcium and phosphorus digestibility have been designed to allow modelling of their interactions.

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LIST OF ABBREVIATIONS

aCa/P	available Ca/P
ADFI	average daily feed intake
AID	apparent ileal digestibility
BFB	bone-free body
BP	body protein
BWG	body weight gain
Ca	calcium
CP	crude protein
dCa/P	digestible Ca/P
DCP	dicalcium phosphate
DFI	desired feed intake
DM	dry matter
DMI	dry matter intake
EFFBP	empty, feather-free body protein
EL	endogenous loss
FI	feed intake
MCP	monocalcium phosphate
MDP	monodicalcium phosphate
MSP	monosodium phosphate
PP	phytate-P
nPP	non-phytate P
oP	organic phosphate
pcdP	precaecal digestible P
P_i	inorganic phosphate
rCa/P	retainable Ca/P
RBV	relative bioavailability
SID	standardised ileal digestibility
tCa/P	total Ca/P
TID	true ileal digestibility

CHAPTER 1. INTRODUCTION

Calcium (Ca) is an essential macro-mineral in the diets of animals. It is required for the mineralisation of bones, where 98-99% of the Ca in the body of the broiler is to be found, mostly in the form of hydroxyapatite crystals ($Ca_{10}(PO_4)_6(OH)_2$) (Veum, 2010). Deficiency, whether the result of inadequate levels in the diet or malabsorption, may result in skeletal disorders such as rickets, tibial dyschondroplasia and painful lameness (Angel, 2007). Extracellularly, Ca is usually present in its ionic form (Ca^{2+}), bound to proteins or bound to anions (Adedokun and Adeola, 2013). Ca is critical in biochemical pathways as diverse as blood clotting, nerve impulse transmission, hormone secretion and muscle function (Anwar and Ravindran, 2016).

In animals that lay hard-shelled eggs, such as birds, Ca acquires a further evolutionary significance. Modern laying hens must secrete Ca equivalent to twenty times their body Ca into eggshell in their annual laying cycle and hence rely on a very effective system of Ca homeostasis (Miller, 1992). Growing broilers have the capacity for rapid assimilation of Ca into the bones, maintaining a tightly controlled level in the plasma (Dacke *et al.*, 2015).

Phosphorus (P) plays a vital role in metabolic processes, structural compounds (e.g. phospholipids) and in bones, where up to 80% of the P in broilers is present (Veum, 2010). Elsewhere in the body, it is present in inorganic form, or bound to proteins, lipids (as in cell membranes) and in nucleic acids (Adedokun and Adeola, 2013). Its concentration within cells and in extracellular fluids is tightly maintained so that biochemical processes can function optimally (Li *et al.*, 2017c).

1.1. Ca and P in broiler nutrition

The most common ingredients of broiler diets (maize, wheat and soya) provide very little Ca for the growing bird's needs. In nature, the bird might be expected to seek mineral sources separate from its basal diet if this were deficient, but in commercial practice complete feeds are supplemented with mineral Ca. This is often in the form of limestone (calcium carbonate, $CaCO_3$). Other sources include dicalcium phosphate (DCP) and monocalcium phosphate (MCP). By-products such as bone meal provide a readily available source of minerals but are not favoured in some markets due to their animal origins.

A large proportion of the P present in plants is found in salts of phytic acid. This compound is the form in which P is stored and therefore particularly prevalent in seeds, which form the basis of most commercial poultry diets (Bradbury *et al.*, 2017). Most phytate-P (PP) is not broken down in the bird's digestive tract and hence as much as 60% of the P in the diet may not be available to meet the bird's requirements (Li *et al.*, 2016a). Supplementation with mineral phosphates may make up the shortfall, but this non-phytate P (nPP) is a costly and non-renewable source of P. Furthermore, P that is not utilised by the birds poses a pollution hazard when poultry litter is disposed of, either as fertiliser or in land fill (Selle and Ravindran, 2008; Ray and Knowlton, 2015).

1.2. Phytase in broiler diets

Exogenous enzymes provide a means of increasing the amount of plant P that is available to poultry. Microbial phytases, produced by fungal and bacterial species such as *Aspergillus niger* and *Escherichia coli,* first became commercially available in the Netherlands in 1991 and the last thirty years have seen the widespread adoption of these additives in poultry and pig diets (Selle and Ravindran, 2008). Phytase hydrolyses phytate and releases P for absorption and use by the animal. Some studies have found additional "extra-phosphoric" benefits, such as improved amino acid digestibility (Sommerfeld *et al.,* 2018a). Walk *et al.* (2014) found that broilers showed a significant improvement in feed conversion ratio and body weight gain (BWG), unrelated to P nutrition, when fed phytase-supplemented diets. They proposed that this was in part due to the release of inositol during phytate hydrolysis.

"The extent of research conducted on different minerals and vitamins is often in direct proportion to their economic value or to the likelihood of encountering a dietary deficiency in practical diets" (National Research Council, 1994). Research in Ca and P nutrition in broilers has been driven by environmental concerns, but more strongly by interest in phytase. Many of the published papers report on trials which test a negative control (low P and/or Ca), a positive control (usually recommended levels of P and Ca) and the negative control with phytase added. This methodology has produced hundreds of papers which almost universally show that phytase improves growth, feed efficiency, P and Ca digestibility and retention. However, our understanding of the broiler's response to these minerals is limited to the narrow range of dietary levels tested and no insight is gained into intermediate points between the positive and negative values. Insight into the interactions between Ca, P and phytase, and hence feeding birds more precisely to meet their requirements, is not supported by this approach. Response curves are required if optimal feeding strategies are to be discovered.

In spite of the established benefits of microbial phytase supplementation, the extent of release of phytin-bound P may depend on a number of factors (Cowieson *et al.*, 2016; Walk and Bedford, 2020). One of these is the inability of currently available exogenous phytases to completely hydrolyse phytate to *myo*-inositol and free P. The final dephosphorylation of lower molecular weight esters must be carried out by endogenous enzymes, thus introducing a number of further variables into the process (Sommerfeld *et al.*, 2018a). Ca plays a particularly important role in determining the effectiveness of phytase supplementation, but its role is not clearly understood. While Ca supplementation in the absence of exogenous phytase supplementation appears to improve the breakdown of phytate in the crop, this effect disappears with the addition of phytase to the diet (Sommerfeld *et al.,* 2018a). Supplementary Ca and P may inhibit degradation of phytate by exogenous phytase. Tamim *et al.* (2004) suggested that this may be explained by the complexes which Ca forms with phytate and that are insoluble at higher pH levels. Other effects of Ca may include those on gut pH when it is provided in the form of $CaCO_3$ and its role in competition for the active sites of phytase.

It is apparent that in order to realise the full benefits of phytase supplementation, with the aim of achieving "phytate-free nutrition" (Cowieson *et al.,* 2016), the role of added Ca and P and the ratio between these two nutrients must be better understood. The contribution of different phytate esters and *myo*-inositol to improved broiler nutrition also require further investigation as it is evident that extra-phosphoric effects are not yet fully understood.

1.3. Research question

What are the relationships between dietary Ca and P and broiler performance, nutrient digestibility, bone growth and phytase efficacy and can these relationships be captured in a simulation model which provides an effective tool for feed formulation, maximising bone growth while minimising P in the excreta?

1.4. Research objective

The main objective of this research was the development of a simulation model that provides a framework to integrate the results of Ca, P and phytase response trials. This would allow these nutrients to be manipulated when formulating feeds for the broiler industry, thereby achieving predictable results, so that birds can reach their potential for growth while minimising environmental impacts.

1.5. Research methodology

This research project was based on a conceptual model of Ca and P flows in the broiler (Figure 1.1). It describes the flow of minerals through the broiler body and proposes relationships between different variables that influence the amount of Ca and P that is available to the bird at different points along this path.

The development of a simulation model which incorporates the interactions between Ca, P and phytase provided a structured approach to the development of a better understanding of these factors.

Through a review of the literature, research questions were developed that, if answered quantitatively, would allow this complex system to be modelled. Following this, a systematic literature review was conducted to ensure that as much of the available data as possible was incorporated into the initial model. This process revealed a dearth of studies but provided indications of the underlying theory that needed to be taken into account in developing the model. From the response studies in which independent and dependent variables matched those required for the model most closely, estimates were made of model parameters. Publications in which the feeds were formulated to meet the birds' needs, and that reported the changes in protein, Ca and P content in the broiler body with age, were identified. These indicated the relationship between these components during growth. Subsequently, studies in which the Ca and P levels in the feed varied, and in which body composition was reported, were used to evaluate the mode

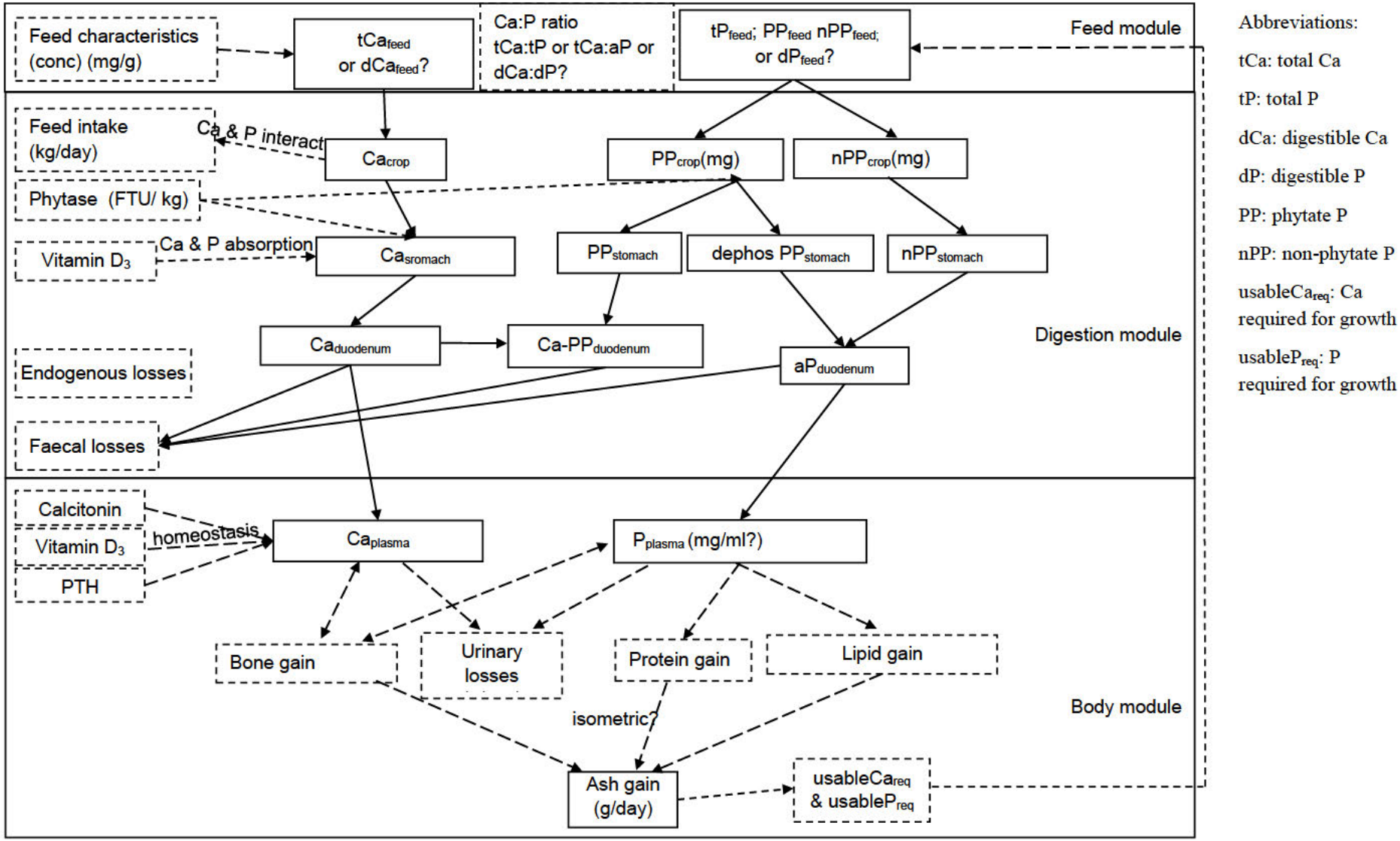

Figure 1.1 Conceptual model of calcium and phosphorus in the broiler

The development of a simulation model provided direction for both this project and future research. It was found that much of the data required for a working model was unavailable and crucial information was missing from the studies that were used. This could be seen as a weakness in the modelling approach, but in fact reveals its strength as a tool for optimal experimental design.

The variables which needed to be understood to allow the development of the model were:

- The potential rate of accretion of Ca and P in the growing broiler.
- The relationship between the growth of the mineral content and the growth of protein in the broiler body.
- The effects of changing Ca and P levels in the diet on the accretion of these minerals in the broiler body.
- The effects of changing levels of Ca, P (both PP and nPP) and phytase on the absorption of minerals from the gastrointestinal tract. Inorganic Ca and P were modelled in the digestive system prior to modelling the influence of phytate.

Factors that were not considered and were flagged for further research and improvements in the model. as a result of the modelling exercise included:

- The appetite of broilers for Ca and P when offered diets differing in Ca, PP, digestible P (dP) and phytase and the effect of these nutrients on feed intake (Wilkinson *et al.*, 2014c; Abdollahi *et al.*, 2016).
- High dose phytase effects with a view to achieving phytate-free digesta (Cowieson *et al.*, 2011a; Walk *et al.*, 2014; Lee *et al.*, 2018).
- The effect of age on the Ca-P-phytase interaction in the gastrointestinal tract and on the proportions of these minerals in the broiler body e.g. in very young chicks (days 1- 4) which have access to high levels of Ca from the yolk sac (Angel *et al.*, 2005a).
- Incorporation of the effect of particle size as it interacts with phytate, inorganic P and phytase and the use of highly soluble Ca (Bradbury *et al.*, 2017).
- Interactions between dietary Ca, P and phytase and energy and amino acids.

The model evaluation process identified gaps in the theory of Ca and P utilisation in the growing broiler. Hence it provided suggestions for further research. The modelling process guides experimental design for further quantification and theory testing.

The implementation of the model relied heavily on an established model of protein growth and various estimates and analyses in the literature. No experimental work could be carried out due to circumstances beyond the researcher's control. As a result, the contribution of this research is a preliminary model and framework within which a planned research programme is structured. It is anticipated that this programme will initiate the process of improving the model. This will in turn raise questions requiring further

experiments in a process of optimal design for the development of theory and practical applications for poultry production.

1.6. Discussion

It has been shown that the protein maintenance and growth requirements of the broiler can be supplied by the diet provided the digestibilities of the dietary ingredients are taken into consideration. However, the picture is somewhat more complicated with respect to mineral nutrition. The chemical interactions in the digestive tract and the interdependence in the body tissues make it more difficult to draw a direct line between growth and intake. However, the potential accretion rate and maintenance requirements still provide a logical starting point for a model. Modelling the constraints on the ability of the bird to reach its potential was the challenge of this research.

CHAPTER 2. SUPPLYING CALCIUM AND PHOSPHORUS FOR THE BROILER'S NEEDS: LITERATURE REVIEW

Decades of research into Ca and P nutrition have not produced a consensus on the appropriate levels of these nutrients in broiler diets (Wilkinson *et al.*, 2014c). Conclusive research is made particularly difficult by the relationship between the two minerals, which results in interactions during digestion, growth and metabolism. Homeostatic mechanisms maintain tight control of plasma levels, particularly of Ca, and allow accretion and resorption from bone as one or other mineral is required for body functions (Proszkowiec-Weglarz and Angel, 2013; Proszkowiec-Weglarz *et al.*, 2019). Hence, the ratio between Ca and P in the feed has been found to be significant as well as their concentrations (Bradbury *et al.*, 2014). It may be difficult to distinguish if a study that aims to investigate Ca/P ratios is effectively investigating the response to changing levels of one mineral while the other is kept constant (e.g. Amerah *et al.*, 2014; Anwar *et al.*, 2016b). Similarly, a study that aims to assess the digestibility of a feed ingredient by including graded levels in the diet may be confounded by the effects of changing levels of one or both minerals (Ca and P) and even changing protein or amino acid levels which affect growth and hence requirements for the minerals.

The proportion of the ingested Ca and P that is retained by the animal is dependent on a range of properties of the feed and the animal consuming it: Ca, nPP, PP and phytase levels in the feed, the physical properties of the feed, the size and age of the bird, its previous exposure to different levels of Ca and P in the feed or even its exposure to disease (Adedokun and Adeola, 2013).

Researchers use a plethora of methods to measure the value of feeds in terms of the availability of Ca and P. Trials that measure the performance of broilers under different feeding regimens abound and these give an indication of the factors that may affect absorption and growth, but do not necessarily quantify the amount of mineral which is retained by the bird. Relative bioavailability is a term used to describe an index that includes more than one measure of performance: commonly BWG and tibia or toe ash (Li *et al.*, 2016b). This is measured against a standard mineral source, such as calcium carbonate ($CaCO_3$) or monosodium phosphate (MSP). Digestibility is usually assessed using a marker and the proportion of mineral that is absorbed up to a certain point (usually the second half of the ileum) is calculated. Taken in conjunction with the feed intake the quantity of absorbed Ca or P may be calculated, but often the two measurements are not taken over the same time period: for example, feed intake may be measured weekly, and the digestibility trial conducted over two or three days. Furthermore, the minerals excreted in the urine are not measured so that requirements for growth cannot be quantified by this measure.

In this chapter, the literature concerning the Ca and P nutrition of broilers will be reviewed. This has a broad scope, while the rest of the study focusses more closely on the modelling process and the research questions that support model development.

2.1. Measurements of mineral availability

Rodehutscord and WPSA (2013) defined available P (aP) as "the part of dietary total P that, at marginal level of supply can be utilised to cover the P requirement of the animal". Historically, various measures of mineral availability have been proposed, some of which conform to this definition and others may not, but nonetheless have practical applications. Rodehutscord and WPSA (2013) described the efforts of a World's Poultry Science Association (WPSA) working group to arrive at a standard method for evaluating the availability of P for poultry. He listed various measures which appear in the literature: non-phytate P (nPP), digestible P (dP), absorbable P, retainable P (rP), utilisable P (uP), relative bioavailable P (RBV), precaecal digestible P (pcdP) and ileal available P. Studies also distinguish between apparent digestibilities and true digestibilities, where the latter takes endogenous losses into account (Anwar and Ravindran, 2016).

The diversity of terms and abbreviations used in the literature create challenges when comparing different studies. Table 2.1 summarises these for reference. X denotes the chemical symbol of Ca or P.

Table 2.1 Terms and abbreviations used in mineral digestibility studies

Abbrev. used in this text	Alternative terms	Alter-native abbrev.	Reference
nPP	non-phytin/phytate phosphorus		Li *et al.* (2017b) Proszkowiec-Weglarz and Angel (2013).
	non-phytate phosphorus	NPP	Adedokun and Adeola (2013) Coon *et al.* (2007) Leske and Coon (2002) Plumstead *et al.* (2008) Akter *et al.* (2016)
aP	available phosphorus		Adedokun and Adeola (2013)
	available phosphorus	avP	Leytem *et al.* (2008b)
	relative biological value	RBV	Coon *et al.* (2007)
	relative biological availability	RBA	Coon *et al.* (2007)
PP	phytate / phytin phosphorus		Li *et al.* (2017b)
X_I	mineral in feed (mass or concentration)		Liu *et al.* (2019) Mutucumarana *et al.* (2015a)
	P ingested in feed (g/kg DM)	P_{I-DMI}	Abbasi *et al.* (2018)
X_O	mineral output (mass or concentration)		Anwar *et al.* (2016b) David *et al.* (2019) Liu *et al.* (2019) Mutucumarana *et al.* (2015a)
	total P flow in digesta/excreta (g/kg digesta/excreta)	P_{O-DMO}	Dilger and Adeola (2006)
	total P flow in digesta/excreta (g/kg dry matter intake)	P_O-DMI	Abbasi *et al.* (2018)
	total nutrients excreted (g/kg DMI)	TNE_g	Leytem *et al.* (2008a)
	nutrient output per kilogram of DMI at the terminal ileum	$PcNE_g$	Plumstead *et al.* (2008)

ELX	endogenous losses of mineral X		
	precaecal endogenous loss of mineral	pcelX	Kupcikova *et al.* (2017)
	ileal endogenous calcium losses	IECaL	Anwar *et al.* (2016b) David *et al.* (2019)
	endogenous phosphorus losses	EPL	Adebiyi and Olukosi (2015) Liu *et al.* (2012) Perryman *et al.* (2016b)
TIDX	true ileal digestibility of mineral X		
	precaecal digestibility coefficient of phosphorus	pcdcP	Rodehutscord *et al.* (2017) Witzig *et al.* (2018)
	true P utilization (precaecal or retention)	TPU	Dilger and Adeola (2006)
	apparent percentage of precaecal nutrient digestibility	$PcND_{\%}$	Plumstead *et al.* (2008)
	true precaecal digestibility	TPD	Abbasi *et al.* (2018)
	coefficient true ileal P digestibility	TPD	Adebiyi and Olukosi (2015)
	true ileal (precaecal) P digestibility	TIPD	Perryman *et al.* (2016a)
	true ileal digestibility coefficient	TIDC	Anwar *et al.* (2016b) David *et al.* (2019) Mutucumarana and Ravindran (2016)
AIDX	apparent ileal digestibility of mineral X		Ravindran *et al.* (2008) Bradbury *et al.* (2018)
	apparent ileal digestibility coefficient	AIDC	Anwar, et al. (2016b)
	apparent ileal phosphorus digestibility	AIPD	Perryman *et al.* (2016b)
	apparent P utilization (precaecal or retention)	APU	Dilger and Adeola (2006)
	apparent precaecal digestibility	APD	Abbasi *et al.* (2018)
	coefficient of ileal apparent digestibility	CIAD	Abdollahi *et al.* (2013)
	coefficient of apparent ileal digestibility	CAID	Mtei *et al.* (2019b) Abdollahi *et al.* (2015)
dX	ileal digestible mineral X (g/kg DMI)		
	precaecal digestible mineral	pcdX	Bikker *et al.* (2016)
	precaecal nutrient absorption (g/kg DMI)	$PcNA_g$	Plumstead *et al.* (2008)
	digestible P	DP	
RX	retention coefficient of mineral (g X in diet)		
	absolute retention value	ARV	Coon *et al.* (2007)
	total tract retention	TTR	Abbasi *et al.* (2018)
	apparent total tract retention	ATTR	Bikker *et al.* (2016)
	coefficient of true total tract P retention	TPR	Adebiyi and Olukosi (2015)
	apparent total tract retention coefficient	ATTRC	Anwar *et al.* (2017)
	overall percentage of nutrient retention	$TNR_{\%}$	Plumstead *et al.* (2008)

rX	retainable mineral (g/kg feed)		
	retainable phosphorus	RP	Coon *et al.* (2007)
	apparent P retention	APR	Liu *et al.* (2019) Perryman *et al.* (2016b)
	standardised P retention	SPR	Liu *et al.* (2019)
	net phosphorus utilisation	NPU	Hurwitz (1964)
	apparent total tract digestibility coefficient	ATTDC	Leytem *et al.* (2008a)
	total retention of dietary nutrients (g/kg DMI)	TNRg	Plumstead *et al.* (2008)

In order to describe the requirements of birds and the potential of feed to meet these requirements, it would be helpful to develop a more clearly defined terminology and standardised assay protocols for these characteristics (Adedokun and Adeola, 2013).

It is conventional to express retention and ileal digestibility as percentages of the mineral in the feed. As a result, the actual mass of retained Ca and P is not immediately apparent. Although these values may be calculated from the feed intake and feed analysis data, if they are provided, little attention is paid to them even though they may hold the key to understanding bird responses to changing diets.

2.1.1. Calcium and phosphorus bioavailability

Requirements for Ca and P have historically been based on relative bioavailability (RBV), which uses performance as an indicator of utilisation. This has meant that a considerable number of studies have measured changes in various performance criteria as levels of Ca and P in the diet changed. Shastak and Rodehutscord (2013) provided a comprehensive review of the history of this approach to comparing feedstuffs. Coon *et al.* (2007), for example, used a calculation of percentage P availability based on an index which takes into account tibia ash, weight gain and feed conversion.

This approach does not measure mineral absorbed by the bird but rather bird performance when compared with a standard ingredient that is considered to be 100% available (Coon *et al.*, 2002; Anwar and Ravindran, 2016). DCP has been used as a standard in P bioavailability trials (Nelson *et al.*, 1990; Ravindran *et al.*, 1995; Coon *et al.*, 2007) and $CaCO_3$ in Ca bioavailability trials (Sa *et al.*, 2004). While this index method, developed by Sullivan (1966), has practical utility for feeding broilers and attempts to balance the producer's objectives, it does not assess the amount of mineral in the feed that is used or excreted by the bird. Furthermore, the bioavailability is assessed relative to a standard mineral source which is assigned a value of 100% RBV. This does not imply that all of the P in the standard is available for absorption by the bird. As a result, this measure is of little use for modelling purposes and is also limited to the conditions (e.g. bird age, other ingredients in the diet) which prevailed during the trial (Shastak and Rodehutscord, 2013).

Early studies of the relationship between Ca and P in the diet highlighted the importance of the ratio between these two minerals. Simco and Stephenson (1961) discussed this at length, and cited Bethke *et al.*

(1928) and others who proposed Ca/P ratios as high as 4:1, with Ca inclusions often over 2% in feed. Their own study appeared to dispute these proposed requirements as they found that including both Ca and P at 0.5% was adequate to maximise growth, feed utilisation and toe ash. In a later study, in which a polynomial regression approach was used to model the relationship between Ca, P and BWG, Twining *et al.* (1965) recommended a total Ca (tCa) concentration of 0.7 to 0.8% and 0.65% total P (tP). However, they conceded that further research was required to determine whether additional Ca would deliver further gains. They showed that narrower Ca/P ratios had a detrimental effect on growth: Ca levels could be dropped without depressing growth provided P was lowered as well. Although the authors acknowledged that the P availability of the diets used in this study was unknown, they included a maize/soya basal diet of 0.41% tP with no added nPP, so that the recommended tCa/nPP ratio may have been close to 2:1.

By the 1980s, researchers began to report results in terms of aP, while tCa remained the standard measure. The genetic potential for growth in broilers had increased significantly and conflicts arose between the requirements for maximum growth and the leg strength to support it. Hulan *et al.* (1985) noted that when a tCa/aP ratio of 1.9 in the starter phase and 2.5 in the finisher was used, growth was maximised but so was tibial dyschondroplasia. Higher ratios improved leg function but impaired growth. A ratio of approximately 2 between Ca and nPP has persisted in broiler feed formulations, perhaps as a result of the ratio between these two minerals in bone. However, with the increasing use of phytases for the dephosphorylation of PP, this ratio has undergone repeated downward revision. Nevertheless, many contemporary studies follow a tCa/nPP ratio of 2 in their diets. Many more recent bioavailability studies have included phytase among the treatments as the use of phytase has provided the impetus for the rise in research into Ca and P.

2.1.2. Ileal or precaecal digestibility

Working Group 2 of the World Poultry Science Association (WPSA) set out to develop a protocol for P evaluation, to measure this value for a range of raw materials and to model the requirements of poultry for this available P (Rodehutscord and WPSA, 2013).

This group recommended pcdP (equivalent to ileal digestibility), as a standardised measure of available P. Digestibility may be expressed in absolute terms (g absorbed from the digestive tract) or, more commonly, relative digestibility (coefficients).

$$\text{digestibility coefficient} = g_{digestible\ mineral} / g_{total\ mineral\ in\ feed} \qquad (Eq.\ 2.1)$$

Apparent digestibility may be calculated as 1 – apparent indigestibility

$$\text{indigestibility} = g_{mineral\ in\ digesta} / g_{total\ mineral\ in\ feed} \qquad (Eq\ 2.2)$$

A marker such as titanium dioxide is usually added to the feed and a sample of both feed and digesta allows the use of the formula for apparent ileal digestibility (AID) coefficient:

$$AID_X = 1 - \left[{}^{M_I}/_{M_O} \times {}^{X_O}/_{X_I} \right] \qquad (Eq.\ 2.3)$$

where M represents the marker, X represents the mineral, subscript *I* represents concentration in the diet and *O* concentration in the terminal ileal digesta with all concentrations expressed in the same units, e.g.% or g/kg dry matter (DM).

To calculate the true digestibility of a mineral from its apparent digestibility, it is necessary to account for the endogenous losses, which increase the amount of mineral present in the digesta.

Ileal endogenous losses (IEL) of Ca and P (e.g. in g/kg DM intake) may be calculated when a Ca and P free diet is fed (Anwar *et al.*, 2016b):

$$IEL_X = X_O \times \left(\frac{Ti_I}{Ti_O}\right) \quad (Eq. 2.4)$$

True ileal digestibility coefficient is then

$$TID_X = AID_X + \left({}^{IEL_X}/_{X_I}\right) \quad (Eq. 2.5)$$

Endogenous losses include basal endogenous losses, which arise independently of the diet, and endogenous losses that are specific to the ingredients used (González-Vega and Stein, 2016). If only basal and not specific losses are taken into account (e.g. when the Ca and P free diet method is used) then this should be termed a standardised digestibility. Specific losses are seldom measured and standardised ileal digestibilities have become widely accepted.

Endogenous Ca losses, a large proportion of which are due to bile secretions, are higher in pigs than in poultry, with figures of 84 to 131 mg/kg dry matter intake (DMI) recorded for the latter and from 123 to 670 mg/kg DMI for the former (Anwar and Ravindran, 2016; González-Vega and Stein, 2016; Anwar *et al.*, 2017; Zhang and Adeola, 2017; Anwar *et al.*, 2018; David *et al.*, 2019). However, a much higher value, comparable to those found in pigs (253 mg/kg DM) was obtained when a corn-based, purified diet (0.19 g/kg Ca) was fed in one of these trials (David *et al.*, 2019).

P exhibits endogenous losses up to 354 mg/kg DMI (Anwar and Ravindran, 2016; Mutucumarana and Ravindran, 2016). This endogenous P, secreted back into the small intestine, makes up a small but significant part of the total P in the digesta: in these trials test diets contained approximately 5 000 mg/kg DMI tP. True P digestibility was therefore approximately 7% higher than the apparent digestibility.

TID values include an allowance for endogenous losses in their calculation, while apparent values do not and hence the amount of nutrient absorbed is underestimated in the latter (Anwar and Ravindran, 2016). When TID is measured using the regression method, with graded amounts of the test ingredient in the diet as the sole source of the mineral, endogenous losses are represented by the intercept of the regression line, while the slope represents the TID. If apparent values are measured by the direct method, endogenous losses must be subtracted from the mineral content of the ileal digesta before true ileal digestibility can be calculated (Cowieson *et al.*, 2019). Usually, a diet free of the mineral under consideration is fed to measure

endogenous losses and hence specific losses are not measured, so that the calculated digestibility is more accurately termed a standardised digestibility.

While TID values are acknowledged as the ideal measure of mineral availability, it is difficult to find repeatable values. Rodehutscord *et al.* (2017) conducted comparative assessments on the same experimental diets in 17 different experimental facilities and found that significant variation arose between the measurements at different facilities. It was not possible to pinpoint age at start of trial, age of sampling, sex, average daily feed intake (ADFI) or BW at slaughter as factors causing these differences and it was speculated that starter diet composition (before feeding test feeds) and management practices should be standardised. A regression of pcdP on phytate disappearance suggested that this was the largest contributor to digestibility.

When the test ingredient is added to a basal diet in increasing amounts in the regression method of retention or TID determination, this often changes the protein level of the diet as well as the mineral content (Abbasi *et al.*, 2018). The test ingredient may replace an ingredient such as corn starch. The regression slope may be affected by the interactions between protein level and mineral. Some studies add casein or egg albumin to maintain growth during the test period (Liu and Adeola, 2016; Abbasi *et al.*, 2018). While it would seem to be logical to add this protein source to maintain constant protein levels, often it is simply added at the same level in all test diets (Liu and Adeola, 2016; Abbasi *et al.*, 2018). These authors did not find an effect on digestibility but did find improved growth where birds were fed more soya-bean meal or casein.

Furthermore, the level of the mineral itself and its concentration relative to its co-dependent (Ca to P or vice versa) are likely to affect absorption. The direct method, in which the test ingredient provides the sole or at least major source of the nutrient in the diet at a level close to the commercial inclusion level may be the best measure of digestibility (Anwar *et al.*, 2016c). These authors cited their earlier work which produced comparable results for the regression and direct methods for measuring TIDCa. However, this value would need to be modified when conditions are not identical to the test conditions in which it was measured. Estimating these modifications and applying them to the diet as a whole may be the most useful way of supplying the needs of the broiler more precisely.

Extensive work has been done at Massey University to determine TID values for a number of important Ca sources (Anwar *et al.*, 2016a; c; 2018; David *et al.*, 2019). The digestibilities were often considerably lower than expected and varied widely between and within trials.

If AID is measured by the direct or difference methods, endogenous losses must be taken into account (Anwar *et al.*, 2015). Researchers have measured these values for P in poultry (Dilger and Adeola, 2006; Mutucumarana *et al.*, 2014b; c; 2015a; b; Mutucumarana and Ravindran, 2016). Anwar *et al.* (2018) found that the value of this figure for Ca (determined using a Ca- and P-free diet) changed (from 84 to 124 mg/kg DMI) as the adaptation period to the diet was increased.

Anwar *et al.* (2018) used the direct method of digestibility measurement where the test ingredient provided the sole source of Ca, and diets were formulated to contain 9g/kg of Ca. They found that the digestibilities of various feed ingredients were low (0.24 to 0.33) and speculated that this might be due to the high nPP levels which resulted in low Ca:nPP ratios (0.70 to 1.43) which were unavoidable as the test ingredient was the only Ca source in each diet. They also speculated that adaptation periods might affect digestibility values as seen in some other studies (Plumstead *et al.*, 2008; Walk *et al.*, 2012b).

The most accurate method of P digestibility measurement is the regression method (Rodehutscord and WPSA, 2013). It has been shown that the direct method produces similar results for Ca digestibility, but this may not be the case when it is not possible to maintain constant Ca/P ratios (2:1) (Anwar *et al.*, 2016c). However, the costs associated with the regression method due to the number of treatments required may be the reason that the direct method is usually preferred and standardised digestibilities are widely accepted.

The available mineral content of the diet is the sum of the mineral content × digestibility coefficients of the ingredients. For this additivity to exist, it is necessary to use true pre-caecal or ileal digestibility values rather than apparent values. This is because the basal endogenous losses of the mineral must only be included once in the weighted sum calculation.

Although it has been proposed that true Ca digestibility values are additive in broiler diets (Zhang and Adeola, 2018), these trials were conducted using semi-purified diets and using the same series of Ca levels in the individual ingredient (limestone and DCP) and the mixed diet. Hence other possible influences on digestibility, such as bird age and Ca, nPP, PP and phytase concentrations in the mixed diets were controlled. Further investigation is therefore needed if standardised digestibilities are to prove useful in practice.

AIDP in complete diets, varying in PP concentration, without the addition of phytase, ranged from 28% (Ravindran *et al.*, 2006) to 66% (Walk *et al.*, 2012b). Cations in the diet, particularly Ca, play a major role in this variability. The formation of Perryman *et al.* (2016b) found that the ileal digestibility of diets varied with the length of pre-assay feeding period, possibly as a result of the adaptive mechanisms discussed below in sections 2.4.3 and 2.5.6.

2.1.3. Total retention

Coon *et al.* (2002) suggested that the retention of minerals is a more meaningful measure of requirements than digestibility or availability, but these confound the effects of the availability of nutrients from the feed and the urinary excretion of nutrients that are absorbed but not required. Bioavailability assays that compare feedstuffs to a standard mineral source do not assess the amount of mineral which is actually retained by the bird, and which is therefore its true requirement for growth. Similarly, the amount of mineral absorbed, which is measured by digestibility assays, is subject to further adjustment and may not reflect the requirement of the animal. The kidney plays a particularly important role in the homeostasis of Ca and P, under the control of various mechanisms including parathyroid hormone (PTH) and vitamin D_3. Precaecal

digestibility estimates have been shown to have a more direct relationship with P concentrations in the diet than retention estimates (Plumstead *et al.*, 2008; Rodehutscord *et al.*, 2012; Walk *et al.*, 2012b). Hence, they may reflect the properties of the diet rather than the needs of the animal.

Leske and Coon (2002) commented on the confusion which arises out of the terminology used to describe measures of P digestibility. It was proposed that retainable P (rP) be "defined as the difference between the amount of phosphorus from a source ingested and the sum total that is voided from the gastrointestinal and urinary tracts at a particular phosphorus intake and bird development". It is important to note that it was recognised that retention is affected by the two variables, growth and intake, so that these should be specified when any rP value is measured. This can be expressed as a proportion of the P ingested so that

$$rP\ (\%) = \frac{total\ P\ ingested - total\ P\ excreted}{total\ P\ ingested} \times 100 \qquad (Eq.\ 2.6)$$

It was further suggested that it would be useful to measure the retention of PP and nPP for a better understanding of the P supplied by different feed ingredients.

Hurwitz (1964) pointed out that a measure of true P utilisation is needed, that takes into account faecal and urinary endogenous losses. It was suggested that if body weight and feed intake are the same, endogenous losses could be assumed to be constant. Hence the change in apparent P retention occurring when two different levels of P are fed would represent the change in net P utilisation. This is one of the early examples of the use of the regression method in P nutrition research. Importantly, it was also pointed out that final body P = initial body P + apparent P retention. Body P was estimated using a relationship between tibia P and body P (see section 2.6.2.3), so that tibia P mass was regressed against P content of diet.

In a study of P availability in broilers, van der Klis and Versteegh (1996) proposed that a standard assay be used for feedstuffs. It was noted that PP had previously been assumed to be unavailable while nPP was assumed to be fully available but that this assumption is incorrect. Their method calculates the mass of P retained by the bird over a three-day period, from 21 to 24 days of age. Their assay is summarised in Table 2.2.

This protocol was proposed to determine the maximum digestion of PP, which is expected to take place at low tP levels in the diet. Values for rP derived in this way are still being used for mineral phosphates and feedstuffs of animal origin (CVB, 2021). However, corrections are applied to account for the lower digestibility of PP at standard dietary mineral levels (3.0 dP/kg and 6.8 g Ca/kg).

Ca retention values are not used in feed formulation, but a standard assay would be required if this were desirable.

Conflicting results are reported in studies measuring retention and digestibility in broilers (see section 2.5.3). It is possible that it is the interactions between Ca, P and phytase in feed that produce these different patterns.

Table 2.2 P availability protocol suggested by van der Klis and Versteegh (1996)

Birds	Male broilers
Basal diet ingredients	low P ingredients such as starch, glucose syrup, soya oil, demineralised whey and cellulose synthetic amino acids, vitamins and minerals other than P added to meet the birds' nutrient requirements should contribute no more than 10% of the P in the test diets (ignored when calculating availability)
P and Ca content	P standardised to 1.8 g/kg calculated available P tCa standardised by the addition of limestone to 5.0 g/kg.
Feed form	3 mm pellets, no steam
Housing	metabolism cages at a stocking density of 33.33 birds per square metre.
Feeding programme	test diets fed from 10 days of age
Balance period	21 to 24 days of age
Measurements	mass of P intake from the feed and P excretion in droppings
Other	water and feed are provided ad lib.

The aim of this modelling exercise is to make sense of the data and to explain the apparent contradictions. These may arise from different levels of nutrients in feed as well as different ratios between them. Furthermore, the convention of reporting percentage utilisation figures rather than absolute values may be obscuring trends that arise from the true requirements of the animal and its ability to meet its needs from its diet.

2.2. Terminology

Table 2.3 summarises the terminology and abbreviations used in this study. True pre-caecal digestibility (tpcd) is the measure of the availability of P for absorption recommended by the WPSA working group (Rodehutscord and WPSA, 2013). Most absorption is completed by the end of the ileum, and the action of microbes in the large intestine does not influence this measurement (Shastak *et al.*, 2012b). The use of lowercase letters for measures of availability, as preferred by the WPSA working group, is not used here because it was felt that the use of capital letters is more consistent with common abbreviations for measures of energy (GE, ME) (e.g. Zhang and Adeola, 2018) and digestibility of amino acids (e.g. Walk and Rao, 2019). Where descriptors indicate the proportion in feed, to distinguish these from measures of the proportion of nutrient, lower case letters were used. nPP as the abbreviation for non-phytate phosphorus was preferred to the commonly used NPP so that only the chemical descriptors are abbreviated with uppercase letters.

Table 2.3 Standardised terminology and abbreviations used in this study

Term used in this thesis	Abbreviation	Definition
non phytate phosphorus	nPP	P not bound in phytate, whether in mineral, plant or animal ingredients
phytate phosphorus	PP	P present in phytic acid, phytin or other phytate compounds, contributed by plant ingredients
mineral concentration in feed	X_I	mineral in the feed as a proportion of the dry matter in the feed
mineral concentration in digesta or excreta	X_O	mineral in the digesta, faeces or faeces + urine as a proportion of the dry matter in the excreta
precaecal endogenous mineral losses	ELX	mass of mineral that enters the digestive tract as a result of bodily functions
true/apparent precaecal digestibility coefficient	T/AIDX	proportion of mineral in feed absorbed by the bird (TAID) or apparently absorbed (AID)
true/apparent precaecal digestible mineral	dX	g digestible mineral/g feed
retention coefficient	RX	proportion of mineral in feed retained by the bird
retainable mineral	rX	g retainable mineral/g feed

2.3. Calcium and phosphorus in feed

Various conventions are used when describing feed ingredients and feeds in terms of their mineral content. While the chemical composition can be measured, nutritionists have moved towards translating the quantities and forms of Ca and P into measures that take their availability into account.

2.3.1. Chemical characteristics of feed ingredients

2.3.1.1.Calcium

Total Ca concentrations in feedstuffs range from 0.02% in cereals to 0.7% in plant protein sources. Additional, inorganic Ca supplies over 80% of the Ca in many broiler starter feeds (Anwar and Ravindran, 2016). Limestone is the most widely-used source (Lee *et al.*, 2021) but Ca is also supplemented by inorganic P sources such as DCP, monodicalcium phosphate (MDP) and MCP (David *et al.*, 2019; Lamp *et al.*, 2020). Ca makes up 400 g/kg of pure $CaCO_3$, but its concentration varies in limestone from different sources, with an average value of 380 g/kg (National Research Council, 1994; Anwar *et al.*, 2016a). Particle size and solubility also vary but these are not sufficient to assess an ingredient's potential availability to poultry (Zhang and Coon, 1997; Anwar *et al.*, 2016b). This may also depend on other factors in the diet

(e.g. anions or other chelating agents that react with Ca) and in the digestion process (e.g. transit time). While efforts are being made to develop a digestible Ca (dCa) system for broilers, tCa is usually used in feed formulation.

2.3.1.2.Phosphorus

P in feedstuffs may be reported as tP. Alternatively, it may be presented as aP (% or g/kg feed) (National Research Council, 1984) or with an availability coefficient relative to a standard as found in the INRA tables of feed composition (Sauvant *et al.*, 2004). More recently, P has been listed according to its form, whether bound in phytate (PP) or not (nPP), or by reporting tP and one of these values, allowing the other to be obtained by subtraction, as is found in the INRAE-CIRAD-AFZ Feed tables (Tran *et al.*, 2017) and the NRC tables (National Research Council, 1994). rP or dP may also be used, based on experimental measures of P absorption or retention (CVB, 2021).

Plants store P in organic form as phytate ((myo-inositol hexaphosphate or 1,2,3,4,5,6-hexakis (dihydrogen phosphate) or IP_6). The molecular formula of phytic acid is $C_6H_{18}O_{24}P_6$ with molecular weight 660 g/mol. P is found at a concentration of 282 g/kg in phytic acid (Selle *et al.*, 2009). IP_6 is usually present in feedstuffs as a salt, typically of magnesium and potassium, or may be present as a chelated molecule with protein or starch. The term phytin is sometimes used for these complex molecules (Angel *et al.*, 2002), although phytin may be more narrowly defined as the calcium-magnesium salt of phytic acid (Selle *et al.*, 2009). PP must be broken down into inorganic phosphate (P_i) if it is to be available to the broiler.

Calculated from the difference between tP and PP, nPP includes inorganic and organic forms (Rodehutscord and WPSA, 2013). P_i may precipitate in the gastrointestinal tract (GIT), for example in the form of calcium orthophosphate ($Ca_3(PO_4)_2$) but is generally considered to be readily available for absorption from the GIT. It is mostly supplied by mineral P sources added to the broiler diet. Other organic phosphates (oP) (e.g. hydroxyapatite in bone meal) may be readily available to poultry, although less so than P_i (van Harn *et al.*, 2017). Further nPP may be supplied by plant ingredients.

In the National Research Council recommendations, nPP replaced available P in feed ingredient composition tables without changes to the values (Applegate and Angel, 2014). However, the two terms should not be synonymous, since the chemical composition of nPP in feedstuffs varies, as does its availability for absorption, and a significant proportion of the PP in feed may be broken down and become available to the bird (Rodehutscord and WPSA, 2013).

Anwar and Ravindran (2016) noted that Ca in feed ingredients is assumed to be 100% available. This has not been a question of particular interest as long as Ca has been a low-cost ingredient in broiler diets and before the use of phytase became widespread. However, it is now clear that the chemical analysis of feedstuffs and the description of requirements in terms of tCa, PP and nPP is insufficient for the precise supply of nutrients for the animal's needs.

2.3.2. Effect of calcium and phosphorus on feed intake

If, as proposed by Emmans (1987), animals eat to achieve a genetically determined potential growth rate of a particular nutrient (x), a desired feed intake (DFI) can be calculated from the quantity of nutrient required to achieve this at a given time and the concentration of the nutrient in the feed. The first limiting nutrient is that which is most deficient relative to the requirement for it. This results in a daily DFI (g/d) for a given diet being calculated as follows:

$$DFI = {}^{RQ_1}/_{FC_1} \qquad (Eq.\,2.7)$$

Where RQ_1 is the requirement for the first limiting nutrient in the diet (units/d) and FC_1 is the concentration of that nutrient in the feed (units/kg). This feed intake may be constrained by factors such as feed bulk and ambient temperature. This effect of dietary composition on feed intake must be implemented through physiological mechanisms and these signalling pathways are gradually being clarified (Richards and Proszkowiec-Weglarz, 2007). If this holds true for a particular nutrient (as it has been shown to do for amino acids), then the consumption of feed should increase when the first limiting nutrient is in short supply, as the bird endeavours to eat enough to meet its requirement.

Nutritional geometry studies of Ca and P have indicated that broilers do not eat to satisfy a need for P but that they may respond to lower Ca in the diet by increasing FI (Twining *et al.*, 1965; Bradbury *et al.*, 2014). The interaction between the two minerals may confound this effect. A lower level of Ca (0.5%) in the feed led to a poorer feed conversion efficiency (gain/feed, FCE) in the earlier study, suggesting a higher feed intake, and the Ca X P interaction was insignificant (Twining *et al.*, 1965). However, the highest tCa concentration was 0.8% and it was in the range 0.8 to 1.2% that Bradbury *et al.* (2014) noted a decline in feed conversion efficiency (FCE) at low nPP levels but not at higher nPP levels, resulting in a significant interaction between Ca and P. They proposed that birds will defend a need for Ca even if this means consuming more P than they require. When offered a separate Ca source, birds increase their consumption of it as the nPP content of their main feed increases, indicating that they are able to maintain a balance between the two minerals (Wilkinson *et al.*, 2014a). This tends to suggest a feedback mechanism from bone, where Ca and P growth is interdependent.

In contrast to the expected increase in FI when P is deficient, which is the bird's response to a limiting amino acid or energy, low P seems to depress appetite (Dilger *et al.*, 2004; Rousseau *et al.*, 2016). A meta-analysis of P utilisation in broilers suggested a quadratic relationship between the nPP level in the diet and FI, but this was not a strong relationship (R^2=0.63) (Létourneau-Montminy *et al.*, 2010). Ca concentration in the diet had a negative effect and phytase a positive effect on FI. There was a highly significant, negative interaction between nPP and phytase (P<0.001) and a less significant, positive one between nPP and Ca. Upregulation of anorexia-related melanocortin receptors in the hypothalamus of birds on extremely low nPP diets (1.2 g/kg) was recently reported (Aderibigbe *et al.*, 2022). This was not linked to changes in the intestine and hence the possible involvement of a bone-derived hormone, lipocalin, was proposed.

2.4. Absorption of calcium and phosphorus from the digestive tract

In his doctoral research, van der Klis (1993) conducted an extensive study of the chemical composition of the gut contents and the absorption of minerals. The importance of the pH, osmolarity and viscosity of the digesta were emphasised: these were found to affect both the rate and extent of Ca and P uptake. In a discussion of the transport of minerals across the gut wall, it was proposed that active, transcellular, carrier-mediated transport predominates in animals on a low level of mineral in the diet and passive, paracellular diffusion predominates in birds fed a high mineral diet. However, subsequent research has shown that the picture is more complicated than this.

2.4.1. Calcium absorption in the intestine

Approximately 90% of the Ca absorbed from the GIT is absorbed in the small intestine (Wasserman, 2004). Early work using yttrium-91as a marker indicated that net secretion of Ca occurred in the duodenum when dietary levels were 1.08% (Hurwitz and Bar, 1970). Ca absorption occurred in the jejunum and upper ileum, in this *in vivo* study on three-week-old broilers. This finding was supported by a study of rats in which the majority of the Ca absorption took place in the distal jejunum and ileum when Ca levels were high, due to the predominance of passive absorption, and duodenal absorption predominated when Ca levels were low (Bronner, 2003). In contrast, most Ca absorption took place in the duodenum and jejunum in spite of a fairly high Ca level (1.18%) in another study which used six-week old broilers (van der Klis *et al.*, 1990). The active and passive mechanisms of Ca absorption are illustrated in Figure 2.1.

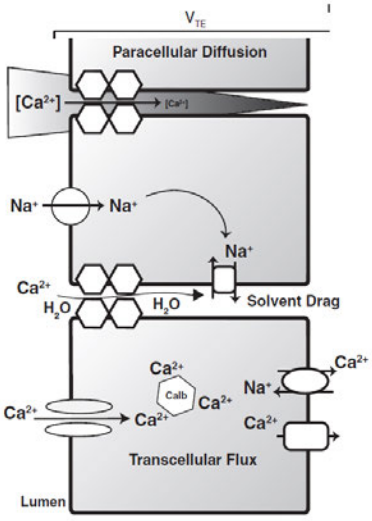

Figure 2.1 Active (transcellular) and passive (paracellular) transport transport of Ca across epithelia

Source: Alexander *et al.* (2014)

Paracellular absorption of Ca across the epithelium of the intestine occurs either by passive diffusion through the tight junctions between cells or due to solvent drag by this route. In mice, this process has been found to be controlled by claudin proteins that alter the permeability of the tight junctions, under the

influence of the active form of vitamin D_3 (Fujita *et al.*, 2008). Beggs (2021) confirmed the role of claudins in increasing the permeability to Ca of the intestine of young animals and also discovered a role for these proteins in promoting Ca absorption in the large intestine. This was particularly important when small intestinal absorption was interrupted through interference with various carriers, further evidence of the adaptive response that maintains the balance of Ca in the body. In rats, paracellular absorption was impeded by the intestinal epithelium, so that it took place at a rate approximately 50 times slower than that which would be expected if there were no barrier between the intestinal lumen and the blood plasma, even at very high dietary Ca levels (Duflos *et al.*, 1995).

Transcellular absorption follows the electrochemical gradient into cells in a carrier-mediated process, involving transient receptor potential channels (TRP), most likely TRPV6, but also involving other carriers (Dimke *et al.*, 2011). Beggs (2021) established the role of Cav1.3 channels in the absorption of Ca into enterocytes in the jejunum and ileum of preweaning mice. TRPV6 was found to be the primary route of apical entry into the duodenal enterocytes, and this persisted in older animals. However, it was the absorption in the jejunum (TRPV6 and Cav1.3 mediated) and ileum (mediated by Cav1.3 and an additional, unknown carrier) that was critical to bone mineralisation in the postnatal animal. This recent work suggests that similar investigations may be warranted in poultry, to understand how the very young chick meets its high demand for Ca.

To complete the transcellular absorption, calcium binding proteins (e.g. calbindin) transport Ca across the cell for active extrusion from the cell at the basolateral membrane (Alexander *et al.*, 2014). While paracellular flux may be into or out of the intestinal lumen, the mechanism of transcellular flux means that this is unidirectional: always absorption (Alexander *et al.*, 2014).

A decline in paracellular absorption occurs over the lifetime of humans, and changes in the fraction of dietary Ca that is absorbed reach a maximum at 5 weeks of age in rats, before declining (Beggs, 2021).

A deterministic model may need to consider digestion and absorption separately. The former is a chemical process in which minerals are made available to the animal, while the latter is tightly controlled through the animal's homeostatic mechanisms.

2.4.2. Phosphorus absorption from the intestine

P absorption continues throughout the small intestine although the proportions absorbed in different sections have yet to be determined (Rodehutscord *et al.*, 2012). Active transport of P is important at normal P levels in rats (Eto *et al.*, 2006) and chick embryos (Cao *et al.*, 2020).

Hurwitz and Bar (1970) concluded that P absorption takes place in the upper jejunum. However, levels of nPP were fairly high (tP at 0.77%, mostly supplied by dicalcium phosphate). Liu *et al.* (2016) conducted *in vivo* studies of ligated small intestine loops from 22-day-old broilers. They concluded that P absorption

occurred principally in the duodenum, through an active, saturable, carrier-mediated process, while passive diffusion predominated in the jejunum and ileum.

Much of the understanding of P absorption through epithelia is based on mouse models (e.g. Hilfiker *et al.*, 1998; Eto *et al.*, 2006) and less is known about P_i transport in poultry (Proszkowiec-Weglarz and Angel, 2013). Mammalian studies have suggested that Type IIb Na-dependent phosphate cotransporters (NaPi-IIb) are the primary route of absorption of P in the intestine (Hilfiker *et al.*, 1998), with transcellular permeation dominating under normal physiological conditions (approximately 2mV transmucosal potential difference) (Eto *et al.*, 2006). Shao *et al.* (2019) demonstrated this mechanism with broiler intestine and confirmed that NaPi-IIb expression is greater in the duodenum than in other sections of the small intestine. However, Olukosi (2016) pointed out that the shorter length of the duodenum results in a greater proportion of P absorbed in the jejunum, where the transit time is longer. Shao *et al.* (2019) also demonstrated a role for Type III Na-dependent phosphate cotransporters (PiT-1) particularly where vitamin D_3 is deficient and particularly for P absorption in the duodenum. In experiments conducted with a tP level of approximately 5 g/kg and phytate at 3 g/kg, Zeller *et al.* (2015a) showed that 34% of the total P was removed by the end of the jejunum and 57% by the terminal ileum.

The ability of the bird to invoke different absorption sites and pathways under different conditions suggests that it has the ability to adapt to variable dietary regimes. This has been borne out by studies of depletion and repletion showing that early mineral depletion can be compensated for by greater retention in later phases (Yan *et al.*, 2005b; Rousseau *et al.*, 2016).

2.4.3. Physiological control of absorption

The control of the absorption of Ca and P in the GIT relies on complex, interdependent physiological mechanisms. These are linked to reabsorption in the kidney to maintain mineral balance (Beggs, 2021).

PTH and vitamin D_3 (cholcalciferol) play a vital and interrelated role in both Ca and P homeostasis. Vitamin D is converted to 25-OH-D_3 (calcidiol) in the liver and this is the main circulating form of vitamin D_3 (Warren and Livingston, 2021). All three aspects of transcellular transport, uptake from the GIT lumen, transport across the cell and extrusion, are influenced by the active form of vitamin D_3, 1,25-$(OH)_2D_3$ (calcitriol) (Hoenderop *et al.*, 2005). Increased PTH levels increase the enzyme activity that leads to the formation of active vitamin D_3 in the kidney, and this acts on the intestinal enterocytes to increase the transcellular uptake of Ca^{2+} when plasma Ca levels drop.

Calcitonin may influence intestinal absorption of Ca (through the influence of Ca on vitamin-D_3), renal excretion and bone resorption but the exact role of calcitonin is still unclear (Hirsch and Baruch, 2003; Felsenfeld and Levine, 2015). The action of calcitonin on osteoclasts, has been investigated in chicks, with varying results (Nicholson *et al.*, 1987; Hall *et al.*, 1994). Hirsch *et al.* (2001) hypothesised that calcitonin might have a role in conserving P during periods between meals, by causing the deposition of calcium phosphate on the surface of bones. This type of mechanism could be important when Ca and P are

consumed separately but must be assimilated together into the bone as hydroxyapatite. The sodium-dependent phosphate transporter (NaPi-IIb) gene plays a critical role in P absorption and is regulated by both P concentration in the lumen of the intestine and vitamin D_3 (Huber *et al.*, 2015; Shao *et al.*, 2019). This gene expression is upregulated in the jejunum and ileum when the diet is severely deficient in nPP (Adedokun and Adeola, 2013). This may explain the improved apparent ileal digestibility of P observed at low P levels in the diet.

Shao *et al.* (2019) demonstrated that mRNA expression in the duodenum and jejunum was affected by nPP concentration in the diet, suggesting an adaptive mechanism whereby birds on low P diets may increase absorption of P. Similarly, Bar *et al.* (1990) found that calbindin, mediated by vitamin D_3, is in increased when dietary Ca and P are low. This effect of dietary Ca would be expected, increasing Ca absorption, but the increase in calbindin with low P is likely to be an indirect one (see section 2.5.2). Morrissey and Wasserman (1971) demonstrated that the adaptation to a low Ca diet is implemented within 1.5 days (the first measurement) and increases to a maximum at 8 days. The birds were 16 days old at the start of their trial (see Figure 2.2). Ca_{47} absorption in the control group decreased after 21 days of age (5.5 days of trial) while it continued to rise in the low Ca group.

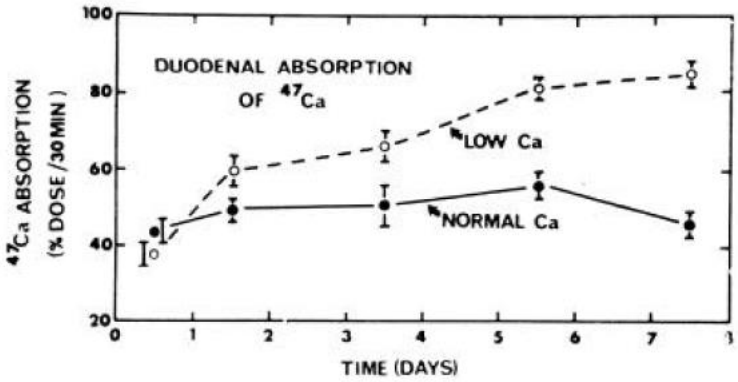

Figure 2.2 Changes in duodenal absorption of Ca in chicks aged 16-24 d
Source: Morrissey and Wasserman (1971)

NaP-IIb expression reaches its maximum at approximately 15 days in broiler chicks (Olukosi, 2016). Yan *et al.* (2005b) demonstrated that broilers can compensate for low Ca and P levels in starter diets by increasing Ca and P absorption and by laying down more minerals in bone in the grower phase. This was supported by other studies that showed changes in the expression of genes that encode Ca and P transporters in the intestine of chicks persisting after increased mineral supply (Ashwell and Angel, 2010; Rousseau *et al.*, 2016). Changes to the P absorption capacity of the intestine are slow compared with the rapid renal response to changes in plasma P, supporting the theory that the kidney is responsible for the regulation of this mineral (Murer *et al.*, 2000). A recent study has cast doubt on this strategy for reducing P in poultry diets (Valable *et al.*, 2020), but the authors also pointed out that a confounding factor in studies measuring ileal digestibility with low nPP levels may be the increased hydrolysis of phytate by endogenous enzymes,

and that this may have meant that P was not limiting in the diets they used. More efficient mineral utilisation after a period of deprivation may also be a result of increased renal tubular reabsorption rather than increased intestinal absorption (Valable *et al.*, 2018), highlighting the importance of studies in which transporters in the intestinal epithelium are studied.

It is therefore apparent that the broiler has the capacity to regulate absorption to meet its requirements for Ca and P. This implies that digestibility of minerals will be affected by the physiological state of the animal, and it follows logically that digestibility will vary with age. Recent studies at Massey University in New Zealand suggest that this is so (David *et al.*, 2020). Morgan *et al.* (2015) investigated endogenous phytase activity and found that this increased with age, suggesting that phosphorus availability may also change.

The complexity of the absorption mechanisms for Ca and P suggest that it may be difficult to arrive at reliable digestibility figures for feed ingredients. The quantities of available minerals that are absorbed from the intestine are likely to be affected by bird as well as feed factors. In practice the term digestibility is used to refer to the proportion of an ingested nutrient that is absorbed up to a certain point in the digestive tract. This convention will be followed below.

2.5. Dietary factors affecting Ca and P absorption

Interactions between Ca and P and other minerals in the diet affect the availability of minerals to the bird. Furthermore, physical characteristics of the feed may also play a role.

2.5.1. Calcium and phosphorus concentration in diet

The relationship between the level of the Ca and P in the diet and their digestibility (g absorbed/g consumed) not been clearly established. If the bird were able to respond rapidly to a deficient supply of mineral and increase absorption, the digestibility would be higher at lower levels and would decrease as the level in the feed increased. However, if properties of the feed limit the absorption, then digestibility will remain constant.

2.5.1.1.Calcium

Anwar *et al.* (2017) measured both TID and apparent retention of Ca in broilers and found that these measures were strongly correlated. This suggests that the broiler has the ability to extract from the feed what it requires, rather than absorbing Ca indiscriminately and excreting the excess. Retention was on average 5.25% higher than TID. Similarly, van Krimpen *et al.* (2013) calculated digestibility from ileal contents and from excreta collection and found that the latter was higher (43 vs. 39%), indicating absorption from the hindgut and little urinary excretion. This is supported by a range of studies cited by Wideman Jr (1987) showing that, under normal conditions and with Ca concentration in feed 10 g/kg or less, 96-99% of Ca filtered in the glomerulus is reabsorbed from the renal tubule. Only those birds on the high Ca, low P diets excreted significantly more Ca than on the other diets, possibly suggesting that they absorbed Ca to meet their bone growth requirements but were unable to deposit this as bone due to the shortage of absorbed

P (Wideman *et al.*, 1985). Birds on the high Ca, normal P diets did not show an increase in Ca excretion, suggesting that these birds did not absorb more than their requirement of Ca and what they did absorb was retained in bone.

The results of experiments to determine the effect of changing dietary Ca levels on Ca digestibility have been variable. In some, as the concentration of Ca in the diet increased, the % absorption decreased (Al-Masri, 1995; Sebastian *et al.*, 1996; Plumstead *et al.*, 2008; Powell *et al.*, 2011) while in other studies, Ca digestibility remained unchanged (Mutucumarana *et al.*, 2014a) and in some, increasing Ca levels increased digestibility (Walk *et al.*, 2012a; Rousseau *et al.*, 2016). These conflicting results may be a result of the interactions between Ca and other dietary factors, such as PP, nPP and phytase, with few studies using a multiple regression approach to analyse these, particularly with regard to Ca absorption (van Krimpen *et al.*, 2013). Study duration varied, resulting in different periods of adaptation to trial diets. This has been shown to affect digestibility in P studies (Perryman *et al.*, 2017a). Al-Masri (1995) found that while relative Ca retention declined as the concentration of Ca in the feed increased, the mass of Ca retained remained between 191 and 201 mg/b/d, averaged over a 15-day period starting at 14 days of age. Most other studies do not report the absolutevalue of mineral absorbed or retained (e.g. in g) but rather report the mineral absorption relative to its content in the feed (percentage of feed mineral or digestible mineral as a percentage of the total feed).

Calculation of absolute Ca absorption normalised to body mass (g/kg BW per d) was the approach used in a meta-analysis of Ca utilisation in pigs in which a positive, linear effect of tCa in diet on Ca absorption was established (Misiura *et al.*, 2018). In another pig study, Zhang and Adeola (2017) noted that the apparent total tract digestibility of Ca did not change with the level in the diet. However, they did note that although Ca levels in the feed were above the recommended level in the highest Ca feed, intake was below the recommended amount due to restricted feeding. Dacke *et al.* (2015) warned that the Ca physiology of birds differs markedly from that of mammals. Many of its particular characteristics relate to the bird's ability to lay hard-shelled eggs and hence studies of pigs should be applied to poultry with caution.

2.5.1.2.Phosphorus

With a constant Ca/aP ratio of 2.2, the ileal digestibility of P decreased as aP in the diet increased until mineral supply exceeded the recommended levels by 25%. (van Krimpen *et al.*, 2016). This study suggested that birds were able to regulate their supply at sub-optimal aP levels, most likely by means of increased phytate degradation in the digestive tract, which was demonstrated at 21 d. When aP in feed exceeded the recommended amount by 37.5% (9.3 g/kg tP, 13.4 g/kg tCa), digestibility increased to the same level as when the supply was 25% less than recommended (6.4 tP g/kg, 7.7 tCa g/kg), suggesting that the control of absorption at the intestinal level may be overwhelmed at very high mineral levels in feed. Dietary P level and precaecal digestible P showed a changing relationship in a study conducted by Bikker *et al.* (2016), but they were able to fit a broken-stick model to the relationship, with a break-point of 0.58 % dietary P and digestibility of 88% below this value and 20.1% above it. They also maintained a fairly constant Ca/P

ratio in the diet (1.1 to 1.2 tCa/tP) but used lower mineral levels (from 2.26 g/kg to 8.6 g/kg tP with 0.85 g/kg PP). Furthermore, the very low levels of phytate reported in these diets would not provide significant additional amounts of P, thus reinforcing the conclusion of van Krimpen *et al.* (2016) that phytate degradation was responsible for increased P absorption in their study, where PP levels were approximately 2.3 g/kg.

Under normal PTH levels, P excretion is around 60% of filtered P, but Wideman *et al.* (1985) showed that pullets on a normal Ca, low P (1% Ca, 0.4% P) or high Ca, normal P (3.25% Ca, 0.6% P) diet reduced inorganic P excretion when compared with a control. Rodehutscord *et al.* (2012) fed graded levels of tP, from 3.12 to 8.14 g/kg feed, maintaining a tCa/tP ratio of approximately 1.8 and found that digestibility was constant but retention was reduced above 5.2 g/kg tP, with P excretion increasing in a non-linear way above this inclusion level. This, and the results in the previous paragraph that highlight the role of phytate in variations in digestibility, suggest that renal control of P levels in the body may predominate over the control of intestinal absorption.

2.5.2. Interactions between Ca and P in the digestive tract

Ca may form complexes with phytate anions in the broiler gut, making both the bound Ca and the PP unavailable to the bird (Nelson *et al.*, 1968; Angel *et al.*, 2002). Higher concentrations of Ca^{2+} ions and hence higher Ca^{2+}/phytate molar ratios increase phytate complex formation (Selle *et al.*, 2009). Ca-phytate complex formation is accelerated above pH5 (Angel *et al.*, 2002), and, compounding this problem, a higher pH in the gizzard is a consequence of higher Ca levels when Ca is supplemented in the form of limestone (Selle *et al.*, 2009; Walk *et al.*, 2012a). A molar ratio of approximately 5:1 Ca/phytate, or 5:6 Ca/PP, as is found in Ca_5K_2Phytate, binds Ca and PP in a mass ratio of 1.08 g/g (Ca/phytate mass ratio = 0.31). This may suggest that a safety margin Ca inclusion of $1.1 \times PP$ be added to diets (Nelson, 1984 as cited in Selle *et al.*, 2009) However, a range of complex ions may be formed such as $\mathrm{CaPhytateH_5^{5-}}$, $\mathrm{Ca_2PhytateH_4^{4-}}$ and $\mathrm{Ca_3PhytateH_3^{3-}}$ (Crea *et al.*, 2006) and Ca may also form complexes with inorganic dietary phosphates.

The interactions described above give rise to some of the effects of Ca and P on one another's digestibility in the broiler. Thus, tP and PP digestibility is decreased by an increasing level of Ca in the diet when nPP is constant (Plumstead *et al.*, 2008). The ratio between the two minerals is also of significance, as a higher Ca: nPP diet reduces the digestibility of P (van Krimpen *et al.*, 2013; Wilkinson *et al.*, 2014c). Similarly, Amerah *et al.* (2014) found that as the Ca/nPP ratio increased, both P digestibility and bone ash decreased. In a study of P retention, Al-Masri (1995) found that, at nPP levels of approximately 0.4%, P retention declined as the Ca/nPP ratio increased from 1.6 to 4.05. While this could be ascribed to greater binding of phytate by Ca, it could also be the result of inorganic complex formation, since PP would not be expected to contribute much to digestible P at the level of nPP that was fed. Some studies have even shown that higher Ca levels increased phytate P disappearance (Applegate *et al.*, 2003; Tamim *et al.*, 2004). Abdollahi

et al. (2016) studied the effect of different Ca inclusion levels, with or without phytase, on various digestibility measures, including total tract retention of Ca and P. A separate Ca source was also offered to the birds. The tP level was 5.7 g/kg and the nPP level 3 g/kg. Increasing Ca levels in the feed, and hence reducing separate Ca intake, decreased P digestibility where phytase was absent.

Effects of the Ca/nPP ratio on Ca digestibility are variable, with increasing values between 1.5 and 2.5 causing a significant reduction in one study (Anwar *et al.*, 2016b), while Ca digestibility remained unaffected in another in which the ratio of Ca/aP (calculated) ranged from 1.8 to 2.7 (Amerah *et al.*, 2014). Anwar *et al.* (2016a) conducted studies investigating the digestibility of Ca and reported that it is affected by P concentration. Ca ileal digestibility (CaID) in birds aged 21-24 days was investigated, using different types of limestone. In this study birds were fed each limestone diet with 0.12 or 4.48 g/kg nPP and Ca digestibility was improved when P was added. The tCa/nPP ratio in the positive P groups was 2:1 and CaID ranged from 0.54 to 0.61. This may be a result of improved bone mineralisation and hence greater Ca absorption. A study in which Ca digestibility was measured at 8, 16, 24, 32, 40, 48, 60, 72 and 96h after the test diets were first fed, demonstrated that measures of aCa change as birds adapt to different diets (Angel *et al.*, 2013).

The picture is further complicated by the increased uptake of Ca when P is extremely low, most likely due to the unfulfilled requirements of bone mineralisation and increased Ca excretion. This was observed when high Ca levels (10.8 g/kg) and low nPP levels (2.7 g/kg) were fed (Wilkinson *et al.*, 2014c). Similarly, Powell *et al.* (2011) showed the highest utilisation of Ca at 0.2% nPP and 0.67% Ca, while the addition of phytase produced a similar pattern but higher peak utilisation. It may be difficult to disentangle the interactions between Ca and P in the GIT and the homeostatic mechanisms that influence absorption.

2.5.3. Calcium and phosphorus sources

Ileal digestibility has been proposed as a suitable measure of mineral availability (Rodehutscord and WPSA, 2013) and several studies have evaluated this metric for Ca and P in feedstuffs. However, a great deal of variation in Ca and P availabilities have been observed in plant, animal and mineral sources (Blair *et al.*, 1965).

2.5.3.1.Plant sources

Much of the P present in plant material is in the form of phytate and little of this is available to the growing chick (Akter *et al.*, 2016). Ca in plant feedstuffs is also too low to supply the broiler's needs (González-Vega and Stein, 2014). The Ca and P levels in some common ingredients used in broiler feeds are shown in Table 2.4.

A collaborative study between 17 research stations evaluated the ileal P digestibility of samples of the same soyabean meal (Rodehutscord *et al.*, 2017). The protocol was not completely standardised and, notably, the treatment of birds before the assay was not prescribed. The digestibility, measured using the regression

method, varied from 19 to 51% of total P, with all but the lowest of these showing a good fit when the linear regression analysis was carried out. The disappearance of IP_6 was analysed at four stations and was found to correspond to the digestibility measurements. The authors surmised that differences in the pre-assay treatment of birds, both in terms of feed (adaptation to lower levels of Ca and P, coccidiostats, compensatory growth, phytase) and housing (floor pens or cages) or genetics (bird strains) may have contributed to the variability in phytate hydrolysis, resulting from variability in phytase, whether endogenous (epithelial secretions) or from the gut microbiota.

Table 2.4 Minerals in plant feedstuffs

Sources: Sauvant *et al.* (2004); Nahm (2007, after Cromwell (1980)); González-Vega and Stein (2014)

Feedstuff	Total P (%)	PP (% of tP)	PP %	nPP (%)	Ca (%)
Maize	0.26	75	0.20	0.06	0.04
Grain sorghum	0.28	70	0.20	0.08	0.03
Barley	0.34	55	0.19	0.15	0.07
Wheat	0.32	65	0.21	0.11	0.07
Oats	0.32	55	0.18	0.14	0.11
Soyabean meal	0.62	60	0.37	0.25	0.03
Cottonseed (full fat)	0.63	80	0.50	0.13	0.16
Canola meal	1.14	60	0.68	0.46	0.83
Wheat bran	0.99	80	0.79	0.20	0.14
Rice bran	1.61	85	1.37	0.24	0.08

Evaluation of the AID (Ca and P) of diets containing different cereals (corn, wheat, oats and barley) showed that the digestibility of PP varied from 3 to 42% and was affected by the type of grain and its interaction with the level of P in the diet (Leytem *et al.*, 2008a). The ileal Ca and P digestibilities between the diets with different grains, however, did not differ significantly except between the oats and maize diets, with the latter having the lowest digestibility values (46% for Ca and 57% for P). Wheat was found to have an average P digestibility of 64%. In contrast, the TIDP of wheat, determined by the regression method, was found to be 46.4% in another study and the TIDP of soybean meal was much higher, at 79.8% (Mutucumarana *et al.*, 2014c).

Different approaches to diet formulation in digestibility studies results in very different TID measurements (Mutucumarana *et al.*, 2015a). Unsurprisingly, Ca/P ratios in the diets were found to play a role (Liu *et al.*, 2013), but the influence of protein levels in the diet is less clear (Liu and Adeola, 2016). For example, without an additional source of protein and using graded amounts of the test ingredient, the diets formulated to measure the P digestibility of maize differ considerably from those of soybean meal in their protein level and hence BWG over the experimental period, and this is likely to make comparisons between digestibility

coefficients derived under these conditions difficult (Mutucumarana *et al.*, 2015a). When the test ingredient is the only contributor of Ca and P and the direct method is used, diets inevitably contain different, and sometimes extremely deficient, levels of mineral and this will influence the results: hence Rutherfurd *et al.* (2002), who used this method, measured far greater phytate hydrolysis and hence P digestibility compared with a study in which more balanced diets were used (Leske and Coon, 1999). The interaction between nPP level and phytate hydrolysis would be expected and must affect the results of the assay (Dilelis *et al.*, 2021a).

2.5.3.2.Animal sources

Poultry by-product meal (PBPM), meat and bone meal (MBM) and fish meal are rich sources of minerals (see Table 2.5), and the availability of these minerals is generally considered to be high. Indeed, the standardised ileal digestibility (SID) of P in PBPM was recently found to be 93-96% (Dilelis *et al.*, 2021b). That of different MBM samples ranged from 49% to 69% in this study which was comparable with an earlier one in which the range was 44% to 62% (Mutucumarana and Ravindran, 2016). This variability probably reflects the variable composition of the feedstuff. Fish meal had a P digestibility of 52% when the direct method was applied and 26% with the regression method, reflecting the challenges in determining a single digestibility measure. Measured by the direct method, Ca digestibility was only 29% in PBPM and 24% in fish meal (Anwar *et al.*, 2018).

Table 2.5 Calcium and phosphorus concentration in feed ingredients of animal origin

Source: González-Vega and Stein (2014, after National Research Council (2012))

Source	Ca (%)	P (%)
Fish meal	4.28	2.93
Meat and bone meal	10.94	5.26
Poultry by-product meal	4.54	2.51
Whey powder (dried)	0.62	0.69

2.5.3.3.Mineral sources

Mineral supplements are usually added to broiler feeds to achieve the dietary levels necessary for growth. Table 2.6 demonstrates that both composition and digestibility vary between studies.

Walk *et al.* (2021) ascribed the challenges in changing to digestible Ca feed formulation in poultry to the variability in digestibility values. This arises from properties of the mineral sources themselves (purity, particle size, solubility), assay methodologies (e.g. adaptation period), diet composition (e.g. phytate content, Ca/P ratio) and bird characteristics (e.g. age, mineral status).

Table 2.6 Mineral sources and mineral digestibilities

Mineral source	Mineral concentration (%)		Ileal digestibility (%)		Age (d)	Source
	Ca	P	Ca	P		
Defluorinated phosphate (DFP)	30.6	18.2				feedtables.com
	32.3	19.0		31.5	28	Bikker *et al.* (2016)
Dicalcium phosphate	27.2	20.4				feedtables.com
				29	21/35	Shastak *et al.* (2012b)
	24.8	18.8		69.3	29	Trairatapiwan *et al.* (2018)
	28.2	18.4		59.0	28	Bikker *et al.* (2016)
	27.5	19.0	69.8	78.6	24	van Harn *et al.* (2017)
	17.8	19.5	28.0		24	Anwar *et al.* (2018)
	25.3		67.1		27	Zhang and Adeola (2018)
	26.0		36.0		24	David *et al.* (2019)
Limestone	35.0		53			Walk *et al.* (2021)
	36.4		63.7		27	Zhang and Adeola (2018)
	40.0		55.0		24	David *et al.* (2019)
Monocalcium phosphate	16.7	22.4				feedtables.com
	14.5	22.0		64.6	29	Trairatapiwan *et al.* (2018)
	18.0	21.8		78.3	28	Bikker *et al.* (2016)
	18.2	22.0	69.7	81.7	24	van Harn *et al.* (2017)
	15.5	22.1	33.0		24	Anwar *et al.* (2018)
	17.4		48.0		24	David *et al.* (2019)
Monodicalcium phosphate	17.3	22.2				feedtables.com
	15.5	21.1		60.2	29	Trairatapiwan *et al.* (2018)
	21.7	20.7		70.7	28	Bikker *et al.* (2016)
Monosodium phosphate anhydrous (MSP_a)	0.00	25.5		67	21	Shastak *et al.* (2012b)
				70	35	
Tricalcium phosphate	34.2	17.7				González-Vega and Stein (2014)

2.5.4. Particle size of mineral source and feed

Findings on the effect of Ca source particle size on Ca availability and on poultry performance seem contradictory (Coon *et al.*, 2002). While smaller particle size has been shown to improve growth when diets are low in Ca, suggesting greater Ca bioavailability (Hillman *et al.*, 1976; Guinotte *et al.*, 1991; Majeed *et al.*, 2020), other studies have shown improved growth and bone mineralisation with larger particle size versus finely ground limestone (McNaughton, 1981; Koreleski and Swiatkiewicz, 2005). Increased Ca digestibility and retention with larger particle size in both limestone and oyster shell Ca sources was confirmed in a series of experiments (Anwar *et al.*, 2016b; Anwar *et al.*, 2017).

This improvement may be explained by changes in gizzard pH and P availability (Naderinejad *et al.*, 2016; Kim *et al.*, 2018). Furthermore, lower solubility may contribute to longer retention time in the gizzard (Zhang and Coon, 1997). These authors also noted decreased *in vivo* solubility of limestone as particle size decreased, in contrast to the relationship for *in vitro* solubility. They suggested that with larger particle size

and longer retention in the gizzard at a lower pH, greater dissociation of calcium carbonate might occur, producing more available Ca for absorption (Manangi and Coon, 2007). The interaction between Ca and P in the digestive tract may also be affected by the particle size of the Ca source: the higher solubility of smaller particles may limit phytate hydrolysis and hence reduce P availability, even in the presence of exogenous phytase (Kim *et al.*, 2018). The use of a highly soluble source of Ca as a possible method of reducing Ca concentration in the diet was investigated (Bradbury *et al.*, 2017; Bradbury *et al.*, 2018). Contrary to expectations, this decreased feed intake and BW gain in broilers in the starter phase, possibly through the mechanisms described above. McNaughton *et al.* (1974) suggested that medium particle size (0.4 to 0.6 mm) offers the greatest weight gains and level of tibia ash.

Effects on digestibility may be seen with increased particle size of other feed ingredients: more finely ground maize in the diets of finisher broilers has been shown to decrease the digestibility of Ca in some experiments (Kilburn and Edwards, 2001; Mtei *et al.*, 2019a) but not in others (Amerah and Ravindran, 2009) and the P in coarser soybean meal is more efficiently utilised (Kilburn and Edwards Jr, 2004).

2.5.5. Choice feeding

Broilers have demonstrated their ability to learn to use a separate source of Ca to supplement their diets when feeds contain low levels (Wilkinson *et al.*, 2011; Abdollahi *et al.*, 2015).

Joshua and Mueller (1979) found that broilers housed in groups developed a Ca appetite which allowed them to supplement a Ca deficient diet (1.2 g/kg) with $CaCo_3$ (0.25-0.6 mm particle size) to provide themselves with adequate levels of Ca (approx. 0.55 g Ca/b/d) for tibia ash growth (lower in deficient diet but not significantly so). This behaviour appeared to be learned as the effect was inhibited when birds were housed individually. In a more recent study, the delay in consuming additional Ca in the first week was attributed to a learning effect but may also have been due to a lower requirement (Abdollahi *et al.*, 2016). Birds on low Ca diets (1.3 and 4.3 g/kg) consumed additional Ca of approximately 0.2 g/b/d in the first week, 0.8 g Ca/b/d in the second week and 2 g Ca/b/d in the third week. tCa intake was highest with 4.3 g/kg feed at 0.31 g Ca/b/d from 1-7 d, 0.97 g Ca/b/d from 8-14 d and 2.2 g Ca/b/d from 15-21 d. At lower levels, birds consume and retain less Ca, but P digestibility is maximised. Wilkinson *et al.* (2014b) arrived at a similar conclusion. On the lowest Ca level (3.5 g/kg) in their study, chicks consumed 0.45 g Ca/day, whereas on dietary levels of 5, 7.5 and 10g/kg Ca, birds ate to provide themselves with approximate 0.6 g Ca/day regardless of basal diet Ca concentration (1-21 days of age).

The consumption of supplementary Ca is rapidly reduced by the injection of PTH or intravenous administration of Ca (Lobaugh *et al.*, 1981). Birds responded within 150 minutes, which suggests that the appetite for Ca plays an important role in homeostasis when there is opportunity for the consumption of a separate Ca source. Separate Ca does not seem to interact with phytate P in the same way dietary Ca does, and Abdollahi *et al.* (2016) attributed this to different times of consumption. However, particle size could also be a contributing factor. The reduced P digestibility associated with the higher levels of Ca in the

complete feed was not apparent in retention of P, except at the two higher levels of Ca and without phytase. Possibly the lower absorption was mitigated to some extent by increased renal reabsorption of P. The increased Ca intake, greater retention of Ca and higher digestibility of P contribute to the impression that separate Ca feeding has the potential to improve mineral nutrition. Furthermore, reducing dietary Ca from 12.3 g/kg to 3.5 g/kg was found to increase ileal digestibility of DM, N and all amino acids except tyrosine by approximately 8%, in spite of the availability of separate Ca and hence unimpaired Ca intake (Wilkinson *et al.*, 2014b).

2.5.6. Prior feeding regime

It has been proposed that broilers may exhibit improved efficiency of Ca and P utilisation and hence reduced P excretion if they are subjected to reduced levels of P in their diet during the early stages of growth (Létourneau-Montminy *et al.*, 2008).

Birds fed a low Ca and P diet in the starter phase exhibit compensatory body weight and bone mineralisation gains when fed a grower diet with normal mineral levels (Yan *et al.*, 2005b; Létourneau-Montminy *et al.*, 2008; Rousseau *et al.*, 2016; Valable *et al.*, 2020). However, one study demonstrated that bone mineralisation was lower at 32 days in birds fed a low Ca and P starter (Yan *et al.*, 2005b). Overall feed consumption was not affected by the treatments in most studies, so that the birds fed the control diet in both starter and grower phases exhibited the highest consumption of Ca and P (Yan *et al.*, 2005b; Létourneau-Montminy *et al.*, 2008; Valable *et al.*, 2020). The desired effect of reducing P consumption seemed to have been achieved through improved efficiency of Ca and P utilisation in birds deprived of these minerals in the starter phase. Not only were ileal digestibilities higher in the deficient diet birds but more PP was hydrolysed when this was measured at the end of the period of deprivation (Yan *et al.*, 2005b). Only in one study were improvements in mineral utilisation efficiency shown to persist beyond the end of the period of deprivation (Rousseau *et al.*, 2016).

The accelerated mineralisation of the skeleton after a period of deprivation demonstrated in these studies suggests that broilers are striving to achieve a certain, predetermined trajectory of bone growth and that they will retain a greater proportion of the mineral in the diet if it is necessary to do so.

2.5.7. Phytase

Microbial phytase is added to broiler diets in order to improve the availability of PP. Phytate hydrolysis produces inorganic phosphates that may be absorbed from the GIT. The benefits of phytase inclusion in broiler diets are well established and this additive is widely used in the poultry industry. Plant phytase is present in certain feed ingredients and endogenous phytases produced by the GIT microbiome and intestinal mucosa break down phytate and lower inositol phosphates (e.g. IP5) under favourable conditions (Applegate *et al.*, 2003). Kemme *et al.* (2006) found that breakdown in the small intestine was particularly

significant for IP3 and IP2: they proposed that exogenous phytases would have been degraded by this stage of digestion and that intestinal phosphatases were responsible for this phenomenon.

2.5.7.1.Measures of phytase activity

In many studies, performance metrics are compared between birds with and without added exogenous phytase. For example, a meta-analysis of 15 experiments demonstrated improvements in growth and tibia ash when diets were supplemented with phytase (Létourneau-Montminy *et al.*, 2010). P equivalency is often used in feed matrices to indicate the increase in nPP which results from a given dose of phytase. This is usually measured indirectly: a low P diet with added phytase is fed and bird performance (BWG or tibia ash) is compared with that of birds fed graded levels of nPP (Adedokun *et al.*, 2004; Han *et al.*, 2009; Li *et al.*, 2015b).

The response to phytase is affected by dietary Ca and nPP levels: when P is deficient or when higher levels of Ca are provided in the diet, birds show a more marked response to phytase (Driver *et al.*, 2005). The level of PP in the diet will also affect the quantity of nPP released by phytase and this will affect the results of P equivalency calculations (Manangi and Coon, 2008).

Phytase efficacy changes with age: the response to added phytase is greater in younger birds (Li *et al.*, 2018; Babatunde *et al.*, 2019). It was suggested that this is due to poorer digestion of P by younger birds, which have less well-developed digestive tracts and hence produce less endogenous phytase enzyme. As a result, more phytate is bound by Ca and is unavailable to the bird.

The interactions described above call into question the usefulness of phytase equivalencies for nPP, since these are based on comparison with standardised diets with particular Ca/P ratios and nutrient contents. Different amounts of nPP will be released when diets deviate from these (Qian *et al.*, 1997). Alternatively, P equivalency values for phytase may be obtained by measuring ileal digestible P and analysing the regression of this variable on phytase intake, but this more direct measure is not common (Adeola and Walk, 2013; Dersjant-Li and Kwakernaak, 2019). Furthermore, this measure was also influenced by Ca levels in the diet: when these were 5 or 6g/kg from highly soluble calcium (HSC), P equivalencies of 1.701 or 1.561g respectively were calculated for 1000 FTU/kg (Adeola and Walk, 2013). Driver *et al.* (2005) argued forcibly that that phytase equivalency varies as Ca and P levels vary (see Section 2.5.7.2 below) and that maximum bird performance may not represent the greatest phytase response.

The laboratory method for determining phytase efficacy, described by Engelen *et al.* (1994) does not provide a useful measure of availability *in vivo*. Phytase is incubated with sodium phytate and the phosphate that is liberated is bound to a reagent and assessed using colorimetry. One phytase unit (FTU) is defined as "the amount of enzyme that liberates 1 µmol of inorganic orthophosphate/min under test conditions (pH 5.5; temperature 37°C; and substrate concentration, sodium phytate [$C_6H_6Na_{12}O_{24}P_6$.$10H_2O$] at 0.0051 mol/L)". Commercial phytases vary in their pH optima and assays must be adapted accordingly. Phytase interactions with Ca, P and other feed characteristics must also be accounted for when feeds are formulated.

2.5.7.2.Phytase interactions with dietary calcium and phosphorus

Several studies have shown that Ca digestibility is improved by the addition of phytase to the diet (Adeola and Walk, 2013; Kaczmarek *et al.*, 2016; Kim *et al.*, 2018; Moss *et al.*, 2018) but this is not always the case (Amerah *et al.*, 2014; Davin *et al.*, 2020). More Ca may be absorbed when phytate is degraded and the formation of Ca-phytate complexes is reduced (Qian *et al.*, 1997). Limestone may reduce exogenous phytase efficacy if the pH in the crop is increased (Selle and Ravindran, 2007). Furthermore, intestinal phytase activity has been shown to be reduced when the Ca level in the diet is increased (Applegate *et al.*, 2003; Tamim *et al.*, 2004). An increase in the ratio between Ca and P resulted in poorer bird performance than when narrower ratios were fed, with supplementary phytase in both diets (Qian *et al.*, 1997). In contrast to this, Driver *et al.* (2005) demonstrated that higher Ca levels improved the efficacy of phytase. It was pointed out that studies may focus on absolute performance rather than the response to phytase, which is greater when Ca is high and nPP is low. Furthermore, the absolute levels of minerals in the diet are significant as well as the ratio between them.

The effect of nPP levels on phytate efficacy has been demonstrated (Driver *et al.*, 2005; Olukosi and Fru-Nji, 2014; Zeller *et al.*, 2015b): birds fed a lower nPP diet demonstrated a greater improvement in P digestibility with added phytase when compared with than those fed a diet with a higher nPP inclusion rate. Zeller *et al.* (2015a) examined the disappearance of phytate in a basal diet and in diets with added phytase and varying amounts of added MCP. The reduction in released P_i from phytate degradation as MCP levels increased may have been a result of changes in the proportions of lower inositol esters: hence, the added nPP may be affecting the release of P through its effect on endogenous phytase production as well as on the action of exogenous phytase. Furthermore, superdosing with phytase led to more complete breakdown of IP6 to IP3 and IP2 while with 500 FTU/kg IP5 was the predominant form. Mineral P inhibits phosphatase breakdown of IP6 due to product inhibition (Zeller *et al.*, 2015b).

Amerah *et al.* (2014) evaluated the interactions between bacterial phytase and the Ca/aP ratio by assessing the effects on ileal phytate degradation and mineral and amino acid digestibility. The formulated Ca/P ratios were 1.43, 2.14, 2.86 and 3.57. These were achieved by maintaining a constant aP level (0.28% as formulated) and varying the amount of Ca with the addition of limestone. Analysis showed higher Ca and PP levels than expected. Furthermore, it was not clear what assumptions were made regarding the availability of PP but it would seem that approximately 30% digestibility was assumed, based on the diet composition described. Since nPP was 1.9g/kg (analysed), Ca/nPP ratios ranged from 2.68 to 6.84. The results showed a 40-50% phytate degradation in the diets not supplemented with phytase, which might be expected at such low nPP levels as a result of increased endogenous phytase activity. Phytate degradation was increased with the addition of phytase, as was P digestibility but not Ca digestibility. Increasing the Ca/aP ratio led to a linear decrease in P digestibility but did not affect Ca digestibility. The only interaction noted between Ca/aP ratio and phytase was on bone ash, which was decreased at high Ca/aP ratios without phytase but increased at high Ca/aP ratios when phytase was added. This is in contrast to a study in which

a three-way interaction between Ca, nPP and phytase affected Ca and P digestibility. These were reduced in high Ca/low nPP diets with added phytase (Akter *et al.*, 2017). These studies are representative of the challenges that researchers face when analysing and interpreting Ca/P/phytase studies: interactions are common and nutrient digestibilities may be affected by physiological factors as well as the chemical interactions between dietary components.

While Adeola and Walk (2013) found no changes in ileal digestibility of DM, energy and N with different levels of P, Ca and phytase, other studies have found that phytase improves amino acid digestibility (Amerah *et al.*, 2014; Li *et al.*, 2015a; Sommerfeld *et al.*, 2018a; Babatunde *et al.*, 2022).

2.5.8. Vitamin D

Vitamin D_3 (calcitriol) is involved in Ca and P homeostasis and vitamin D receptors (VDR) are found in a range of body tissues, including the intestinal mucosa, renal epithelia, parathyroid gland and bones (Proszkowiec-Weglarz and Angel, 2013). Plasma Ca and P are controlled through the mechanisms of intestinal absorption, bone resorption and renal tubular reabsorption. Vitamin D plays a critical role in all of these processes.

Vitamin D_3 influences the active, transcellular absorption of Ca in the intestine by increasing the calcium-binding protein, calbindin (Adedokun and Adeola, 2013). The passive, paracellular pathway of Ca absorption may also be facilitated by vitamin D_3, through its effect on the claudins of the tight junctions between enterocytes (Fujita *et al.*, 2008). Sodium-dependent phosphorus co-transporters (NaP-IIb and PiT-1) that allow the active transport of P across the intestinal mucosa are also influenced by vitamin D_3 (Shao *et al.*, 2019).

Baker *et al.* (1998) found that chicks fed diets deficient in P showed positive responses in body-weight gain and tibia ash to increasing levels of vitamin D_3. Birds on diets adequate in P, even if low in Ca, did not derive any benefit from vitamin D_3 levels above 5 µg/kg. Similarly, Bar *et al.* (2003a) showed that 25-Hydroxycholecalciferol, a metabolite of vitamin D_3, improved BWG and tibia ash when P was deficient but did not mitigate the effects of Ca restriction.

2.5.9. Electrolyte balance

The balance of cations and anions in the diet is another approach to quantifying the chemical interactions (Gorman and Balnave, 1994). While these authors found that supplementation of metabolisable anions and cations may affect broiler performance, they did not find a useful balance equation to capture this information. The dietary electrolyte balance (DEB) may affect the availability of Ca and P and hence affect mineral availability, particularly the mineralisation of bones (Ravindran *et al.*, 2008; Araujo *et al.*, 2012).

2.5.10. Chelating agents

Various chelating agents in addition to Ca, including cations such as Cu, Fe, Mg, K and, in particular, Zn may reduce the availability of P through the formation of complexes with phytate (Maenz *et al.*, 1999; Banks *et al.*, 2004). Phytate may also form complexes with proteins and amino acids, affecting their availability in broiler diets (Ravindran *et al.*, 2000).

It is apparent that a complex interaction of bird and dietary factors affect the amount of Ca and P that are available to the bird from its gut contents.

2.6. Calcium and phosphorus in the broiler body

Broilers require Ca and P for tissue growth. A certain amount may also be required for maintenance and obligatory excretion in the urine (see sections 2.7 and 2.8). The minerals must be delivered through the wall of the GIT into the blood plasma at a rate sufficient to provide for these body functions. How the bird deals with a deficiency or excess of minerals must also be understood if modelling is to succeed.

Various approaches have been used to model Ca and P in monogastric animals. Kebreab *et al.* (2009) produced a dynamic and deterministic model for Ca and P which based rates of utilisation of these minerals for bone deposition on available levels in blood plasma. They also modelled Ca mobilisation from bone, as this dynamic relationship is critical in the daily cycle of egg-shell development. This "push and pull" from the plasma is appropriate in a layer model, but a broiler model, in which growth is the critical output, may require a calculation of desired bone growth to provide a "pull" for required Ca and P, with plasma levels assumed to be constant (Létourneau-Montminy *et al.*, 2015). This may also have a basis in the physiology of Ca homeostasis, with the plasma pool of Ca clearing into the skeleton every few minutes (Shaw *et al.*, 1989). Symeou *et al.* (2014) and Létourneau-Montminy *et al.* (2015) used the increases in Ca and P in body tissues during the growth of pigs and the requirements for maintenance as an estimate of the true requirement for these minerals.

2.6.1. Ca and P growth in the whole broiler body

If the true requirements for Ca and P in the growing broiler are to be modelled, it is necessary to know how the quantities of each of these minerals in the broiler body change over time, and to be able to relate this to some measure of bird body size.

It is reasonable to expect that the increase in Ca and P in the animal body will have a predictable relationship with BWG, since these minerals have particular roles in the tissues that make up the bird body in which they occur and might be expected to form a certain proportion of each of these (WPSA, 1985). However, modelling studies suggest that body protein may be a better reference for growth, since excess lipid gain may be due to an imbalance between energy and protein or between amino acids. This would lead to a change in the proportion of ash, and hence its component minerals, in the whole body, as minerals are present in lipid tissue only in small quantities. It has been shown that protein growth follows a predictable

growth curve, depending on the genotype of the broiler under consideration (Emmans, 1995; Gous *et al.*, 1999). Furthermore, it is reasonable to assume that bone growth is consistently related to the growth of the muscles attached to it and the mass of protein in the bone itself under ideal conditions. This does not exclude the possibility that bone mineralisation may be compromised under conditions of deficiency and this relationship may change. Few studies of broilers have measured Ca and P growth at the same time as protein to allow the relationship between these variables to be analysed.

The trajectory of the increase in bone mass is perhaps less likely to be influenced by short-term changes in lipid mass. It has been shown that birds strive to regain their ideal protein to lipid ratio by adjusting their consumption of higher or lower protein feeds, where available (Gous and Fisher, 2011). However, it is not known if similar mechanisms exist to restore the relationship between minerals and protein.

Few studies have measured the growth of Ca and P in the broiler by analysis of total body composition. Serial slaughter and analysis of Ca and P allows the direct measurement of how much of these minerals is deposited in the body. The dearth of studies may be due to the work involved (De Groote and Huyghebaert, 1997) and the practical challenges of obtaining a homogeneous, representative sample of body material for analysis (Shastak and Rodehutscord, 2013). However, this approach would provide the best indication of the ability of birds to utilise nutrients in the diet under different feeding regimes and would also indicate the potential growth of broilers under ideal conditions. If protein growth were also analysed, the relationship between Ca and P and protein could be assessed.

An early study of the availability of Ca from different sources reported eviscerated body ash between 3.09 and 3.54% BW at 4 weeks of age (135 to 199 g BW) when Ca and P levels in the diet were 0.9% and 0.7% respectively. The Ca proportion of ash was between 25.73 and 27.33% (0.8 to 0.97 % of body weight) (Blair *et al.*, 1965). This is somewhat higher than the broiler body composition data cited in WPSA (1985), which proposed that body Ca declined from 0.68% at 3 weeks of age to 0.66% at 8 weeks and P from 0.49% to 0.47 % in the same period. When carcasses are analysed at a single age, this cannot provide information regarding the growth curves of Ca and P or their relationship to protein or body weight over time.

In the 1980s, Hurwitz and Plavnik (1986) carried out a study of the mineral content of the growing broiler. This is summarised in Table 2.7.

The change in the Ca/P ratio was ascribed to the changing proportions of P in soft tissue and bone as bone is mineralised in early life. The body protein (BP) content of the carcasses in the same experiment was reported elsewhere (Plavnik and Hurwitz, 1983).

Table 2.7 Change in proportions of Ca and P in broiler carcass

Sources: Hurwitz and Plavnik (1986); Plavnik and Hurwitz (1983)

		g/kg BW defeathered			
Age	BW	Ca	P	BPr	Ca:P
1	39	3.19	3.65		0.87
4	79	4.33	4.34		1.00
7	118	5.55	5.33	158	1.04
14	283	6.87	5.87	159	1.17
21	530	5.87	5.23	161	1.12
28	842	6.01	5.51	162	1.09
35	1203	6.81	6.46	163	1.06
42	1588	8.16	7.05	165	1.16
49	1999	7.43	5.98	166	1.24
56	2203	9.44	6.09	167	1.55

Using the same data, the relationship between the proportion of Ca in the empty, feather-free carcass and age (time) was estimated (Hurwitz *et al.*, 1987b). The equation of best fit was for the fraction of carcass calcium (Ca_c) was

$$Ca_c = 2.72 \times 10^{-3} + \frac{t}{(1.01 \times 10^8 + 206t)} \qquad (Eq.\,2.8)$$

where time (t) is measured in seconds.

Only one study was found in which body Ca, P and protein content from serial slaughter was reported in the same paper (Caldas *et al.*, 2019). Birds were fed a standard corn/soybean meal ration as mash. Crude protein (CP), Ca and nPP were 21.5%, 0.9% and 0.45% respectively in the starter and declined to 18.2%, 0.76% and 0.38% in the post-finisher ration. Only the Gompertz function parameters for these three components were reported, and not the treatment means. However, it is possible to compare these functions to ascertain if allometry between the components exists and to estimate the parameters of this allometry.

The form of the Gompertz function that was used was to calculate the mass of component Y at age t was

$$Y = a * e^{-e^{-b*(t-c)}} \qquad (Eq\ 2.9)$$

where a = asymptote (mature mass of component y), b = rate of maturing and c = inflection point (age at which growth is a maximum).

The birds in this experiment were deprived of feed for 6 hours prior to slaughter. The carcasses, including feathers and remaining visceral contents, were homogenised and analysed.

2.6.2. Ca and P growth in bones

When considering changes in Ca and P content in the broiler, the measurement of the absolute quantities of these minerals is of importance.

Most of the skull and furcula of the bird are formed intramembranously (Franz-Odendaal, 2011), but the rest of the skeleton develops through endochondral ossification, in which hyaline cartilage is laid down first and then replaced by mineralised tissue (Allen and Burr, 2019). This takes place from the middle (diaphysis) outwards to the ends (epiphyses) of long bones. During this process the epiphyseal plates develop, and these are responsible for the longitudinal growth of the bone, which varies considerably among the bones within a growing individual (Allen and Burr, 2019). The process of bone modelling includes the formation of mineralised bone by osteoblasts and the resorption by osteoclasts, occurring in a coordinated manner on the bone surface to both increase bone mass and shape bones.

Most of the Ca and a large proportion of the P in the broiler body resides in the mineralised bone. While the mass of some bones (femur and tibia) has been shown to change in proportion to BW as birds grow (Applegate and Lilburn, 2002), it is unlikely that the composition remains unchanged. The protein in bone is largely collagen (98 to 99 %) (Araujo *et al.*, 2012). This fibrous protein provides the matrix within which mineralisation of bone occurs. The non-collagenous proteins play a role in the regulation of metabolism, including the calcification of bone (Rath *et al.*, 2000). A distinction must be drawn between the mass (protein and mineral) and size (length and volume) of bones, since these change at different rates relative to BW (Applegate and Lilburn, 2002).

One of the principal reasons that researchers have studied Ca and P is to improve skeletal function in broilers. Measurement of bone mineralisation (proportion of ash) is commonly used to assess skeletal health (e.g. Atteh *et al.*, 1989; Shafey *et al.*, 1990; Angel *et al.*, 2005b; Coon *et al.*, 2007; Araujo *et al.*, 2012; Gautier *et al.*, 2018).

2.6.2.1.Proportion of ash in bone

Published studies reveal a wide variation in the proportions of ash in bone, with numerous different techniques used for dissection and preparation of bones (Field, 2000). The ash content of tibiae with cartilage caps is approximately 120 g/kg less than that of tibia shafts alone (Fritz and Halloran, 1943). Wise (1970) found that when broilers were fed a commercial starter diet from hatch to 6 weeks, ash as a proportion of dried tibia remained at a relatively constant 585 g/kg. The bones were not defatted as became common practice in subsequent studies and which would increase the proportion of ash further. In contrast, in another early study in which whole carcass bone was analysed, it was found to contain approximately 177 g/kg protein, 570 g/kg moisture, 162 g/kg lipid and 87 g/kg ash in 56-day old broilers (Broadbent *et al.*, 1981). Presumably the remaining 5 g/kg is carbohydrates such as those required for the formation of advanced glycation end products (Rath *et al.*, 2000). This analysis is equivalent to 658 g/kg protein and 323 g/kg ash in dry, fat-free (DFF) bone. In this study, carcasses were dissected into meat, skin and bone, and bones were not boiled. This would presumably leave cartilage with bone, potentially reducing the proportion of ash.

Ash in dry, defatted bone without attached tissue has become a standard measure in recent years and the tibia is the most commonly measured bone (see Chapter 4) . Ash content in excess of 640 g/kg has been reported at 21 d (Ravindran *et al.*, 1995) and at 14 and 28 d (Barshan *et al.*, 2019) while others found values to lie between 371 and 415 g/kg at 17 days old (Shafey *et al.*, 1990) and 290 to 510 g/kg at 22 days of age (Persia and Saylor, 2006) with varying levels of Ca and available P in the diet. It seems that ash as a proportion of bone mass varies markedly with diet.

It is uncommon to dissect and analyse the entire skeleton (Angel, 2007). Most of those cited above analyse only tibia ash and this only at the end of the experiment, at a single age. Serial measurements of tibia ash were collected in one study (Skinner and Waldroup, 1995). Although patterns in ash content may be discerned by comparing results from different studies, it is rarely possible to derive age response curves from these data. Only a few studies have measured mineral content at intervals throughout the growing period and they suggest an initial increase in the proportion of ash in the skeleton followed by a levelling off (see Figure 2.3). A quadratic function appears to provide a reasonable fit to these data.

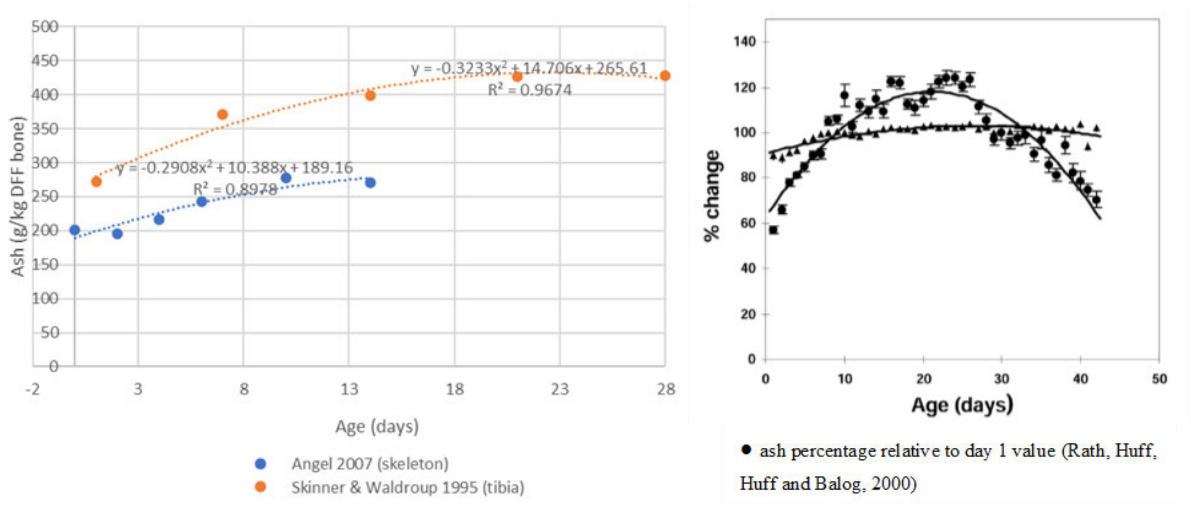

Figure 2.3 Change in bone ash content with age in broilers reared on standard feeds

Applegate and Lilburn (2002) analysed the femurs and tibias of broilers at 1, 8, 15, 21, 28, 35, and 43 d of age. Apart from changing diets for 4 phases, no further details of the feeds were provided. The ash content of dry, defatted tibia and femur samples reached a plateau at 21 d but the tibial diaphysis increased in ash content relative to the femoral diaphysis. This may have been confirmation of the greater sensitivity of the femur to dietary changes that was observed in earlier studies (Itoh and Hatano, 1964; Dilworth and Day, 1965; Moran and Todd, 1994).

Toe ash and foot ash are alternative measures of bone mineralisation (Ravindran *et al.*, 1995; Garcia and Dale, 2006). While some studies have shown that this measure is as sensitive to changes in dietary P as tibia ash (Yan *et al.*, 2005a; Garcia and Dale, 2006), ash may appear to be proportionally lower in the foot (see Figure 2.4). However, this may be due to challenges associated with cleaning the bones of the foot of soft tissue (Shastak *et al.*, 2012c).

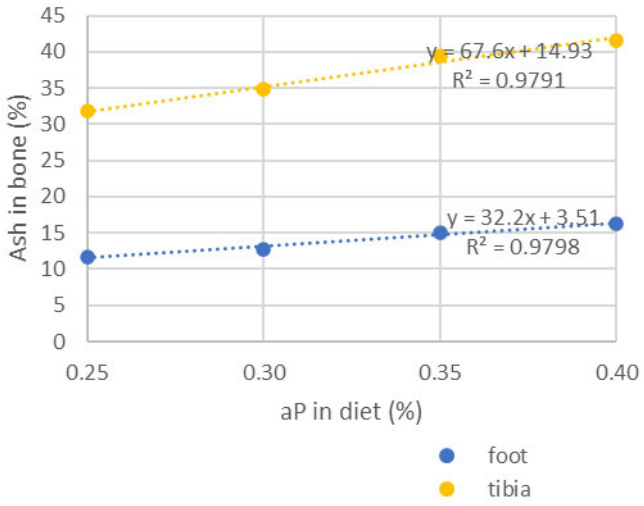

Figure 2.4 Foot and tibia ash at 14 d in response to aP

Source: Garcia and Dale (2006)

Cox and Balloun (1971) suggested that the proportion of total skeleton bone ash in body weight could be calculated from femur ash weight in laying hens using the regression equation $y=5.581+12.66x$ where x is the ash weight of a single femur bone in g/kg BW. Where the sampled bone mass is recorded, absolute bone ash values for the tibia can be calculated from the relative masses of ash that are usually reported, but a reliable method for relating this to whole skeleton ash in broilers has not been established.

2.6.2.2.Effects of diet on ash

Powell *et al.* (2011) found that increasing the Ca content of diets with 2 g/kg nPP resulted in a linear decrease in BWG and tibia ash and quadratic effects on percentage tibia ash. Digestibility studies show that the bird relies more heavily on PP under these conditions and that Ca binds this (see section 2.5.2). Thus it is not surprising that the addition of phytase resulted in increases in BWG and tibia ash with the same diet. The greater improvements at higher Ca levels suggests that sufficient P is released by phytase to match the bone mineralisation potential of the available Ca. Similarly, Simco and Stephenson (1961) conducted a study in which tCa/tP ratios varied from 0.625:1 to 2:1, but the PP% was approximately 0.28% so that the lowest tCa/nPP ratio was 1:1. They observed depression in growth with low P levels (0.28% nPP) and a tCa/nPP ratio above 3.5:1. No phytase additives were available at that time.

Adeola and Walk (2013) reported that as P levels from potassium phosphate increased there was a quadratic increase in tibia ash content, similar to the observations of Kornegay *et al.* (1996). Phytase provided a similar effect. This suggests that there is a levelling off of the rate of increase in ash as P levels increase. It was also found that the proportion of ileal digestible P deposited in ash was lower at lower Ca and higher phytase inclusion rates. This reflects the need for both Ca and P in bone formation. It was proposed that 5 g of soluble Ca (7 g tCa) and 1000 FTU/kg offered an optimum balance between Ca and P. Tibia ash was proposed as the most sensitive measure of determination of P bioavailability. Regression coefficients for

ileal digestibility and bone mineralization provided an estimate of the efficiency of use of ileal digestible P. This was 92.8% with 6 g/kg HSC and 1000 FTU/kg. This points to an obligatory urine loss of P.

A nutritional geometry approach has been used to investigate the relationships between Ca, nPP and skeletal health (Driver *et al.*, 2005; Bradbury *et al.*, 2014). Tibia ash, gait score, bone abnormalities and the latency to lie (LTL) test were used to assess the latter. This approach uses different concentrations of Ca and of nPP (4-5 of each in these studies) and then applies different combinations of these two minerals within each treatment. (Bradbury *et al.*, 2014) concluded that both very wide (high Ca, low nPP) and very narrow Ca/P (low Ca, high nPP) ratios are deleterious to skeletal health. Although nPP influenced performance more than Ca, as was also seen in a study of rats (Shapiro and Heaney, 2003), it was concluded that birds defend their Ca requirement even if it means over-consuming P but not vice-versa. This may reflect more finely tuned homeostatic mechanisms for Ca regulation.

Zeller *et al.* (2015b) found that mineral P increased BW gain and feed consumption and decreased the feed:gain ratio. Supplementation with phytase improved all three measures and the highest BW gain and lowest feed to gain ratio were found with supplemented MCP and superdosed phytase.

The effects of dietary Ca, P and phytase levels on bone cannot be interpreted in isolation from the age of the bird: these effects are the result of the interaction between the digestibility of the diet and the requirements of the bird for optimal bone mineralisation. When both digestibility and bone ash are analysed, the requirements for bone growth may be more readily discerned (e.g. Adeola and Walk, 2013).

2.6.2.3.Amount of Ca and P in bone

If the effect of diet on the Ca and P content of the broiler is to be simulated, it is important first to know what these values are in a bird growing to its genetic potential: the potential Ca and P deposition must be modelled.

An early study of a small number of pullets at the onset of lay showed remarkably consistent values for Ca in bone ash, with a mean of 370 g/kg (Taylor *et al.*, 1960). Field (2000) proposed that Ca be used as an indicator of bone in meat (*bone % = Ca %×5*) because it is present as a constant proportion of bone ash (37%). However, the studies on which this relationship was based mostly involved mature animals.

Huyghebaert (1996) used the calculation $body\ weight \times 0.0055$ to calculate P mass in the body of 21- and 42-day old broilers but did not reference this calculation. Hurwitz (1964) analysed carcass and tibia P and found a strong correlation between them, with body P = 19.6 × tibia P. This was unaffected by variations in dietary P and hence would provide a useful parameter for calculating whole body P. However, this relationship was not confirmed in modern broilers and was found to vary with age and diet (Shastak *et al.*, 2012a). The slope of the linear regression of whole-body P retention on tibia P was 17.7 with an R^2 value of 0.97. Although the relationship was not one of direct proportion, if the initial whole tibia P mass were known this relationship might be useful.

Caldas *et al.* (2019) reported that the rate of maturing for body protein, ash, Ca and P were similar, suggesting allometric relationships between them. Furthermore, the similarity between the Gompertz parameters for protein and Ca suggested an isometric relationship, with Ca at 4.3 g/kg protein and P at 3.6 g/kg protein. Since most Ca is found in the skeleton, this also suggests that the mineralised skeleton was growing isometrically with body protein. This is not borne out by a study of the growth of the broiler skeleton that found that the skeleton decreased from 214 to 175 g/kg BW between hatch and 14 days of age (Angel, 2007). However, the allometric relationships were not analysed and the inclusion of feathers and gut contents are likely to limit the usefulness of these results. Furthermore, body protein was not analysed so that it was not possible to assess the allometric relationship between ash, Ca, P and body protein growth.

2.6.2.4. Ca/P ratio in bone

Because Ca and P are bound together in hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) in bone, deposition of this tissue is expected to require both minerals in a fixed ratio (Létourneau-Montminy *et al.*, 2015). However, it has been noted that selected strains of broiler may exhibit Ca/P ratios differing from the expected molar ratio of 10:6 (1.67:1, mass ratio 2.16:1) in bone. This may also change over the growing period when birds are fed a balanced diet, or when the diet is deficient in one or other mineral. Patterns in mineralisation such as this may be due to changing supply of minerals relative to requirement. When bone mineral varies from the stoichiometry ratio of hydroxyapatite, it usually contains proportionally less Ca, so that the molar ratio between Ca and P is less than 10:6 (Glimcher *et al.*, 1981). This is evident in many studies (Skinner and Waldroup, 1995; Angel, 2007; Suchý *et al.*, 2009; Browning and Cowieson, 2014). This may be explained in terms of a lower degree of mineralisation of the bone (Sanchez-Rodriguez *et al.*, 2019). This results in variation in the quantities of other ions, both cations (e.g. Na^+ , Mg^{2+} and Sr^{2+}) and anions (e.g. HPO_4^{2-} and CO_3^{2-}) that may occupy sites in the crystal lattice (Browning and Cowieson, 2014; Von Euw *et al.*, 2019). It was proposed that significant amounts of HPO_4^{2-} are found on the surface of bone mineral crystals, resulting in an average chemical composition of bone mineral with a molar ratio for Ca: (P+C) of between 1.2 and 1.5. – the proposed formula for a 2-year-old sheep gives a Ca/P molar ratio of 7.5:5.4, which represents a mass ratio of 1.8 (Von Euw *et al.*, 2019).

Williams *et al.* (2000b) observed that Ca concentration in bone increased from 131 mg/g at 4 days to 185 mg/g at 40 d. A Ca/P molar ratio that varied from 1.3 to 1.7 was recorded. In a more recent study, the molar Ca/P ratio of cortical bone in the tibias of male Ross broilers decreased from 1.68 (mass ratio 2.15) at hatch to a minimum of 1.6 (mass ratio 2.06) at 7 days before increasing to reach 1.67 at 37 days (Sanchez-Rodriguez *et al.*, 2019) (see Figure 2.5).

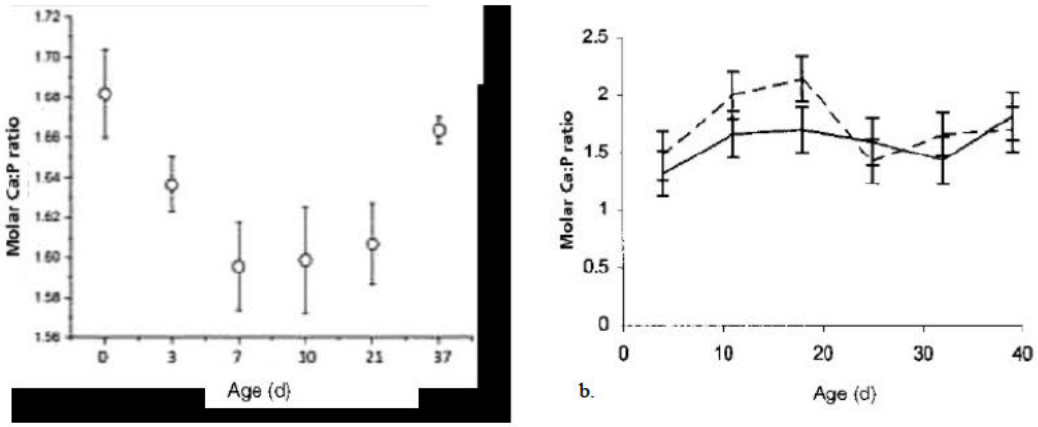

Figure 2.5 Change in bone Ca/P molar ratio with age

Sources: a. Sanchez-Rodriguez *et al.* (2019) b. Williams *et al.* (2000b)

Glimcher *et al.* (1981) indicated that younger animals exhibited a higher proportion of HPO_4^{2-} in bone, resulting in a lower Ca/P ratio. This can be seen in chicks between 0 and 14 days (Angel, 2007), at 28 days (Skinner and Waldroup, 1995) and at 4 and 25 days (Williams *et al.*, 2000a). Amorphous calcium phosphate (ACP) (Ca/P_i molar ratio of 1.35) and possibly octacalcium phosphate (OCP) are precursors to hydroxyapatite (Glimcher, 2006; Von Euw *et al.*, 2019). ACP is thought to be transported in blood in the form of nanoclusters with proteins and has been shown to crystallise when in contact with existing hydroxyapatite as the proteins are unable to penetrate the collagen fibrils (Nudelman *et al.*, 2010; Lenton *et al.*, 2020). The existence of these nanoclusters would account for the supersaturation of biofluids with Ca and P (Anderson, 1991; Kerschnitzki *et al.*, 2016) and may present a challenge to a simple solubility model of mineral exchange between the body compartments of GIT, blood and bone. A mass ratio as high as 2.27 has been reported in 42-day old birds (Muszyński *et al.*, 2018).

2.6.3. Proportions of soft tissue and bone

P is found in muscle tissue where it is independent of Ca for many of its functions and this forms the bulk of the remaining 20 to 40% of P in the body. This concentration is fixed and hence changes in body P concentration are due to variable amounts in bone and the proportions of soft tissue and mineralised bone in the body (Khaksarzareha *et al.*, 2017). P in muscle has been recorded at 2.2 (older birds) - 3.0 (younger birds) g/kg fresh weight (Grey *et al.*, 1983), while in fat tissues 0.013 g P /kg was reported (Farmani and Rostammiri, 2015).

Eits *et al.* (2002) studied the relationship between protein and ash in broilers where protein: energy ratios varied from the ideal and where feed intake was restricted. They analysed defeathered carcasses and organs separately and confirmed an isometric relationship in birds slaughtered at 200, 800 and 1600 g body weight. Their allometric calculations suggested an ash: protein ratio of 0.15. However, they found that the proportion of ash in the organs increased as the proportion of ash in the carcass decreased to achieve this isometry. When feed intake and protein to energy ratios depressed protein growth, however, they found that ash growth in the carcass was not depressed in proportion to protein, although the two remained in the

same proportions in the internal organs. They suggested that this indicates changes in the muscle to bone ratio. 14% of the body protein was found in the organs of 800 g birds (18.1 g out of a total 128.7 g for carcass + organs) and 12% in the 1600 g birds. The proportion of ash in the skeleton increases with age (Angel, 2007) so that the decrease in ash as a proportion of protein is most likely due to an increased proportion of muscle tissue in the carcass, in which the proportion of ash is considerably lower than it is in bone.

In order to develop a theory of mineral growth for broilers (a deterministic rather than an empirical approach) it was necessary to understand how the different tissues in the broiler body grow, as well as knowing how chemical composition changes (Zoons *et al.*, 1991). It is often stated that the modern broiler exhibits a disproportionate growth of muscle (meat) compared with unselected, slower growing chickens, leading to undue stress on the skeleton. However, few studies have verified this claim.

Although the proportion of ash in bone is higher at 400 – 600 g/kg dry, fat-free tissue (Edwards Jr, 1988; Angel, 2007) than in muscle at 10 – 12 g/kg (Grey *et al.*, 1983), it performs critical functions in muscle. The growth of both of these tissues is affected by P deficiency during the growing period but the mechanisms and proportions of the prioritisation of P is unclear (Bertram, 1995).

One of the few studies in the literature that quantified the growth of the dissected body over the life of the broiler showed that the proportion of lean muscle in the broiler increased from 30.9% of the body weight at 1 week old to 47.4% at 6 weeks (Figure 2.6) (Murawska *et al.*, 2011). Giblets (heart, gizzard and liver) decreased from 8.2 to 3% in week 6 and skin and subcutaneous fat increased from 7.85 to 12.63% at week three before stabilising at 12.5% of the body weight. It would be necessary to take the proportion of ash in these tissues into account when trying to understand changes in ash: protein ratio. Bones decreased from 11.6% of body weight at 1 week to 9.1% in week 8 and remained at this level until 10 weeks of age. As shown by Skinner and Waldroup (1995) ash, Ca and P increase as a proportion of bone mass as the bones become more mineralised with age. Hence it is possible that the proportional decrease in skeleton mass accounts for the proportional increase in lean mass in terms of ash content. It is also possible that changes in the mass of internal organs contribute to an overall isometric relationship between ash and protein.

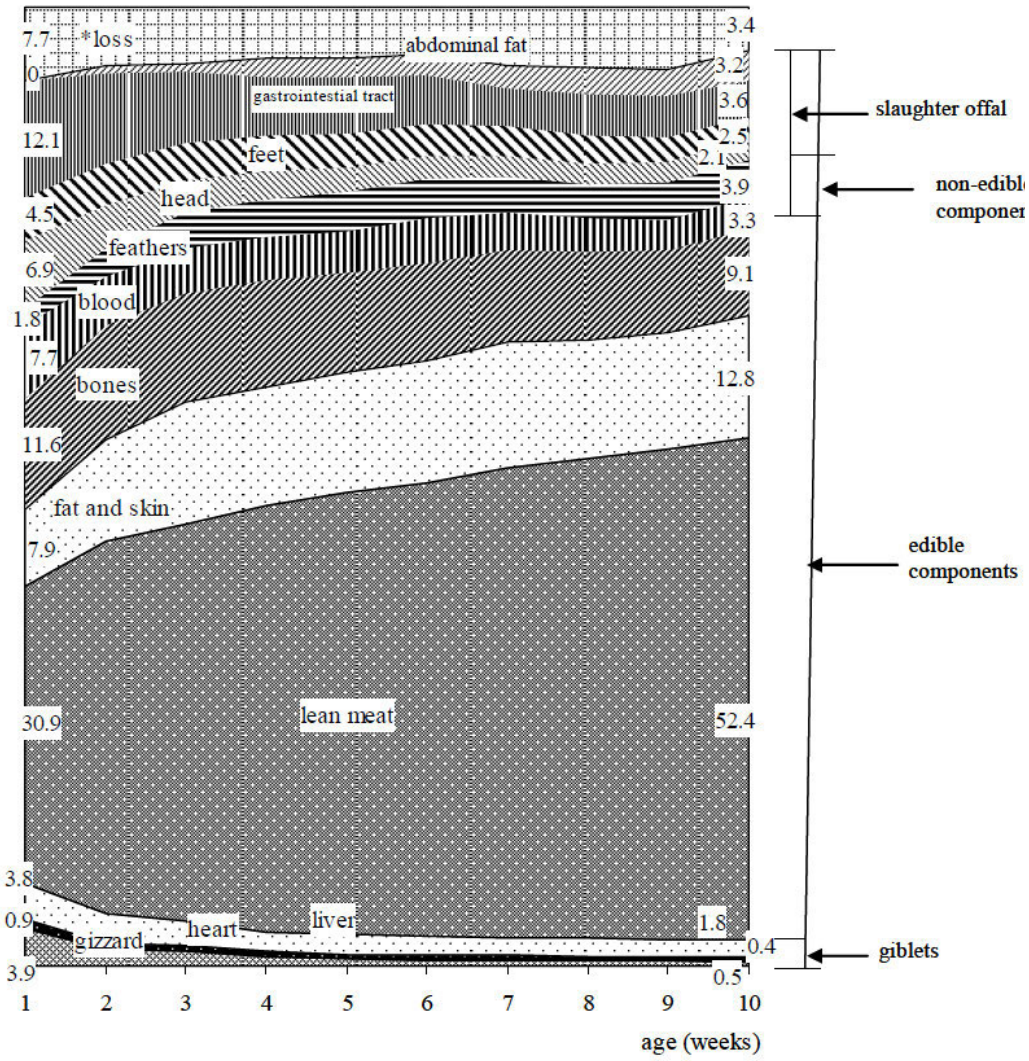

Figure 2.6 Percentage content of body components from week 1 to week 10 of age (birds slaughtered at the end of the labelled week with 12 hr fast before slaughter)

Source: Murawska *et al.* (2011)

2.7. Maintenance

The calculation of maintenance requirements for amino acids is well-established (Burnham and Gous, 1992; Fisher, 1998) but little is known about these values for Ca and P. Using a similar approach to the amino acid convention, a coefficient was applied to the degree of protein maturity in a model of P in pigs to calculate the mass of P required for maintenance at time t as follows:

$$P'_{maintenance}(t) = d \times \frac{N^*(t)}{{N^*}_m{}^{0.27}} \frac{kg}{d} \qquad (Eq.\,2.10)$$

where $N^*(t)$ is the body protein at time t and ${N^*}_m$ is the body protein mass at maturity and d is a scaling coefficient (Misiura *et al.*, 2020).

WPSA (1985) suggested Ca and P maintenance requirements of 0.05 g and 0.03 g/kg BW/d, assuming that there would be turnover of 1% of body contents (see section 2.6.1). Over a number of days, this publication used 60% of the BW change to calculate average BW, rather than 50% when calculating maintenance. As this included faecal endogenous P, it was suggested in the CVB system for aP, published in the Netherlands, that the following equation be used to calculate P for maintenance over a period (in mg):

$$P_{maintenance} = 0.014 * \{(BW_2 - BW_1) * 0.6 + BW_1\} * (t_2 - t_1) \qquad (Eq. 2.11)$$

where BW = BW in g (van der Klis and Blok, 1997).

This formula was translated by Kebreab *et al.* (2009) into 14 mg P /kg BW/d and 55 mg Ca/kg live weight per day in their layer model. However, the approach used by Misiura *et al.* (2020) is a more sound one, since an isometric relationship between ash and body protein has been demonstrated (see section 2.6.3) while the ash in the whole body is influenced by changes in lipid content.

2.8. Urinary excretion

If the urine output of birds is to be measured, colostomised animals are required since the urine and faeces are otherwise mixed in the excreta. However, it must be borne in mind that caecectomised birds are not in a normal physiological state and results may be affected by this.

A study of mineral urinary excretion, designed to measure changes during heat stress, applied feeds with 1.25% Ca and 1.02% tP to 7-week old, colostomised broilers (Belay *et al.*, 1992). Ca excretion under thermoneutral and heat distressed conditions was 7.7 mg/kg BW/d while P excretion was 23 mg/kg BW/d under thermoneutral conditions, rising to 93 mg/kg BW/d under heat distress. Manangi *et al.* (2018) showed that under conditions of P deficiency, 40- to 50-day-old broilers retain all but a small amount of ingested nPP and only excreted 0-15 mg/d, whereas they excreted 235 mg/d at the highest dietary nPP level (Figure 2.7).

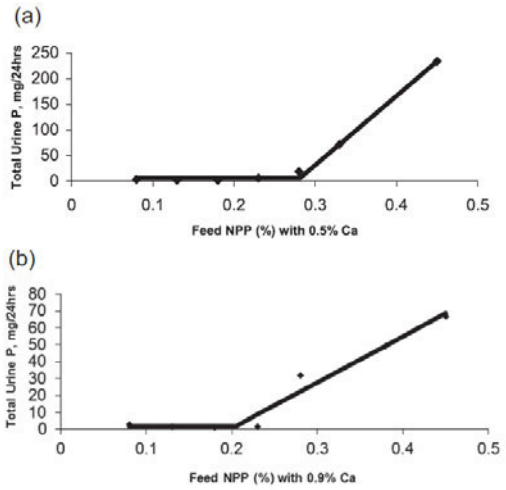

Figure 2.7 Urinary excretion of P in 40-day-old (a) or 50-day-old colostomised broilers (b)
Source: Manangi *et al.* (2018)

Ca excretion in the urine decreased as nPP in the diet increased, presumably as increasing amounts of Ca were retained in bone, bound to P (Figure 2.8). Above a 0.3% nPP, Ca excretion is constant and negligible. This supports the idea that the bird absorbs Ca from the GIT to meet its bone mineralisation requirement and Ca is only excreted in large quantities if there is insufficient P to allow bone mineralisation. The excessive Ca urinary excretion (400 mg/d) when low nPP levels and high Ca levels are fed as compared with 150 mg/d when the Ca/nPP ratio is narrower, implies that excessive Ca is absorbed from the GIT. This extreme situation indicates a breakdown in the normal regulatory system and is also seen in the high Ca digestibility observed under these conditions by Wilkinson *et al.* (2014c).

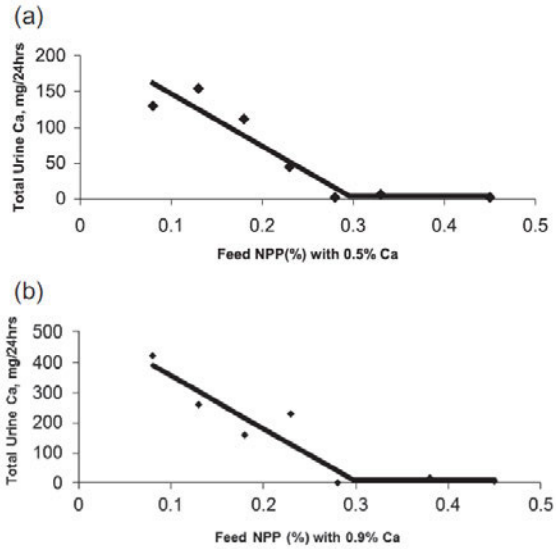

Figure 2.8 Urinary excretion of Ca in 40-day-old (a) or 50-day-old colostomised broilers
Source: Manangi *et al.* (2018)

Indications of urinary losses of Ca and P may be given by digestibility and retention estimates carried out on the same trial (Shastak *et al.*, 2012b)

2.9. Retention data as an indication of growth

Retention measurements indicate how much of the ingested feed remains in the body after faecal and urinary losses have occurred. Although it can be equated to growth if absolute retention values can be calculated, it is more commonly reported as a proportion of the same mineral in the diet or as a digestible mineral as a proportion of feed. Retention is influenced by dietary factors, which may limit the mineral available for metabolism and growth, and animal requirements. Hence the interpretation of retention studies requires an understanding of both these processes.

Ca or P response studies in which retention is measured often suggest a quadratic relationship such that increasing mineral content increases the amount of mineral retained up to a certain level, beyond which the quantity of mineral retained plateaus or decreases, the latter possibly due to a mineral imbalance. This "break-point" is used to indicate mineral requirements.

Retention is commonly measured as a relative value, using an indigestible, non-absorbable indicator to quantify the proportion of mineral fed that is excreted. Retention is then calculated as the difference between ingested and excreted mineral. Feed intake data may be absent or recorded over a longer period than the retention assay: excreta collection typically takes place over a two- to three-day period while feed intake is recorded weekly. Therefore calculating the absolute retention, and hence growth is not always possible.

Studies in which N retention was also recorded could provide an indication of mineral: body protein ratios (Gautier *et al.*, 2017). A further insight that might be derived from studies in which Ca and P retention are both measured is the proportional growth of BFB and Bone: as in body composition, this ratio becomes lower as the proportion of P in BFB increases, implying poorer bone mineralisation. In a second experiment reported by Gautier *et al.* (2017), mineral retention remained constant (1.88-1.92 g/bird Ca and 1.27-1.25 g/bird P for a 7-day period) as the Ca level in the diet rose from 5.1 to 10.4 g/kg at a constant tP level, but at 19.3 g/kg Ca in the feed, the bird appeared to retain very little P (0.41 g) while retaining excessive Ca (3.13 g), resulting in a retained Ca: retained P ratio of 7.71. This suggests an extreme P deficiency, possibly as a result of precipitation in the GIT, and raises the question of where the Ca is stored.

Retention figures are affected by both growth and digestion processes, as in the situation described above, so must be treated with caution. However, assessed with the absolute retention values, they can suggest proportions of P in BFB and Bone with a balanced diet and indicate where the system breaks down.

While retention studies which allow the researcher to determine the mass of mineral retained in the body provide an indication of growth, they do not distinguish between minerals required for different tissues. However, the different proportions of Ca and P deposited into bone and soft tissue, particularly the very low levels of Ca in soft tissue, means that the ratio between retained Ca and P should give an indication of the proportions of bone and muscle tissue growth under limiting conditions (Bertram, 1995). Ca retention would be a sufficient indicator of bone growth if the changes in Ca/P ratio in bone can be modelled.

2.10. Discussion

Once it has been firmly established what quantity of each nutrient is required to allow the body of the broiler to grow optimally for the particular production system, it becomes imperative to deliver these nutrients with as little waste as possible. In order to do so, the nutritionist would ideally be able to calculate how much of the nutrients fed to the animal will pass through the wall of the GIT and be available for use by the animal and how much of this will be excreted in the urine. Practically, it is difficult to separate nutrients that are defaecated by the bird from those excreted in the urine. Furthermore, there are indications in the literature that the mechanism by which the bird meets its Ca needs differs from that which it uses to meet its P needs. Since these two minerals must be considered together, the particular means by which the bird rids itself of unneeded nutrient becomes less important than the overall quantity retained and utilised for growth and maintenance.

Preliminary searches for data that would allow the digestion, absorption and retention of Ca and P by the broiler to be modelled did not return the necessary parameters. It was decided to conduct a broad, systematic literature review to establish if appropriate studies had been reported and to generate a database of published data from which more specific information might be derived.

CHAPTER 3. MODELS OF CALCIUM AND PHOSPHORUS IN MONOGASTRIC ANIMALS

A model is a simplified representation of the real world which allows the scientist to express theory in such a way that it can be tested (through experiments) and lead to an improved understanding of the system under consideration (France and Dijkstra, 2006). This process suggests where there are gaps in the body of knowledge: modelling can be used to direct research and guide experimental design. It may also allow the user to predict the response of a system to different treatments (Emmans, 1995) and hence be of practical use in suggesting the optimal inputs to a commercial operation.

In this chapter the application of models to animal nutrition will be discussed and the published models of Ca and P in monogastric nutrition will be reviewed. A conceptual model for the present study will be proposed, comprising three components that will be considered in the modelling process.

3.1. Modelling approaches

France and Dijkstra (2006) identified three modelling approaches: mechanistic, empirical and teleonomic. These can be distinguished by their basis in the hierarchical nature of organisation in biology.

Since each level of organisation (e.g. progressing from cellular to tissue to organ levels of organisation) shows responses that are based on processes at the lower levels of this hierarchy, theory may be built at the lower levels in order to predict responses at higher levels. This is the basis of mechanistic models, which "seek to understand causation" (France and Dijkstra, 2006). In these models, linear programming may be used for optimisation (e.g. least-cost feed formulation) while differential equations form the basis of the rate:state formalism which allows deterministic models to describe how variables change over time. When a differential equation is expressed as an integral equation it allows the prediction of a stepwise (for example, daily or hourly) change in a state variable based on the underlying parameters which determine the rate of change.

Empirical models, on the other hand, use experimental data to quantify and hence to describe relationships between variables at a single level of organisation. Statistical techniques, such as multiple regression analysis, are used. This may produce a mathematical relationship such as equation 3.1 which estimates a dependent variable D (digestibility)

$$D = \alpha + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \cdots + \beta_n x_n \qquad (Eq. 3.1)$$

where α is the intercept and x_i are a number (n) of independent variables or predictors, each associated with a partial regression coefficient or parameter (β_i) (Dhanoa *et al.*, 2008). These models are extremely

useful and widely used, but predictions are often limited to the conditions under which the empirical data was collected (France and Dijkstra, 2006).

Teleonomic models are seldom used in physiology since they simulate goal-directed behaviour where constraints at higher levels of organisation direct behaviours at lower levels (France and Dijkstra, 2006). However, Martin and Sauvant (2010) used this approach to model nutrient partitioning over the life cycle of dairy cows. Their model proposed that the animal's compartmentalisation of energy for different functions was dictated by an overarching goal of maximum lifetime reproduction (generation of viable offspring). They developed a model that considered the components of this goal, one of which is growth. Modelling that is based on the theory of feed intake to achieve growth and production goals could similarly be considered teleonomic. This may also have relevance for modelling the way in which broilers prioritise minerals for different body tissues: a subset of the growth component could include goals for bone growth and soft tissue growth.

In practice, the parameters of mechanistic models are often quantified using experimental data (e.g. Wellock *et al.*, 2006). These authors described some precautions which should be taken to mitigate the limitations which might be introduced by relying on the empirical elements. These included using only the results of strictly controlled experiments and using the most biologically precise variables possible.

3.2. Existing models of Ca and P

Models of Ca and P digestion, absorption and retention in both pigs and poultry were considered since these represent two important monogastric livestock groups.

3.2.1. Hurwitz et al. (1983 – 1987)

A model of Ca homeostasis in the broiler was developed, in which the intestine, bone and kidneys modulated plasma Ca levels (Hurwitz *et al.*, 1983). Later, a description of growth was added to this model (Hurwitz *et al.*, 1987b). These authors contended that, although 99% of the body Ca is in the bone, the critical role of this mineral in diverse metabolic functions has resulted in the prioritisation of the maintenance of Ca levels in body fluids, if necessary at the expense of bone through the mechanism of resorption (Hurwitz *et al.*, 1987a).

Growth of body weight was predicted using a Gompertz equation. Ca, however, was modelled as a function of age and hence was predicted using equation 3.2.

$$Ca_c = U_1 + \frac{t}{(U_2 + U_3 t)} \qquad (Eq. 3.2)$$

where Ca_c = Ca as a proportion of feather-free body at time t (in seconds) $U_1 = 2.72 \times 10^{-3}$, $U_2 =$ 1.01 x 10^8 s and $U_3 = 206$.

Figure 3.1 illustrates this relationship. It suggests that a rapid initial increase in Ca as a proportion of EFFB is followed by a levelling off after the first two weeks. This may correspond with the mineralisation of the skeleton after hatching.

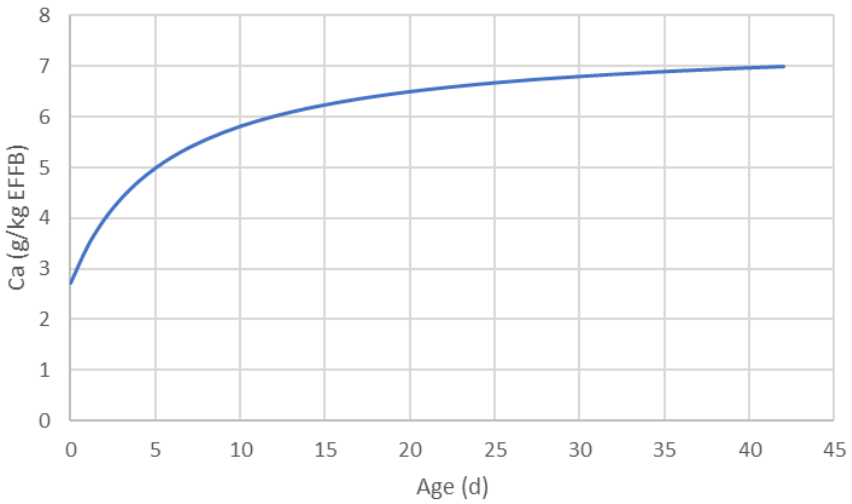

Figure 3.1 Change in Ca as a proportion of EFFB with age

Source: Hurwitz *et al.* (1987b)

P was not considered in this model. However, this research group conducted trials in which carcass analysis was carried out (Plavnik and Hurwitz, 1983; Hurwitz and Plavnik, 1986). Their publications offer some of the scarce data for Ca and P content of the whole carcass.

From these data, the relationships between age and Ca and P as a proportion of body protein were calculated. Body protein contents (g) for days 1 and 4 were calculated from a simulation using the EFG broiler model and as these data were not reported in Plavnik and Hurwitz (1983). The resulting values are shown graphically in Figure 3.2 a . The values for the first two weeks have been plotted in Figure 3.2 b.

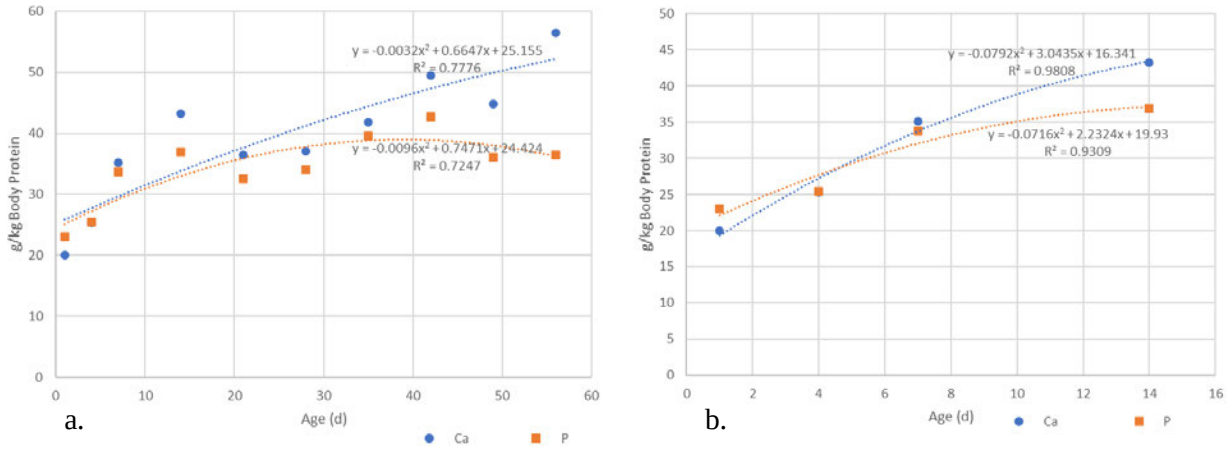

Figure 3.2 Relationship between Ca and P and body protein in the EFFB with age

Source: after Plavnik and Hurwitz (1983); Hurwitz and Plavnik (1986)

Neither logarithmic nor quadratic functions were well fitted to these data over the 56-day period of the trial. Ca and P as a proportion of body protein appear to fluctuate. However, a quadratic function can be fitted to the data from the first two weeks, at the end of which the ratio appears to be reaching a plateau. This phenomenon was described in pigs in Mahan and Shields Jr (1998). In this study, ash, Ca and P were expressed as proportions of empty, fat-free body weight. This calculation includes ash and water in the denominator, but if the proportions of these are known, the relationship to protein can be established. The protein/ash ratio increased between 0 and 20 kg mass (Figure 3.3). In this period, Ca and P increased as proportions of empty, fat-free body, with weaning occurring at 8.5 kg. From 20 kg to 75 kg, Ca and P formed constant proportions of empty, fat-free body, but increased from 75 to 145 kg.

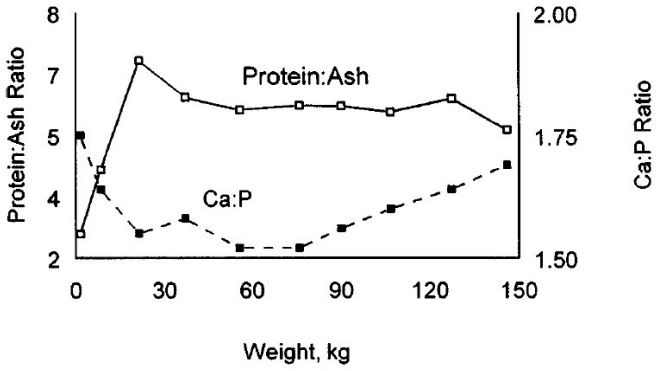

Figure 3.3 Changes in body protein:ash and Ca:P of pigs from birth to 145 kg BW

Source: (Mahan and Shields Jr, 1998)

The diets provided in Plavnik and Hurwitz (1983); Hurwitz and Plavnik (1986) were formulated to NRC (1977) specifications and were changed at 4 weeks. It could be speculated that the Ca and P requirements for bone formation were not met by the initial diet between 14 and 28 days, resulting in lower mineral masses as a proportion of body protein. Following the change of diet, there appears to be an increase in mineral/protein ratios.

The Ca/P ratio in this study also changed with age (Figure 3.4).

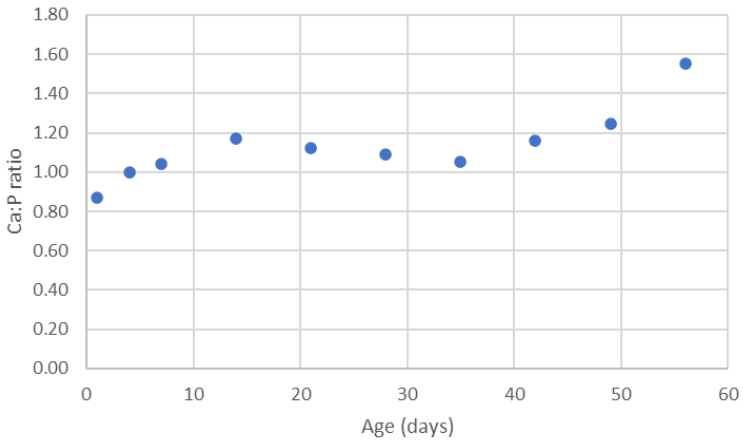

Figure 3.4 Relationship between Ca/P ratio and age in male broilers

Source: after Hurwitz and Plavnik (1986)

This suggested a change in the distribution of P through the body, with a greater proportion in muscle in the first 10 days and an increasing proportion in bone after 42 days.

When the natural logarithms of Ca and P are plotted against the natural logarithm of body protein, the resulting graphs do not suggest isometry between the minerals and protein as the slopes are not equal to 1 (Figure 3.5).

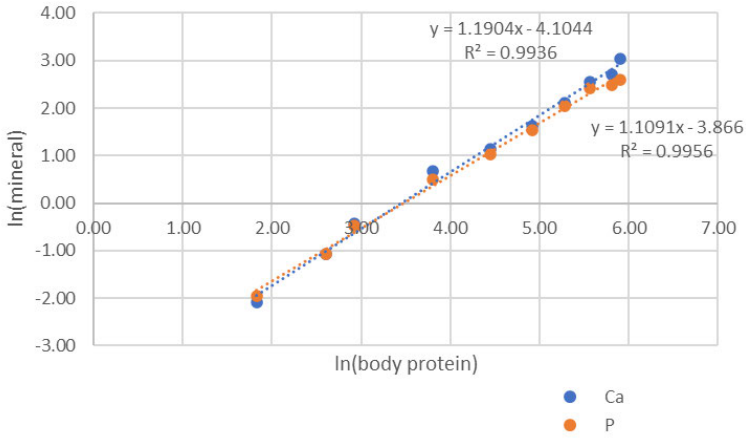

Figure 3.5 Relationship between natural logarithms of Ca and P and body protein in the EFFB of White Rock broilers

Source: after Plavnik and Hurwitz (1983); Hurwitz and Plavnik (1986)

The deterministic model of Ca homeostasis developed by Hurwitz *et al.* (1987a) was parameterised for a White Rock genotype very different from today's broilers. It provided a framework within which regulating mechanisms might be clarified and calcium flows quantified. However, it has limited applicability to strategies for broiler feeding:

- Calcium intake was modelled as dependent on energy requirement, with this driving feed intake. This is a flawed premise, as it is more often a first-limiting amino acid that determines feed intake in practice.
- Two components of Ca absorption from the intestine are proposed, one regulated by vitamin D and one independent of vitamin D, but the influence of components of the diet and the bird's characteristics were not considered.
- The model was designed to quantify Ca flows as a function of the hormones involved: this is different from a teleonomic approach where the goal of bone growth drives calcium homeostasis, with hormones playing a subordinate and enabling role.

This model had the potential to deepen understanding of the interaction of various mechanisms affecting Ca accretion in the broiler. Its development required the assimilation of existing knowledge and it guided a program of research that is still relevant today: it is an exemplar of the usefulness of models in a research setting.

3.2.2. Fernández 1995

A study conducted at the National Institute of Animal Science in Tjele, Denmark applied graded levels of Ca and P in the diets of growing pigs, while maintaining a constant Ca/P ratio The initial experiment was a balance trial in which intake, faeces and urine were assessed for Ca and P and net absorption (apparent digestibility) and retention were calculated (Fernandez, 1995a). In a further trial, radio isotopes were used to monitor the accretion of Ca and P in bone and their excretion in urine (Fernandez, 1995b). A model of Ca and P flows was then developed (Fernandez, 1995c).

This research suggested equations for the relationship between intake and retention for pigs of different liveweights (Fernandez, 1995a). Larger proportions of mineral intake were retained in larger pigs. This suggests that animals have the ability to draw more from their feed to satisfy changing requirements. The idea that older animals have a reduced capacity to absorb Ca and P was rejected and it was suggested that previous studies have introduced a confounding effect from the mineral levels in the feed. It was proposed that values for the usable minerals in feed be linked to the developmental stage of the animal. However, these relationships were not explored with a range of ages or body weights, so that predictive equations for values other than 35 and 65 kg were not generated.

It was proposed that active transport of Ca across the intestinal wall accounts for the curvilinear nature of the relationship between intake and absorption, since active transport is a saturable process.

Only small amounts of Ca were excreted in the urine except at the lowest level of Ca and P, at which level it was surmised that the low level of P was the driving factor behind greater excretion of Ca. This phenomenon has been seen in poultry, but with high Ca and low P levels in the diet (Wilkinson *et al.*, 2014c). The amount of Ca absorbed corresponded closely to the amount retained, which was not the case for P. Similarly, Anwar *et al.* (2017) found a strong correlation between Ca digestibility and retention. Renal excretion played a more significant role with P. It was concluded that "under normal intake conditions the dietary Ca input is regulated solely at the digestive tract, while the utilization of consumed P, unlike Ca, is regulated through renal action and indicates that the intestinal absorption of P is not regulated to the same degree as Ca". Similarly, Dimke *et al.* (2011) comment that only 1-2% of Ca that passes into the nephron is excreted: almost all is reabsorbed in both passive and active processes. PTH plays an important role in this conservation of Ca.

The injection of radioisotopes of Ca and P provided measurements of endogenous faecal losses, absorption corrected for endogenous losses, and bone accretion and resorption of minerals (Fernandez, 1995b). It was pointed out that a large pool of minerals in the skeleton provides a buffer for the maintenance of virtually constant levels in the blood plasma. Hence the skeletal levels will respond to dietary changes through the mechanisms of bone formation and resorption. These changes were described in terms of mineral balance, with a positive balance representing skeletal growth and a negative balance, skeletal reduction.

Importantly, it was found that pigs have a constant bone accretion rate regardless of mineral intake or even absorption, suggesting that this is physiologically predetermined. It was shown that bone resorption for the maintenance of metabolic processes compensated for lower amounts of Ca absorbed from the intestine, and as Ca absorption from the intestine increased, resorption decreased. This might be problematic at higher mineral intake levels as the accretion/resorption cycle is important for bone modelling. It appeared that pigs as they were bred in the mid-1990s could not develop bones at the rate required to support their protein growth (Fernandez, 1995c). It was suggested that there may be a mineral intake level which supports resorption for normal bone development, and this would likely be between medium and high levels of mineral intake. Better measures of bone development might be devised, and these traits selected for.

The model developed from the above studies considered only pigs at two body weights (35 and 65 kg) and two dietary mineral levels. It demonstrated some of the limitations of a purely empirical model: it provided a picture of Ca and P flows under the conditions applied in the experiments but did not allow predictions of system behaviour under different conditions. Nonetheless, some of the findings did indicate underlying physiological phenomena that could be of importance in the development of a mechanistic model.

3.2.3. Létourneau-Montminy *et al.* (2011)

This physicochemical model explored the fate of P in the digestive tract of broilers as it is released from phytate in the proximal GIT (crop, proventriculus and gizzard), absorbed in the small intestine and precipitated in the higher pH conditions that prevail in the distal small intestine. The role of phytase and Ca in these processes was incorporated into the model.

Inputs to the model were the different forms of Ca and P in the diet: PP, NPP of animal origin, nPP and Ca of plant origin, and nPP and Ca of mineral origin. The outputs were minerals absorbed into the body from the proximal and distal small intestine, and those passing into the large intestine and hence excreted.

The various forms of the minerals were represented as pools in four different compartments for broilers: the crop, proventriculus-gizzard, proximal small intestine, and distal small intestine. Once in the model compartments, P could be PP (soluble or non-soluble) or nPP (soluble or non-soluble). Different pH conditions and retention times were associated with each compartment, based on data reported in previous studies. Endogenous P was a further input to the soluble nPP in the proximal small intestine.

The following processes were modelled: solubilisation of PP, nPP and Ca, hydrolysis of PP by phytases, absorption of soluble phosphate and Ca, and precipitation of insoluble Ca/P compounds. Although the time-step applied in the simulation was one hour, outputs were calculated on a daily basis. Figure 3.6 is a representation of the model.

Phytate hydrolysis by phytase was modelled according to a Michaelis-Menten equation. As phytate was hydrolysed by phytase, a proportion of the non-soluble PP became soluble and hence susceptible to hydrolysis. This solubilisation process was modified by Ca which reduces the solubility of PP.

All nPP ingested entered the pool of non-soluble nPP and was then partially solubilised. This process was modelled using solubilisation coefficients for the different input forms of nPP, determined from previous studies.

Absorption from the proximal small intestine was modelled in two parts: an active, saturable process represented by a Michaelis-Menten equation and a passive absorption process represented by a linear function of the soluble nPP pool. The parameters for these were estimated from studies using isolated jejunal loops in pig. Similar work has subsequently been done by Liu *et al.* (2016), and equations were fitted for P absorption from the duodenum, jejunum and ileum. At the time of model development, such data were not available for broilers. If this approach were applied to a deterministic model of Ca and P absorption, the absorption coefficients and the Michaelis-Menten constants might have to be compared at different ages and after different prior feeding regimes. This could be a challenging aspect of a deterministic model: the model discussed in this section was parameterised for a 50 kg pig and a 21-day-old broiler. In doing so, the authors acknowledged that most broiler data available from the literature were collected at this age. Even for this purpose, information was scarce and pig data only were used for some aspects of the model, such as the hydrolysis of phytate by phytase.

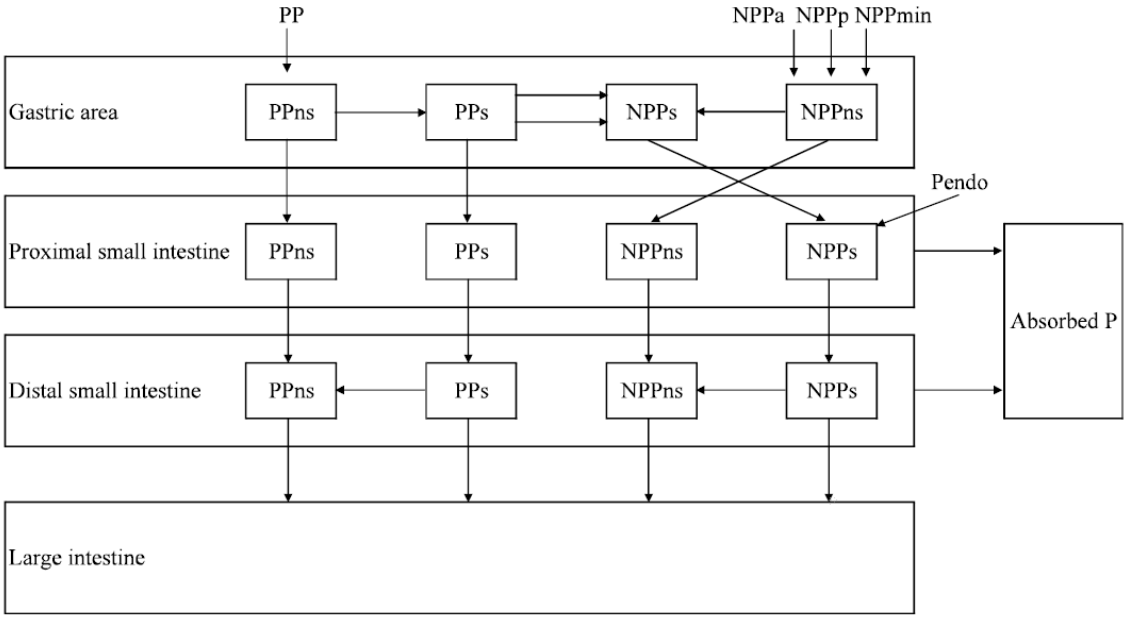

Figure 3.6 Model of phosphorus in the gastro-intestinal tract of the pig indicating the flows and of phytate phosphorus (PP), non-phytate phosphorus (NPP) in their non-soluble (ns) and soluble (s) forms

Source: Létourneau-Montminy *et al.*, 2011

Validation using pig studies showed that the model adequately predicted P digestibility in pigs. However, the authors noted that changes in transit time of the digesta and pH variations would affect the results.

Furthermore, the regulation of absorption would need to be addressed if the model were to be used to predict the P and Ca available to the animal from its diet.

This model offers an intriguing and truly deterministic approach to modelling digestion and absorption. Attempts to establish parameters for it would no doubt require a series of experiments that would increase the body of knowledge on this topic enormously.

3.2.4. Létourneau-Montminy *et al.* (2015)

Létourneau-Montminy *et al.* (2015) developed a dynamic model of Ca and P interactions in the pig. It was highlighted that a range of objectives are necessary in the production of pork, such as profitability, animal welfare and the environment. They noted that the ratio between bone and lean growth changes over the growth phase of the animal's life. This suggests that a model would need to treat these two tissues separately. Hence these authors suggested understanding of the growth of bone and muscle tissue as the starting point for establishing requirements. Furthermore, different genotypes have different P requirements due to different lean and bone growth potential. Compensatory bone mineralisation causes changes in requirements in Ca- and P-depleted animals. The relationship between protein growth and bone mineralisation changes in any circumstances other than the ideal.

Feed intake was an input to the mineral model, with this approach based on the InraPorc model (van Milgen *et al.*, 2008). Values were calculated according to the net energy requirement of the animal, as determined by its body weight. this is in contrast to models in which the first-limiting nutrient, usually an amino acid, is used to calculate desired feed intake (Gous *et al.*, 1999; Wellock *et al.*, 2003; Fisher and Gous, 2008; Symeou *et al.*, 2014).

The model comprised three modules: digestion (nutrients absorbed + nutrients in faeces), soft tissue (lipid + protein) and ash (in soft tissue + in bone + urinary excretion). Extracellular minerals are maintained at near constant levels and hence these were treated as zero pools.

3.2.4.1.Digestion

The digestibility module is described as a simplification of the deterministic model described in section 3.2.3 (Létourneau-Montminy *et al.*, 2011). The inputs are: PP, nPP of plant origin, nPP of mineral origin, nPP of animal origin, exogenous microbial phytase, endogenous plant phytase and tCa.

After faecal endogenous losses were accounted for, apparent total tract digestibility coefficients (ATTD) were applied to each of the mineral inputs to calculated absorbed P. These digestibilities were considered to be additive, so that each component was multiplied by its corresponding digestibility coefficient and the products summed to calculate the absorbed P. However, an interaction between Ca and nPP was included. Phytase was incorporated through the use of equivalencies which calculate the amount of inorganic Ca and P that can be replaced by microbial or plant phytase. Equation 3.3 calculated the mass of absorbed P.

$$absorbed\ P\ = -(faecal\ ELP\ \times\ DMI)\ +\ (ATTD\text{-}PP \times PP)$$

$$+(ATTD\text{-}nPP_{plant/animal/mineral} \times nPP_{plant/animal/mineral}) - (Ca\text{-}nPP \times nPP \times Ca)$$

$$+P\ equivalency\ microbial\ phytase + P\ equivalency\ plant\ phytase \qquad (Eq.\ 3.3)$$

Basal faecal endogenous losses were calculated at 0.19 g P/kg DMI. The ATTD coefficients applied to nPP and PP from plants were 0.73 and 0.21, respectively. ATTD coefficients for DCP, MCP, NaCP, animal sources & lactoserum were obtained from the INRA tables of composition (Sauvant *et al.*, 2004). The effect of Ca on the availability of nPP for absorption was modelled using a coefficient of –0.0333.

Calcium ATTD was calculated by meta-analysis, with a quadratic function fitted to the data. Hence quadratic and linear parameters were estimated (–0.02 and 0.83 respectively). This variable was referred to as "soft" because of the low P levels in the studies used to estimate it. This aspect of the model produced the poorest results while P values were better modelled. Absorbed Ca was further modified by the interaction between Ca and PP. Equation 3.4 demonstrates this calculation.

$$absorbed\ Ca\ = -(faecal\ ELCa\ \times\ DMI)\ +\ (ATTD\text{-}ca\ Linear\ \times\ Ca) +$$

$$(ATTD\text{-}Ca\ quadratic\ \times\ Ca^2) - (Ca\text{-}PP\ \times\ Ca\ \times\ PP)$$

$$+\ Ca\ equivalency\ microbial\ phytase\ +\ Ca\ equivalency\ plant\ phytase \qquad (Eq\ 3.4)$$

Basal faecal endogenous losses were calculated at 0.139 g Ca/kg DMI. The quadratic and linear parameters for Ca intake were estimated at –0.02 and 0.83 respectively. The coefficient of $Ca\text{-}PP$ was –0.32, applied to this product.

Absorbed P and Ca were measured in g/day. P and Ca in faeces were estimated by subtraction from feed intake.

3.2.4.2.Soft tissue

This module used the InraPorc model to represent the growth of protein and lipid (van Milgen *et al.*, 2008). Feed intake and growth parameters are inputs to this model. Feeds are characterised using faecal (total tract) digestibility coefficients and metabolic energy (ME) values as they are usually understood for the purposes of feed formulation, with no attempt to model interactions between nutrients or animal effects on nutrients. ME and net energy (NE) from excess protein, fat, starch sugars and digestible fibre were calculated using conversion factors. Obligatory urinary energy excretion and that from deamination of excess amino acids was taken into account. Potential protein deposition was described by a Gompertz function, parameterised by the mean protein deposition during the period of study rather than the mature weight. Body protein (lean mass) and lipid deposition were predicted. This deterministic model is an example of a rate:state formalism (see section 3.1) in which state variables (e.g. body protein, body lipid) are expressed in g and rate variables, in this instance with a time step of one day, are expressed in g/d (e.g. body protein gain, lipid gain).

3.2.4.3.Ash

A Ca/P ratio in bone of 2.2 was assumed, and in the whole body of 1.65. The bone ash/soft tissue ash ratio in pigs fed to maximise bone mineralisation is 78:22 (Nielsen, 1973 cited in Létourneau-Montminy *et al.*, 2015). In this model, the percentage of all Ca in the body which is found in bone was set at 99%, and approximately 70% of body P was in bone. Bone Ca/P ratios vary in broiler studies (see section 2.6.2.4) and lower ratios of Ca/P in the EFFB are observed during growth (Hurwitz and Plavnik, 1986; Caldas *et al.*, 2019).

It was proposed that the concentration of P in muscle and fat is constant, so that Ca and P accumulation in soft tissue is directly proportional to accumulated protein and lipid. Concentrations in lean tissue were reported as 0.59% P, 0.02% Ca and 2.39% miscellaneous minerals and in lipid tissue 0.05% P, 0.01% Ca and 0.25% miscellaneous minerals. These values were described as, for example, *proportion of P in muscle*, in the table of model parameters and were derived from pig dissection data and were values reported on a dry matter basis in the source data (Nielsen, 1973). The *proportion of protein in muscle* was 0.58 and presumably here this does not refer to a percentage, so that a P to protein ratio of 0.0102 might be inferred. As-is values for the protein and mineral content of muscle have been reported in poultry (Singh and Essary, 1974; Grey *et al.*, 1983). These imply slightly higher mineral to protein ratios. For example Grey *et al.* (1983) reported the protein content of breast muscle to be approximately 24% (vs 19.4 % in the pig data used in this model) and the P content, which decreased with age, at 0.2-0.3%. This results in a P to protein ratio of approximately 0.012 and a Ca to protein ratio of 0.00043 in muscle tissue. These ratios are lower in skin for P to protein (0.008) and higher for Ca to protein (0.0012) so that extrapolating from muscle to body protein, much of which is found in other tissues (including bone), must be approached with caution.

It was assumed that the bird prioritises P for soft tissue growth over bone retention. This effect was negligible for Ca. Hence this model assumed that Ca or P deficiencies are reflected almost entirely in impaired bone growth.

Potential Ca in bone was calculated using whole body Ca to potential protein ratio (0.05) from Pomar *et al.* (2006), with the assumption that 99% of body Ca is found in bones (Crenshaw, 2001). This is a parameter that could vary with genotype and hence is an input into the model. Actual Ca growth in bones considered both the available Ca and the balance between Ca and P, with a fixed ratio of 2.2 between these two minerals. If the diet resulted in a Ca deficit in bone, but supply increased subsequently, the retention was increased to the same degree as the magnitude of the deficit of the potential Ca in bone. This would result in a decreasing deficit over time until the Ca in the bone reached its potential value. Thus Ca, and hence P retention in bones is independent of the actual body protein, unless this is less than 70% of potential body protein. Below this value, bone growth is suppressed, reaching zero growth when protein growth is zero.

Urine endogenous losses of Ca and P were 2.0 and 0.5 mg/kg BW respectively. In addition to this, minerals absorbed but not retained due to excess supply or an imbalance in the supply for bone growth were added to the urinary excretion in the model.

3.2.5. Lautrou et al. (2019)

This paper reported further developments in the model which was described in the previous section. The distinctive feature of this model was the prediction of the actual deposition of Ca and P in bones independently of body protein. However, potential deposition was still linked to body protein and this was seen as problematic, because an analysis of protein and minerals in a previous study was reported to contradict this proposition (Couture *et al.*, 2018).

The data used to arrive at this conclusion were the combined results of three published studies (Langlois *et al.*, 2016a; b; Gonzalo *et al.*, 2018). These data were averaged for control (*témoins*) and deficient (*carencés*) feeds at 4 different body weights. If the reported means for protein and ash for the control group are logarithmically transformed, they can be plotted as shown in Figure 3.7. The slope of the trend line is close to 1 and suggests isometry between body protein and bone ash, contrary to the conclusion drawn in Couture *et al.* (2018).

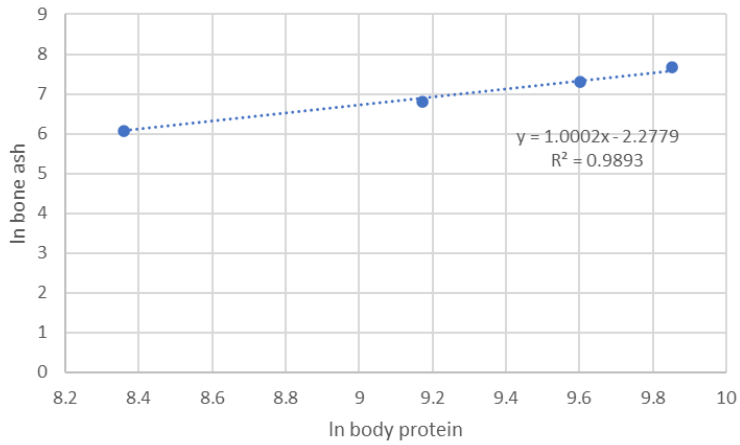

Figure 3.7 Relationship between body protein and bone ash in pigs of body mass 28 to 121 kg
Source: after Couture *et al.* (2018)

In revising the pig model, DXA measurements of lean, fat and bone ash were once again used, and converted to protein, lipid, Ca and P (Lautrou *et al.*, 2019). The calculation of Ca and P from ash was based on data from 90 kg pigs, in which ash was 33.85% (averaged across sexes), Ca formed 12.33% and P 5.95% of bone DM. The treatment across all studies in the database in which ash was maximised was selected for calculation of potential Ca deposition in g/d. This treatment provided 135% of Ca and dP.

A quadratic function was fitted to the bone Ca deposition against body weight for this treatment and, when compared with the exponential function fitted to body protein against body weight, it was concluded that

there was no relationship between bone mineral growth and soft tissue growth (see Figure 3.8). However, allometric relationships were not investigated.

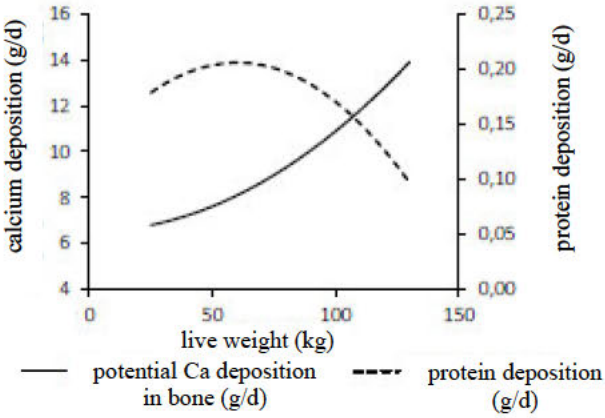

Figure 3.8 Potential deposition of body protein and bone Ca as a function of live weight in pigs
Source: Lautrou *et al.* (2019)

Modelling mineral growth as a function of body weight may be confounded by the short-term fluctuations in body lipid that occur during the growth period when energy and/or protein do not meet requirements exactly. Furthermore, as body weight is the sum of the chemical components of the body, there is greater logic in modelling the relationships between these components rather than with the whole of which they form a part. For allometry to hold true, the chemical components under consideration must have the same rate of maturing. This has been found to be true for protein, a genetically determined amount of lipid, water and ash. They will be present in the mature body in fixed proportions, but the proportions may change during growth if the components are allometrically, but not isometrically related. Hence their relationships to body weight, which is the sum of the components, would be expected to change in different ways over the growth period. An allometric relationship is to be expected between Ca and P and body protein, since ash (of which they form a large proportion) has been shown to have an isometric relationship with body protein.

3.2.6. Khaksarzareha *et al.* (2017)

This conference paper described the parameters of a factorial approach to feeding P, in which the sum of the requirements for growth and maintenance provides the requirement for aP. Maintenance requirements were equated to endogenous losses. However, these were mostly estimated from retention trials and hence included urinary losses. A regression of endogenous losses (EPL) (g/d) against BW (kg) produced the quadratic equation below. This was used to calculate maintenance requirements.

$$EPL\ (g/d) = 0.0034 + 0.0185\ BW - 0.0021\ BW^2 \qquad (Eq.3.5)$$

Retention data were used to calculate optimal body P deposition values. The treatments considered contained Ca within 15% of the Ross 308 recommendations and P levels that provided an average Ca/tP

ratio of 1.4. Regression analysis suggested a linear relationship between cumulative body phosphorus deposition (BPD in g) and cumulative BWG (in kg) as follows

$$BPD = 4.68BWG \qquad (Eq. 3.6)$$

Contour plots of body P deposition with dietary tP on the horizontal axis and tCa on the vertical axis were constructed from 41 publications. The data were separated into treatments with and without phytase. The application of these relationships in conjunction with the body deposition and maintenance calculations could form the basis of a purely empirical model for estimating the dietary requirements for Ca and P.

3.2.7. Symeou, Leinonen and Kyriazakis, 2014

A model that predicted P intake, digestion, retention and excretion in growing pigs was developed. This aimed to *translate total dietary P (tP) into digestible P (dP)... simulate dP retention… estimate P excretion in terms of both insoluble …and soluble* P. Hence the model outputs are P digested, P retained, P excreted (in urine, faeces, soluble and insoluble) and final body P composition (kg). Symeou *et al.* (2014) calculated dP_{req} (digestible P requirements) and dP_{input} (P digested from the lumen and available for retention), measured in g/day.

3.2.7.1. Digestion

PP, nPP and plant phytase inputs to the digestion module were derived from the INRA table of feedstuffs (Sauvant *et al.*, 2004). The model also included inputs of exogenous phytases from *A. niger* (3-phytase), *E. coli*, (6-phytase). The digestion process that was modelled was the release of nPP from phytate by these phytases and endogenous phytases produced in the small intestine. The influence of dietary Ca concentration on phytate dephosphorylation in the small intestine was considered. Phytate dephosphorylation was modelled in the stomach compartment, where pH is favourable to this process.

Endogenous phytases were of small- or large-intestinal origin. The reduced plant phytase activity that results from high pelleting temperatures was calculated from the decrease in faecal P digestibility of feed ingredients with high phytase activity (e.g. wheat) (Sauvant *et al.*, 2004) and assuming absorption of 80% of the nPP produced by phytate hydrolysis. It was assumed that no Ca-PP complexes are formed in the stomach in spite of the potential for changes in pH to enable this.

Dephosphorylation of PP was quantified as kg/kg PP using the first order kinetics equation

$$PP_{dephos} = K_{max}.(1 - e^{-R \times FTU}) \qquad (Eq\ 3.7)$$

where K_{max} is the maximum phytate dephosphorylation (total amount of susceptible phytate) and R the rate parameter. Both of these depend on the source of phytase. These equations were fitted from the results of phytase response trials which included very high doses (superdosing) of phytase (Adeola *et al.*, 2006; Kies *et al.*, 2006) for the exogenous phytases and from Sauvant *et al.* (2004) for plant phytases.

Broiler values were used to establish values for endogenous small intestinal phytases, taking into account dietary Ca levels (Plumstead *et al.*, 2008).

$$SI_{dephos} = 0.261 - (0.0158 * Ca) \quad (Eq. 3.8)$$

where, SI_{dephos} is the amount of PP dephosphorylated per unit of PP that enters the small intestine (kg/kg PP), and Ca is the dietary Ca in (g/kg diet). Although the phytate-binding effect of Ca was modelled, the formation of inorganic calcium phosphate in the small intestine was not, since it was assumed that sufficient PP would be available to bind Ca ahead of nPP.

The values for dephosphorylation in the large intestine (LI_{dephos}) were estimated using data from Sandberg et al. (1993) who used ileum-cannulated pigs. nPP released in the large intestine in this way was not absorbed.

3.2.7.2.Feed intake

Although FI was not predicted to increase when P was the first limiting nutrient in the diet, the depression of FI when diets are severely deficient in P was noted. Nonetheless, desired feed intake was calculated based on energy or protein requirements divided by net energy or digestible protein content of the diet. The only constraint on FI considered was bulkiness of feed.

3.2.7.3.Retention

When digested P intake is less than that required by the pig, and P is the first-limiting nutrient, all digested P (nPP + PP_{dephos}) is assumed to be retained in the body after maintenance requirements are met.

Maintenance requirement for P (in g) was calculated as

$$P_{maint} = p.Pr.{P_m}^{-0.27} \quad (Eq. 3.9)$$

where *p* has a value of 0.1293 g/day (for all genotypes) and Pr and Pr_m are the actual and mature protein weights in kg. In calculating *p*, it was necessary to use an allometric relationship between Pr and BW in order to use data from Jongbloed (1987). The efficiency of utilisation of digested P for maintenance (e_{maint}) was assumed to be 1. The efficiency of dP utilization for growth (e_{growth}) is calculated from the regression of net P retention (g/day) against dP intake and was assumed to be 0.94 (Rodehutscord *et al.*, 1999; Pettey *et al.*, 2006; National Research Council, 2012).

Maximum (potential) P retention $(maxP_{ret})$ in g/d was modelled as isometric with potential body protein (PrR) (g/d) so that

$$maxP_{ret} = k_2 * PrR \quad (Eq\ 3.10)$$

The isometric coefficient (k_2) has a value of 0.0337 in this model. This relationship was assumed to be constant, even when this mineral is deficient (Rymarz *et al.*, 1982; Jongbloed, 1987; Hendriks and Moughan, 1993; Mahan and Shields Jr, 1998). This is an assumption that was abandoned in later revisions

of the model. It was noted that National Research Council (2012) suggested an allometric relationship between body P and body N, described by a quadratic equation.

Parameters from Wellock *et al.* (2003) were used to calculate potential growth of the pig and digestible P requirement (g/d) required P was calculated as

$$dP_{req} = \frac{P_{maint}}{e_{maint}} + \frac{max\ P_{ret}}{e_{growth}} \qquad (Eq.\ 3.11)$$

Faecal P may be soluble or insoluble, however P absorbed but not utilized is excreted in the urine in soluble form. In this model soluble P losses (sP_{losses}) were calculated as follows

$$sP_{losses} = P_{maint} + NPP_{indig} + inefP_{ret} + \max\left[\left(dP_{intake} + dP_{req}\right), 0\right] \qquad (Eq\ 3.12)$$

where NPP_{indig} was the proportion of nPP in the digesta that is not absorbed (20%), $inefP_{ret}$ is 10% of the absorbed P (which presumably includes the inefficiency of dP use for growth), dP_{intake} is the P available for retention and dP_{req} is the digestible P requirement (g/d), presumably expressed as a negative amount.

Insoluble P comprises IP6, lower phytate esters and divalent cations, which are not modelled. This suggests that it is calculated from PP less any dephosphorylated P.

3.2.8. Misiura *et al.* (2020)

This study described the further development of the model in the previous section, with a particular emphasis on modelling the growth of P in the pig body. Meta-analyses were used to answer the following questions with regard to pigs fed P deficient diets:

Q1. Do they modify their feed intake (FI)?

Q2. How is the relationship between bone mineralisation and muscle tissue affected?

Q3. Are the P intake resources allocated differently within the body?

3.2.8.1.Feed intake

Feed intake (FI) responses to changes in P supply were investigated with a meta-analysis of fifteen studies. Each study included at least four different levels of P, fed *ad libitum* to grower and finisher pigs. Standardised total tract digestible (STTD) P was estimated from the feed ingredients in most studies and feed intake was scaled to body weight to allow a linear mixed effects regression model to be fitted to the data. This did not reveal a significant effect of STTD P on FI.

3.2.8.2.Relationship between protein and P growth

A meta-analysis confirmed that the relationship between P weight and protein weight was not significantly different from isometry when nutritionally balanced feeds (including sufficient P) were fed. However, if

P-deficient feeds were fed the allometric exponent was significantly less than 1 and an allometric relationship between body P and body protein was indicated.

Although an insufficient number of studies was available to establish the relationship between body P and body protein with protein deficient feeds, the relationship between body ash and body protein varied between nutritionally balanced, P deficient and protein deficient diets. The estimated allometric coefficient was significantly greater than 1 when protein was deficient and significantly less than 1 when P was deficient, indicating that the isometric relationship breaks down in both these cases.

3.2.8.3.Proportion of P in bone tissue

The authors introduced a partition of minerals between bone and soft tissue to the original model. 11 studies were identified in which the P in bone (P_{bone}) in the whole, empty body could be calculated. P in bone was not measured directly, but the measurement of empty body Ca in these studies allowed the calculation of P_{bone} as follows:

$$P_{bone} = \frac{0.99 \times Ca}{2.16} \ (kg) \qquad (Eq. 3.13)$$

This was based on the assumption that 99% of body Ca is in bone and that Ca and P are deposited in bone at a fixed ratio of 2.16.

Regression analysis was used to investigate the relationship between P_{bone} as a percentage of body P and P expressed in g/kg EBW. analysed to determine if P_{bone} formed a constant proportion of body P. This hypothesis was rejected as the proportion of P in bone increased as EBW increased. The fitted relationship was used to estimate the initial weight of P in bone from the initial EBW and the initial total body P. In the model, the soft tissue received priority for P supply and P was only allocated to bone once this requirement was met. Protein growth was restricted when the P supply fell below the amount required for maximum soft tissue growth.

Rather than modelling P, Ca and other minerals and using the sum of these to estimate ash (Létourneau-Montminy *et al.*, 2015), ash growth was calculated using three different allometric equations, depending on whether the feed was balanced, deficient in P or deficient in protein. The coefficients of these equations are summarised in

Table 3.1 Allometric coefficients for the calculation of ash deposition in the pig body from protein deposition

Source: (Misiura *et al.*, 2020) supplementary materials

protein and P in feed	a	b
balanced	0.19	1
protein deficient	0.208	1.08
P deficient	0.186	0.873

The model discussed in sections 3.2.7 and 3.2.8 is based on a proven theory of protein and lipid growth, and P and ash growth were added in a logical and rigorous manner. The release of P from phytate was modelled deterministically. A single coefficient was used to describe the efficiency with which nPP is absorbed from the digestive tract (Symeou *et al.*, 2014) and this may require modification. Furthermore, Ca is considered only insofar as it affects phytate digestibility and P deposition in bone. However, a meta-analysis published subsequently (Misiura *et al.*, 2018) provided a comprehensive analysis of the interactions that affect the absorption and retention of Ca and this could form the basis for model of Ca digestibility, using an empirical approach. This might encounter challenges in predicting absorption at ages other than those commonly tested in digestibility trials: the homeostatic control of Ca absorption at the intestinal wall may result in changes in Ca absorption with changes in the physiological status of the animal.

Comprehensive, deterministic models of calcium and phosphorus in pigs and chickens would contribute to the effective management of mineral inputs and outputs from production systems. Existing models are mostly in the field of pig nutrition, but broiler models could follow some of the principals suggested by these. Further investigation of the relationship between protein and minerals in the bird body, the ability of birds to recover bone mineral levels after a period of depletion and the deterministic modelling of interactions in the digestive tract are likely to be required.

3.3. Models of broiler production

Gous and Berhe (2006) indicated that the large number of variables to be considered, and hence experiments required, when modelling a broiler population to optimise profitability precludes the use of an empirical approach. Broilers from different breeding companies have different genotypes and hence different responses to nutrients. Furthermore, changes in the genetic potential of broilers rapidly renders experimental data obsolete (Gous, 2014). If a model is to be versatile and adaptable, it is necessary for it to have, as its foundation, theories of animal processes such as growth.

3.3.1. Prediction of growth in a broiler model

A modelling approach that predicts potential protein growth using a Gompertz equation can be applied to a range of different genotypes and both sexes by varying the equation parameters. The growth of other body components can be calculated from feather-free body protein using allometric relationships and taking into account lipid deposition as it is affected by diet (Danisman and Gous, 2013). The prediction of the growth of portions of the carcass such as breast and thigh is important where these must be optimised for maximum profit. Gous (2014) noted that while the allometry was surprisingly consistent across sexes and genotypes in the past, the model parameters for growth rate and weight at maturity need to be verified regularly as breeding produces birds with changing potential for growth.

A critical aspect of the broiler model described by the authors above is its ability to predict feed intake (Gous, 2014). This is based on the theory proposed by Emmans (1981) which suggests that an animal seeks

to consume sufficient feed to grow at an inherent potential growth rate. This growth comprises protein, ash, water and a certain minimum amount of lipid. The desired feed intake is that which provides the bird with sufficient quantities of the first-limiting nutrient in the diet and can be calculated as

$$F^* = {}^{n}/_{N} \qquad (Eq.\,3.14)$$

where F^* is the desired feed intake, n is the mass of the first limiting nutrient required by the bird for the particular period and N is the concentration of that nutrient in the feed. This theory, asserting that animals eat to satisfy their requirement for the first-limiting nutrient, contradicted the belief, still popular in some circles today, that animals simply eat to satisfy their energy requirement. In his seminal paper, Emmans (1981) firmly rejected this idea, citing evidence that birds become fat on protein-deficient feeds due to overconsumption of energy. Similarly, Kyriazakis (1997) pointed out that animals appear to eat to satisfy long-term goals that contribute to fitness for survival and reproduction, rather than eating to maximise energy intake as a short-term goal. This effect is less certain when feeds are deficient in a vitamin or mineral rather than one or more amino acids (Emmans, 1981).

Lipid growth, in addition to the minimum that is associated with potential protein growth, occurs as a means of storing energy when there is an excess in the feed consumed above that required by the bird, the amount of heat that is present in the urine and that lost to the environment (Emmans, 1981).

Gous and Berhe (2006) described an optimisation tool which incorporates a feed formulation module, a growth model and an optimisation procedure. The growth model uses the least cost feed as an input and predicts the performance of a flock of broilers under certain environmental conditions. The output of the model provides input to the optimisation module and a circular feedback mechanism is established. Nutrient requirements for animals cannot be fixed as the curvilinear response curves shift with changes in output. The optimum value on the curve will change as the cost of inputs and value of outputs to the farming system change (Morris, 2006). Furthermore, environmental objectives can be addressed with an optimisation approach: N excretion is addressed in the existing model. In the case of P, a proposed goal is to maximise growth while minimising P excretion.

3.3.2. Digestion models

Simulation models of digestion in animals may attempt to model the breakdown, absorption and transit of the digesta in a series of compartments which represent different sections of the GIT (Fernandez, 1995c; Bastianelli *et al.*, 1996; Kebreab *et al.*, 2009; Dias *et al.*, 2010). These models usually have as their inputs the quantity, composition and frequency of feed intake by the animal (Bastianelli *et al.*, 1996). Dynamic differential equations describe the fate of nutrients as they move from one compartment to another or out of the modelled system. Challenges include the estimation of the flow rate of digesta, which may be affected by feed characteristics such as particle size and liquid viscosity (Bandemer and Schaible, 1942; van der

Klis *et al.*, 1990; van der Klis, 1993). Feeding patterns and gut fill over time would also be expected to influence nutrient absorption (Buyse *et al.*, 1993).

Digestibility coefficients for amino acids in feed ingredients have been successfully used in feed formulation for decades. However, this model may not be suitable for minerals such as Ca and P, for which the absorption and utilisation mechanisms are physiologically more complicated (Adedokun and Adeola, 2013). Some authors have found that the ability to utilise P has a genetic component (Zhang *et al.*, 1998; Beck *et al.*, 2016) while others point to the effects of early P deprivation on P absorption and utilisation in the older bird (Létourneau-Montminy *et al.*, 2008). Several studies describe interactions between Ca and P levels in the diet (e.g. Fernandez, 1995b). In attempts to model the flow of Ca and P in the animal, empirical digestibility values are commonly used to calculate the amounts of Ca and P that are absorbed from the small intestine (Bastianelli *et al.*, 1996; Kebreab *et al.*, 2009).

3.3.3. Feed intake models

Feed intake is usually an input into models of minerals in monogastric animals (Fernandez, 1995c; Lopes *et al.*, 2009; Dias *et al.*, 2010). However, it is important to be able to predict the effects of changes in dietary minerals on feed intake and two recent pig models include this (Létourneau-Montminy *et al.*, 2015; Misiura *et al.*, 2020). The EFG model predicts feed intake from the first-limiting amino acid in the feed when energy is not limiting (Emmans, 1981) and mineral models that have this functionality will be more accurate in predicting growth. The EFG model predicts increased feed intake at marginal deficiency of the first-limiting nutrient but decreased feed intake when the nutrient is severely deficient, as growth rate is significantly constrained (Gous, 2007). The feed intake responses of broilers to different degrees of deficiency of Ca and P will be of relevance to future models.

3.4. A model of calcium and phosphorus in the broiler

Modelling is an effective way of highlighting gaps in the body of knowledge. The present study was part of a wider investigation of Ca, P, phytate and phytase interactions in broilers and as such it plays a role in directing this ongoing research.

A conceptual model was first developed (see Figure 1.1), indicating the factors that may need to be taken into account in the ultimate model, and the approach to modelling that would be applied. Work on the model raised further research questions. As in any model, assumptions about the relationships between some variables were necessary and the process of refinement of the model to produce more and more accurate outputs will be ongoing.

3.4.1. An extension of the EFG broiler model

The EFG broiler growth model provided the basis on which the sub-models of mineral nutrition were built. It predicts protein growth from a Gompertz equation with parameters that are genotype specific. Feathers are modelled with different parameters since their growth does not have an allometric relationship with

body protein growth and the amino acid profile of feathers differs significantly from that of feather free body (FFB) protein. Lipid, water and ash growth were already intrinsic to the model and certain constraints, such as the effects of feed bulk, temperature and vaccination have been incorporated.

The broiler growth model forms part of an integrated broiler feed management system. This includes the Winfeed least-cost feed formulation system, the growth model and the optimisation module. The mineral model was designed to work with the first two of these, with the potential for future integration of variables such as bone mineralisation and phosphate excretion into the optimiser.

Figure 3.9 shows a conceptual diagram of the model with its links to the EFG system.

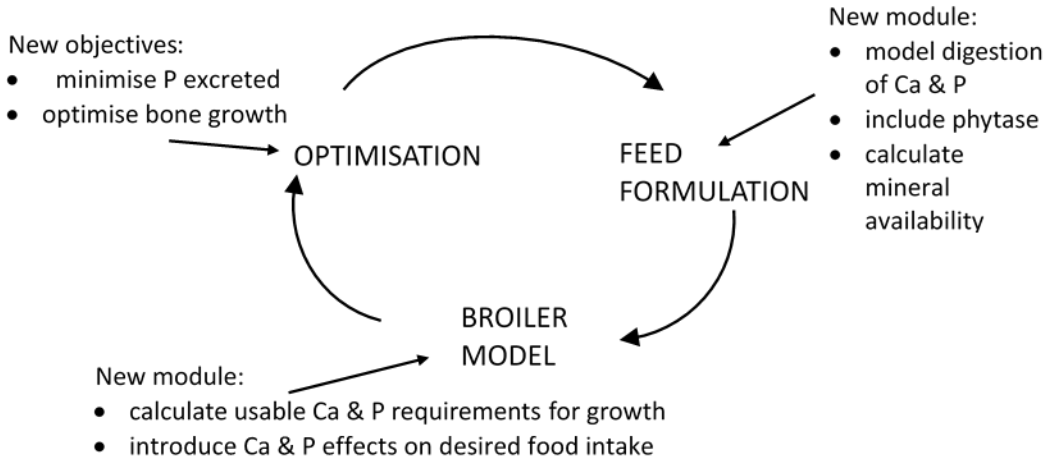

Figure 3.9 Integration of mineral model with existing broiler management system

3.4.2. Feed module

The feed module is required to describe the nutrients provided to the bird in its feed. This is an output of the least-cost feed formulation program. This already included tCa and aP. In conformity with the convention of the NRC nutrient requirements for poultry, the aP previously in the feed matrix was equivalent to nPP (National Research Council, 1994). The feed matrix was modified to include the feed ingredient values for tP, nPP and PP as well as tCa. Phytase was added to the matrix (RONOZYME® HiPhos) with an activity level of 10000 FYT/g (One phytase unit (FYT) is defined as the amount of enzyme that releases 1 μmol of inorganic phosphate from phytate per minute under reaction conditions with a phytate concentration of 5.0 mM and pH 5.5 and temperature 37 °C) (European Food Safety Authority, 2012). Levels of these nutrients in a formulated feed then provided the feed characteristics that are inputs to the digestion module, along with feed intake from the broiler growth model.

3.4.3. Mineral digestion module

The digestion module processes the input variables to determine the quantities of Ca and P that are available to the bird for absorption across the wall of the GIT.

This module takes into account the feed composition, the feed intake of the bird, and levels of Ca, PP, nPP and phytase to simulate the interactions of minerals with one another and with other components of the feed to produce quantities of Ca and P that are available for absorption into the bloodstream from the GIT. This simulation mimics the processes of dephosphorylation by phytase, precipitation of phytate and Ca as insoluble compounds and binding of inorganic phosphates by Ca and other nutrients. It also simulates the absorption of soluble Ca and phosphates as chyme passes along the small intestine.

The outputs of this module are available Ca (aCa) and available P (aP) in mg/bird/day.

3.4.4. Growth module

The growth module is based on the EFG broiler growth model. This can simulate the growth of a single bird or a flock subjected to the constraints of the nutrients in the feed. In order for body protein and bone to grow, sufficient amino acids, energy and minerals must be supplied. The bird attempts to grow at a rate determined by its genotype, laying down protein, lipid, minerals, water and ash. This ideal growth is compromised when nutrients are provided in insufficient amounts. The addition of Ca and P to the model is the contribution of this project. When these minerals are deficient in the diet, the bird's bone growth is constrained initially. More extreme deficiency of P compromises whole body growth through constraints on protein growth. Following a period of deficiency, the bird strives to return to the isometric relationship with its actual body protein.

3.5. Discussion

While models of energy and protein growth in monogastric farm animals are well established, mineral models are still being developed. The physiological influences on the requirements for Ca and P are complex so that innovative ways must be found to capture the interactions inside the GIT and to simulate partitioning between bone and soft tissue in the body.

Pig mineral models are well advanced, and a great deal of progress has been made over the past 8 years (Symeou *et al.*, 2014; Létourneau-Montminy *et al.*, 2015; Misiura *et al.*, 2020; Lautrou *et al.*, 2021). Work in broilers may have been hampered by a dearth of body composition studies in which Ca and P were analysed. Although there are more publications in which protein, lipid, water and ash are quantified, ash is often calculated by subtraction. Furthermore, assuming fixed proportions of Ca and P in ash may obscure the actual relationships between one mineral and another and between these minerals and protein. Before embarking on the lengthy experiments that are required to establish the Ca and P growth from hatch to maturity, it was decided that a preliminary model based on published data might be developed. This would guide the design, for example in terms of feeding strategy, of future experiments. A systematic literature review was chosen as a suitable approach to assessing the current body of knowledge that would be needed to build such a model.

CHAPTER 4. SYSTEMATIC LITERATURE REVIEW

An abridged version of this chapter was published under the title "Constraints on the modelling of calcium and phosphorus growth of broilers: A systematic review" (Salisbury *et al.*, 2021) (see Appendix 2). While the factors affecting Ca and P nutrition and growth of broilers were discussed in Chapter 2, this chapter describes a methodical approach to identifying those studies that might provide the data required to model this system.

Simulation models have enabled nutritionists to move away from the concept of using fixed amino acid values (termed 'requirements') when formulating feeds for broilers, by taking account of the interactions between the bird, the feed and the environment (Gous, 2014; Sakomura *et al.*, 2015). Mineral nutrition may also be improved by modelling such interactions. This would allow nutritionists to consider P and Ca together, since they interact in the GIT and in the skeleton. For example, the Ca/P ratio in broiler diets is known to be of significance, but it is often arrived at heuristically, based on experience of bird performance, rather than determined from modelled interactions. In the last twenty-five years, research in P nutrition has focussed on exogenous phytases that can improve the availability of P. However, there is a risk that studies continue to confirm the benefits of these additives without improving our understanding of mineral digestion and growth. Modelling has the potential to uncover gaps in the body of knowledge and may serve a purpose in directing research, beyond the purely commercial imperative.

Early studies of the growth of broilers with respect to minerals have seldom been replicated, despite significant changes in broiler genotypes in the last thirty years (Caldas et al. 2019; Hurwitz and Plavnik 1986). A substantial proportion of the Ca and P in broilers resides in the skeleton and its growth is not well documented (Angel 2007).

Challenges to implementing a modelling approach in Ca and P nutrition arise from the chemical interactions between them during digestion and growth (see Figure 4.1 and Chapter 2). However, it is the complex relationship between these two minerals and other nutrients that makes modelling the most promising approach to improving Ca and P nutrition.

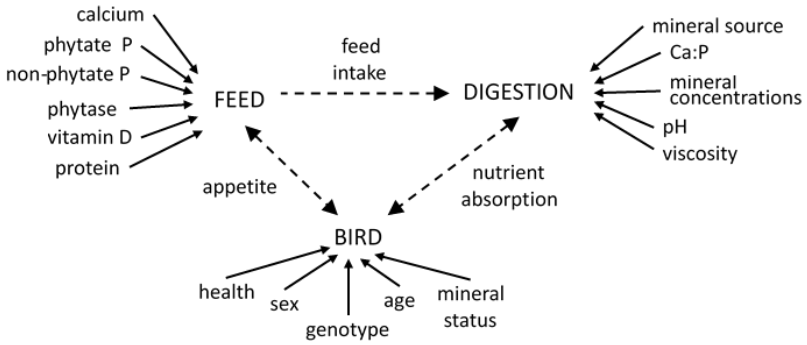

Figure 4.1 Factors affecting calcium and phosphorus digestion and growth in broilers

Deterministic modelling was chosen to provide a framework within which the fate of Ca and P in the broiler might be understood and quantified.

In practice, providing nutrients for the needs of a flock of broilers is often conceptualised as a supply-driven process that follows the direction of nutrient flow and time: Feed is manufactured, based on standardised requirements. It is presented to the animals *ad libitum,* and they consume a certain amount of it. Some of this feed is available at the wall of the GIT and the birds absorb this and meet or fail to meet their requirements for growth (see Figure 4.2).

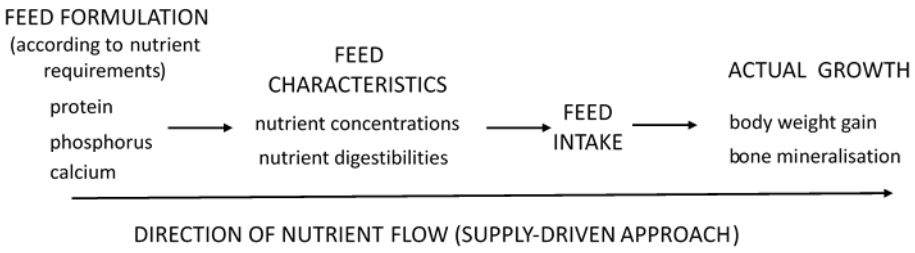

Figure 4.2 Feed formulation in practice

In contrast, a deterministic model takes a demand-led approach (see Figure 4.3), first considering the potential growth of the animal, which is dependent on its genotype. The nutrients required to support this growth are quantified and hence the optimum amounts to be absorbed across the wall of the GIT are determined. The lumen of the GIT acts as a reactor that makes nutrients available for absorption. In amino acid nutrition, standardised digestibility coefficients can be used to describe the availability of the various amino acids in each ingredient. The sum of the products of these with the concentrations of the amino acids and proportion of the ingredient in the diet provides an estimate of the available amino acids in the complete diet. Feed intake is regulated to provide sufficient digestible amounts of the first-limiting nutrient in the feed – usually an amino acid. The ability of the bird to consume feed to meet its needs determines feed intake but if other constraints (such as bulk or temperature) limit its ability to consume sufficient amounts of the feed that is presented to it, growth is, in its turn, compromised.

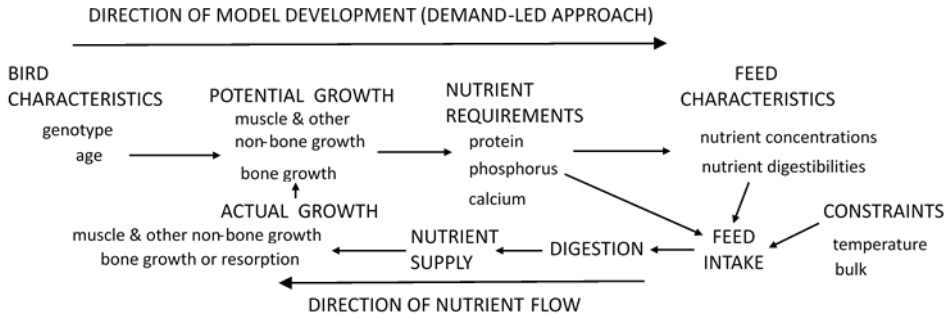

Figure 4.3 Proposed modelling process for Ca and P digestion and growth in broilers

While the modelling approach has been used successfully to optimise broiler nutrition for protein and energy (Emmans, 1981; Bastianelli et al., 1996; Gous et al., 1999; Eits, 2004; Sakomura et al., 2005;

Gous, 2014), the principles described above may not be sufficient to model mineral nutrition. It is not clear to what extent the bird has the ability to regulate feed intake if a mineral is first-limiting in the diet (Misiura et al., 2020). Feed intake may actually be inhibited by a diet that is deficient in P (Létourneau-Montminy et al., 2010). The critical role that P plays in metabolism (for example, the role of ATP) may mean that the bird is unable to metabolise other nutrients when there is insufficient P in the diet and hence feed intake may be reduced. Furthermore, the bird has the capacity to increase the flow of some minerals across the wall of the GIT into the bloodstream according to its needs, through the mechanism of active transport (Huber *et al*., 2015). This challenges the notion of a constant digestibility value for minerals in a given feed ingredient and the additivity of the digestible portions in a formulated diet. The fate of nutrients once they are absorbed into the body must also be understood: current models take into account that while amino acids are absorbed if available, they are differentially processed (into proteins or fat) according to the requirements for growth (Gous *et al.*, 1990). Conversely, absorbed Ca and P that is not immediately required by the bird for muscle, bone growth or metabolic activities is mostly excreted.

The modelling approach has proved its value in improving the precision of broiler nutrition. It has also guided research in this field to answer the critical questions that provide a better understanding of the underlying mechanisms of growth and nutrition. The integration of a broiler model with feed formulation and broiler enterprise optimisation has allowed producers to improve margins while minimising nitrogen pollution (Gous, 2014).

If the problem of mineral nutrition to allow precise feeding, thereby reducing cost and environmental impacts, may best be addressed through a modelling approach, what information is required to develop such a model (or set of models) and to integrate this with an existing system of broiler management? The following are suggested as the minimum data that are needed:

- *The growth of minerals in the broiler body under ideal and non-ideal conditions must be quantified.*
- *The availability of nutrients ingested by the birds to meet the requirements for growth, maintenance and endogenous losses must be understood and quantified.*
- *Mechanisms that regulate feed intake and mineral absorption must be taken into account.*

These provided the objectives for a systematic literature review. It is possible that, over the last sixty years, the necessary data have been published. To allow the literature to be assessed in an unbiased way and to ensure the inclusion of as much of the available data as possible, it was decided that a systematic literature review would be an appropriate approach.

4.1. The role and nature of the systematic literature review

A systematic literature review (SLR) is a tool used to investigate all of the research which is relevant to a particular problem (Sargeant and O'Connor, 2014). Unbiased assessments of the current state of

knowledge on a particular issue have become particularly challenging as the number of scientific articles has increased in recent decades (O'Hagan *et al.*, 2018). An SLR provides a more repeatable and scientific method to *identify, asses and synthesize* the available information (Sargeant and O'Connor, 2014, p.3).

The SLR method used in this study adopted a practicable approach, using two citation index databases to search for articles. This was followed by a scan of the abstracts of all articles returned by the searches. An iterative process of elimination narrowed the collection of articles down to those reporting studies of broilers that included variables relevant to the objectives. The full text of the remaining articles was examined to determine if the data could support a deterministic model.

Although an SLR is often conducted to answer a very specific question, this paper seeks to explore the state of the body of literature with respect to a set of questions with a specific objective: modelling Ca and P in the broiler.

4.2. Search terms

Key words taken from the research questions were used to develop the search terms to be used on the chosen data bases. To ensure that the "controlled vocabulary" of the literature was used, keywords found in review studies published in World's Poultry Science Journal were examined. The search used was "calcium AND phosphorus" and a scan of the abstracts excluded layer and broiler breeder studies, other types of poultry and non-nutrition studies. Of the 46 reviews returned by the search, 11 were focussed on mineral nutrition in broilers. The keywords from these reviews are summarized in Table 4.1. Phytase, phytate or phytic acid appear in 9 of them, reflecting the dominance of studies of this enzyme in the research on Ca and P. It was not considered necessary to include this in the systematic search: this literature will be captured in a search that includes phosphorus. The keyword that appeared repeatedly and was directly relevant to the research questions was digestibility.

Reference to the research questions suggested that further terms would be necessary to capture the information required to build a model. Growth and body composition, particularly skeletal development in the case of Ca and P, must be understood. These minerals must be absorbed and retained after they have been digested. The third research question requires information on feed intake, although this is regularly reported in nutrition studies.

Table 4.1 WPSJ review articles on calcium and phosphorus nutrition in broiler

Authors	Keywords
Cowieson and Bedford (2009)	phytate; carbohydrase; endogenous losses; amino acids; energy; poultry
Cowieson *et al.* (2009)	phytate; phytase; endogenous losses; amino acids; energy; poultry
Cowieson *et al.* (2011a)	phytase; dosage; phytate; inositol; monogastrics
Nahm (2007)	phase feeding; phosphorus excretion; calcium and phosphorus ratio; phytic phosphorus; vitamin D; total phosphorus; phytase; total tract digestibility
Rodehutscord and WPSA (2013)	phosphorus; availability; determination; standard protocol; broiler; phytase; digestibility; broilers
Sebastian *et al.* (1998)	digestibility; growth; microbial phytase; phytic acid; poultry
Shafey (1993)	available phosphorus; bone; calcium; chickens; growth; phosphorus
Shastak and Rodehutscord (2013)	phosphorus; availability; evaluation; approaches; history
Singh (2008)	phytic acid; phytase; phosphorus; protein; digestibility; pollution; poultry
Vieira *et al.* (2018)	chelator; feed acidification; feed enzyme; pH; phosphorus; phytate
Wilkinson *et al.* (2011)	broilers; calcium; choice feeding; phosphorus; phytase; phytate

The final composite search that was applied to the Web of Science and Scopus citation index databases was: (*Calcium* OR *Phosphorus*) AND *broiler** AND ((*digest** OR *absor** OR *retention* OR *utili?ation*) OR "*feed intake*" OR ("*body weight*" OR *growth*) OR *skelet** OR *"body composition"*).

4.3. Refinement of reference collection

Papers returned by the searches covered a broad range of topics related to Ca and P in poultry and other species. The searches (reference information and abstracts) were imported into EndNote reference manager, which facilitates the identification of duplicates and allows the organisation of references into subject groups.

4.3.1. Removal of extraneous papers

Once references without abstracts and duplicates from the two online databases had been removed, the titles and abstracts of all the remaining papers were scanned. Filters were applied to identify those which might provide data that are directly relevant to this project. These filters are summarised in Table 4.2.

Table 4.2 Summary of filters applied to the database

Criterion	Included	Excluded
Bird type	broiler	other species, layers. broiler breeder
Study type	nutrition	endocrinology, pathology, pharmacology, toxicology
Test diets	soybean/corn/wheat	alternate grains (e.g. barley, DDGS, triticale)
	phytase, vitamin D	alternate protein sources (rapeseed meal, peas)
	sources of Ca and P	additives, minerals other than Ca & P

Only papers that reported on broiler studies were included. Eliminated for this reason were mostly papers on layers, broiler breeders, turkeys, ducks and geese. While these papers might provide insights into some physiological mechanisms, they would not record absolute mineral measurements that could be used in a broiler model.

Furthermore, the search found papers on aquaculture and the application of poultry litter to soils, and these were eliminated in the same scan. The next cull was of papers which studied broilers but in the fields of toxicology, pathology, environmental factors (temperature, cage enrichment, litter management) and those nutrition papers unrelated to Ca or P (lipid and amino acid studies). A number of papers on aflatoxicosis, heavy metal toxicity, water quality and pathologies such as necrotic enteritis were eliminated at this stage. The only significant deficiency condition considered was tibial dyschondroplasia.

The abstracts of papers reporting on studies of supplements other than phytase and vitamin D were scanned to identify any that reported on effects on Ca and P retention, and these were not deleted from the EndNote library. Those eliminated covered such a wide range of additives that it seemed unlikely that any comparison would be possible, and any results were likely to be confounded by the effects of the additives. A few were retained in the Physiology group if they appeared to offer an insight into factors other than Ca, P, nPP, phytase or vitamin D that might affect Ca and/or P metabolism. These were retained for informative, rather than quantitative analysis purposes. To ensure clarity further, studies in which the diets were based on grains other than wheat and maize were removed. The abstracts of these studies were examined, and the studies were reclassified rather than eliminated if they appeared to offer an insight into another aspect of Ca and P nutrition, such as the action of phytase or bone growth. Most had as their primary goal testing different inclusion levels of the alternative feed ingredients, often against a corn-soy control diet.

The number of papers removed at each stage of the filtering process is shown in Table 4.3. The initial search was carried out in July 2019. The search was rerun in July 2020, adding a further 39 relevant papers to the database.

Table 4.3 Number of references returned by databases and eliminated (July 2019 search)

Number of results				Eliminated				Total left
Web of Science	Scopus	Duplicates	Total without duplicates	No abstract	Species / Bird type	Study field	Supplements/ ingredients	
2218	1651	1073	2796	160	689	440	530	977

4.3.2. Keyword assignment and categorisation of broiler nutrition papers

The nutrition papers that remained were initially categorised by the information in their keywords and from their abstracts, into Endnote groups, as shown in Figure 4.4. Full text articles of as many as possible of the references were added to the database as file attachments. It is interesting to note that almost exactly half of the studies which remained at this stage examined the use of phytase. This supports the contention that the introduction of this enzyme to broiler diets has driven the increase in research into Ca and P nutrition.

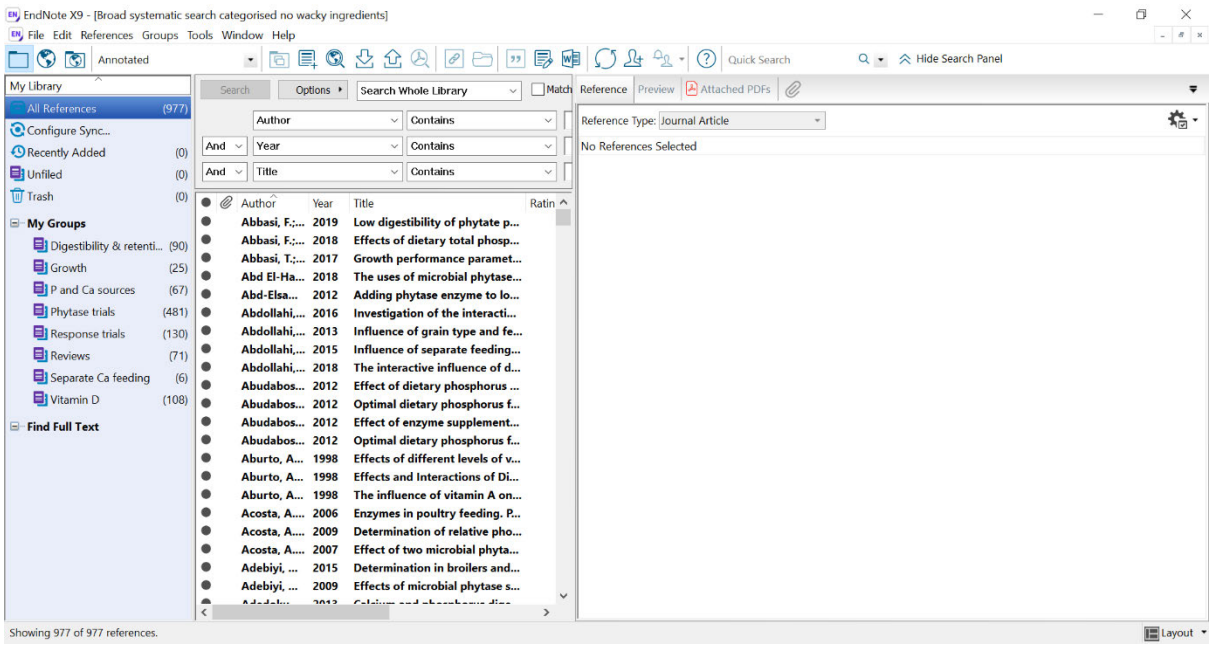

Figure 4.4 Initial categorisation of papers in EndNote

The categories were developed in an attempt to group together the papers most useful in answering each research question. The categories were modified as a closer examination of the papers proceeded. Papers were also moved between categories as more information than was apparent in the abstract became available from the full text. EndNote makes it possible for references to appear in more than one group. This was particularly useful with regard to the phytase studies. 155 studies tested a positive control, a negative control and the negative control plus phytase which meant that they were not useful for establishing Ca and P response relationships. However, there were also studies that included a range of Ca and/or P levels with and without phytase. These might provide valuable data with respect to the research questions.

The abstract of every remaining paper was scrutinised more closely to identify those in which both the independent and dependent variables were chosen so that the results could be used for a broiler model of Ca and P. For instance, if several different levels of Ca and P were fed but only BWG, FI and tibia ash were measured, this would provide insufficient information. Similarly, if the diets consisted of a positive control, negative control and phytase only, ileal digestibility values would not be useful for establishing absorption responses to changing Ca and P levels. The full text was also studied if the abstract was ambiguous (e.g. in the use of terms such as utilisation or digestibility). Keywords related to the research questions were assigned to each paper in EndNote to facilitate the categorisation of the studies for their usefulness in different aspects of the mode. These are summarised in Table 4.4 and give an indication of the nature of the studies that were commonly encountered in this systematic search.

Table 4.4 Keywords assigned to Ca and P studies

Keyword	Study characteristic
Independent variables:	
phosphorus response/ calcium response	diets contained three or more levels of P/Ca
age response	measurements taken at three or more ages (growth and development studies)
calcium: phosphorus	at least three different Ca: P ratios tested in diets in conjunction with responses
phytase	two dietary levels of phytase (usually 0 FTU/kg and a commercially recommended level)
phytase response	diets contained three or more levels of phytase
PC, NC Phytase	positive control (adequate Ca & P) negative control (low Ca & P), negative control plus one level of phytase
enzyme combination	phytase fed in combination with other enzymes e.g. xylanase, carbohydrase
vitamin D response	diets contained three or more levels of vitamin D
phosphorus source/ calcium source	tests different P/Ca sources by substitution or regression methods
particle size	feed or limestone particle size varied in diet
pelleting	diets varied in feed form (mash, crumbles or pellets)

Dependent variables:	
body composition/ body protein/ body ash/ body phosphorus / body calcium	whole body analysis carried out and protein/ash/ P/ Ca analysed, with or without feathers, empty body weight or full body weight reported. carcass composition without internal organs included
bone development	some measure of bone growth recorded with measurement at more than two ages
tibia ash	mass or proportion of ash in tibia measured
tibia phosphorus/ tibia calcium	P/Ca proportion of tibia mass or tibia ash measured
amino acid retention/ phosphorus retention/ calcium retention/ nitrogen retention	excreta collection and analysis of AAs/P/Ca/CP (total or sample with marker), retained proportion of AAs/P/Ca/CP ingested calculated
plasma phosphorus OR plasma calcium	serum/plasma P/Ca measured
digesta viscosity	viscosity of digesta measured
GIT pH	pH of digesta measured
phosphate transporter	responses in NaPi-IIb and other transporters measured
endogenous phosphorus / endogenous calcium	endogenous phosphorus/ calcium losses measured
phosphorus excretion/ calcium excretion	P/Ca excretion but not retention measured
phosphorus digestibility OR calcium digestibility OR amino acid digestibility	collection of ileal contents with marker, P/Ca/AAs absorbed calculated as a proportion of P/Ca/AAs ingested
Other keywords	
review	review of the literature
modelling	papers on modelling in animal nutrition were included as the literature search was expanded

Almost exactly half of the studies (481 out of 977) selected from the 2019 search examined the use of phytase. This supports the contention that the introduction of this enzyme to broiler diets has driven the increase in research into Ca and P nutrition. 163 studies tested a positive control, a negative control and the negative control plus phytase which meant that they were not useful for establishing Ca and P response relationships. However, there were also studies that included a range of Ca and/or P levels with and without phytase. Adding “phytate” OR “phytic acid” to the search in July 2020 added five review papers ((Selle *et al.*, 2000; Cowieson, 2005; Choct, 2006; Selle *et al.*, 2012; Moss *et al.*, 2019) six papers offering insights into the nutritional physiology of phytate digestion (Onyango *et al.*, 2006; Liu *et al.*, 2008; Cowieson *et al.*, 2011b; Truong *et al.*, 2014; Gonzalez-Uarquin *et al.*, 2020; Greene *et al.*, 2020) and eight phytase studies to the database.

4.4. Selecting papers to answer the research questions

Papers were assessed to ascertain if the variables contributed to clarifying different aspects of each research question, so that the different modules of the model could be developed.

4.4.1. Growth of minerals in the broiler body under ideal and non-ideal conditions.

To quantify growth responses, a range of dietary mineral levels must be fed, and measurements must be taken at different ages. Whole body composition or absolute mineral retention at a series of different ages could be used to model mineral growth in the broiler.

A study could be used for growth model development if it met the criteria summarised in Table 4.5.

Table 4.5 Selection of studies for development of growth model

Diets	Measurements
Ca, P and CP analysed	Feed intake AND excreta mass reported for total retention of P, Ca and protein
	OR
	Whole body composition, empty, organs included, ideally defeathered
Vitamin D level adequate	BWG reported until at least 56 days of age
Fed ad lib	Sampling at more than two ages
Ca & P levels in diets consistent from day old	At least two measurements in first three weeks

4.4.1.1.Body composition

12 studies out of the entire database included whole body composition analysis (see Table 4.6)

Only two of these met the criteria for model development. Caldas *et al.* (2019) calculated Gompertz growth function parameters for protein, Ca and P growth and from these the allometric relationship between protein and each mineral could be estimated. The feathers were included in the carcass analysis and this protein would have to be removed using known feather growth calculations. Hurwitz and Plavnik (1986) reported the mineral content of defeathered birds but the genotype used in this study is likely to be different from the modern broiler.

Table 4.6 Body composition studies

Authors	Sampling	Diets	Body composition analysis	Meets criteria?
Broadbent *et al.* (1981)	no, 56 day old	standard broiler starter and finisher	eviscerated, dissected	No. Only one slaughter age
Caldas *et al.* (2019)	1, 4, 7, 12, 17, 22, 27, 33, 39, 47, 54 and 60	Cobb standard starter, grower, finisher, withdrawal	EBW with feathers	Yes. Feathers must be removed
Hamdi *et al.* (2015b)	14 d	3 levels Ca 4 levels P	whole body, possibly without right tibia and with gut contents Tibia ash	No. Only one slaughter age
Hemme *et al.* (2005)	10d, 16 d	control plus 3 P sources	g/kg DFF body ash, Ca, P	No. Only two slaughter ages, commercial starter to 10 d
Hurwitz and Plavnik (1986)	1,4,7,14,21,…70 d	standard NRC diets	mEq/kg defeathered carcass	Yes. Response to varying Ca & P not studied
Kadim *et al.* (2005)	42 d	unknown diets, organic acid trial	% DM, defeathered whole carcass	No. Only one slaughter age
Niess *et al.* (2005)	21, 35, 42 d	unknown diets, non-essential AA trials	whole body incl. feathers, g/kg fresh and DFF	No. Only one slaughter age up to 21 d
Olukosi and Adeola (2008)	0, 21 d	positive control, negative control, phytase, xylanase	growth of protein, fat, ash, Ca, P	No. Only two slaughter ages
Schoner *et al.* (1993)	0, 14 d	4 levels Ca 4 levels P Phytase	whole body incl. feathers, retention of ash, Ca and P	No. Only two slaughter ages
van Krimpen *et al.* (2013)	1750 g	3 levels Ca 2 levels P	whole body with calculated feather amounts added back feathers,	No. Only one slaughter age
Williams *et al.* (2000b)	4, 11, 18, 25, 32, and 39 d	Ross standard starter and grower	DFF tibia and muscles analysed, Ca and P determined	No. Whole body not analysed
Zelenka (2012)	sample every two days up to 22 d	standard starter	EB with feathers analysis to 3 weeks, exponential curves fitted	No. Only allometric and exponential growth coefficients reported.

4.4.1.2.Calcium and phosphorus retention

Measurements that determine the amount of Ca and P that is retained by the broiler at a series of ages would indicate the growth of these minerals in the body. Retention studies for broilers may be performed using a marker in the feed that indicates the proportion of ingested mineral that is retained. If the diet

composition and feed intake are known for the same period as excreta collection, absolute retention can be calculated. An alternative method is complete excreta collection, analysis and comparison with intake. This provides a more direct measure of the mineral that remains in the animal but depends on accurate measurement techniques.

The EndNote library was examined for studies in which both P and Ca retention were measured at a series of different ages. Diets were either standard levels of Ca, P, protein and energy or varying mineral levels in otherwise standard diets. Out of 27 studies that met these conditions, only two measured retention more than once and these did so twice (Plumstead *et al.*, 2008; Jamroz *et al.*, 2011).

4.4.1.3.Bone growth

Létourneau-Montminy *et al.* (2015) and Misiura *et al.* (2020) modelled bone growth separately from soft tissue growth in pigs. To implement this for broilers, the growth of minerals in the skeleton would have to be quantified. Angel (2007) commented that no previously published study provided insights into mineral deposition in the skeleton in the first two weeks of the broiler's life. In this study, birds were sampled at 0, 2, 4, 6, 10 and 14 days of age and the ash, Ca and P in the skeleton were analysed. To model skeletal growth, the Ca and P content of the skeleton would have to be analysed at least weekly beyond this age, but these data have not been published.

In the EndNote library, 29 studies were identified in which particular bones, such as the femur and tibia, were analysed for ash, Ca and P. In 15 of these, tibiae were analysed at a single age (21 d in 60% of these) and a further 7 at two or three different ages (Itoh and Hatano, 1964; Barreiro *et al.*, 2009; Barreiro *et al.*, 2011; Bello *et al.*, 2019). This left seven studies in addition to Angel (2007) in which bones were serially sampled and which might therefore be used to model bone mineral deposition if a relationship between the bones sampled and the skeleton can be established. These studies are listed in Table 4.7.

Only one study provided diets with two different Ca and P levels (Jamroz *et al.*, 2011). This means that ideal bone growth might be estimated from the other studies, but bone growth under Ca or P deficiency, excess or imbalance would still be a matter for conjecture.

Table 4.7 Bone growth studies

Authors	Age	Treatments	Measurements
Angel (2007)	2-14 d	Standard diets	skeleton ash, P, Ca
Han *et al.* (2015)	7, 14, 21, 28, 35, and 42 d	Standard diets (0.99% rCa, 0.45% nPP)	tibia ash, P, Ca femur ash, P, Ca metatarsus ash, P, Ca
Jamroz *et al.* (2011)	1, 7, 14, 21, 28 d	2 levels of Ca 2 levels of P	tibia ash, P, Ca
Li *et al.* (2017a)	0, 7, 14, 21, 28, 35, and 42 d	Standard diets	tibia ash, P, Ca
Müller *et al.* (2012)	7, 14, 21 & 42 d	Electrolyte balance, 6 levels	femur ash, P, Ca, protein
Sanchez-Rodriguez *et al.* (2019)	0, 3, 7, 10, 21, 37 d	Standard diets (NRC requirements)	Ca:P in tibia tibia phosphate
Skinner and Waldroup (1995)	0, 7, 14, 21, 28, 35, 42, 49 56 d	Standard diets	tibia (with cartilage) tibia ash, P, Ca
Williams *et al.* (2000b)	4, 11, 18, 25, 32, and 39 d	Standard feeds	tibia ash, P, Ca 3 sections

4.4.2. Availability of ingested calcium and phosphorus

True amino acid requirements, as determined from growth models rather than standardised tables, can be met most precisely when the digestibilities of the amino acids in dietary ingredients are known and when feeds are formulated accordingly (Ravindran *et al.*, 2017). If Ca and P are to be modelled, then it is essential to know how much of the dietary mineral intake is available to the bird for metabolism and growth. Robust, repeatable digestibility coefficients for the Ca and P in different feedstuffs and conclusive evidence of the additivity of calculated digestible Ca and P in complete diets would be the simplest way in which availability could be calculated. These could be included in the matrix of a feed formulation program.

An alternative approach that has been implemented in two pig models in recent years includes standard digestibilities for some common mineral sources but uses more general rules for the availability of P in other feed ingredients and hence adjusts digestibility for the diet as a whole (Létourneau-Montminy *et al.*, 2015; Misiura *et al.*, 2020). Interactions between Ca and P, and the effect of exogenous phytases are also modelled. This approach produces an available output of P from the complete feed rather than the cumulative, additive input of the feed ingredients.

The research objective of the digestion component of this modelling study was to understand and quantify the availability of nutrients ingested by the birds to meet the requirements for growth, maintenance, and endogenous losses. Studies of the digestibility of Ca and P sources, or that measured the digestibility of complete diets with changing levels of Ca and/or P might shed light on this problem. The criteria used to select studies for closer examination are summarised in Table 4.8.

Table 4.8 Selection of studies for development of digestion model

Diets:	Birds:	Measurements:
• different sources of P or Ca OR P or Ca response tested • graded levels of source fed (regression method) OR at least three levels of P or Ca • semi-purified (P and Ca free) diets OR maize-soyabean or wheat-soyabean basal diets • no additives except phytase • feed intake for the assay period reported • feed composition reported • adequate protein, energy and vitamin D fed, not varied with treatments	• 35 days or younger • male and/or female, • modern, selected broiler strains	• ileal or precaecal digestibility of Ca AND P • sampling from second half of ileum • indicator in diet, concentration in feed and ileal contents analysed • digestibility reported as proportion of total mineral in feed

The systematic literature review returned a few mineral and animal source studies in addition to those described in section 2.5.3.3. Two additional studies were published after the systematic review was completed, and these were added to the EndNote database. Some further details about the trials are included in Table 4.9.

Table 4.9 Ca and P source studies: animal and mineral sources

Authors	Sources	Dietary Ca P CP (g/kg)	Age (d)	Regression measurements
Barshan *et al.* (2019)	• DCP • bone meal	• 10-11.5 • 7.2-8.4 • 203-213	25-26	AID
Bikker *et al.* (2016)	• MCP • DCP • MDCP • DFP	• 4.4 • 3.2-3.4 • 181	28	one level of inclusion: common intercept
Cowieson *et al.* (2015)	• KH2PO4 • MCP • DCP • TCP	• 9 • 5.2-5.3 • 216	4-25	AID
Davin *et al.* (2020)	2 limestone sources	• 7.7-9.7 • 5.3-7.0 • 214	1-21	AID 2 levels of Ca and P
Dilelis *et al.* (2021b)	• 3 MBM sources • 3 PBM sources	• 8.78 • 4.19 nPP • 233	19-22	TID P direct method p-free diet
Hamdi *et al.* (2015a)	• limestone, • Ca chloride • protected TCP	• 5.7-8.5 • 0.67-0.95 • 220	1-14	AID Ca, P 4 levels of P

Ketels and de Groote (1988)	• MCP • DCP • DCP.H_2O • 3 X MBM	• 11 • 1.1-4.5 • not shown	1-21	6 levels of P
Kim *et al.* (2019b)	4 sources of limestone	• 6.3-6.9 • 3.01 • 170.1	23-24	AID Ca, P
Lamp *et al.* (2020)	• MCP • DCP • MDCP • DFP	• 7 • 2.2 nPP • CP not shown	1-21	AID P direct
Munoz *et al.* (2020)	• MBM • plasma protein	• 0.8-4.1 • 0.7-3.4 • CP not shown	17-21	TID P regression 2 basal diets
Mutucumarana *et al.* (2015b)	3 samples of MBM	• 8.03-9.89 • 3.57-4.45 • 117-145	21-28	TID P regression method
Mutucumarana and Ravindran (2016)	4 different samples of MBM	• 1.4-8.73 • 1.11-5.15 • 10.72-42.88	28-31	TID P direct method
Omara *et al.* (2020)	• DCP • $CaPO_4$ nanopowder	• no analysis	0-14	AID P 4 levels of P
Paiva *et al.* (2013)	• limestone • seaweed	• 6.3-9.7 • 8.2-8.4 • 227-248	0-7	AID Ca, P
Rezvani *et al.* (2019)	• $CaCO_3$ • oyster shell • egg shell	• 0.78 • 0.32 aP • 189	11-?	AID Ca
Shastak *et al.* (2012b)	• MSP (3 wk) • DCP (3 wk) • MSP (5 wk) • DCP (5 wk)	• 8.2-12.8 • 4.3-5.9 • 255	16-20 d 30-34 d	TID P
Trairatapiwan *et al.* (2018)	• MCP • MDCP • DCP	• 3.9-5.0 • 3.0-4.0 • 210 (calc)	21-29 d	TID P
van Harn *et al.* (2017)	• MCP • DCP • bone meal	• 5.0-5.6 • 4.3-4.8 • 224-228	14-24	TID P one level common intercept
Walk *et al.* (2012b)	• MCP • HSC	• 5.8-10.6 • 5.9-6.9 • 248-253	0-21	AIDP, Ca
Wang *et al.* (2022)	• 5MCDP sources • 3 DCP sources	• 9.0 • 5.3 • 230	21-26	AID P substitution
Zhang and Adeola (2018)	• limestone • DCP	• 3.2-5.7 • 4.3-6.6 • 165-177	22-27	TID Ca

The TIDP values of some mineral sources from the studies in are summarised in Table 4.10. It is apparent that there is considerable variability in digestibility values. This is in agreement with the findings of Rodehutscord *et al.* (2017) that P digestibility measurements are not consistent, even if the ingredient under consideration is unchanged and analyses are performed in the same laboratory.

While studies reported bone meal digestibility ranging from 42 to 69%, this may be a reflection of product variability as well as different trial conditions (Mutucumarana *et al.*, 2015b; Mutucumarana and Ravindran, 2016).

Table 4.10 Digestibility of mineral sources

Authors	MCP	DCP	MDCP	DFP	MSP
Bikker *et al.* (2016)	78.3	59.0	70.7	31.5	-
Shastak *et al.* (2012b)					
3 weeks	-	30.0	-	-	67.0
5 weeks		25.0			54.0
Trairatapiwan *et al.* (2018)	64.6	69.3	60.2	-	-
van Harn *et al.* (2017)	88.5	82.4	-	-	-
Ketels and de Groote (1988)	34.7	41.1	-	-	-

The variability in TIDP and TIDCa of animal and mineral sources undermines their usefulness in feed formulation. If robust digestibility values can be obtained only under strictly repeatable experimental conditions, it is doubtful whether they can accurately predict the mineral available to the bird in a commercial feeding setting.

The systematic literature review returned 15 studies of Ca and P digestibility in plant ingredients (Table 4.11). An additional study was included that was published after the systematic literature review.

Table 4.11 Ca and P source studies: plant sources

Authors	Sources	Ca, P and CP in diet	Age	Measurements
Adebiyi and Olukosi (2015)	• wheat-DDGS	• 2.0-4.2	14-21 d	TID P
David *et al.* (2021)	• soybean meal • canola meal	• 1.73-1.98 • 4.6-5.3 • 175-290	retention	TID Ca, P direct method
Ingelmann *et al.* (2019)	• 4 maize sources •	• 4.99-5.64 • 3.20-3.79 •	20-28 d	AID Ca, P PP disappearance
Kim *et al.* (2019a)	• HP sunflower meal • MSP	• 0.98-1.17 • 0.4-0.52 • 19.9-26.2	14-21 d	AID P, Ca graded levels of P, TID not calculated
Kupcikova *et al.* (2017)	• wheat • maize	• 3.68-4.96 • 2.26-3.74 • 184-246	24-30 d	TID P regression method 3 levels of P, varying Ca:P

Leytem *et al.* (2008a)	• maize • wheat • barley • high fat-low lignin oats	• 7.2-10.4 • 5.2-8.3 • 206-232	0-21 d	AIDP, PP Ca
Mutucumarana *et al.* (2014c)	• wheat • sorghum • soybean • DDGS	• 0.96-3.46 • 0.57-4.51 • 21-261	21-28 d	TID P regression method
Mutucumarana *et al.* (2015a)	• maize • soybean	• 0.49-5.6 • 0.56-4.52 • 17-317	21-28 d	TID P regression method (constant vs varied protein levels)
Olukosi *et al.* (2015)	• rapeseed meal	• 5.9-8.9 • 5.0-7.4 • 188-275	21-26 d	TID P regression method (varied protein)
Perryman *et al.* (2017b)	• maize	• 1-12.2 • 0.7-3.3 • 210	19-21 d	TID P regression method diets isonitrogenous and isocaloric, 3 collections
Rodehutscord *et al.* (2017)	• soybean meal	• 4.57-6.78 • 3.02-4.59 • 231-339	sampling at 21-28 d	TID P regression method protein and Ca varied
Rutherfurd *et al.* (2002)	• soyabean • wheat • maize • rice bran	• 0.3-3.1 • 2.0-18.3 • 76-182	fed for 2h on d 7	TID P direct method P-free diet
Shastak *et al.* (2014)	• maize • wheat	• 4.25-5.97 • 2.38-3.02 • 179-213	16-22 d	AID Ca, P TID P regression method
Trairatapiwan *et al.* (2019)	• maize • soyabean meal	• 3.3-5.2 • 2.5-4.0 • 180-308	22-29 d	TID Ca, P regression method
Witzig *et al.* (2018)	• wheat • triticale	• 2.50-5.96 • 2.50-4.36 • 217-272	20-26 d	TID P regression method 2 levels of inclusion
Zaefarian *et al.* (2013)	• maize • wheat	• 11.3-11.4 • 8.0-8.2 • 256-273	21 d	AID Ca, P

The variation in ileal digestibility coefficients for common plant P sources is considerable. This is in spite of assays mostly being carried out at similar ages (20-28 days of age). Both the differences inherent in the ingredients and differences in the physiological status of the birds are likely to contribute to this. Similarly, Ca digestibility studies have produced different digestibility coefficients for the same ingredient (Angel *et al.*, 2022), but here assay methodology may also have been a contributing factor.

If the alternative approach is to be followed, formulated diets might be defined in terms of their chemical composition and then a reliable method devised for calculating the available Ca and P from these and

some attributes of the bird (actual requirements). This approach has been considered in pigs, with absorption and retention outputs in g Ca/kg BW/d (Misiura *et al.*, 2018). Independent variables were tCa, tP, NPP and PP intake (g /kg BW/d) and phytase (FTU/kg BW/d). The interactions between the various dietary components (Ca, nPP, PP and phytase) were quantified. Studies that could be used for this analysis would have different levels of Ca and/or P and/or phytase. Comparable ileal digestibility methods would be required. Studies meeting these criteria that were identified are summarised in Table 4.12. Four studies that were identified after the systematic review was complete were also included.

Where phytase was included at two levels and the zero inclusion was part of a factorial design, this is described as "3 levels of phytase" since the zero level of inclusion provides another data point.

It is apparent that there is a wealth of data on the effect of different levels of Ca, P and phytase on digestibility. These provided the basis of a meta-analysis to establish appropriate regression coefficients for an empirical model of Ca and P availability. In most of these trials AID of nutrients was reported as the digestibility variable, and where TID was measured, AID values were also tabulated. Source studies were therefore included where both AIDCa and AIDP were reported and where different mineral levels were used in the feed. Endogenous losses were considered in the model as a function of DMI, so that the response of digestibility to changes in nutrients would be the same, regardless of digestibility measure.

Table 4.12 Ca and P response studies

Authors	Age (d)	Treatments	Measurements
Adeola and Walk (2013)	24	2 levels Ca 4 levels P 3 levels phytase	AID Ca, P tibia ash
Akhavan-Salamat *et al.* (2011)	35	1 level Ca 3 levels P	AID Ca, P, CP tibia ash, Ca, P
Akter *et al.* (2017)	1-24	3 levels Ca 2 levels P 2 levels phytase	AID Ca, P, CP RCa, P
Amerah *et al.* (2014)	21	4 levels Ca 2 levels phytase	AID Ca, P, CP
Babatunde *et al.* (2022)	12-23	2 levels Ca 4 levels tP/PP 5 levels phytase	AID Ca, P, N Foot ash
Bikker *et al.* (2016)	24, 28	4 levels of P 4 sources of P	TID Ca, P R P, Ca: 26, 27, 28 d
(Bowen *et al.*, 2022)	44	2 levels Ca 2 levels P 4 levels phytase 2 phytase sources	AID Ca, P tibia ash

Bradbury *et al.* (2017)	1-14	2 levels Ca 2 sources Ca 2 levels phytase	AID of P, Ca, other minerals Foot ash
Bradbury *et al.* (2018)	1-28	2 levels Ca 2 sources Ca 2 particle sizes 2 levels phytase	Ileal digestibility of P, Ca, other minerals Foot ash
Dersjant-Li *et al.* (2018)	10, 21, 42	5 Ca levels 3 (nP)P levels 2 phytase levels	AID Ca P, R Ca, P tibia ash, Ca, P
Dersjant-Li and Kwakernaak (2019)	21	2 Ca levels 4 P levels 5 phytase levels	AID Ca, P R Ca, P tibia ash
Fallah *et al.* (2020)	1-21	5 levels Ca 5 level P	AID Ca, P tibia, toe ash
Hamdi *et al.* (2015a)	14	4 P levels 3 Ca sources	AID Ca, P tibia ash
Jendza *et al.* (2006)	21	4 P levels 5 phytase levels	AID P, N
(Kiarie *et al.*, 2015)	22	2 Ca levels 2 P levels 6 phytase levels	AID Ca, P, Mg, Na, amino acids
Kim *et al.* (2018)	28	4 Ca levels 2 particle sizes 2 phytase levels	AID Ca, P
Kim *et al.* (2019a)	21	4 P levels 2 phytase levels	AID P
Leytem *et al.* (2008b)	21	4 levels Ca 3 levels phytate P	AID P
Li *et al.* (2014)	20	4 levels Ca 2 levels P	AID Ca, P
Li *et al.* (2018)	9, 21	3 levels Ca 3 levels phytase	AID Ca, P
Mutucumarana *et al.* (2014a)	21-27	3 levels Ca 1 level P	AID Ca, P, N, fat
Perryman *et al.* (2016a)	25-26	5 levels Ca 4 levels P	TID Ca, P
Plumstead *et al.* (2008)	21	4 levels oCa 3 levels of PP	AID Ca, P, PP
Powell *et al.* (2011)	21	3 levels Ca 2 levels phytase	AID Ca, P
Ravindran *et al.* (2006)	21	3 levels Ca 3 levels PP 4 levels phytase	AID Ca, P, AAs
Rousseau *et al.* (2016)	21, 35	3 levels of P 3 levels of Ca dietary change 21d	AID Ca, P

Santos *et al.* (2008)	21, 35	4 levels of P 4 levels of Ca 4 levels phytase	AID Ca, P, CP, amino acids
(Sommerfeld *et al.*, 2018b)	22	4 levels of P 4 levels of Ca 4 levels phytase	AID Ca, P tibia ash
van Krimpen *et al.* (2013)	35	3 levels Ca 2 levels P	AID Ca, P
van Krimpen *et al.* (2016)	10, 21, 30 and 38	6 levels Ca 6 levels P	AID Ca, P
Walk *et al.* (2022)	11-24	5 levels Ca 1 level P	AID Ca, P
(Walters *et al.*, 2019)	14, 28	2 levels Ca 2 levels P 7 levels phytase	AID Ca, P tibia ash, Ca, P
Wilkinson *et al.* (2014a)	35	2 levels Ca 4 levels P	AID Ca, P, CP
Wilkinson *et al.* (2014c)	28	13 levels Ca 14 levels P	AID Ca, P, N, amino acids and DM
Wilkinson *et al.* (2014b)	21	4 Ca levels Separate Ca source	AID Ca, P, N, amino acids and DM
(Woyengo *et al.*, 2010)	22	2 levels Ca 2 levels P 4 levels phytase 2 feed forms	AID Ca, P, amino acids
Zhang and Adeola (2018)	27	3 levels Ca 3 levels P	AID Ca, P
Ziaei *et al.* (2008)	21	4 levels Ca 2 levels P	AID Ca, P

4.4.3. Feed intake

Almost all of the Ca and P response studies identified in the systematic literature reported feed intake and dietary Ca and P levels. This was a broader subset of the response data than the digestibility studies, because even studies reporting only BWG and FCR could be used to assess the effect of minerals in the diet on feed intake. Rigorous selection criteria were applied to ensure that studies were comparable: only those with analysed levels of Ca and P and reporting feed intake weekly were used.

4.5. Discussion

This systematic review of the literature revealed that the research necessary for the development and evaluation of a model of Ca and P growth in the broiler has not been published.

Serial slaughter and analysis of Ca and P provide an indication of the quantity of these minerals that is deposited in the body. It was apparent that such body mineral composition studies are rare. The dearth of studies may be due to the work involved (De Groote and Huyghebaert 1997) and the practical

challenges of obtaining a homogeneous, representative sample of body material for analysis (Shastak and Rodehutscord 2013). In spite of a paucity of data, the studies identified by the systematic search were used to develop a preliminary model of Ca and P growth in broilers. This will be refined as further data become available from ongoing research, while providing an indication of where that research should be focussed.

The model is based on an isometric relationship between body protein and Ca and P under ideal conditions, as suggested by Caldas *et al.* (2019). Isometric coefficients derived from the body mineral and protein data in Hurwitz and Plavnik (1986) and Plavnik and Hurwitz (1983) were used for confirmation, although changes in genotype between these studies and Caldas *et al.* (2019) are likely. The carcass measurements in this study included feathers so that the protein in these had to be estimated and removed to calculate isometric coefficients between empty, feather-free body protein and Ca and P. Further serial slaughter data are required for more accurate parameterisation.

The model calculates actual growth from the available nutrients in the diet. Mineral retention values reported in the literature are almost exclusively conducted at a single age in each trial. This meant that no data were available to measure Ca and P changes over time when these minerals were present in the diet at varying levels. However, these data may be useful in evaluation of the broiler model.

Soft tissue and bone are modelled separately so that different priorities can be given to these tissues under dietary mineral constraints as proposed in previous pig models (Létourneau-Montminy *et al.* 2015; Misiura *et al.* 2020). However, insufficient data were available to simulate the prioritisation of these tissues under conditions of deficiency. A naïve system in which P is only allocated for bone mineralisation once full requirements for soft tissue are met was implemented. Protein growth and feed intake are constrained if the requirement for P for the bone-free body (BFB) is not met.

The model will be evaluated using data from the bone growth studies identified from this review, once the relationship between minerals in individual bones and whole skeleton is established.

Digestibility coefficient values for Ca and P in feed ingredients are not robust or repeatable. A deterministic, physico-chemical model that quantifies the soluble Ca and P that can be absorbed from the GIT is the ultimate goal but an empirical model that describes the interactions between ca, nPP, PP and phytase to calculate absorbable minerals was an interim measure that allowed the available Ca and P to be approximated and the growth model to be evaluated.

No consistent effect of dietary Ca or P on feed intake could be established. The known depression of appetite when P is deficient was incorporated through the suppression of protein growth and the resultant reduction in desired feed intake.

It is apparent that there is scope for further research that would improve Ca and P models. Trials in which the Ca and P levels in the diet are varied and soft tissue and whole skeletons are sampled at

intervals through the growing period will enhance our understanding of the prioritisation of bone and muscle growth under deficiency conditions. Feathers differ from the empty broiler body in amino acid profile and in their rate of maturing and are modelled separately as a consequence. A model that separates bone and soft tissue growth may incorporate a similar approach: the amino acid profile of feed for optimal skeletal muscle growth may not be identical to that for optimal bone growth. Different types of bone, whether cortical or trabecular, may respond differently to available mineral and amino acid supplies. The relationship between feed amino acid profile and diet Ca and P supply and skeletal muscle growth, bone growth and the mechanical properties of bone has not been explored fully.

The development of a deterministic model suggests avenues for research that would provide insights into the fate of Ca and P in the broiler body. The precision feeding and optimisation capabilities of an integrated model can address concerns about the depletion of non-renewable phosphate reserves and pollution of the environment by excreted P.

CHAPTER 5. MODEL DEVELOPMENT

Gous *et al.* (1999) pointed out the difficulties which nutritionists face in attempting to establish the nutrient requirements of broilers as genetic selection produces birds with increased growth rates. It was proposed that: "A well-founded and accurate theory of growth, body composition, and feed intake would allow requirements to be predicted.". This approach resulted in the development of the EFG broiler growth model, based on a set of propositions that form the basis of this theory (Emmans, 1987).

1. "A given animal at a given lipid-free weight has a potential lipid-free growth rate which it seeks to attain."
2. "A given animal at a given lipid-free weight seeks a particular level of fatness."
3. "The potential growth rate, and the fatness that the animal seeks, both depend only on what kind of animal it is, and its degree of maturity; they are inherent."
4. "An animal seeks from its environment the resources that it needs to attain its potential growth rate and the fatness that it seeks."

The present study sought to add a model of Ca and P in broilers to this growth model. This chapter describes ten propositions that formed the basis of such a model and the parameters required to implement it.

5.1. Protein growth

Proposition 1: The potential growth of protein and minerals in the feather-free body and feathers is genetically determined.

In the EFG model, the genotype is described in terms of its potential protein growth and feathering rates. The normal, or ideal, growth of other components, such as lipids, are calculated using allometric coefficients. The requirements for various body components can be ascertained, based on their growth in the body, maintenance requirements (including endogenous losses) and the efficiency with which nutrients are used for growth and maintenance.

The Gompertz function can be used to describe the potential protein growth in the empty, feather-free body (EFFB) and in feathers. Although alternative growth curves have been used for this purpose, for example, the logarithmic (e.g. Zelenka, 2012) and von Bertalanffy (Teleken *et al.*, 2017) equations, Emmans (2022) has argued that the Gompertz equation, with just three parameters, each of which having biological meaning, is the most appropriate equation to use for this purpose.

The Gompertz function may take the form

$$W_t = W_m \times e^{-e^{-B(t-t^*)}} \qquad (Eq.\,5.1)$$

Where W_t (kg) is the weight of the chemical component at age t

W_m (kg) is the body component weight at maturity

B (/d) is the rate of maturing of the component and

t^* (d) is the inflexion point or age (time) at which the growth rate is at a maximum

Since $ln\frac{\left[-ln\left(\frac{W_0}{W_m}\right)\right]}{B} = t^*$, where W_0 = weight at $t = 0$, this equation may also be written

$$W_t = W_m.e^{-e^{ln\left[-ln\left(W_0/W_m\right)\right]-B.t}} \quad (Eq\ 5.2)$$

and the absolute growth rate of an animal at weight W is

$$dW/dt = B.W.ln(W_m/W) \quad (Eq.\ 5.3)$$

W/W_m may be described as the degree of maturity (u) and hence equation 5.3 may be written

$$dW/dt = B.W.ln(1/u) \quad (Eq.\ 5.4)$$

An allometric relationship exists between different components of the body, whether chemical components or body parts, if they have the same rate of maturing (B).

The allometric relationship may be expressed as

$$y = a.x^b \quad (Eq.\ 5.5)$$

where y is the weight of one component of the body at time t, x is the weight of another component and a and b are constants, the allometric coefficient and allometric exponent respectively (Huxley, 1932).

Hence, if an allometric relationship between the weights of two components exists, a linear regression may be fitted such that

$$\ln(y) = ln(a) + b.ln(x) \quad (Eq.\ 5.6)$$

where a is the intercept and b the slope of the fitted line.

If the allometric exponent is 1, then there is an isometric, or directly proportional, relationship between x and y.

The parameters of the Gompertz equation for ideal EFFB protein (EFFBP) growth and feather protein (FP) growth have been determined for different broiler strains. Allometric relationships between EFFBP and lipid, water, and various body parts have been established (Hancock *et al.*, 1995; Gous *et al.*, 1996; Danisman and Gous, 2011).

The EFG model for the potential growth of the chemical components of a broiler formed the basis of the preliminary modelling work on Ca and P growth which was carried out in this project. An Excel spreadsheet was created with the parameters (rate of maturing, mature mass and initial mass) of the Gompertz equations for EFFBP (BP_0, BP_m and B_{BP}) FP growth (FP_0, FP_m and B_{FP}) (see Table 5.1) for the calculation of the potential growth of these components.

Table 5.1 Gompertz parameters for empty feather-free body protein and feather growth

EFFBP		FP	
BP_0 (kg)	0.005	FP_0 (kg)	0.0001
BP_m (kg)	1.4	FP_m (kg)	0.315
B_{BP} (/d)	0.044	B_{FP}(/d)	0.052

Allometric coefficients for the calculation of the weights of water, lipid and ash in the defeathered body were also entered (Gous *et al.*, 1999). These are summarised in Table 5.2. The allometric equations were of the form

$$W = a * {W_{FFBP}}^{b} \qquad (Eq\ 5.7)$$

such that a and b are the allometric coefficient and exponent respectively and W is the mass in g of the chemical component under consideration. W_{FFBP} is the mass of body protein in g at that age . Since ash growth has been shown to be isometric with protein growth, b is 1 for this component.

Table 5.2 Allometric coefficients for calculation of body components from body protein

Component	*a*	*b*
Water	3.4	0.88
Lipid	1.2	1.49
Ash	0.165	1

Model implication 1: Protein growth in EFFB and feathers was modelled using Gompertz functions with established parameters for different genotypes. Water and lipid were modelled using allometric relationships with EFFBP and with established coefficients for different genotypes.

5.2. Ash, calcium and phosphorus growth

Proposition 2: Under ideal conditions, there is a constant ratio (isometry) between EFFBP and body ash, Ca and P

It has been reported that the potential growth of ash in the broiler is isometric with the growth of EFFB protein, with a coefficient of 0.165 g ash/g body protein (Gous *et al.*, 1999). Eits *et al.* (2002) observed that although isometry held true for the carcass and organs combined, it was a result of an increasing ash weight/protein weight in the organs and a decreasing ash weight/protein weight in the carcass. An isometric coefficient of 0.15 g ash in defeathered carcass+organs /g body protein was reported (Eits *et al.*, 2002). Body composition was analysed at 200, 800 and 1600 g BW, so no sampling was done under 10 days of age. This is the period in which other studies suggest that the isometric relationship is uncertain (Hurwitz and Plavnik, 1986; Angel, 2007). Plotted values were averaged over 9 diets with different protein to energy ratios, fed at two FI levels. Ash weights for different dietary treatments were corrected to mean protein weights, and protein weights for different treatments were not reported, so that it was not possible to infer the effect of feed on the ash/protein relationship. Thus, this isometric

relationship has only been confirmed when the bird receives a balanced feed and not under conditions where either minerals or protein are deficient in the feed. In poultry (Murawska *et al.*, 2011) and pigs (Pettey *et al.*, 2015) the ratio of viscera to carcass decreases over the growth period, and this would also contribute to a constant average ash proportion. Shastak *et al.* (2012a) analysed whole carcass ash, Ca and P in 21- and 35-day-old broilers on different dietary P levels in a study which sought to establish a relationship between tibia P and whole-body P. The ratio of whole-body P to tibia P decreased with age, perhaps as a result of changing proportions of BFB and bone. The whole-body Ca/P ratio increased from 1.2 at 21 d to 1.4 at 35 d, which would also indicate an increased proportion of bone mineral. It was proposed that this was due to a deficiency in Ca and P supply for the rapid bone growth in the first 3 weeks. Work in pigs has suggested that the isometric relationship between body ash and body protein changes when the feed is unbalanced either for protein and energy or for protein and P (Misiura *et al.*, 2020).

The mineral composition of whole-body ash has not been established throughout the growth of broilers. Few studies have calculated the proportions of whole-body Ca and P and protein in defeathered carcasses with gut fill removed (Hurwitz and Plavnik, 1986; Niess *et al.*, 2005).

Caldas *et al.* (2019) included feathers and visceral contents in the analysis of whole-body protein, lipid, water, ash, Ca and P. Birds were sampled for chemical analysis between 1 and 60 days of age. The results of this study were reported as Gompertz parameters for the growth of these components (see Table 5.3). For accuracy, the mature weight of each component in the Gompertz equation should be obtained by extrapolation of observations made to about 15 weeks of age. Few studies have extended to this age, resulting in dubious estimates of mature weights. Longer trials include those that extended to 60 d of age (Caldas *et al.*, 2019) or 70 d of age (Hurwitz and Plavnik, 1986), but others were terminated at 1 600 g BW (Eits *et al.*, 2002) or even at 22 d of age (Zelenka, 2012).

The rate parameters calculated by Caldas *et al.* (2019) were similar for the components, suggesting allometric relationships between them. Furthermore, the similar values of t* for protein and Ca suggested an isometric relationship. Since most Ca is found in the skeleton, and P in the skeleton grows in proportion to Ca, this also suggests that the skeleton mineral mass was growing isometrically with body protein.

As it was the only study in which Ca, P and protein were analysed to 60 d of age, the isometric coefficients for Ca and P were initially developed from this study. Values for body protein, ash, Ca and P in g were extracted from this study. It was necessary to simulate FP growth from the known Gompertz parameters of the Cobb male broilers used in the study and to remove this from the feathered body protein weights.

Table 5.3 Parameters of the Gompertz equation for chemical components of the broiler body (including feathers and visceral contents)

Source: Caldas *et al.* (2019)

Response variable (g)	B	X_m	t^*
Body weight	0.047	5465	33.5
Protein	0.049	1001	34.5
Ash	0.051	108	31.7
Ca	0.047	27	34.4
P	0.048	21	33.5

B is the rate of maturing of each component, while X_m represents the weight of each of the components at maturity and t^* is the age at which growth rate is greatest.

A linear regression of the natural logarithms of the mineral masses on the natural logarithms of FFBP mass, suggested an isometric relationship between Ca, P and ash with FFBP (see Figure 5.1), since the slopes of the graphs are close to unity. Standard errors were 0.00842 for ash, 0.0447 for Ca and 0.0335 for P.

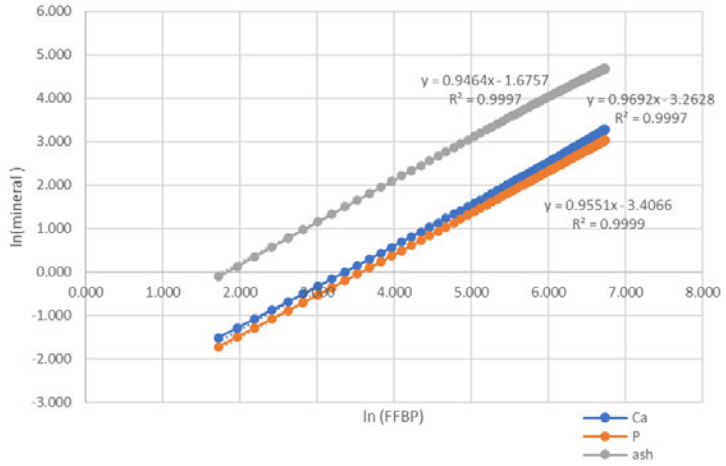

Figure 5.1 Results of the linear regression of ln(Ca), ln(P) and ln(ash) on ln(FFBP)

Source: after Caldas *et al.* (2019)

The isometric coefficients (*a* values in equation 5.5) were calculated to be 0.0312 g Ca/g FFBP, 0.0246 g P/g FFBP and 0.1314 g ash/g FFBP (see Figure 5.2).

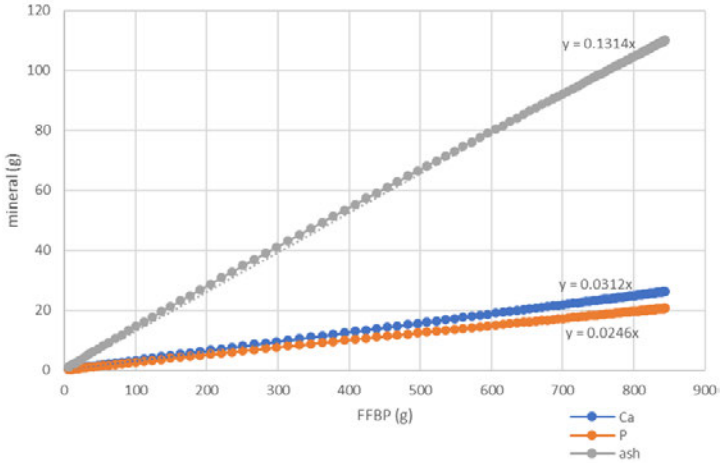

Figure 5.2 Estimation of the parameters of the allometric relationship between Ca, P and FFBP

Source: after Caldas *et al.* (2019)

This implies a Ca/P ratio in the broiler body of 1.27.

The ash content of 13% of FFBP is lower than the studies mentioned above. This could represent a genotype difference, or a change in ash content over time as broilers are selected for muscle mass. Alternatively, this could be a result of lower dietary Ca and/or P than required for the bird to reach its potential. The inclusion of visceral content was disregarded in the further use of these coefficients but could have influenced their values. Higher ash content in the bones of birds unselected for growth when compared with selected birds has been reported (Williams *et al.*, 2000b).

When the values for Ca and P in g/g EFFBP from the mean values reported in this study are plotted against age, the resulting graph suggests some changes in these ratios over time that may not be captured by the isometric model (see Figure 5.3).

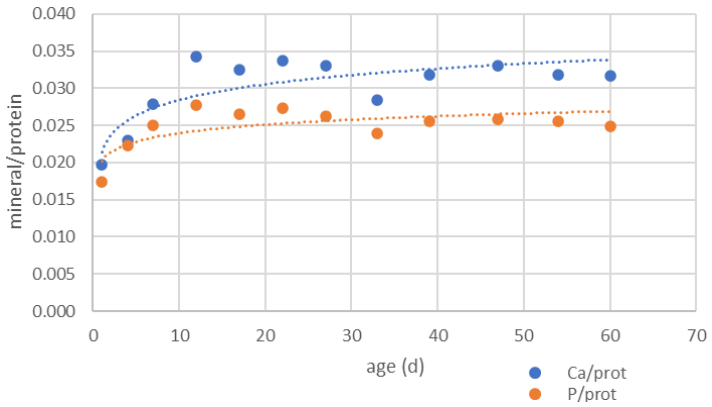

Figure 5.3 Changes in Ca, P relative to EFFBP over time

Source: after Caldas *et al.* (2019)

This reflects the initial increase in the proportion of Ca and P to body protein that was observed by Hurwitz and Plavnik (1986), with a logarithmic function providing a better fit than a linear function. Williams *et al.* (2000b) reported a rapid increase in tibiotarsal ash from days 4-11 and Angel (2007) made a similar observation in whole skeletons. The pre-starter period may require a different approach from the calculation of requirements in the older bird, but trials will be required in which optimal feeding is ensured, and in which body and bone protein, Ca and P are measured frequently, if this is to be modelled accurately.

Niess *et al.* (2005) proposed that the broiler body contains 6.1 g Ca/kg fresh BW. At 16.5% protein in the EFFB, this suggested a Ca content of 0.037 g/kg EFFBP. 5.1 g P/kg fresh BW was reported, and this would imply a coefficient of 0.031 g P/kg EFFBP, with a Ca/P ratio of 1.20. This study only analysed birds at 21, 35 and 42 days.

Hurwitz and Plavnik (1986) measured Ca and P contents of the empty, feather-free carcasses weekly from day-old to 70 days of age. In order to use these data for the model, protein weights were assigned

according to the reported body weights and the proportions of protein described in their earlier report on the same trials (Plavnik and Hurwitz, 1983). The relationship between Ca and body protein showed a linear increase in the first week but from week 2 to 8 the proportion of Ca vs body protein levelled off but varied between 0.042 and 0.046. The isometric coefficient calculated by fitting a horizontal linear regression line to these data was 0.046 for Ca on body protein. The decrease in this value between the earlier and later publications may be indicative of a better developed skeleton in the bird of the 1980s, and therefore the lower value from Caldas *et al.* (2019) was used in this study. Similarly, calculations from Hurwitz and Plavnik (1986) (section 3.2.1) showed the isometric coefficients to be 0.041 and 0.035 for Ca and P respectively vs FFBP when the allometric exponent was adjusted to 1.

Studies have also shown isometric relationships between protein, Ca and P in pigs (Symeou *et al.*, 2014). These are summarised in Table 5.4.

Table 5.4 Isometric coefficients for Ca and P in pigs

Source	ash/protein (g/g)	Ca/protein (g/g)	P/protein (g/g)
Rymarz *et al.* (1982)	0.194	0.057	0.034
Hendriks and Moughan (1993)	0.198	0.048	0.032
Mahan and Shields Jr (1998)	0.179	0.055	0.035
Wiseman and Mahan (2010)	-	0.039	0.026
Pettey *et al.* (2015)	0.156	0.021	0.017

An interesting phenomenon is the decline in ash/protein, Ca/protein and P/protein observed between 1982 and 2015. The Ca/P ratio declined from 1.7 to 1.2 over the course of these studies. It may simply be coincidental, but it suggests increased BFB relative to bone. Symeou *et al.* (2014) proposed a coefficient of 0.034 g P/g BP. If the Ca/P ratio in the whole body is no less than 1.25, this P/BP ratio would suggest that the Ca coefficient must be at least 0.042 g Ca/g BP.

Model implication 2: The potential whole-body isometric coefficients were chosen to be 0.13 g ash/g EFFBP, 0.0312 g Ca/g EFFBP and 0.246 g P/g EFFBP

5.3. Proportions of Ca and P in bone and bone-free body

Proposition 3: Under ideal conditions, BFB Ca and bone Ca, BFB P and bone P, and bone Ca and bone P remain in the same ratios to one another

This proposition requires that absorbed minerals are allocated to BFB and bone in the same proportions throughout the growing period. This allows the isometric coefficients for the whole-body Ca and P to be divided in these proportions for the calculation of potential growth. This assumption was necessary due to the complete absence of published studies in which broilers were dissected and protein, Ca and P analysed serially until maturity. It is important to note that this proposition does not imply that the weights of bones and BFB remain in proportion, or even that ash in bone and BFB remain in proportion, since Ca and P in g/kg bone mass or g/kg ash may change. However, these elements may provide indications of whether this proposition is likely to require modification in future iterations of the model.

The isometric relationship between EFFB protein and ash implies either that:

1. the proportions of bone and BFB mass and the proportions of ash in them remain constant, since the ash content of these two tissues is different, or
2. changes in the ash contents of these tissues balance changes in their proportions by mass.

The proportion of ash in the skeleton increases with age, according to Angel (2007). It has already been suggested that an overall body isometry between chemical components may disguise changes in the proportions in different body parts (Eits *et al.*, 2002). Murawska *et al.* (2011) dissected broiler chickens at intervals and plotted the changes in the proportions of different body components (see Figure 5.4).

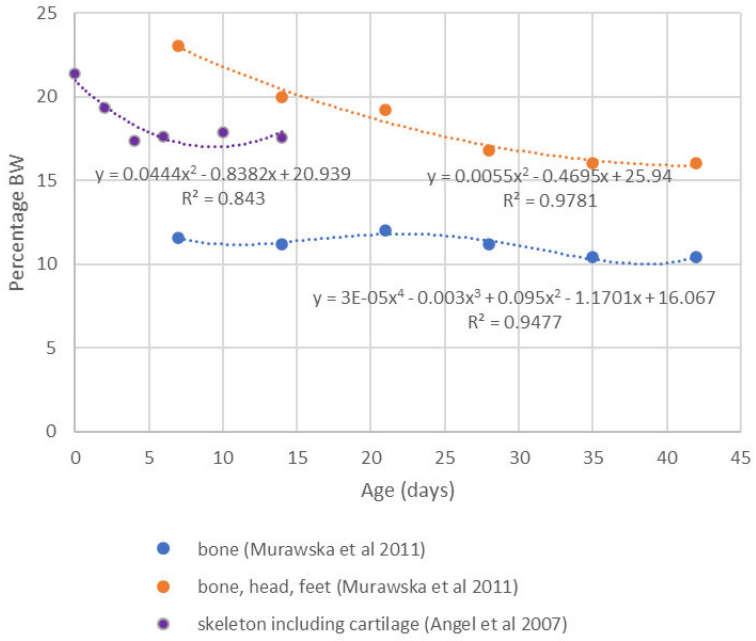

Figure 5.4 Changes in bone mass as a proportion of body mass

Source: after Angel (2007); Murawska *et al.* (2011)

Although detailed figures are hard to extract from this publication and the changes in percentage loss (evaporative loss, lung and trachea loss) appeared significant, the pattern of changes offers some indication of how a model that separates soft tissue and bone ash could be underpinned by differential effects on these tissues. In a study of the first 14 days post-hatch, Angel (2007) found that the skeleton made up approximately 20% of body mass, but a slight drop in this proportion with age is consistent with the findings of Murawska *et al.* (2011) in their study of changes in the proportions of different components of the broiler body over time.

Proportions of skeleton and BFB appear to change in the first three weeks while many components stay relatively stable in proportion to body weight after this period. In one study, cartilage was not removed, which may have resulted in a lower ash measurement (Angel, 2007). Head and phalanges were included.

The downward trend in skeleton mass as a proportion of body mass that can be discerned in the first week of life is accompanied by an increase in the proportion of ash, Ca and P in the skeleton (Skinner

and Waldroup, 1995; Angel, 2007) which may account for a constant proportional relationship between body protein and minerals (see Figure 5.5).

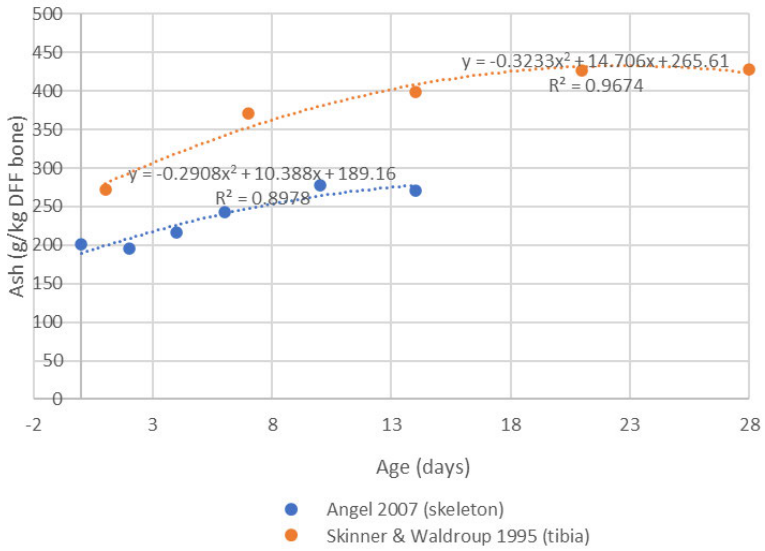

Figure 5.5 Changes in bone ash with age

Source: after Skinner and Waldroup (1995); Angel (2007)

An analysis of the total mass of protein, Ca and P in bones and BFB at intervals during the growth of the broiler would improve our understanding of the relationships between these variables. Angel (2007) described such an experiment, but only reported the results of the first 14 days. Body protein was not reported and nor was whole-body Ca and P. When body protein values were estimated from BW, using the EFG model, the bone Ca and P ratios to these values were lower than expected but increased over this period (see Table 5.5)

There is some doubt about the proportion of P found in the BFB. Although Veum (2010) proposed that this was 20% of the body P, and has been cited to this effect (Proszkowiec-Weglarz and Angel, 2013). few studies have measured it. The Ca/P ratio derived from studies in which Ca and P are measured in the whole body suggests that a greater proportion of body P is found in the BFB.

Table 5.5 Relationship between EFFBP and skeleton Ca and P from 1-14 d

Source: after Angel (2007)

Age (d)	Minerals in bone (g/g EFFB)			
	Ash	Ca	P	Ca:P
0	0.0517	0.0169	0.0115	1.73
2	0.0447	0.0152	0.0104	1.73
4	0.0413	0.0154	0.0102	1.78
6	0.0483	0.0188	0.0123	1.80
10	0.0600	0.0228	0.0156	1.72
14	0.0567	0.0237	0.0159	1.76

Bone mineral varies from the stoichiometric ratio of hydroxyapatite, usually containing less Ca so that the molar ratio between Ca and P is less than 10:6 (a 2.16 mass ratio) (Glimcher *et al.*, 1981) (see section 2.6.2.3). This may occur as a result of substitutions for Ca in the hydroxyapatite matrix or free phosphate on bone surfaces.

If a 2:1 Ca/P ratio in bone ($Ca_{BON}: P_{BON}$) is assumed, and if it is assumed that 99% of Ca is in the bones, then a Ca/P ratio of 1.6 suggests that 79% of P in the body is found in the bone. If the range of values for $Ca_{BON}: P_{BON}$ is 1.8 to 2.2 it results in the proportions in Table 5.6.

Table 5.6 Range of values for bone P in the empty broiler body

Ca:P in EFFB	Proportion of P in bone		
	Min $Ca_{BON}: P_{BON} = 2.2$	Average $Ca_{BON}: P_{BON} = 2.0$	Maximum $Ca_{BON}: P_{BON} = 1.8$
1.6	0.73	0.79	0.90
1.5	0.69	0.74	0.84
1.4	0.64	0.69	0.79
1.3	0.60	0.64	0.73
1.2	0.55	0.59	0.67
1.1	0.50	0.54	0.61
1.0	0.45	0.50	0.55
0.9	0.41	0.45	0.50

The value of Ca:P changes during growth in the studies of Hurwitz and Plavnik (1986) and Caldas *et al.* (2019), shown in Table 5.7. These suggest that under 50% of body P is found in bone at 4 days of age. This is supported if the values of minerals in g/g EFFBP calculated from Angel (2007) are calculated as a proportion of the coefficients for EFFB minerals in section 5.2.

There is no substantiated allometric relationship between the growth of feathers and the growth of body protein (Gous *et al.*, 1999). In modelling the relationship between body protein, Ca and P, the question arises whether this might also be the case for skeletal growth. In extending the model described in Létourneau-Montminy *et al.* (2015), Lautrou *et al.* (2019) proposed that bone mineral growth is independent of body protein. Hence calcium was modelled as a function of age.

Logically, the absence of a physiological link between body protein growth and mineral growth seems unlikely. It presents a vision of an animal that deposits minerals in bones that are not growing in size or protein mass.

Table 5.7 Values for Ca:P in the empty broiler body

Age (d) Hurwitz/Caldas	Hurwitz and Plavnik (1986	Caldas *et al.* (2019)	Olukosi and Adeola (2008)	Niess *et al.* (2005)
1/1	0.87	1.14	-	-
4/4	1.00	1.03	-	-
7/7	1.04	1.12	-	-
12/14	1.17	1.24	-	-
17/-	-	1.23	-	-
22/21	1.12	1.23	1.27	1.36
27/28	1.09	1.26	-	-
33/35	1.06	1.19	-	1.40
39/42	1.16	1.24	-	1.35
47/49	1.24	1.28		
54/56	1.55	1.25		
60		1.27		

Collagen is the principal protein present in bones and is present in many other tissues, contributing substantially to the total, defeathered body protein. Bone mineralisation depends on the presence of a collagen matrix in which hydroxyapatite may accumulate. Factors such as physical activity have been shown to have an impact on bone growth through the mediation of skeletal muscle (Bain and Watkins, 1993). It seems logical that, as in fat deposition, an ideal mineralisation will follow protein growth in order to provide support for the soft tissue mass of the body, while under conditions of mineral restriction or deficiency the body may distribute these minerals differently in order to maintain an optimal protein growth while still providing skeletal support. Mineralisation under conditions of deficiency may follow a different pattern from the potential and it may be necessary to disaggregate this from the growth that occurs in association with tissue protein growth. P plays a critical role in both bone and muscle tissues, for example in its role in phospholipids in cell membranes. The bird must balance this with the requirement for P to be bound with Ca during bone mineralisation.

As a result of these inconsistencies, it was decided to model Ca and P independently of ash and to modify ash mass by subtracting any shortfalls in these minerals at the end of each cycle.

Model implication 3: The isometric coefficient of Ca is divided proportionally between BFB and bone (1% and 99% respectively) and the isometric coefficient for P is divided proportionally between BFB and bone such that a mass ratio of 2:1 exists between Ca_{bone} *and* P_{bon}*. This results in isometric coefficients as follows: 0.00031* Ca_{BFB}*/g EFFBP 0.03089 g* Ca_{bone}*/g EFFBP, and 0.00915 g* P_{BFB}*/g EFFBP and 0.01545 g* P_{bone}*/g EFFBP .*

5.4. Calcium and phosphorus requirements in broilers

Proposition 5: True requirements for Ca and P comprise the potential growth in BFB and bone for the time period under consideration, endogenous losses in the GIT which are dependent on DMI, and maintenance losses which are proportional to the product of metabolic protein mass at maturity and degree of protein maturity.

5.4.1. Endogenous losses

Endogenous losses (EL) are frequently reported in g/kg DMI (Anwar and Ravindran, 2016; González-Vega and Stein, 2016; Anwar *et al.*, 2017; Zhang and Adeola, 2017; Anwar *et al.*, 2018; David *et al.*, 2019). Values vary depending on whether they are estimated from the intercept of the regression of indigestible mineral on ingested mineral or from the ileal mineral when a Ca- and P-free feed is fed (Anwar *et al.*, 2018). When different feed ingredients are tested for ileal digestibility, EL losses vary widely (see Table 5.8). It has also been noted that widening Ca/P ratios in the feed result in reduced P absorption and endogenous excretion. Furthermore, Perryman *et al.* (2017b) reported that maintaining a Ca/P ratio in test diets when applying the regression method resulted in negative endogenous losses, while maintaining a low Ca level (35%) resulted in positive values ranging from 102-167 mg/kg DMI. It was suggested that the ability of Ca to bind P, including endogenous P, affects the AIDP, particularly at lower levels of P. A more complex model of endogenous losses may be required. Given this variation, it was considered that expressing these values in mg/g FI would not result in a material change to the results. Further research will be required to determine if it is possible to estimate endogenous losses more accurately.

Table 5.8 Endogenous losses of Ca and P

Source	Method/ingredients	Ca (mg/g DMI)	P (mg/g DMI)
Anwar *et al.* (2018)	regression method	-0.023	-
	Ca and P-free diet	0.086	
David *et al.* (2019)	purified diet	0.131	-
	corn-based feed	0.253	
David *et al.* (2021)	grower diet	0.236	0.310
	finisher diet	0.029	0.130
Dilelis *et al.* (2021a)	regression method	-	-1.062
	Ca and P-free diet		0.127
Dilger and Adeola (2006)	regression method	-	0.235
Mutucumarana *et al.* (2014c)	regression method	-	-0.009-0.609
Shastak *et al.* (2014)	regression method	-	0-0.230
Trairatapiwan *et al.* (2019)	regression method	0.580	0.610-1.540
Zhang and Adeola (2018)	regression method	0.223	-

5.4.2. Maintenance

As with a calculation used to determine the energy required for maintenance, and assuming that no Ca or P is required for the maintenance of body lipid, estimates of maintenance requirements for Ca and P might be considered to be proportional to body protein weight (Emmans, 1987). Symeou *et al.* (2014) used a calculation based on the scaling rule used by Brody which related maintenance at mature size to $BW^{0.73}$. For growing animals, this is modified by the degree of maturity (u) so that the maintenance requirement may be calculated as

$$X_{maint} = m * BP * BP_m{}^{-0.27} \quad (Eq\ 5.8)$$

where m is a maintenance coefficient, BP is the mass of body protein and BP_m represents the maximum or mature body protein mass. For pigs, the coefficient applied for P was $m = 0.1293$ (Symeou *et al.*, 2014). This was adopted for broilers. A coefficient proportional to the ratio of Ca/P in BFB (0.034) was applied to calcium, as its role in metabolism requires smaller amounts.

5.4.3. Requirements

The mass of Ca and P required by the birds each day was then expressed as a dietary requirement. The broiler model predicts feed intake, so requirements are readily expressed (in g/kg feed) as

$$\frac{X_{obligatory\ loss} + X_{growth}}{FI} \quad (Eq\ 5.9)$$

Where $X_{obligatory\ loss}$ and X_{growth} are expressed in mg/b/d and FI is expressed in g/b/d.

Manangi *et al.* (2018) provided graphs to show the relationship between P and Ca levels in the feed and urinary excretion. They showed that P excretion could drop to 0 mg/d if dietary P was extremely low. Although it was not clear if this was physiologically sustainable or if the bird needs to lose a certain amount of P in urine to maintain health, the model allows zero excretion of both Ca and P.

Model implication 4: Calcium and phosphorus requirements are calculated as basal endogenous loss (0.1 mg Ca/g FI and 0.2 mg P/g FI) + maintenance requirement (calculated from current EFFBP and mature EFFBP, with coefficients 0.0044 for Ca and 0.1293 for P) + requirements for growth (calculated from protein gain in the current period, using the appropriate isometric coefficients for BFB and bone (see Section 5.3)).

5.5. Retainable calcium and phosphorus

Proposition 5: Calcium and phosphorus in the ingested feed and that which is digestible before the distal ileum is retainable by the bird with the exception of the amounts required for endogenous losses and maintenance.

Ileal or precaecal digestibility was chosen as the measure of Ca and P availability. Rodehutscord and WPSA (2013) defined available P as *the part of dietary total P that, at marginal level of P supply, can*

be utilised to cover the P requirement of the animal. Available P (aP), and similarly, available Ca (aCa), in g/kg complete diet, are inputs to the growth model that are produced by the digestibility module. Multiplied by feed intake, these provide the amounts of Ca and P available to the animal in g (ACa and AP). Retainable P was defined as *that proportion of dietary total P that is deposited in the body of an animal*. The requirements are for growth, endogenous excretions and maintenance. Hence, once the latter two amounts have been subtracted, ACa and AP are assumed to be retainable for growth (RP and RCa in g).

True ileal (or precaecal) digestible Ca or P (TIDCa or TIDP) is an empirical measure of the Ca or P that is absorbed from the GIT under a certain set of conditions. Apparent ileal digestible mineral is reduced by the endogenous losses into the GIT that increase the apparently indigestible portion of the GIT contents at the end of the small intestine. For the model, this is the appropriate measure as endogenous losses form part of the estimated requirement, and these should not be included twice.

Model implication 5: Retainable Ca and P (g) are calculated as the available Ca and P outputs of the digestion module less the maintenance requirements and endogenous losses.

5.6. Allocation of calcium and phosphorus to bone and bone-free body

Proposition 6: BFB is prioritised over bone in the allocation of retainable Ca and P

Although the broiler may exhibit an isometric relationship between body protein, ash, Ca and P under ideal (potential) conditions, the differential prioritisation of bone and soft tissue growth may result in a breakdown of this ideal relationship when Ca and P supplies are compromised. Thus, nutritional factors may be responsible for fluctuations in proportions of P in BFB and bone, and hence in Ca/P ratio, during growth.

Deviations from the ideal growth rate were modelled in ash, Ca and P as changes in proportion of these components in bones to those in soft tissue, resulting from their different prioritisation in the allocation of retainable Ca and P. A simple series of two pools to be filled sequentially was used. The soft tissue pool was filled first and the bone pool was only augmented once Ca and P for muscle growth was satisfied. This approach to prioritisation was used because it has been shown to be useful in pigs (Létourneau-Montminy *et al.*, 2015). Further research will be required, in which BFB and bone are separated, and mineral content analysed, if a definitive model of prioritisation is to be developed.

Model implication 6: Retainable Ca and P (g) are compared with requirements for BFB growth. If they are greater than these, the requirements for BFB are subtracted from RCa and RP, allocated to the total BFB, and the balance is considered for the growth of bone Ca and bone P. If they are less than or equal to BFB requirements, all available RCa and/or RP is allocated to BFB.

5.7. Retention of calcium and phosphorus in bone

Proposition 7: Retainable Ca and P are retained in bone only if both are present in sufficient quantities to maintain a Ca/P ratio in bone that falls within certain limits.

Retainable Ca and P not required to fulfil the requirements for BFB is considered to be available for bone mineral deposition. However, the amount of Ca and P deposited in bones is constrained by the formation of hydroxyapatite as the principal compound in which these minerals are found and which requires both minerals to be present.

Although it was proposed that the ideal Ca/P ratio in bone is 2.00, rather than the stoichiometric ratio of 2.16 or the lower values often observed (see section 2.6.2.4), under conditions that are not ideal, this ratio may vary.

Model implication 7: Retainable Ca and P (g) not required for BFB growth is compared with the requirements for bone. Minerals are retained in bone (added to the total bone Ca or P mass) up to the potential requirement for bone for the time-period plus any shortfall accumulated in previous periods. However, should the retention of Ca result in a Ca/P ratio in bone in excess of 2.2 g Ca/g P, then only as much Ca as will result in a ratio of 2.2 g Ca/g P will be retained. Similarly, should there be insufficient Ca such that the ratio will drop below 1.8 g Ca/g P, only as much P as will result in a ratio of 1.8 g Ca/g P will be retained.

5.8. Return to isometry after deficiency

Proposition 8: The bird will at all times strive to return to the isometric ratio between Ca and P and EFFBP

In the broiler growth model, when body lipid content is in excess of the genetically-determined amount, the bird will, whenever possible, make use of the stored lipid as an energy source and adjust feed intake accordingly, to enable it to return to the ideal amount of body lipid. In the mineral model, it is assumed that the bird does not adjust feed intake to rectify a deficiency of bone mineral. However, should more mineral become available in the feed, the bird will retain as much as possible towards the shortfall in its body mineral. This may allow the bird to recover from deficiency if feed intake and feed Ca and P are sufficient.

Model implication 8: Retained Ca and P (g) are compared with potential retention (requirement) and any shortfall is added to the requirement for the following period.

5.9. Effect of bone-free body deficit

Proposition 9: Under all conditions, BFB P must remain in the same proportion to FFBP

This proposition rests on the assumption that the metabolic functions of P in the body are closely related to the growth of soft tissue, and hence this element is allocated in a constant proportion to all soft tissues.

Thus, if there is insufficient P for the growth of EFFBP in the following period, EFFBP growth is slowed in proportion to the deficiency in P.

P may be found in muscle at a concentration of between 2.2 and 3.0 g/kg fresh weight (0.002 to 0.003 g/g), with higher levels found in younger birds (Grey *et al.*, 1983). These findings suggest that proposition 9 may be questionable, but muscle cannot simply be equated to protein, and it may be changes in fat and connective tissue that are responsible for declining P levels in muscle. Levels in chicken fat are extremely low, at 0.013 g/kg (Farmani and Rostammiri, 2015).

Vitamin D plays a role in the homeostasis of Ca and P, and its effects on intestine, kidney and bone are well documented. Vitamin D receptors are found in these and many other tissues and vitamin D supplementation has been shown to increase breast meat yield by influencing protein synthesis (Starkey, 2014; Vignale *et al.*, 2015) and this may provide a mechanistic link between bone and muscle growth. P deficiency may affect muscle growth through differential effects on the expression of genes regulating calmodulin/calcineurin and insulin-like growth factor (Schmeisser *et al.*, 2017).

Since muscle growth is compromised by low dietary P, once the BFB P falls below the ideal, potential protein growth is reduced in proportion to this deficiency. This results in a decrease in feed intake and hence in actual protein growth only when available P falls below the proportion in BFB and once bone mineralisation is reduced to zero.

Model implication 9: If P is insufficient for BFB growth, the rate of maturing (B in the Gompertz equation) of EFFBP is reduced in the model as a means of slowing potential protein growth in proportion to the shortfall in BFB P, this having the effect of reducing feed intake.

5.10. Proportion of calcium in bone and bone-free body

Proposition 10: All retainable Ca and P not accumulated in BFB and bone, according to the requirements of these tissues, is excreted.

The logical sequence of the model suggests that Ca and P available from the GIT is absorbed and is:

1. secreted into the GIT for excretion in the faeces (endogenous loss) or
2. used for maintenance or
3. retained in the BFB or
4. retained in the bone or
5. excreted in the urine

However, it is also possible that Ca and P are available for absorption from the GIT but are not absorbed as a result of the regulation of absorption at the intestinal mucosa. This is immaterial in the model because, as in the bird, excreta are mixed so that urinary excreted minerals are combined with faecal excreted minerals.

Model implication 10: The sum of Ca and P retained in the BFB and bone (i.e. total body Ca and P) is subtracted from the Ca and P ingested to obtain excreted Ca and P, which are outputs of the model.

5.11. Implications of the model propositions

The propositions above comprise the fundamental theory applied in building the model. Review of these raises further questions, which provide the impetus for revision of the model. This iterative approach allows an incremental, continuous improvement of the model. It also suggests experiments that could elucidate aspects of the model.

5.11.1. Ca and P in bone and hence body Ca and P may vary in relation to FFBP

The isometric relationship between EFFBP and body Ca and P is assumed only under ideal conditions, with a feed that supplies all the bird's needs for protein, energy, vitamins and minerals. The model allows this isometry to be disturbed when the feed is deficient in Ca and/or P. As bone mineral growth is reduced/slowed, body protein growth continues at the level permitted by the dietary components other than minerals. Thus, the Ca and P in bone, and hence in the whole body, decrease relative to EFFBP.

A deficiency in the current model may be that it does not allow the growth of mineral to continue at a greater rate of maturing than protein growth when this is compromised by low protein or unbalanced feeds. Misiura *et al.* (2020) modelled P growth under conditions of mineral deficiency by using allometry that calculated body P with an allometric exponent less than 1. When protein was deficient but minerals adequate, body P was calculated with an allometric exponent greater than 1, to simulate the changes in ash to protein ratios observed in pig studies.

5.11.2. Deficiency of Ca and P in bone does not affect EFFBP growth

This implies that bone mineral growth could cease completely, and the bird would continue to increase EFFBP. This requires modification. Létourneau-Montminy *et al.* (2015) applied an arbitrary scaling coefficient to reduce body growth when bone minerals fell below 70% of potential and this may be a logical addition to the model. (Itoh and Hatano, 1964) carried out a trial in which a deficiency of vitamin D was shown to affect total Ca in the skeleton: a 40% deficiency of bone Ca was accompanied by a 14 % drop in BW. Feed intake was not reported, but it is apparent that some feedback of Ca deficiency to protein growth is appropriate.

While reduction in bone ash, Ca and P contents under conditions of deficiency is clear, the dearth of whole-body studies means that the effect on soft tissue growth is more difficult to discern. However, this may be implied from changes in the retained Ca/P ratio (Bertram, 1995). Because 99% of the Ca is in the bones, the Ca/P ratio should decrease as bone mineral decreases as a proportion of whole-body mineral. However, this ratio may be misleading in cases where BFB weight is compromised. P growth must be divided in some proportion between bone and BFB and the model assumption that BFB P is

prioritised over bone P has not been tested. The amount of Ca in BFB is small enough that the assumption that this enjoys priority over bone is not material.

The mechanisms and prioritisation rules are not well understood (Bertram, 1995). Poultry studies focus on the mineralisation of bone at lower dietary mineral concentrations. However, many observe that growth is compromised by mineral deficiency (Bar *et al.*, 2003b) and that anorexia develops with low P diets. If the two-pool system were not applied, but some proportional allocation was implemented, there might be implications for the situation in which Ca is in short supply. Under these circumstances, bone would be compromised but more P would then be available for BFB growth and hence overall growth will be less compromised. This might make sense at least in the short term since the ability to compensate for fluctuating Ca demand might be an evolutionary trait of birds.

5.11.3. Effect of Ca and P deficiency on feed intake

In the model, P deficiency influences feed intake through its influence on the rate of maturing of EFFBP. When there is insufficient P to meet BFB requirement, protein growth is slowed and, as a result, desired feed intake is reduced. However, (Bradbury *et al.*, 2014) noted that feed intake increases when Ca in the feed is low, even if this means that nPP is over-consumed, although animals will not over-consume Ca to meet their P requirement when nPP is deficient in the feed. It would be necessary to model other factors that affect feed intake to discern the effects of Ca on feed intake. Once the model produces accurate representations of digestibility and requirements, it will be the ideal vehicle for the consideration of the confounding factors. Experiments to quantify the effects of Ca and P on feed intake could be designed so that they produce accurate estimates.

5.12. Model summary

A summary of the model parameters is presented in Table 5.9 and in Appendix 1. These include the inputs used for the EFG model and those added for the Ca and P model.

A flow diagram representing the growth model is shown in Figure 5.6. The model was originally developed using Excel ® and subsequently incorporated into the EFG software model. A Matlab ® version was also coded, to allow for the testing of different feeding strategies such as separate Ca or low Ca/high P and high Ca/low P feeds in a choice feeding scenario.

The model requirement outputs were initially compared with NRC recommendations and then with retention studies. In the absence of primary data and considering the range of values derived from the literature, it was considered prudent to calibrate the growth coefficients before full model evaluation (see Chapter 6).

Table 5.9 Model inputs for Ross high yield type bird

Inputs		units	value (m/f)
rates of maturing			
B	body rate of maturing	/d	0.45/0.46
B_f	feather rate of maturing	/d	0.06/0.06
BW_m	mature empty body weight	kg	7.65
mature weights			
L_m	mature fat content	%	10
BP_m	mature EFFBP	kg	1.48/1.07
FP_m	mature feather protein	kg	0.32/0.26
LP	mature lipid/protein ratio	-	0.52/1.04
L_m	mature lipid	kg	0.77/1.11
W_m	mature water	kg	4.76/3.45
A_m	mature ash	kg	0.33/0.24
Ca_m	mature Ca	kg	0.039
P_m	mature P	kg	0.03056
allometric coefficients			
a_L	lipid/EFFBP allometric coefficient *a*		1.2
b_L	lipid allometric exponent *b*		0.29/0.51
a_w	water allometric coefficient *a*		3.4
b_w	lipid allometric exponent *b*		0.12
a_{ash}	ash/EFFBP isometric coefficient *a*	g/g BP	0.165
a_{CaBFB}	Ca/EFFBP isometric coefficient *a*	g/g BP	0.00031
a_{Cabone}	Ca/EFFBP isometric coefficient *a*	g/g BP	0.03089
a_{PBFB}	P/EFFBP isometric coefficient *a*	g/g BP	0.00915
a_{Pbone}	P/EFFBP isometric coefficient *a*	g/g BP	0.01545
maintenance coefficients			
m_{Ca}	Ca for EFFBP maintenance		0.0044
m_P	P for EFFBP maintenance		0.1293
endogenous losses			
el_{Ca}	Ca losses to faeces	mg/g FI	0.25
el_P	P losses to faeces	mg/g FI	0.25
Ca/P ratios in bone			
Ca/P_{min}	minimum allowed ratio for bone growth	g/g	1.8
Ca/P_{max}	maximum allowed ratio for bone growth	g/g	2.2

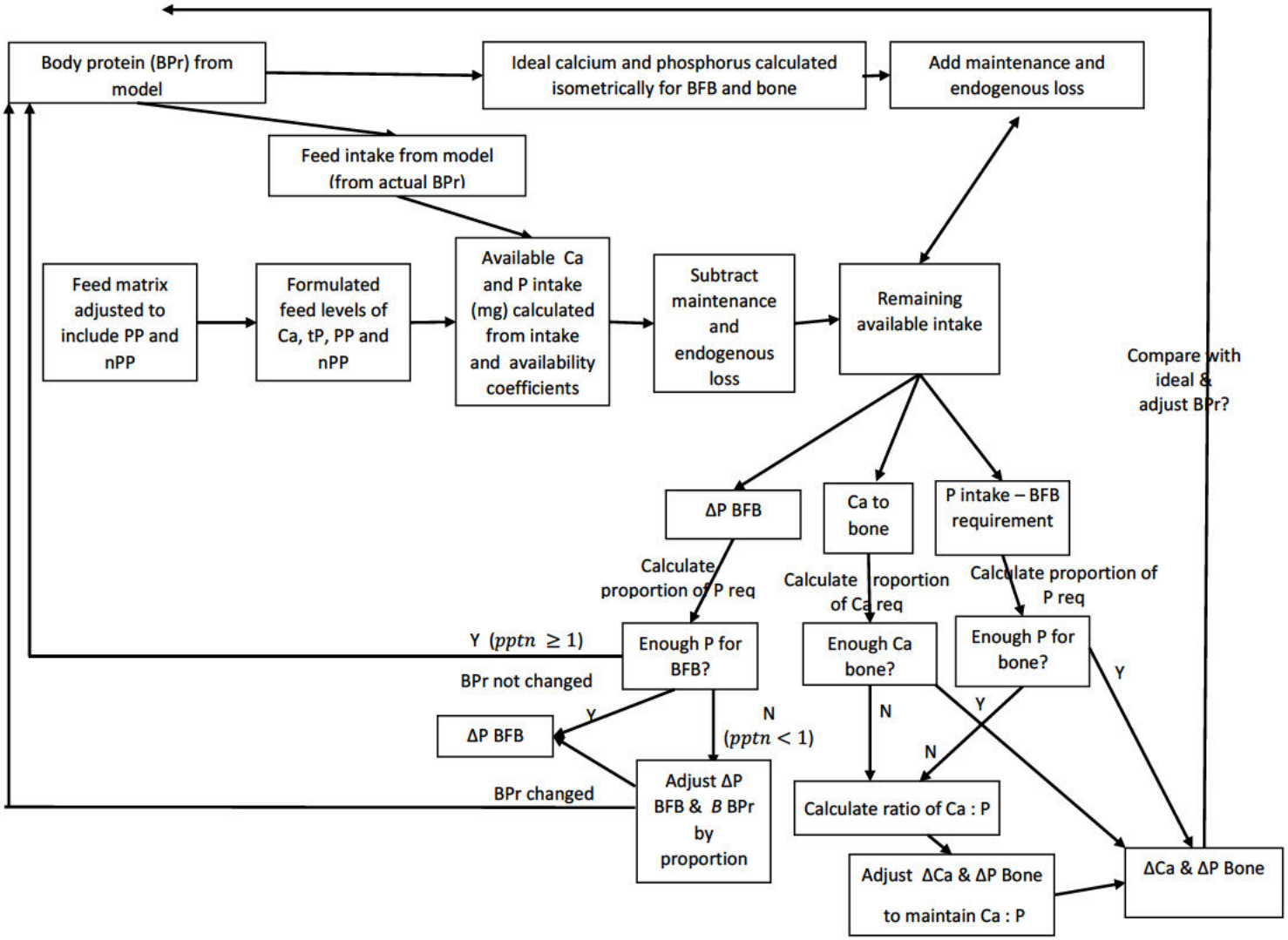

Figure 5.6 Decision and calculation flow of model to calculate calcium and phosphorus retained by broilers during growth

5.13. Comparison of model outputs with NRC recommendations

The values of required Ca and P in the feed are summarised in Table 5.10. These were calculated using the desired feed intake and Ca and P requirements from the model, assuming that a balanced feed was fed and no constraints such as bulk or temperature were imposed. Ca digestibilities from the digestibility model were used to convert the requirements for aCa to tCa. The requirements expressed as g/kg feed as proposed by the National Research Council (NRC) standards were compared with these (National Research Council, 1994).

Table 5.10 Model outputs for available Ca and P requirements

	Model output (g Ca or P/kg FI)				NRC (1994) (g/kg)		
Age (d)	avCa	tCa	avP	tCa:avP	tCa	nPP/avP	Ca:nPP
1	3.9	6.3	2.8	2.25	10	4.5	2.22
7	3.9	6.3	3.3	1.90	10	4.5	2.22
14	3.8	6.2	3.3	1.90	10	4.5	2.22
21	3.3	5.3	3.4	1.56	9	3.5	2.57
28	2.9	4.6	3.5	1.31	9	3.5	2.57
35	2.8	4.5	3.6	1.25	9	3.5	2.57

Both Ca and P requirements from the model are lower than those in the NRC recommendations. Furthermore, the NRC values only reflect nPP, and P available from phytate might supply higher levels to the bird. From 21 to 35 days the model requirements and NRC recommendations are the same for P, but Ca requirements from the model diminish to half of the NRC recommendation by 35 d.

Furthermore, the NRC proposes a Ca/nPP ratio that increases with bird age while the model suggests that this ratio decreases.

5.14. Comparison of model requirement estimates with mineral and protein retention

The results of a trial investigating the effect of Ca concentrations and Ca/P ratio in starter feeds on nutrient retention provided an indication of the variability in the Ca and P in proportion to body protein, and allowed the calculation of the retained Ca/P ratio (Gautier *et al.*, 2017). Feather protein growth of 16.3 g for the male Ross 308 broilers used in this trial was deducted so that the retained protein reflects feather-free body protein (see Table 5.11). The model parameters (isometric coefficients/*a* values) were compared with calculated maximum Ca/FFBP and P/FFBP retention ratios calculated from this study.

The retention ratios ranged from 0.0561 to 0.0577 g Ca/g FFBP retention and 0.0315 to 0.0348 g P/g FFBP retention when feeds were balanced for Ca, P and CP were fed. This suggested that the estimates from Caldas *et al.* (2019) were low, particularly in the case of Ca, since mineral retained is assumed to be deposited in the body and hence should be comparable with measurements of body composition.

Table 5.11 Retention of N, Ca and P in 14- to 21-day-old broilers

Source: adapted from Gautier *et al.* (2017)

Feed composition (g/kg)				Retained g/b (d14-21)			Retained nutrient ratios		
CP	Ca	TP	Ca:P	N×6.25	Ca	P	Ca:P	Ca:N×6.25	P:N×6.25
230	6.1	5.4	1.13	35	2.02	1.10	1.83	0.0577	0.0315
230	8.1	5.3	1.53	42	2.45	1.46	1.68	0.0583	0.0348
230	9.8	5.2	1.88	34	1.90	1.15	1.64	0.0561	0.0341
230	10.5	5.4	1.94	29	0.64	0.97	0.66	0.0222	0.0338
230	14.0	5.5	2.55	37	1.23	1.01	1.22	0.0330	0.0271
230	15.7	5.4	2.91	40	1.08	0.95	1.13	0.0267	0.0237
230	20.9	5.4	3.87	40	2.93	0.93	3.16	0.0742	0.0234

Alternative parameter values were calculated from Hurwitz and Plavnik (1986) and are shown in Table 5.12. In this study, feather-free carcasses were analysed.

Table 5.12 Revised model parameters

coefficient	model value 1 (Caldas *et al.*, 2019)	model value 2 (Hurwitz and Plavnik, 1986)
a_{CaBFB}	0.00031	0.00042
a_{Cabone}	0.03089	0.042
a_{PBFB}	0.00915	0.0011
a_{Pbone}	0.01545	0.021

5.15. Comparison with separate calcium feeding experiments

The requirements for Ca and P from the model were compared with amounts retained in a trial in which separate Ca was offered to the birds (Abdollahi *et al.*, 2016). The P in the experimental feed was 5.2 g/kg. Although there was a range of Ca levels in the feed, this did not reflect the intake as birds were able to supplement the lower Ca feeds with separate Ca. This may have allowed them to balance their Ca and P for optimal retention (growth), which is why this study was chosen for the initial comparison with the model. The original coefficients and adjusted coefficients were used in the model and the Ca and P requirements for the first 21 d were calculated in g.

Figures 5.7 and 5.8 show the quantities of Ca and P retained in the starter period (0-21 d) in the published studies, compared with model predictions. The horizontal graphs of the growth predicted by the model represent the potential (maximum) mineral growth. The maximum retention achieved empirically should not exceed this value. It can be seen that while the revised model parameters estimate retention greater than was measured in these studies, the original parameters produce estimates that are exceeded over a range of mineral levels in the feed and hence are likely to underestimate potential growth.

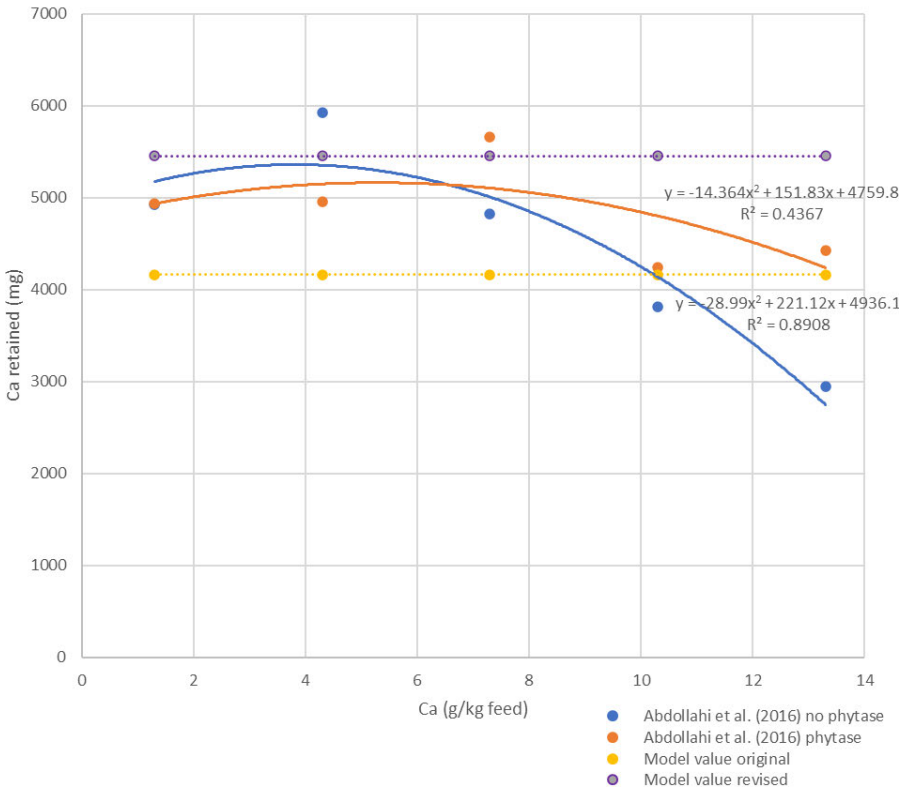

Figure 5.7 Ca retained by broilers in the starter phase

Source: after Abdollahi *et al.* (2016) and model outputs (Table 5.12)

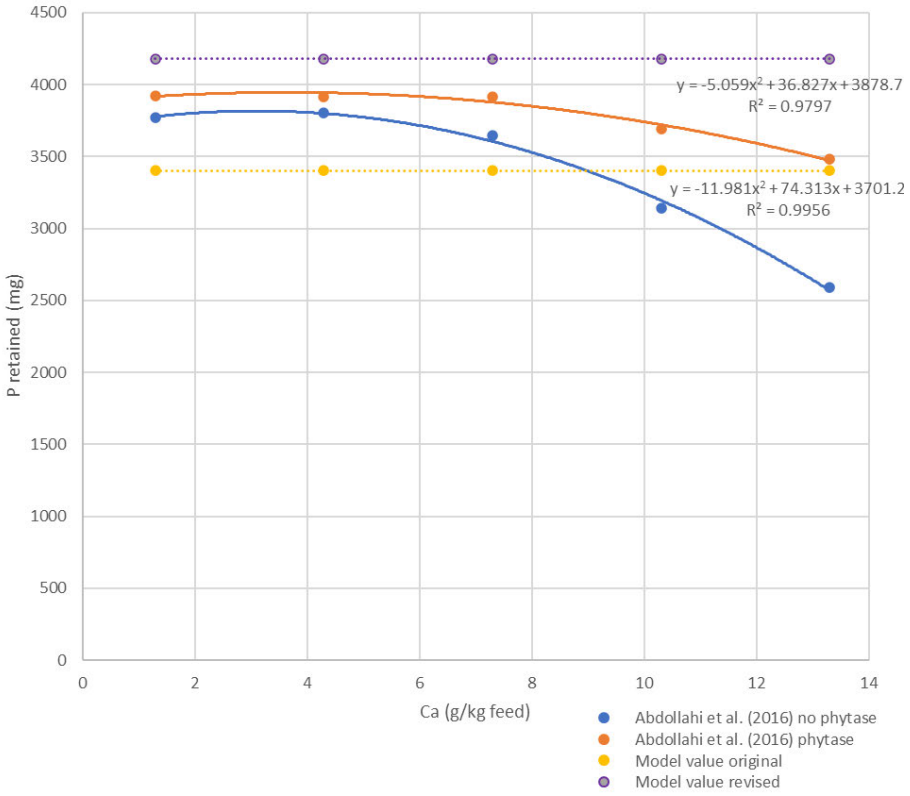

Figure 5.8 P retained by broilers in the starter phase

Source: after Abdollahi *et al.* (2016) and model outputs (Table 5.12)

This study also suggested that the model was originally underestimating the Ca and P requirements of birds growing to their potential, since these should represent the maximum possible mineral that can be retained. The revised coefficients are a better estimate of the retention in this study and were used for further model evaluation.

5.16. Calcium and phosphorus digestibility

The model of growth described in this chapter requires inputs of available Ca and P. These are understood to be the amounts of mineral that can be absorbed from the GIT to meet the bird's requirements for growth, maintenance and endogenous secretions. The chemical characteristics of the feed ingredients will determine the digestibility of the nutrients in them to a certain extent, while the characteristics of the mixed feed will give rise to further digestibility considerations. The feed will interact with conditions in the digestive tract, which are determined by dietary and bird factors, resulting in a proportion of the nutrients in the feed being absorbed across the wall of the tract into the body of the bird. Variability in the digestibility values for feed ingredients (see section 4.4.2) and the importance of interactions between Ca, nPP, P and phytase in the feed influenced the decision to develop a model of these interactions for the complete feed rather than including digestibility values for feed ingredients in the feed formulation matrix.

The Ca-P-phytase interactions in the digestive tract must be modelled if an accurate estimate is to be obtained of the Ca and P that is available to the bird. The ability of the bird to adapt and to extract a greater proportion of the minerals available to it must be considered through adjustments to the digestibility based on age, body mass or body protein and possibly current or previous deficiency.

The focus of this project was on the growth model, but to carry out the evaluation, consideration must be given to the availability of the nutrients consumed by the bird. Two options were considered for the calculation of available Ca and P: a deterministic, physico-chemical model (Létourneau-Montminy *et al.*, 2011) or an empirical model from digestibility values. While the former is considered to be a desirable goal, the latter has been shown to be a feasible and practicable alternative (Létourneau-Montminy *et al.*, 2015; Misiura *et al.*, 2020). These two options are discussed in this section.

5.16.1. Regression models based on digestibility measurements

In Chapter 4, the published literature was reviewed, and a number of digestibility studies were identified that might be useful in the development of regression models of digestibility (see Table 4.12).

Misiura *et al.* (2018) conducted a meta-analysis of Ca digestibility in pigs. The regression indicated significant effects of tCa, PP, nPP (g/kg BW/d) and phytase (FTU/kg BW/d) intakes on absorbed Ca (g/kg BW/d). These fixed effects and the interactions between tCa and exogenous phytase and between exogenous phytase and PP, explained 90% of the variability in Ca absorption. A regression in which variables were expressed on a g/kg DMI was also considered by Misiura *et al.* (2018), but only estimated endogenous losses were reported on this basis. The BW used to scale the variables was the initial BW,

presumably at the beginning of the retention or digestibility assay: serial BW measurements or final BW were recorded in too few studies.

The requirements for inclusion in such an analysis are summarised in Table 5.13.

Table 5.13 Selection of studies suitable for empirical digestion models

Diets:	Birds:	Measurements:
• at least three levels of P or Ca tested • maize-soyabean or wheat-soybean basal diets • no additives except phytase • feed intake for the assay period reported with no changes in feed • analysed Ca and P reported • adequate protein, energy and vitamin D fed	• 35 days or younger • male and/or female, • modern, selected broiler strains • body weight recorded at the time of the digestibility assay	• ileal or precaecal digestibility of Ca AND P

When the studies returned by the systematic search were considered for similar analyses, some differences in the way in which poultry and pig studies are commonly reported became apparent. Feed intake is seldom reported for a short period before slaughter for ileal digestibility determination. These data are often reported weekly, as is BWG, but the performance data do not allow the calculation of absolute absorption of Ca and P, unless it is assumed that digestibility does not change with age. Only 5 of the studies returned by the systematic search recorded BW at slaughter. Hence scaling of digestibility to either BW or feed intake does not seem feasible using published literature.

Ileal digestibility was most commonly measured at 21 days or soon after: 25 out of the 38 studies used birds between 20 and 25 days of age. Digestibility may change with age (see Figure 5.9).

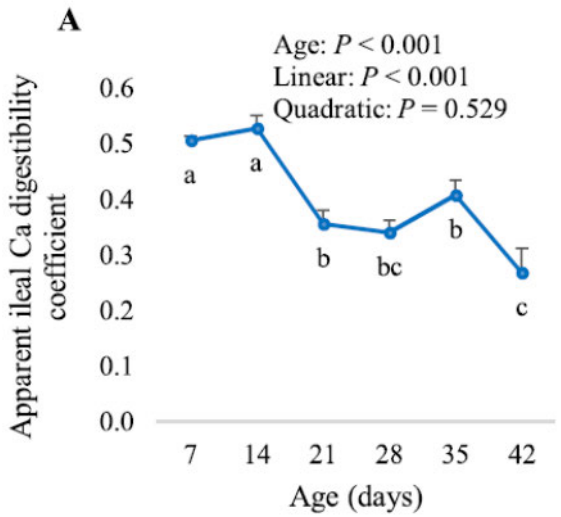

Figure 5.9 Effect of age on apparent Ca digestibility coefficient

Source: David *et al.* (2020)

It is uncertain if age effects on digestibility are due to differences in mineral availability or bird requirements and their effects on absorption (Shastak *et al.*, 2012b). Ileal or precaecal digestibility is usually determined on a single occasion (usually the last day of the trial) so that the interaction with age is usually not explored within an experiment. However, an analysis of Ca and P digestibility at 21 days using published data could draw on the largest number of studies. Similar body weights and feed intakes could perhaps be assumed for birds close in age.

The effect of particle size on digestibility should be included in the analysis for major Ca sources (McNaughton *et al.*, 1974; Guinotte *et al.*, 1991; Kim *et al.*, 2018). Particle size may contribute a greater variation to digestibility than Ca source, with an increase in particle size of limestone and oyster shell from 0.5 to 1 mm increasing digestibility by approximately 50% (Anwar *et al.*, 2017). This would add further complexity to the regression model.

Ideally, empirical models should be applied only in similar conditions to those in which they have been shown to work. If the only goal were to model standard commercial broiler production, these would probably suffice. Hence regression models could provide a bridge so that growth model development can progress.

5.16.2. Mechanistic modelling of Ca and P digestibility

Digestibility depends on the presentation of dissolved minerals at the wall of the GIT and the ability of the bird to transport these minerals across the gut wall into the blood. This ability may be assumed to be a function of the bird's needs for growth and maintenance (and hence depletion of the blood plasma pool). The dissolved minerals will be products of various chemical and physical processes which take place in the gut lumen. It is these processes that should be modelled if digestibility is to be assessed mechanistically.

The gut is a reactor, in which the feed encounters various secretions produced by the epithelial cells of the GIT wall and the accessory organs, such as the pancreas that secretes bile. Reactions may occur as the dry feed becomes mixed with liquid and both endogenous and exogenous enzymes catalyse the breakdown of feed components. To model the fate of Ca and P in this reactor, it would be necessary to quantify the different forms of these minerals and the prevailing chemical conditions, such as pH, in the different parts of the GIT. *In vitro* studies of the digestion of Ca and P and action of phytase in the broiler gut attempt to recreate the conditions in various sections e.g. crop, proventriculus and small intestine (Zyla and Gogol, 2002; Lan *et al*., 2010; Sommerfeld *et al*., 2017). The deterministic model developed by Létourneau-Montminy *et al.* (2011) included simulation of the hydrolysis of phytate by phytase, using a Michaelis-Menten equation and *in vitro* and *in vivo* derived parameters from pigs.

The balance of cations and anions in the diet is another consideration in modelling the chemical interactions in the GIT (Gorman and Balnave, 1994). While supplementation of metabolisable anions and cations was reported to affect broiler performance a useful balance equation to capture this information was not established.

Composition of digesta depends primarily on feed composition. However, this is modified by secretions into the gut and the removal of material across the intestinal wall. The pH of the digesta varies along the GIT due to the secretion of hydrochloric acid in the proventriculus and bile in the proximal section of the duodenum. pH ranges in different sections of the broiler GIT, drawn from Guenter and Sell (1973), Wiseman *et al.* (1956), Farner (1942) and Shafey *et al.* (1991), are shown in Table 5.14.

Table 5.14 pH in different sections of the broiler GIT

Source: van der Klis (1993)

Segment	pH
Crop	4.5 – 5.3
Proventriculus	2.0 – 4.6
Gizzard	2.6 – 4.3
Duodenum	5.5 – 6.2
Jejunum	5.8 – 6.9
Ileum	6.3 – 8.0
Caeca	5.8 – 6.8
Rectum	6.3 – 7.7

A major consequence of changes in the pH of digesta is the change in solubility of the mineral components. This affects their solubility and hence their availability for absorption by the bird. Wiseman *et al.* (1956) suggested that birds may maintain a similar pH profile through the intestine in spite of changes in diet. This assumption is made in the deterministic model of P digestibility of Létourneau-Montminy *et al.* (2011).

Absorption of minerals also requires that digesta be exposed to the gut epithelium. The time during which this can occur depends on the rate of passage of the gut contents through the GIT. Table 5.15. summarises some experimental values that have been recorded for the retention of the digesta in the various sections of the gut.

Table 5.15 Estimated retention times in different sections of the broiler GIT

Source: van der Klis (1993)

Segment	Retention time (min)
Crop	31 – 48
Proventriculus+ Gizzard	33 – 39
Duodenum	5– 10
Jejunum	71 – 84
Ileum	90 – 97
Caecum	119
Rectum	26 – 56

Retention time varies considerably, with the viscosity of the contents shown to influence this variable (van der Klis, 1993).

In order to model the mass and composition of the contents in each section of the gut, intake and retention time must be combined to determine the proportion of intake present in a given section at any time during the feeding period (Sommerfeld *et al.*, 2017; Svihus and Itani, 2019).

Some of the fundamental data, such as transit time and pH in different sections of the GIT have been published. The principles of a deterministic model of P digestibility have been demonstrated (Létourneau-Montminy *et al.*, 2011) and a combined, deterministic model of Ca and P could provide the most versatile option for quantifying the minerals available to the bird. However, the development of such a model will require experimental calibration (e.g. repeated measurements of the quantity and form of minerals excreted by birds). Alternatively, it may be possible to calibrate it by regressing mineral deposition in the body on intake and establishing the quantities available to the bird. This will require a well-established and reliable model of mineral growth and accurate estimates of endogenous losses and maintenance requirements. This thesis forms the foundation of such a model and the wider project will support the development of such a deterministic model.

5.17. Discussion

A model of Ca and P growth in the broiler has been proposed, with ten propositions that will need to be tested both through modelling simulations and further experimental work. The next chapter will describe the calibration of the model and validation exercises to assess its present capacity to predict mineral growth.

CHAPTER 6. MODEL EVALUATION

The previous chapter described a model of the growth of Ca and P in the broiler body, based on an existing model of protein growth and energy. A preliminary calibration compared the model coefficients with the relationship between retained Ca and P and retained protein in broilers. Considering these findings, and the coefficients from pig studies, the allometric coefficients were revised. In this chapter, the model will be calibrated using whole body analyses from the literature and further digestibility and retention studies. This exercise was not intended to produce a final model, but to demonstrate that modelling Ca and P growth in broilers is feasible and could be useful. Further research, as described in Chapter 7, will be necessary if a complete, functional model is to be produced.

6.1. Selection of studies for model evaluation

The systematic literature review (Chapter 4) revealed how few studies of calcium and phosphorus nutrition reported the chemical composition of whole broiler bodies. Only Angel (2007) dissected bodies to analyse the skeleton separately, but in that study body protein was not measured and the cartilage caps were left on the bones, resulting in lower ash measurements than expected.

Of the 10 body composition studies identified in the systematic literature review, two were principally used in the development of the model (Hurwitz and Plavnik, 1986; Caldas *et al.*, 2019). The others were considered for calibration of the model. To be useful for this purpose, the studies would be required to meet the reporting requirements outlined in Table 6.1.

Table 6.1 Selection of studies for calibration of growth model

Diets	Body composition
At least two levels of Ca and P in feed Vitamin D level adequate Ca, P analysed in feed Protein in feed calculated or analysed Fed ad lib	Ca, P and protein analysed in empty body

Body protein content may be predicted using the EFG broiler model, but would also ideally be reported, so that the relationships between protein and minerals in the body can be reliably established.

While the abstracts of all studies in Table 4.6 mentioned the analysis of body composition, including Ca and P, this was not always reported in a way that allowed the required measures to be extracted. In some, the composition of the feed was not reported (Broadbent *et al.*, 1981; Kadim *et al.*, 2005; Niess *et al.*, 2005). In others, while body composition was analysed, it was reported only as retention (Schoner *et al.*, 1993; Hemme *et al.*, 2005). Only two studies returned in the systematic search contained data which might be used in assessing the model (Olukosi and Adeola, 2008; van Krimpen *et al.*, 2013).

While the first of these two studies included protein, Ca and P body composition data, only three treatments, a negative control, positive control and negative control plus phytase were considered useful, since the others included xylanase as well (Olukosi and Adeola, 2008). Hence van Krimpen *et al.* (2013) was the only study initially considered. The experiment is described in section 6.3.

A PhD thesis from Martin Luther University, Halle-Wittenberg was generously shared with us, which contains body composition studies, pre-caecal digestibility and full-excreta-collection retention studies (Dieckmann, 2004). This was the most comprehensive and reliable source of calibration data, particularly with respect to body composition (see section 6.2).

6.2. Dieckmann (2004)

This study sought to optimise P provision in broiler feed, providing for maintenance and growth. It was pointed out that both feed and animal factors must be considered, including P sources, phytate levels, bird age, sex and genetic characteristics. Whole-body analysis and precaecal (ileal) digestibility were chosen to allow the quantification of the P absorbed by the bird and retained in the tissues. Hence the study comprised two trials in which birds were fed diets varying in Ca and nPP. These ran from 1-22 days and from 23-43 days respectively. Birds were slaughtered at intervals and analysed for whole-body ash, Ca and P. Protein, lipid and dry matter contents were reported as averages across the treatments at each slaughter age. A further three P response trials investigated precaecal digestibility (sampling digesta from the ileum) and retention (excreta collection). A last trial investigated the effect of Ca on P retention, with 2 levels of P in the feed.

6.2.1. Body composition studies

6.2.1.1.Study description

Two basal diets, a starter and a grower, were formulated. For each, a proportion of the feed was reserved for a mixture of varying amounts of MCP, limestone and sand, and a constant level of maize starch, so that the protein and energy content of the feeds within each trial remained constant while the Ca and P levels in the feed changed. The basal diet was principally composed of maize, wheat gluten and soybean meal (450 g/kg CP). When the composition was formulated using the Winfeed® feed formulation program from EFG software, the protein levels were close to those analysed (285 g/kg formulated vs 279 g/kg analysed for the starter and 280 g/kg formulated vs 275 g/kg analysed for the grower). The formulated digestible lysine levels were 11.7 g/kg for the starter and 10.2 g/kg for the grower. This was assumed to be the first-limiting nutrient for the calculation of feed intake in the broiler model. The formulated PP value was 1.6 g/kg for both feeds. The analysis showed that this was correct for the grower, but the starter was slightly higher, at 1.7 g/kg.

Seven feeds were prepared, with varying levels of Ca and nPP. The mineral composition is shown in Table 6.2. Variability in tP, reported in the study, can be ascribed to nPP since the same basal diet was used and MCP was the additional source of P. No exogenous phytase was added to the feeds in this study.

Table 6.2 Analysed Ca, tP, and nPP (g/kg) in experimental feeds

Source: adapted from Dieckmann (2004)

	Feed number						
	1	**2**	**3**	**4**	**5**	**6**	**7**
Starter							
Ca	5.04	6.15	6.79	7.59	8.27	8.04	7.94
tP	3.32	4.17	4.87	5.69	7.59	8.25	8.95
nPP	1.62	2.47	3.17	3.99	5.89	6.55	7.25
Ca:tP	1.52	1.47	1.39	1.33	1.09	0.97	0.89
Ca:nPP	3.11	2.49	2.14	1.90	1.40	1.23	1.10
Grower							
Ca	4.43	4.67	5.84	7.17	7.52	7.46	7.32
tP	2.55	3.09	4.01	4.81	5.76	6.79	7.45
nPP	0.95	1.49	2.41	3.21	4.16	5.19	5.85
Ca:tP	1.74	1.51	1.46	1.49	1.31	1.10	0.98
Ca:nPP	4.66	3.13	2.42	2.23	1.81	1.44	1.25

Experiment IA: The starter study comprised 1400 Ross birds, with 5 replicates of each of the 7 treatments for each sex. Floor pens contained twenty birds at the start of the experiment. Live mass and feed intake were recorded weekly. 50 additional birds from each sex were killed at day-old, and five birds from each pen were killed at the end of weeks 1, 2 and 3. Empty body weight, with feathers, was recorded.

Experiment IB: The grower study comprised 350 Ross birds of each sex. They were reared at the same time as the starter experiment, but all were fed diet 6 from the starter trial for the first two weeks and diet 4 until 23 days of age. They were divided into groups of 10 birds of similar BW. 25 birds of each sex were killed to obtain an initial body weight measurement and 5 birds from each of the pens was killed at 42 days of age.

6.2.1.2.Results

The treatments had no significant effects on growth, feed intake or feed efficiency. This indicated that the diets were not extreme, which was desirable for the initial calibration of the model.

Ash, Ca, P and Mg content of the EBW was reported for each treatment. It was noted that these were strongly influenced by the different levels of nPP. Protein, dry matter and lipid content (g/kg EBW) of the empty body were not significantly affected by the feeds. Hence these were reported only as the average over all the treatments at the end of each week in the body of the thesis. However, the raw data were included in the appendices, so that it was possible to calculate mean protein content (g/kg EBW) for each treatment and sampling age.

It was necessary to estimate feather protein using the Ross 308 feather protein calculation in the EFG broiler model, so that the EFFBP could be estimated and hence Ca/EFFBP and P/EFFBP coefficients could be calculated. These were not required for the modelling exercise but provided some validation of the overall isometric coefficients (g whole-body mineral/g body protein) discussed in Chapter 5 (summarised in Table 6.3). It also suggested a possible method of establishing such coefficients (see section 7.1.1).

Table 6.3 Effects of Ca and nPP in feed on body composition of female broilers at days 7, 14 and 21
Source: adapted from Dieckmann (2004)

			End week 1		End week 2		End week 3	
Feed #	**Ca**	**nPP**	**Ca:protein**	**P:protein**	**Ca:protein**	**P:protein**	**Ca:protein**	**P:protein**
1	5.04	1.62	0.0187	0.0179	0.0252	0.0213	0.0242	0.0186
2	6.15	2.47	0.0242	0.0202	0.0318	0.0237	0.0330	0.0244
3	6.79	3.17	0.0303	0.0229	0.0373	0.0267	0.0340	0.0247
4	7.59	3.99	0.0371	0.0262	0.0383	0.0277	0.0386	0.0270
5	8.27	5.89	0.0349	0.0259	0.0399	0.0288	0.0381	0.0255
6	8.04	6.55	0.0373	0.0276	0.0394	0.0286	0.0393	0.0279
7	7.94	7.25	0.0358	0.0263	0.0416	0.0295	0.0402	0.0275

It was not possible to establish any allometric relationships using these data, since this would require body composition analysis to a more advanced age. However, the regressions of Ca and P on body protein for the different nPP treatments in experiment IA were analysed and are shown in Figure 6.1.

The slopes of the regression plots represent the rate at which mineral content in the body increases as protein content increases. If isometry was maintained regardless of diet, this rate would be constant and hence all the coefficients would be the same. However, these regressions suggest that for the first three treatments, body Ca and P were reduced relative to body protein. The slopes of the graphs were less than those of the remainder of the treatments, which were similar to one another. This implies that, with certain feeds, less mineral is deposited in the body relative to protein deposition. Taken in conjunction with the absence of effects on body protein, this provides justification for the decoupling of body protein growth and mineral growth in the model under non-ideal conditions. This is achieved through the separation of BFB and bone growth: while BFB growth is reduced if P is deficient, a deficit of minerals in the bones does not influence protein growth and hence protein, and by extension body weight, can continue to grow normally while bone minerals do not. It is important to note that this does not contradict the notion of isometry between FFBP and Ca and P under ideal conditions.

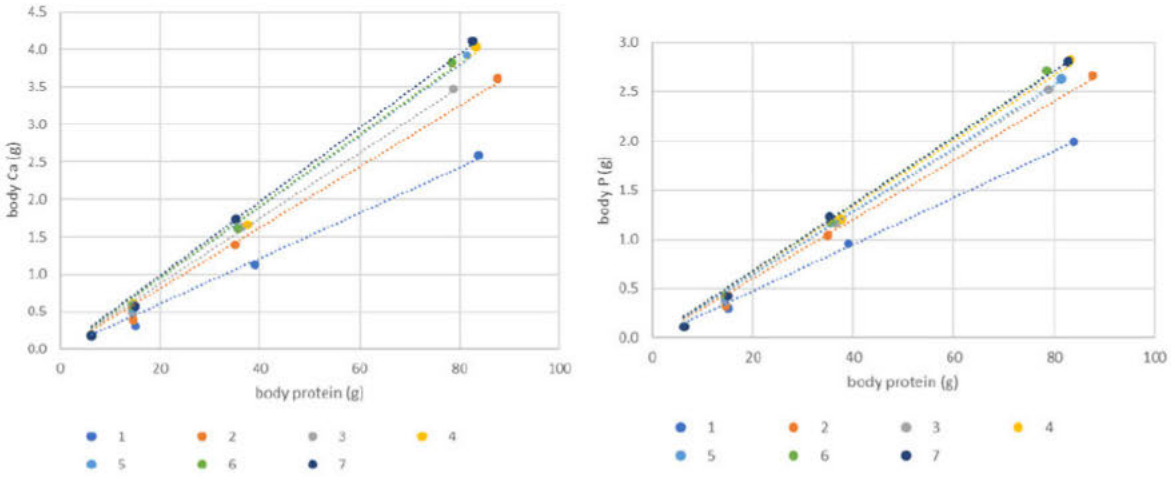

Figure 6.1 Regression of empty body Ca and P in male broilers on empty, feather-free body protein for the first three weeks of life, for 7 levels of nPP in feed

Source: adapted from Dieckmann (2004)

The effect of diet on body minerals is a function of the availability of Ca and P from the diet and the requirements of the animal. The impact of lower nPP levels was mitigated by greater dephosphorylation of PP. It was calculated in this study that, assuming 100% availability of nPP, up to 40% of available P was supplied from PP in the treatments with the lowest level of P (Dieckmann, 2004, p. 43). In contrast, where 80% of P in the diet was in the form of nPP, only half of this was utilised, assuming no PP dephosphorylation. This highlights the need for a model in which both the digestibility of Ca and the different forms of P, and the utilisation of the available minerals in the body are considered. This will allow the effects of different feeding strategies on these two systems to be understood and outputs improved.

The simple linear regression coefficients of the graphs in Figure 6.1 are summarised in Table 6.4.

Table 6.4 Regression slopes for chemical components of growth

Source: adapted from Dieckmann (2004)

	in feed			in growth		
Feed #	**Ca**	**P**	**Ca:nPP**	**Ca:EFFBP**	**P:EFFBP**	**Ca:P**
1	5.04	1.62	3.11	0.0303	0.0237	1.27
2	6.15	2.47	2.49	0.0406	0.0301	1.35
3	6.79	3.17	2.14	0.0438	0.0318	1.38
4	7.59	3.99	1.90	0.0475	0.0334	1.42
5	8.27	5.89	1.40	0.0474	0.0322	1.48
6	8.04	6.55	1.23	0.0478	0.0341	1.40
7	7.94	7.25	1.10	0.0492	0.0339	1.45

As discussed in section 5.2, these values may overestimate growth in the first week after hatch. The male birds at hatch contained 0.0278 g Ca/g EFFBP and 0.0175g P/g EFFBP. By the end of the first week male birds on treatment 1 contained 0.0205 g Ca/g EFFBP and 0.0197 P/g EFFBP. The accretion ratios from the values reported in the study were 0.0134 g Ca gain and 0.0186 g P gain/g EFFBP gain versus 0.0442 g Ca

gain and 0.0348 g P gain/g EFFBP gain for treatment 4. By the second week these values had risen to 0.0312 and 0.0249 g/g EFFBP gain in treatment 1, but it seems clear that Ca, in particular, was severely undersupplied in the first week. Only treatments 5, 6 and 7 appeared to provide for the Ca and P growth close to the values from the regressions in the first week.

The results of the body composition studies described above were captured for use in the training phase of the model calibration, described in section 6.4.

6.2.2. Digestibility studies

In two other studies in this project, precaecal (ileal) digestibility was determined. The feeds were formulated to provide adequate amounts of nutrients other than Ca and P to meet the broilers' requirements.

Experiment IIB: The birds for this experiment were fed a starter diet (GfE, 1999 recommendations, approx. 14.3 g/kg lysine, 10g/kg Ca, 7 g/kg P, 5.2 g/kg nPP) until 9 days of age. They were weighed and transferred to cages in groups of 10 birds. 7 levels of P were fed, with 5 replicates for each of the higher levels and 4 replicates of the lowest level. The analysed feed composition is summarised in Table 6.5.

Table 6.5 Analysed Ca, total P, and non-phytate P (g/kg) in digestibility trial feeds
Source: Dieckmann (2004)

	Feed number						
	1	**2**	**3**	**4**	**5**	**6**	**7**
CP	217	217	217	217	217	217	217
Ca	5.52	7.04	7.45	9.48	9.88	9.50	10.01
tP	3.15	4.05	5.00	5.92	7.01	8.39	10.09
PP	1.7	1.7	1.7	1.7	1.7	1.7	1.7
nPP	1.5	2.4	3.3	4.2	5.3	6.7	8.4
Ca:nPP	3.8	3.0	2.3	2.2	1.9	1.4	1.2

The Ca:nPP ratios in this digestibility assay were similar to those in the starter body composition trial, although higher levels of Ca and nPP were fed in diets 6 and 7 in the digestibility trial. This was considered a useful range of mineral levels with which to determine digestibilities for the body composition simulation.

The male broilers ate the test feeds for 12 days. All birds were killed at 21 days and weighed. The small intestine from Meckel's diverticulum to 2 cm before the opening of the caecum was excised and the contents rinsed out with distilled water. The contents from all the birds in each cage were combined for the determination of pre-caecal digestibility.

Experiment IIC: Feeding began at 17 days in this experiment. Feeds were fed for 11 days, with collection of excreta in the last 5 days for the calculation of mineral retention. Birds were killed at 28 days for the determination of precaecal digestibility.

In this trial, a semi-purified diet was fed, containing maize, potato protein and maize starch. When formulated in Winfeed®, the protein exactly matched the analysed level of 184 g/kg, with PP at 1.5 and tP

at 2 g/kg. This matched the analysed P level of 2.02 g/kg, which was fed in Treatment 1, along with a Ca level of 5.52 g/kg. The mineral composition of the treatments is summarised in Table 6.6.

Table 6.6 Analysed Ca, total P, and non-phytate P (g/kg) in experimental feeds

Source: Dieckmann (2004)

	Feed number						
	1	**2**	**3**	**4**	**5**	**6**	**7**
Ca	3.30	6.32	7.80	9.74	9.47	9.45	9.41
tP	2.02	2.51	3.23	4.71	5.20	5.96	7.14
PP	1.50	1.50	1.50	1.50	1.50	1.50	1.50
nPP	0.52	1.01	1.73	3.21	3.70	4.46	5.64
Ca:nPP	3.11	2.49	2.14	1.90	1.40	1.23	1.10

Digestibility coefficients for Ca and P measured in these two experiments are summarised in Table 6.7.

Table 6.7 Digestibility coefficients (% of intake absorbed) in experiments IIB and IIC

Source: Dieckmann (2004)

		Feed number						
		1	**2**	**3**	**4**	**5**	**6**	**7**
IIB	Ca	80.0 (±1.41)	80.0 (±1.56)	71.8 (±0.80)	57.2 (±2.50)	44.7 (±0.82)	41.1 (±1.75)	37.7 (±1.31)
	P	64.4 (±2.54)	63.9 (±1.52)	62.7 (±0.95)	56.6 (±1.78)	49.8 (±0.79)	51.2 (±2.27)	49.5 (±2.21)
IIC	Ca	74.3 (±1.95)	70.7 (±2.18)	60.8 (±3.06)	43.1 (±5.57)	36.2 (±4.32)	30.3 (±6.28)	31.3 (±5.68)
	P	46.6 (±3.05)	44.1 (±2.75)	47.5 (±0.54)	43.9 (±2.50)	47.4 (±1.63)	44.5 (±2.41)	42.1 (±4.76)

The lower P digestibility seen in experiment IIC was surprising, as almost all P was supplied from inorganic sources. While the basal diet is not expected to influence the digestibility (Shastak *et al.*, 2014), it was considered prudent to apply the model from experiment IIB, in which diets more similar to the diets in experiment IA and IB were fed.

Net precaecal P absorption decreased with increasing P in the feed, from 64 to 50% and Ca net precaecal absorption declined from 80 to 38%. Ca and P levels both changed between feeds, providing an opportunity to determine the effects of these variables and any interactions between them.

The empirical digestibility model proposed for use with the broiler growth model is based on a regression of Ca and P ileal digestibility on Ca, PP, nPP and phytase levels in the feed. The principal objective in the training and validation process described in this chapter was to establish the usefulness of the growth model. Combining this with a simplified digestibility model reduced the uncertainty that arises when trying to separate the effects of interactions in the GIT and Ca and P interactions in the body during growth. In the study described here, no phytase was added to the diets and a single level of PP was applied throughout with the use of the same maize/soybean/wheat gluten basal diet for all treatments. A multiple regression for the digestibility of Ca and P in which only two factors (Ca level and nPP level) were independent variables provided a good fit to the data. Calculations of the digestibility of complete feeds, based on trials with birds of the same genotype and even the same parent stock, could reasonably be applied in the evaluation of the effect of diet on body composition for the model.

A regression analysis of Ca and P digestibility on Ca and nPP levels in the feed produced the following models for experiment IIB:

$$proportion\ of\ Ca\ absorbed\ = 1.149(\pm 0.148) - 0.042(\pm 0.025) \times Ca - 0.045(\pm 0.018) \times nPP\ (Eq.6.1)$$

$$proportion\ of\ P\ absorbed\ = 0.796(\pm 0.072) - 0.020(\pm 0.012) \times Ca - 0.013(\pm 0.009) \times nPP\ (Eq.6.2)$$

There was no significant interaction between Ca and nPP on either dependent variable. These two models were used in the training phase of model adjustment.

6.3. van Krimpen *et al.* (2013)

The effect of changing growth rates (through manipulation of protein levels), different Ca/aP ratios and incremental available P levels on body composition, ileal digestibility and nutrient retention, were tested.

Birds were fed a standard starter feed to 10 days of age, after which the treatments were applied. The calculation of aP was not explained, but reference was made to the CVB feed tables. These contain phosphorus availability values for feed ingredients from digestibility trials and hence, unlike the NRC requirements, aP cannot be equated to nPP. The feeds were therefore captured in the WinFeed ® feed formulation program and the nPP and PP values were derived from the composition (reported in an appendix to the publication). As reported in the study, the calculated values for Ca and P were below the analysed values. The difference was allocated equally to nPP and PP, since its origin was unclear. This resulted in the Ca, nPP and PP values for the grower diets shown in Table 6.8.

Table 6.8 Analysed Ca, total P, phytate-P and non-phytate P (g/kg) in body composition trial feeds
Source: van Krimpen *et al.* (2013)

	Feed number					
	1	**2**	**3**	**4**	**5**	**6**
Ca	3.87	5.98	7.89	5.75	8.44	11.05
tP	5.36	5.37	5.38	6.58	6.53	6.74
PP	2.10	2.10	2.10	2.10	2.10	2.10
nPP	3.26	3.27	3.28	4.48	4.43	4.64
Ca:nPP	1.19	1.83	2.41	1.28	1.91	2.38

Although it was mentioned that samples of birds were killed and dissected at days 1, 10, 35 and 42 (for the standard growth broilers), only the 35-day body composition results were reported. This provided a validation point for body composition. The dissected carcasses were analysed for N, ash, Ca and P.

Main effects rather than treatment means were reported in the tables. The effects of changing Ca/P ratios and incremental aP levels were combined to recreate treatment means. The treatments in which growth rate was slowed and the effects of sex were not modelled.

It was reported that the mass of the skeleton as a proportion of body weight varied from 6.7% when a low Ca/P ratio was fed with a low aP level to 8.6% of EBW when a standard Ca/P ratio was fed with a high aP level. The skeleton was 17-21% of BW in (Angel, 2007). This suggests that the dissection reported in the

study under consideration excluded some bony body parts. This is further confirmed by the high calcium content of the body component described as soft tissue (24.7% of total body Ca) when it is expected that not more than 1 or 2% of Ca will be in the BFB. It might be that heads and feet were combined with the soft tissue. Hence, although trends in skeleton mineralisation could be discerned in the reported data, the proportion of P in BFB and bone would have had to be inferred from EBW Ca/P ratios and skeleton Ca/P ratios rather than directly calculated from the reported data. For this reason, and because the data used in the training exercise did not separate bone and BFB, it was decided that whole body results would be assessed in the validation exercise.

Ileal and faecal digestibility fractions for this study are summarised in Table 6.9.

Table 6.9 Digestibility coefficients (% of intake absorbed) of Ca and P in feeds varying in Ca and non-phytate P

Source: van Krimpen *et al.* (2013)

		Feed number					
		1	**2**	**3**	**4**	**5**	**6**
Ca	ileal	41	44	61	22	35	51
	faecal	39	50	68	21	31	50
P	ileal	42	45	60	38	41	56
	faecal	44	41	39	29	32	33

The discussion of this study emphasised the importance of the Ca/P ratio in the diet. It highlighted the negative effects of a high ratio on the protein content of the broiler body, suggesting that P was limiting for performance. It was proposed that this was a result of the effects on digestibility of P due to the formation of insoluble inorganic calcium phosphate (Ca_2PO_4) in the GIT. Although skeleton as a proportion of EBW and tibia mass were also negatively affected, tibia strength and Ca and P content were highest at the highest Ca/aP ratio. This finding is representative of the phenomena that may be clarified using a model that accurately captures the digestibility of Ca and P and their allocation to bone and BFB.

Although faecal digestibilities were higher than ileal digestibility fractions for Ca in feeds 2 and 3, and for P in feed 1, suggesting absorption in the large intestine, this was not a consistent phenomenon. Hence the whole-body composition at 35 days and the ileal digestibilities reported in this study were used in the validation exercise.

6.4. Model calibration

The model was initially developed in Excel®, but test runs in this format are time-consuming to set up and difficult to compare. The final model will be part of an existing software suite that includes a feed formulation program (Winfeed®), a broiler model of protein and energy that has been validated for commercial use, and an optimiser that integrates the other two functions to allow the producer to meet production, financial and environmental targets (see Figure 3.9). While this readily allows the input of feed compositions from published trials and comparisons of broiler performance with different treatments, the

code is not readily accessible. To facilitate model development and to allow the exploration of more academic feeding options (such as morning and afternoon feeding of different diets (Gous and Du Preez, 1975)), a standalone version of the model, including the potential protein growth component was coded in Matlab® to allow development of the model in a transparent and readily accessible form.

6.4.1. Starter feed trials

Initially, model parameters were adjusted by regression to fit the data, so that the principal differences between the runs (simulations) and the experimental data would be those intrinsic to the structure of mineral model. Adjusted parameters are summarised with the original parameters in Appendix 1. Feed intake and body weight gain reported in (Dieckmann, 2004) were treated in this way. The fit of these to the model with regression-adjusted lysine levels (as the first-limiting nutrient) are shown in Figure 6.2.

Throughout the following figures, colours represent the different feeds used in the body composition experiment. The model values are shown as lines representing model functions, while experimental values are shown as circles of the same colour. Body weight and feed intake were recorded at weekly intervals. These variables were not significantly affected by the treatments, and a single model line is seen: the fitted lines are superimposed on one another and hence only the black line (treatment 7) is visible. The model reduces protein growth only if the provision of phosphorus is below the requirement for bone-free (BFB) body growth. With an excellent fit to the reported bird performance measurements, the behaviour of the model with regard to Ca and P was next considered.

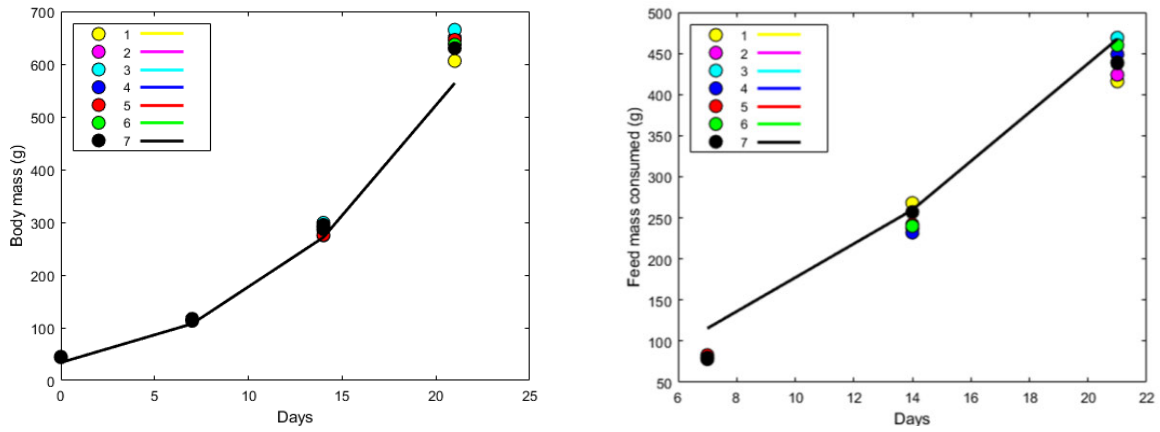

Figure 6.2 Fit of model to experimental body weight and feed intake data

The experimental values of minerals relative to protein mass are recorded in Table 6.3. The modelled and experimental values for Ca and P relative to body weight against time are shown in Figure 6.3. In this figure, the experimental values are represented by dots (values at 7, 14 and 21 as calculated from Dieckmann (2004)). The lines represent the model predictions, with each treatment represented by a different colour that corresponds to those allocated to the experimental values of the same treatments.

Feeds 6 and 7 allowed birds to reach considerably higher proportions than the modelled Ca/BW potential between days 14 and 21, while the model fits the experimental values well at day 7. Feed 5, with a fairly

conventional combination of 9.47 g/kg tCa and 5.20 g/kg tP, follows the modelled Ca body concentration most closely. The model anticipates the poorer performance, suggesting lower bone mineralisation, for feeds 1 and 2 during the period 14-21 days of age. However, it is less successful at anticipating the relatively lower Ca body levels found experimentally with feed 4 at day 14.

It seems that the model tends to underestimate the potential growth of Ca relative to body protein in the second and third weeks of life. As the whole-body Ca/EFFBP coefficient was increased well above that estimated for Caldas *et al.* (2019), this may reflect better mineralisation in birds in this earlier period.

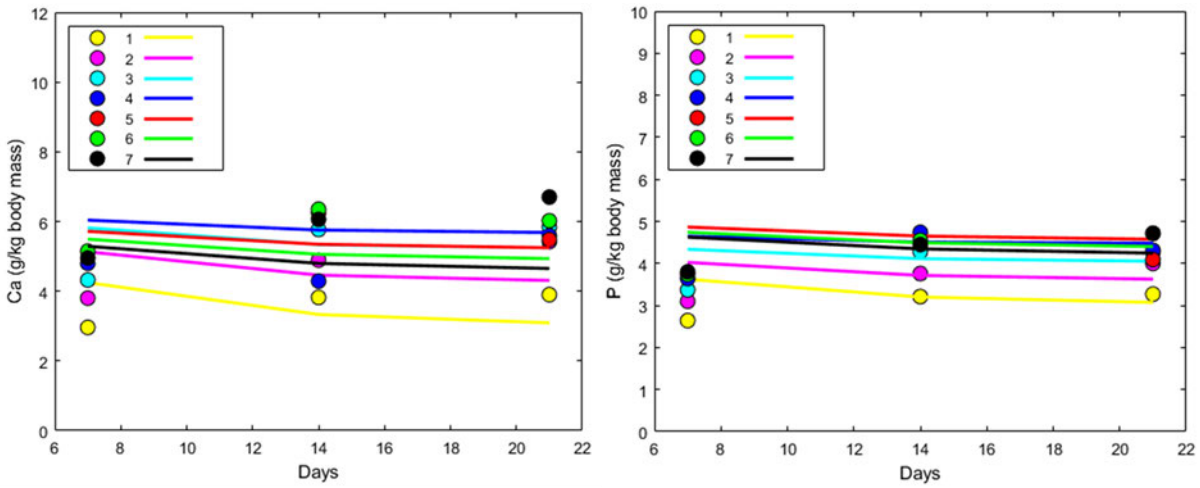

Figure 6.3 Fit of model to experimental Ca and P as a proportion of body mass

The relatively poor bone mineralisation implied by the experimental Ca/BW ratio for feeds 1 to 4 at 7 days is not anticipated by the model. This may be a result of higher requirements in the pre-starter phase or changes in digestibility, as seen in Figure 5.3.

The P/BW relationship is well modelled for most of the feeds at 14 and 21 days. Feed 5 is modelled as close to non-limiting (a horizontal graph) but the experimental concentration at 21 days was slightly lower. The model and experimental values for feeds 4, 5, 6 and 7 are closely clustered. The model does not fit well with the experimental results at 7 days. This suggests that the model is overestimating the requirement for P at this age. The lower experimental values for all feeds may be due to a different relationship between P and body protein in the early period, as suggested in section 5.2, and might be alleviated by higher P levels in the feed in the pre-starter period. Body composition trials will establish more reliable parameters.

In both plots it can be seen that the model anticipates a decline in Ca and P relative to BW on the lower mineral diets, while the experimental data do not agree. This may be a result of variation in digestibility not captured by the model, since only 21-day digestibilities were available and Ca digestibility has been reported to decrease from 7 to 21 days of age (David *et al.*, 2020). Further experimentation will be required to improve the predictive performance of this aspect of the model.

6.4.2. Comparison of modelled digestibility with regression values

In the calibration or training phase of the model, various parameters may be fitted using experimental data. In the previous sections, Ca and P digestibility was modelled. If the amounts of available Ca and P were fitted from the body composition data by regression, the calculated parameters are essentially retention coefficients. For comparison with the modelled digestibilities, this exercise was performed on the calibration data. The coefficients obtained were different, but the results were similar for the lower Ca and P feeds, as seen in Figure 6.4. The higher Ca and P feeds were better fitted by the use of the regressed retention values.

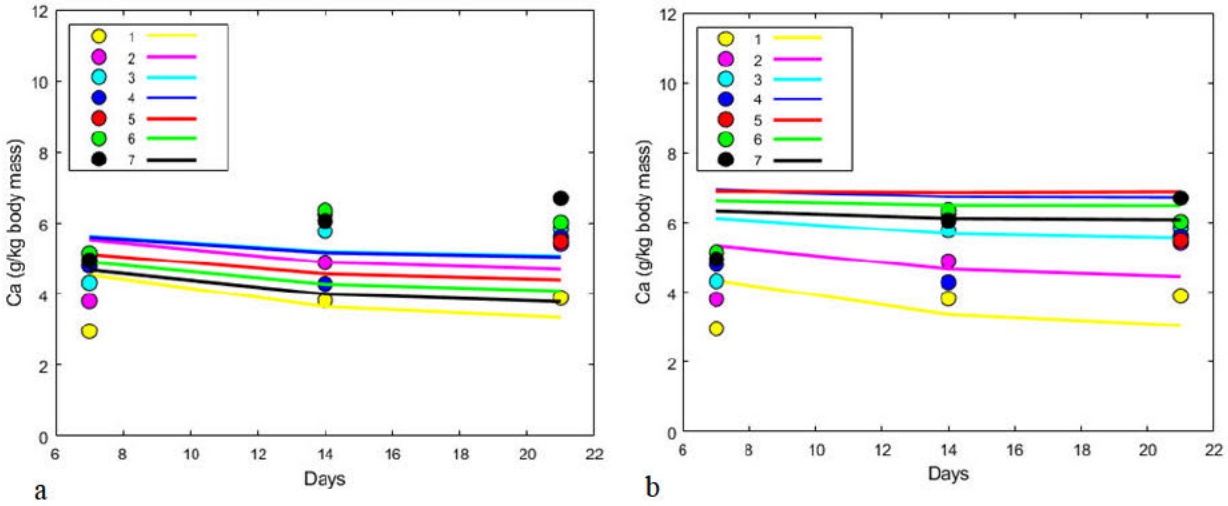

Figure 6.4 Comparison of modelled digestibility (a) with use of regressed retention values (b)

6.5. Model validation

In the calibration/training phase, the model was adjusted to fit the body weight and feed intake data using a regression of the experimental results on the model parameters. Validation requires that simulations are run with these parameters and the fit of the results to the experimental data is assessed.

6.5.1. Grower feed trials (Dieckmann, 2004)

The parameters fitted for the starter period were used for simulations of growth in the grower trial. Only 42-day body compositions were reported, and the feed to day 23 was the same for all treatments. Body weight gain and feed intake plots are shown in Figure 6.5.

The parameter values from the starter feed regression provided a good prediction of the experimental data for BWG and FI in the grower phase.

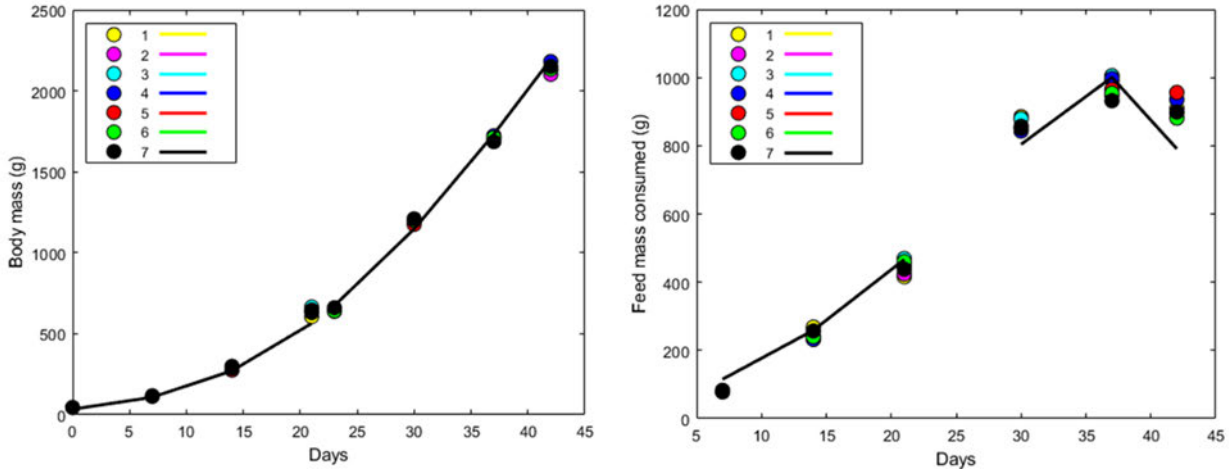

Figure 6.5 Comparison of model with experimental body weight and feed intake data in grower trial

Ca and P masses relative to body weight are shown in Figure 6.6. As seen in Table 6.3, it is to be expected that the ratios between Ca and P and EFFBP, and hence body weight, would be expected to change when Ca and/or P are deficient in the diet. Reduced bone mineralisation, with unimpaired protein gain, gives rise to these changes.

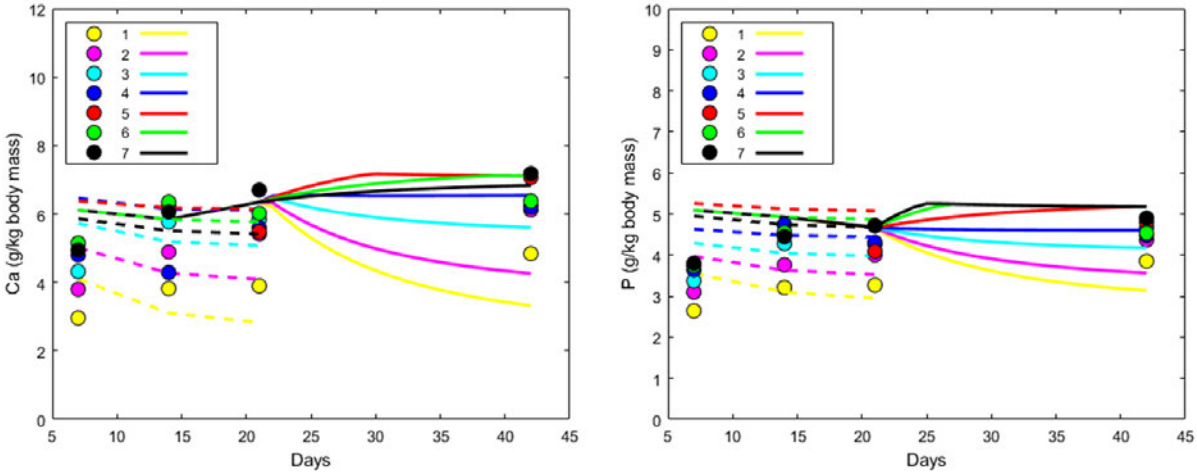

Figure 6.6 Comparison of model with experimental Ca/BW data in grower trial using regression-generated digestibility coefficients

The starter phase graphs are included for comparison purposes (shown as dashed lines). All birds intended for the grower trial ate the same feed in the starter phase, represented by the solid black line up to day 21.

The model appears to be in agreement with the experimental data for the feeds supplying higher amounts of mineral, and this provides support for the whole-body isometric coefficients proposed in section 5.2. However, it did not simulate the effect of the poorer grower diets on 42-day body composition well, underestimating the Ca and P content relative to BW. While the model showed a decline in Ca relative to body mass over the grower phase, this was not observed in the experimental results, which showed an increase in Ca/BW for all feeds. Similarly, the model anticipated a drop in P/BW for feeds 1 to 3 that was not reflected to the same extent in the experimental data. Nonetheless, the relative performance of feeds 1

and 2 was as expected. Once again, the model appears to overestimate the potential P/protein coefficient slightly when the feed is not limiting for either of these nutrients. The simulations in which the regressed retention coefficients from the starter phase were applied to the grower phase are shown in Figure 6.7.

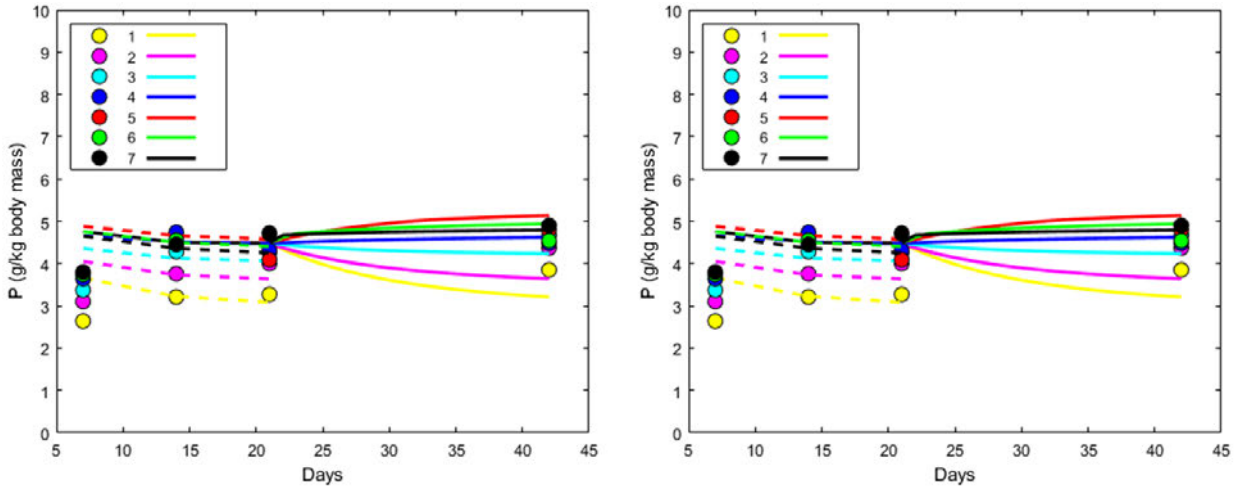

Figure 6.7 Comparison of model with experimental P/BW data in grower trial using regressed retention coefficients

The coefficients derived from the regression of body composition on digestibility in the starter phase produced simulations that were closer to the experimental data, particularly for Ca, but a greater understanding of how the broiler deals with deficiency is required.

6.5.2. Grower feed trials (van Krimpen *et al.*, 2013)

The grower feeds used in the body composition study of van Krimpen *et al.* (2013) (Table 6.8) were applied to the model. Digestibilities were entered directly, since these were determined for birds in the same study (Table 6.9). The standard starter diet (CVB, 2012) was modelled with digestibilities of 46% for Ca and 55% for P. These digestibilities were based on a regression model developed from the digestibility data of (van Krimpen *et al.*, 2016).

The results of the simulation for the 6 feeds are summarised in Table 6.10.

Table 6.10 Body weight and empty, feather-free body protein at 35 days of age for experiment and model comparison

	BW		EFFBP	
Feed	**Exp**	**Model**	**Exp**	**Model**
1	1543	1546	188	250
2	1532	1546	228	250
3	1491	1546	199	250
4	1577	1546	216	250
5	1566	1546	214	250
6	1525	1546	206	250

The Ca/aP ratio was reported to influence BW at 35 d in the original study, with a low ratio causing a 3% reduction in BW. A high aP level increased BW by 2.2%. The model did not reflect these differences. This suggests that the prioritisation of BFB P over bone P may require some adjustment, so that EFFBP growth is reduced before bone growth reaches zero. Similarly, experimental work will be required to establish if a feedback of Ca deficiency on EFFBP growth is required. In the preliminary model discussed here, protein growth is slowed if insufficient P is available for EFFBP growth. If Ca deficiency compromises growth, this suggests that compromised bone mineralisation reduces the rate of maturing of body protein because most Ca in the broiler body is present in the bone. This should be incorporated into future versions of the model if empirical evidence suggests this is the case. However, the average experimental BW of 1539 g is close to the modelled mass. The average experimental EFFBP mass of 208 g is lower than the modelled mass of 250 g. However, this experimental average was calculated by deducting a feather protein mass of 76 g (using the EFG broiler model, Ross 308 birds) from the average protein mass of 284 g, including feathers. Thus the difference could be less significant.

The modelled changes in Ca and P over 35 days are shown in Figure 6.8. The measured masses of Ca and P, based on the reported 35-day body composition and EBW, are represented by markers of the same colour.

It is apparent that the simulated curves for Ca do not coincide with the measured values. The values for P were also unexpected.

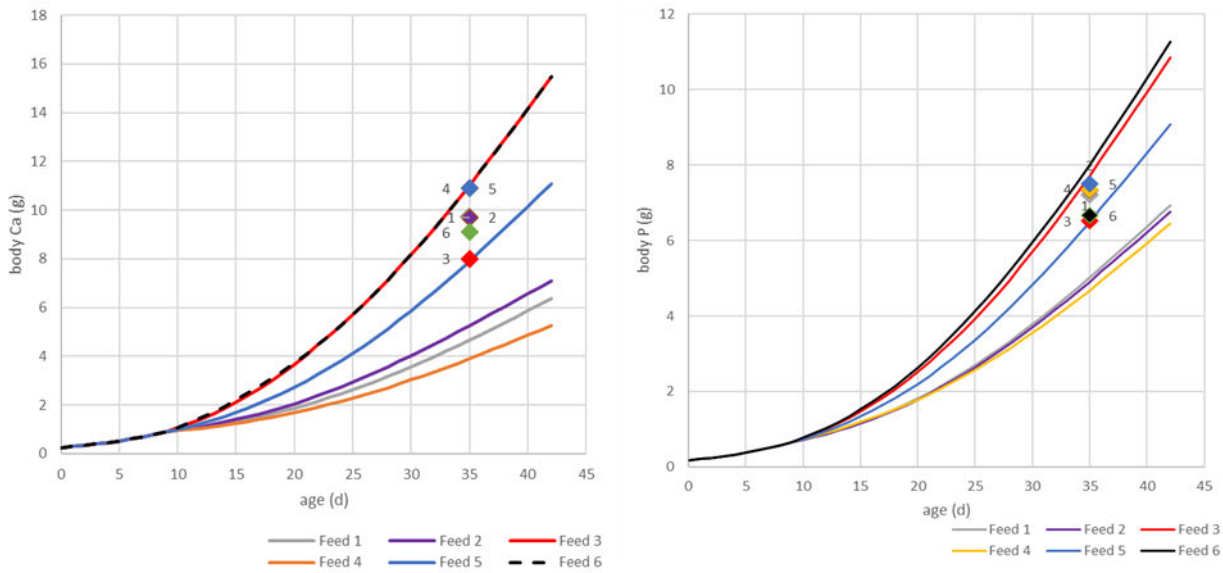

Figure 6.8 Simulation (modelled) and experimental results for growth of calcium and phosphorus using measured digestibilites from the growth study (van Krimpen *et al.*, 2013)

An exercise was conducted to determine if the problem lay with the growth model or the digestibilities reported in the studies. Feed intake was reported only for the period from 10 to 42 days, and hence Ca and P intake (g) could be calculated. Body weight gain between 10 and 42 days was reported in the study, and hence BWG for this period could be confirmed. For this exercise, it was necessary to assume that body Ca

and P (g/kg BW), analysed at 35 days, remained constant from 10 to 42 days and hence Ca and P gain for the period could be calculated. Retention coefficients could then be calculated from body mineral gain/intake. The results of this exercise are summarised in Table 6.11.

Table 6.11 Whole body retention of Ca and P from day 10 to day 42

	gain 10-42 d (g)		intake 10-42 d (g)		retained (gain/ intake)		ileal digestibility (absorbed/ intake)	
Feed	**Ca**	**P**	**Ca**	**P**	**Ca**	**P**	**Ca**	**P**
1	18.04	13.45	12.31	17.05	0.95	0.51	0.41	0.42
2	18.28	13.84	19.08	17.13	0.63	0.53	0.44	0.41
3	14.44	11.73	24.57	16.75	0.39	0.47	0.61	0.63
4	21.36	14.48	18.47	21.14	0.73	0.43	0.22	0.37
5	21.67	14.89	27.20	21.04	0.51	0.45	0.35	0.44
6	17.24	12.66	34.76	21.20	0.33	0.39	0.51	0.53

Comparing the retention fractions with the ileal digestibility fractions, it becomes apparent that the two measures are inconsistent. Whereas retention should be lower than absorption, except where absorption takes place in the large intestine (Shastak *et al.*, 2012b), in this instance the retention values for feeds 1 and 2 and 4 and 5 are noticeably higher than the digestibilities.

These differences may explain some of the anomalies observed in the simulations: the poor simulated performance of feed 4, for example, may be due to the low digestibility inputs and the superior performance of feed 1 over feed 6 for both Ca and P content of the 35-day body in the experiment may also be due to much higher absorption for the low Ca and nPP feed than measured.

An alternative validation exercise used the modelled digestibility coefficients calculated from the regression carried out on the data of Dieckmann (2004) instead of the values reported in the van Krimpen *et al.* (2013) study itself. The Ca and nPP levels in the latter study were used as inputs into the digestibility model developed from the former (see section 6.2.2). The coefficients are shown in Table 6.12, with the coefficients from the study itself repeated for comparison.

Table 6.12 Digestibility coefficients (%) applied in validation exercise

	(van Krimpen *et al.*, 2013)		regression model (Dieckmann, 2004)	
Feed	**Ca**	**P**	**Ca**	**P**
1	41	42	84	68
2	44	45	75	63
3	61	60	67	60
4	22	38	62	68
5	35	41	59	57
6	51	56	47	51

The simulated growth of Ca and P is shown in Figure 6.9. In these simulations, the cluster of values for Ca and P (g) in the broiler body at 35 days is modelled considerably better, as a comparison of the closeness of the simulated lines to the experimental dots in Figure 6.9, as compared with Figure 6.8, shows. This

highlights the importance of digestibility and validates the regression model developed for the calibration exercise.

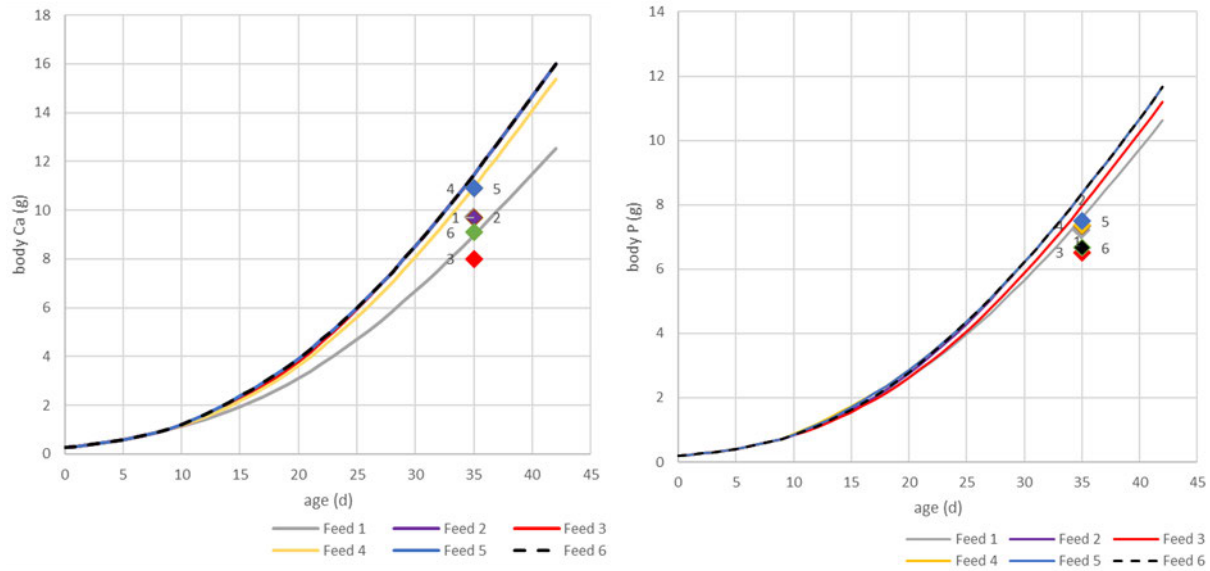

Figure 6.9 Simulation and experimental results for growth of Ca and P using regression model digestibilites

The graphs of the simulated changes in the ratios of Ca and P with EFFBP, with the experimental points marked, are shown in Figure 6.10. In this exercise the digestibilities generated by the regression analysis were used.

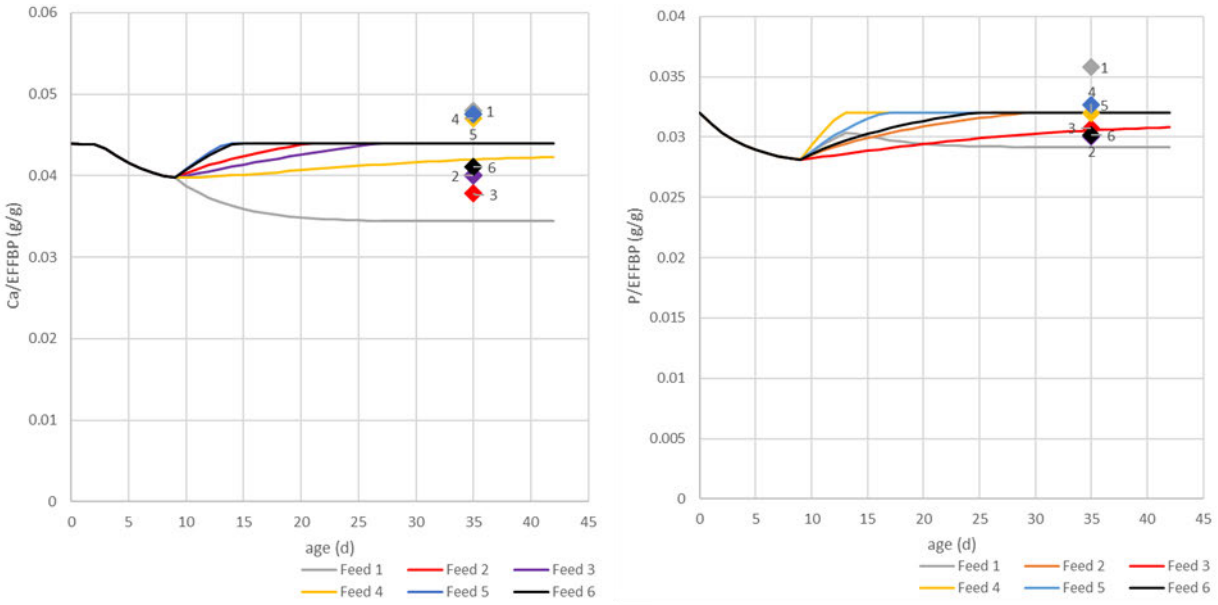

Figure 6.10 Simulation (modelled) and experimental results for calcium and phosphorus/protein ratios using modelled digestibilities and experimental data (van Krimpen *et al.*, 2013)

Even with the higher digestibility generated by the regression model, the experimental Ca and P/protein ratios for the birds on Feed 1 are higher than expected, but examination of the experimental data shows that protein growth was lower rather than the mineral growth higher. Mineral growth was, as shown in Figure

6.9, close to the average of the recorded values for the trial feeds. The adaptive mechanisms which allow birds to increase Ca and P absorption when these mineral are deficient in the diet may be involved, as discussed in section 2.5.6. Certainly it seems that an improved model of mineral availability will be useful.

6.6. Model application

In this section the model will be applied to the problem of deciding the Ca and nPP levels in feeds. This is not intended as a definitive guide but rather as a demonstration of the type of problem that can be explored using the model. It proposes the model as a decision support tool for practical purposes, which would support precision feeding by suggesting Ca and P levels in feed, based on modelled or empirical measurements of growth.

Following the demand-led modelling approach, it was assumed that the ideal or potential bone growth of Ca and P have been established. In practical situations, as tested in Dieckmann (2004), the BFB is supplied with sufficient Ca and P for protein growth to continue unconstrained.

On the supply side, different levels of phytate P and lysine in the feed were considered. Practically, these may be constrained by available raw materials and costs and would be determined in the feed formulation. In the standalone model, lysine is assumed to be the first-limiting nutrient which determines feed intake. Hence the lower the level of digestible lysine, the higher the feed intake and hence, generally, the lower the concentration of Ca and P required in the feed.

Digestibility was determined by the regression model of precaecal digestibility described in section 6.2.2. This was shown to be consistent with retention (section 6.4.2) and is thus supported for use in at least the specific conditions that prevailed in the study. More generalisable digestibility models would follow from calibration and validation against other experimental data sets.

The model was used to solve for the feed Ca and nPP levels that resulted in ideal bone growth with minimal mineral excretion. Ideal bone growth can be achieved with higher levels, but at the expense of financial and environmental goals.

The results of this exercise for 2 levels of PP and 4 levels of digestible lysine are shown in Figure 6.11 and Figure 6.12. They should be read together, as combinations of Ca and nPP that will allow birds to reach their potential mineral growth in bone.

The graphs for 1.7 g/kg of PP reflect the phytate level that was applied in the trials from which the digestibility coefficients were derived.

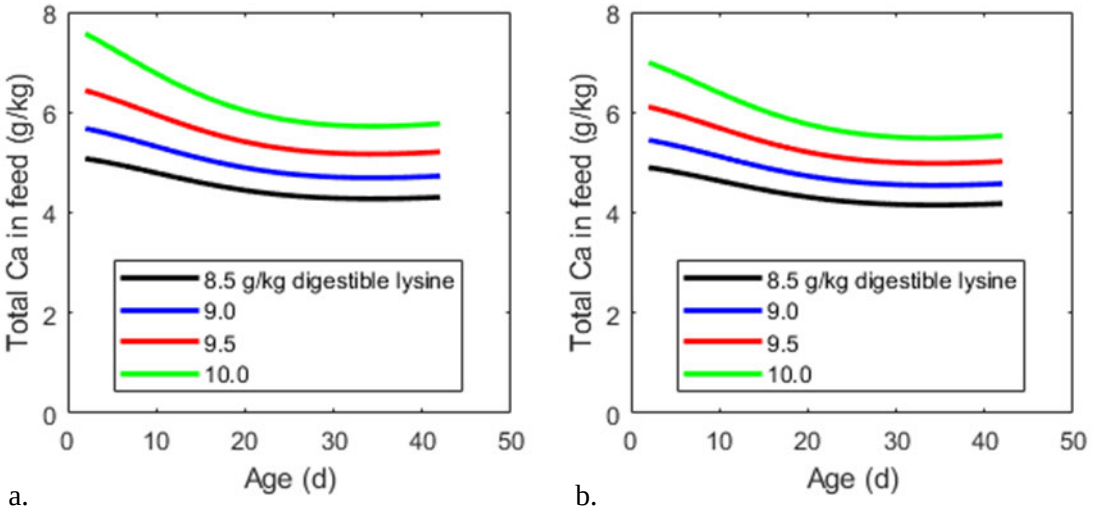

Figure 6.11 Feed concentrations of Ca for ideal bone growth with (a) 1.7 g/kg phytate P in feed and (b) 2.0 g/kg phytate P in feed

The required levels of Ca are lower than might be expected, but higher in the pre-starter phase, particularly with lower lysine levels. This agrees with Angel (2007), who suggested increased mineral concentrations and using highly available mineral sources in pre-starter diets.

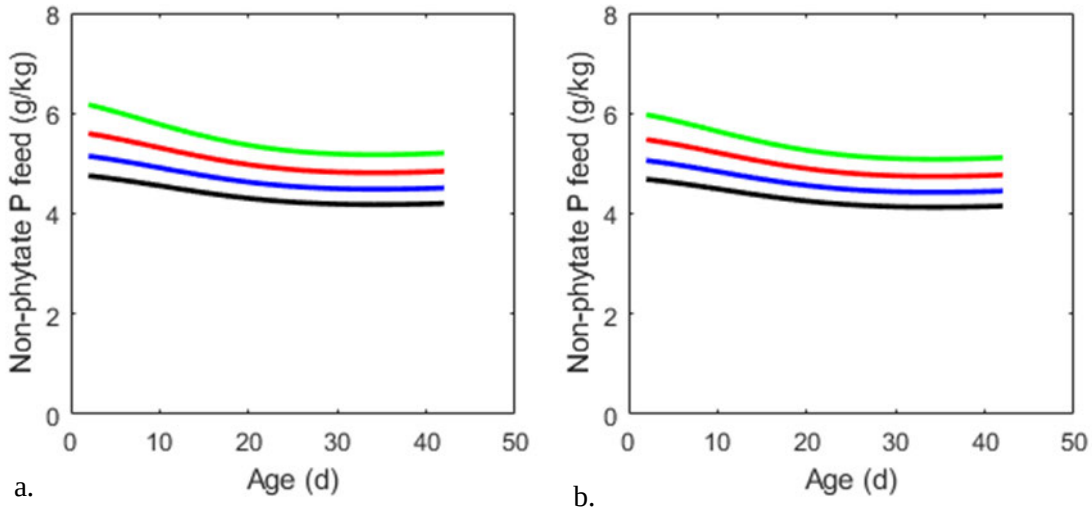

Figure 6.12 Feed concentrations of nPP for ideal bone growth with (a) 1.7 g/kg phytate P in feed and (b) 2.0 g/kg phytate P in feed

Some issues arise when the PP level is changed to 2 g/kg. The digestibility of Ca and P is little changed by the increased PP, because the only coefficients in the regression model were applied to Ca and nPP since the experiments from which it was derived used a fixed PP content. The digestibility of PP, at 1.6 g/kg feed, is assumed to follow the P model digestibility, whereas the level of PP is likely to influence this. Requirements for Ca might be higher if PP has a negative effect on its digestibility. Another factor which would affect the results would be the introduction of phytase to the diet, as is now almost universal practice. This points to the need for a more detailed empirical digestibility model if it is to be applied to a broader range of feeds.

Another outcome from these simulations is a decrease in the mineral/lysine ratio with lysine levels in feed (Figure 6.13).

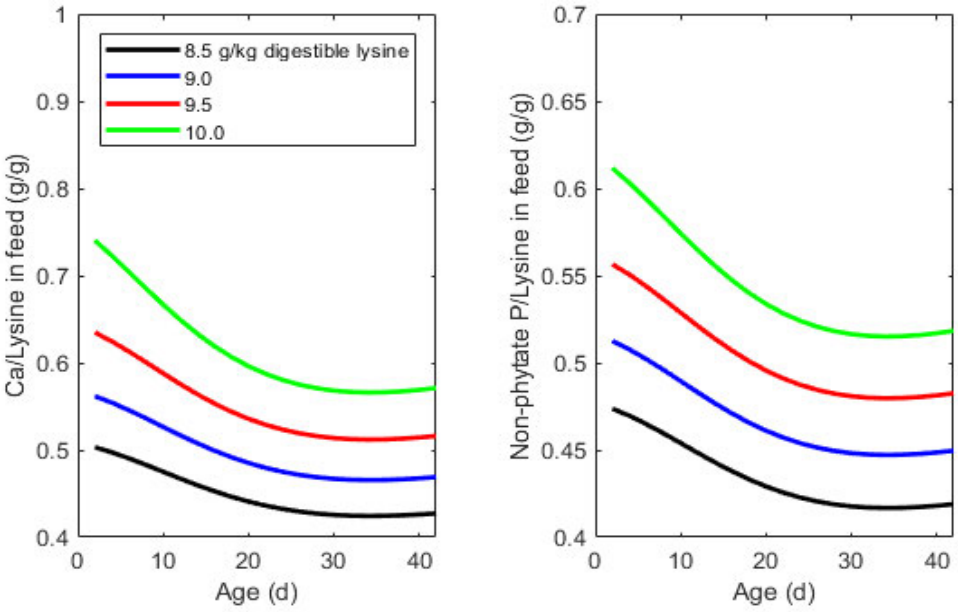

Figure 6.13 Mineral/lysine ratios in feed for ideal bone growth with 1.7 g/kg phytate P in feed

A contributor to this phenomenon is the way in which endogenous losses are modelled. These depend on feed intake, and hence increase as the feed becomes less nutrient dense. This highlights the importance of understanding the implications of different modelling approaches: if endogenous losses were modelled relative to body protein mass, for example, this effect would be reduced.

6.7. Discussion

The two studies that provided suitable body composition data for evaluation of the model allowed calibration and validation for whole-body values. In both cases, these studies included digestibility trials, and this proved invaluable. It allowed the development of a regression model suitable for the specific time and conditions under which the trials were conducted. Direct use of digestibility measurements was less successful with the second validation data set, but the regression model from the calibration proved a better fit. The effect of Ca and P availability on growth was demonstrated.

The model has been shown to provide a reasonable fit with experimental data beyond the prestarter period. This supports the idea of an allometric, if not isometric, relationship between Ca and P and EFFBP. The model did not predict the ability of the bird to continue depositing Ca and P in the body under conditions of deficiency and this suggested changes in digestibility with age and possibly with poorer diets. This merits further investigation.

Ca and P/EFFBP ratios were affected in the experimental data as well as the model simulations, validating the use of a mechanism to allow mineral growth to deviate from isometry with EFFBP and hence with BW. None of the treatments compromised body mass in the model, but a decrease in the experimental EFFBP

mass in the poorest treatments suggested that BFB was affected, albeit to a small extent. The prioritisation of bone and BFB could not be inferred from the results, but this did suggest that a simple pool model, in which BFB is first supplied and bone mineralised thereafter, is not adequate. This is to be expected and will be more accurately modelled once data from dissections becomes available.

A practical application of the model was demonstrated, and this suggests that the model, with further refinement, can provide a valuable tool for producers.

CHAPTER 7. CONCLUSIONS AND FRAMEWORK FOR FUTURE RESEARCH

The conceptual model described in Chapter 2 followed a logical flow of minerals through the bird: from feed intake which can be multiplied by mineral concentrations in the diet to calculate mineral intake, through digestion (with undigested minerals excreted in the faeces), into the body, where minerals are retained for metabolic processes and growth, while any excess absorbed is excreted in the urine.

The modelling approach follows the reverse of this mineral flow: growth of the bird is assumed to drive a mineral requirement and hence growth was modelled first. It is argued below that the bird seeks to meet this requirement in different ways that are facilitated by physiological mechanisms inherent in the animal. Hence there is a "pull" from the body to extract minerals from the digestive tract, but the extent to which requirements can be met is principally constrained by mineral availability at the interface between body and intestinal lumen, i.e. at the gut wall.

In this thesis, it has been argued that modelling can provide a useful tool for broiler producers, allowing them to predict the results of different feeding strategies, to meet environmental targets and to optimise financial returns. A model of protein and energy in broilers, integrated with a feed formulation program and an optimisation module has been applied to commercial operations and has achieved significant improvements in profitability (Gous, 2014). However, this integrated system is the product of years of focussed research, and modelling of mineral growth will require an intensive phase of research and model development if it is to prove as useful in practice. This project has revealed gaps in our understanding of how birds deal with the complex interactions that constrain mineral growth. This underscores the role that models play in guiding research. Rather than repeating studies that provide no novel insights, avenues for future research are constantly revealed. The pieces of the puzzle can be uncovered in carefully designed experiments and contribute to a greater understanding of the whole. This is a process of continuous, incremental improvement that simultaneously increases the scientific validity and commercial value of the model.

This concluding chapter summarises the areas in which insufficient research has been done and suggests some experiments or series of studies that will both enhance our understanding of Ca and P in the broiler body and improve our ability to meet the birds' needs without compromising profitability or environmental targets. In conclusion, an argument for the inclusion of such a model in both commercial and research settings will be presented.

7.1. Current and future research

Some of these experiments have already been carried out. The results will inform further model calibration and development when they become available and hence the model will indicate the further experiments

that will be required. Studies in which serially slaughtered, defeathered, empty bird bodies are analysed for protein, lipid, water and mineral composition are absent from the literature. While Hurwitz and Plavnik (1986) reported Ca and P concentrations in relation to EFFBW, protein content was not reported in the same study. Caldas *et al.* (2019) included feathers in the analysis. Furthermore, only Gompertz equation parameters were provided in this publication. Age-related changes in digestibility have not been clearly established and the potential to scale digestible nutrient absorption, for example to body protein, has not been considered in the literature.

7.1.1. Potential calcium and phosphorus growth

In developing a model of Ca and P in the broiler, in which potential growth is related to body protein growth, it is important to draw a distinction between the ideal, or potential growth that would occur if no limitations were placed on growth, and the actual growth that is constrained by nutrition and environmental conditions.

In this model, potential Ca and P are isometric (in a constant proportion) to potential protein in the broiler body. This is a special case of allometry, but lipid and water both show relationships to protein in which the proportions change during growth, but all components share a common rate of maturing (growth relative to degree of maturity) and reach maturity at the same time. This may be the case for Ca and P, particularly with respect to bones. These might grow rapidly in the initial stage after hatching to provide skeletal support for the young chick. While it has been proposed in some modelling studies that Ca in the skeleton (and hence in the body) of pigs is independent of protein growth (Lautrou *et al.*, 2020), a regression of the log-transformed body protein and minerals in the same study suggested otherwise. Allometric relationships have been established for growth in body weight, lipid and water with protein. Previous studies suggested that potential ash is isometric with feather-free body protein, hence the proposal that Ca and P might show this special case of allometry as well.

It has been proposed that the modern broiler, selected for BWG and hence for soft tissue growth, does not have the skeletal support required for its increased mass (Williams *et al.*, 2004). This would likely be reflected in changes in body mineral content, particularly Ca, relative to protein. The low coefficient of ash with respect to protein, calculated from Caldas *et al.* (2019) (0.13 g ash/g body protein) suggested that this measure may have declined over time, since earlier body composition studies proposed a value of 0.15-0.16 g ash/g body protein (Gous *et al.*, 1999; Eits *et al.*, 2002). The data of Hurwitz and Plavnik (1986) also suggested higher coefficients for Ca and P than those derived from Caldas *et al.* (2019).

An alternative interpretation of studies in which Ca and P, and hence ash, are found to be present in smaller proportions relative to body protein, is that birds are not being fed to meet their potential mineral growth (Thorp and Waddington, 1997). In the absence of any other data, and given that the feeds were formulated to meet the Cobb recommendations (Ca 9 g/kg and nPP 4.5 g/kg in the starter), the isometric coefficients derived from Caldas *et al.* (2019) were assumed to represent potential growth. However, it may be that

birds struggle to meet their real Ca and P needs from a complete diet. While protein growth may be maximised, Ca and P growth may not be able to reach their potential due to the feeding strategy. This means that an experiment to determine the relationship between potential protein growth and potential Ca and P growth might have to be designed differently from one estimating the Gompertz parameters for potential protein growth, for example. A further challenge is the interaction between Ca and P and other dietary factors. This makes it difficult to ensure that Ca and P are not limiting in the diet.

7.1.1.1. Dietary treatments for the estimation of potential Ca and P growth in broilers

Diets must be applied that allow birds to grow to their potential. If it is assumed that potential growth of Ca and P can be maximised along with protein, then birds will be growing to potential when protein growth is not limited and the ratios between minerals and protein are maximised.

Dieckmann (2004) showed that a range of Ca and P/body protein ratios arise when different Ca and P levels are provided in the feed. This is shown in Table 6.3. While it is apparent that low mineral levels compromised bone growth, as shown in poor ash gain and low Ca/FFBP and P/FFBP ratios, BWG is only compromised at 42 days. The mineral levels and resultant mineral/EFFBP ratios suggest a possible strategy for establishing potential growth: feeding a number of different levels of Ca and P and a variety of ratios between them.

Nutritional geometry trials (Bradbury *et al*., 2014), in which birds are serially slaughtered, are presently being conducted at the University of KwaZulu-Natal (UKZN) to establish these relationships. Variability in carcass lipid that arises from unbalanced feeds affects the relationship between minerals and BW. Hence protein and lipid in the carcasses must be measured in addition to Ca and P. A high ratio (0.0513 g Ca/g EFFBP), observed in the body composition validation data set was noted to be the result of a lower protein measurement (van Krimpen *et al*., 2013). The growth of both EFFBP and minerals must therefore be analysed at their potential if reliable relationships are to be established.

Another approach to allowing birds to grow to their potential body protein, Ca and P might be to allow choice feeding and to rely on the birds' innate ability to choose an optimal diet. If birds choose to meet their Ca and P needs from different components of the diet (e.g. low Ca, high P mixed feeds and a separate Ca source) then interactions in the GIT may be minimised. Some retention studies in which Ca and P were offered separately suggest that retention of Ca may exceed the model predicted requirements under these conditions (see Figure 5.7). A number of choice-feeding trials with different levels of Ca and P in the diets have recently been conducted at UKZN and their results are awaited.

Similarly, the addition of high doses of phytase increased Ca retention above modelled requirements during an assay of birds aged 18-20 days (Kornegay *et al.,* 1996). Hence the inclusion of phytase in trials such as those described above may allow birds the opportunity to reach their potential mineral growth.

7.1.1.2.Measurements required for parameterisation of the growth model

Serial slaughter of birds from day-old to maturity (at least to 16 weeks of age) will be necessary if the relationship between Ca and P and body protein is to be modelled accurately.

- Feathers and intestinal contents must be removed before the EFFBW is determined.
- The homogenisation of carcasses is critical so that samples are representative of whole-body composition. The removal of feathers facilitates this process.
- Carcasses should be analysed for protein, lipid, water, ash, Ca and P.
- Frequent sampling (every 2-3 days) during first three weeks of age may reveal changes in Ca and P relative to protein as the skeleton of the chick is rapidly mineralised (see section 5.2). Weekly sampling may suffice for latter part of the growing period.
- Absolute masses of chemical components must be reported, which requires analysis of body composition and measurement of body mass in the same birds.
- It is necessary to establish the potential growth of Ca and P relative to body protein in different sexes and genotypes. This will require numerous serial slaughter experiments.

Serial sampling of feathers is required to measure changes in mineral composition during the growing period. The concentration of N, Ca, and P in feathers has been estimated at 133, 5.0 and 2.7 g/kg respectively (CVB, 2010 cited in van Krimpen *et al.*, 2013). This suggests there is 0.006 g Ca and 0.003 g P/g protein in feathers. This is likely to represent broilers at slaughter age and may vary over the life of the animal. In the present model, this Ca and P is included in the soft tissue component, as coefficients were derived from the relationship between feathered-body Ca and P and feathered-body protein less feather protein. However, if the minerals in feathers are closely associated with feather protein, they should be modelled separately as the feather protein is in the main model. Furthermore, a rigorous study would require separate analysis of feathers and feather-free body.

Dissection to separate Ca and P in bone and BFB is required for accurate modelling of these two components. The separation of BFB and bone in the model maintains the isometry between body protein and Ca and P in the soft tissue (by reducing protein growth proportionally when retainable P is below the requirement for BFB), while allowing the Ca and P in bone to deviate from isometry with body protein and hence produce the changes in the proportions of Ca and P to protein that are seen in the literature. The results reported in Angel (2007) and van Krimpen *et al.* (2013) illustrate the challenges associated with the separation of bone and BFB. In the first of these two studies, cartilage caps were left on the bones and heads and feet were included in the skeleton portion of the body in their entirety. This resulted in reduced concentrations of ash in the skeleton and higher proportions of skeleton to EBW than expected (Murawska *et al.*, 2011). In the second study, some skeletal components, possibly heads and feet, were included in the soft tissue portion or ignored altogether, so that skeleton as a proportion of EBW was low.

- A standardised dissection method is required that includes skull and carpal bones but eliminates soft tissue from the head and feet. Cartilage caps should not be included in the bone portion.

- As-is weights of skeleton and BFB must be ascertained before dry, fat-free analysis of ash, Ca and P.

If a consistent relationship between tibia and skeleton Ca and P could be established, the results of many studies could be re-analysed for nutritional effects on bone growth. Lee *et al.* (2019) reported Ca proportions of ash from 35.8% in ribs to 41.9% in femur. Similar proportions were found in animals fed 60% of the recommended requirements. Tibia Ca formed a lower proportion of ash compared with that of the whole carcass (36.4 vs 36.9% of ash) while tibia P was higher than that in the whole skeleton (19.5 vs 18.7% of ash).

- In experiments to determine whole skeleton minerals, tibias should be sampled and a regression analysis of whole skeleton on tibia Ca and P performed.
- Femur, foot and toe composition could also be considered as these are commonly sampled.

Once the experiments above are completed, it may be possible to use the tibia Ca/P ratio and the EFFB Ca, P and protein to estimate the allometric coefficients for a variety of genotypes, and as genotypes change over time. It is an ongoing task to describe broiler genotypes in terms of their potential protein growth and the potential growth of other components relative to this. Reliable relationships between sampled body parts and whole bird chemical composition would allow us to update these parameters with a less onerous experimental programme than that described above.

7.1.2. Obligatory losses of calcium and phosphorus

In the model at present, endogenous losses are calculated as a proportion of feed intake. Maintenance requirements are scaled to body protein. In the model evaluation, uncertainty about maintenance (see section 2.7) and the influence of this on simulation outcomes made it necessary to set this parameter to zero.

Since it is not possible to establish maintenance requirements directly in a growing animal, in which denying the animal sufficient nutrients for growth results in illness and death before growth ceases, different approaches are required. It is reasonable to suppose that there will be a maintenance requirement for P associated with protein in the soft tissue, and this may be reflected in endogenous losses in the faeces and obligatory urinary losses. It has been proposed that most obligatory Ca loss occurs in the faeces as endogenous losses (Hansard *et al.*, 1954). Manangi *et al.* (2018) showed that both Ca and P urinary losses could reach near-zero levels.

In our model, it is not important where maintenance losses are excreted, whether in the faeces or the urine. Once reliable digestibility values are established, body composition trials in which mineral absorption and body protein and minerals were measured could be used to calculate the linear regression equations of mineral absorption against retained mineral. The intercept would represent the maintenance requirement at zero mineral retention. The values for intake and retention could be scaled to mature $EFFBP^{0.73}$ (Emmans and Kyriazakis, 2001).

7.1.3. Changes in body composition with changes in available nutrients

Experimental work similar to that described for the determination of the potential growth of Ca and P in broilers is to be conducted at UKZN, to quantify various aspects of the effect of changes in the available quantities of these nutrients. This will require a robust assessment of the interactions in the GIT that affect availability, so that these quantities can be assessed accurately. The design of such experiments will be suggested by the model in an effort to estimate the model parameters with the greatest possible accuracy.

7.1.3.1.Allocation of Ca and P between bone and BFB

It is unlikely that this is on a simple pool system as it is currently modelled. If it is established that Ca and P are found in constant proportions to protein in the BFB (van Krimpen *et al.*, 2013), then the changing Ca/P ratio in the body and variation in the Ca/protein and P/protein ratios suggest that these minerals may be well below their potential in bone while the BFB continues to grow. However, the reduced BWG in birds fed diets low in P may indicate that protein growth is compromised before bone P growth reaches zero (Bradbury *et al*., 2014). Furthermore, reduced gain/feed at lower Ca levels in feed may indicate changes in body composition, possibly as protein growth is reduced and lipid growth increases. Modelling the way in which the bird allocates P to the different tissues may be the key to predicting the effects on body weight and bone mineralisation of different feeding regimes in a mechanistic way, i.e. through the underlying relationships between body components during growth rather than attempting to investigate every possible scenario with an empirical approach.

The analysis of body composition, including protein, lipid, Ca and P, when feeds differing in available Ca and P are fed, would indicate how P is allocated to different tissues with changing levels of restriction. If relationships can be established between, for example, tibia Ca and P and bone or whole-body Ca and P, the onerous task of dissection may be avoided. It may be enough to establish the proportional changes in tibia Ca and P when available Ca and P in the feed change to estimate the way in which P is allocated to the bone and BFB. Analysis of body protein would be essential: assumptions about the proportion of protein in BWG mask changes in the lipid proportion.

Nutritional geometry and separate Ca feeding trials to establish potential growth would provide supporting data for modelling the prioritisation of different tissues for mineral allocation. The model bounds for the variation in Ca/P ratio in bone were set at 1.8 to 2.2 to allow some measure of "storage" of one mineral when the other is deficient. This was based on an analysis of studies in which tibia Ca and P were reported and which showed mass ratios from 1.81 (Dersjant-Li *et al.*, 2018) to as high as 2.43 (Williams *et al.*, 2000b). Sanchez-Rodriguez *et al.* (2019) demonstrated changes in the tibia Ca/P ratio with age. It was established that in younger birds (up to 7 d), the lower degree of mineralisation is accompanied by higher concentrations of carbonate ions and lower Ca/P ratios. Further work to compare patterns of Ca/P ratio and ash content to establish potential or ideal bone growth and variability that can be ascribed to diet will allow

more accurate prediction of P distribution between the bone and BFB from representative bones and whole-body composition or retention.

7.1.3.2.Depletion/repletion effects

A fundamental proposition of the model is that the bird strives to return to its potential relationship between Ca and P and protein. If there is a shortfall in body mineral, the bird will retain available Ca and P to achieve this. Since it does not increase feed intake as it would with a limiting amino acid, this can only occur when Ca and P in excess of the desired intake in proportion to the protein growth for the current period is available. It may be that there are limits to the ability of the bird to retain additional mineral, particularly with respect to the bone mineralisation process, which requires resorption and bone formation as bones change in size to support increasing body mass.

Growth trials demonstrating the ability of broilers to recover from a deficiency of Ca and P in early life have been published (see section 2.5.6). Changes in body composition in similar studies will allow adjustment of model parameters to simulate the rate of recovery and extent to which compensatory Ca and P growth occurs. The extent to which depletion diets are deficient will require an understanding of the effects of deficiency on nutrient absorption. Empirical calibration of a deterministic model of the availability of Ca and P from feeds will be necessary to take this into account.

7.1.4. Calcium and phosphorus availability

Without calculating the digestibilities of the diets, the confounding effects of the interactions between Ca and P in the GIT cannot be taken into account. This emphasises the importance of modelling these, as the growth model is refined.

In the preliminary model described in this thesis, the available nutrients were calculated using an empirical, regression model. This has been shown to be effective in modelling pig Ca and P nutrition (Létourneau-Montminy *et al.*, 2015; Misiura *et al.*, 2020). In a commercial setting, a model of this type could be a valuable addition to a feed formulation program: rather than attempting to assign digestibility values to each ingredient and assuming these are additive, the complete feeds could be assessed, and the available Ca and P calculated. However, it is important to bear in mind that empirical models are limited in usefulness to the range of variable values tested in the studies analysed. In broilers, most digestibility studies use broilers at approximately 21 days of age. Feeds are often largely based on maize or wheat and soybean meal. Ca and P are varied by the addition of inorganic sources, usually limestone, MCP or DCP.

The first aspect of digestibility that requires clarification as a consequence of model evaluation (Chapter 6) is the effect of age on the digestibility of Ca and P and its relationship to protein growth (Shafey and McDonald, 1990; Thomas and Ravindran, 2010; David *et al.*, 2020). It was noted that the model predicted a decline in the available Ca and P for accretion in the broiler when levels in the feed were low, but the experimental results suggested otherwise.

As the digestibility of both Ca and P have been shown to decrease as the mineral content of the feed increases it may be useful to consider the absolute amounts of Ca and P that are absorbed. This requires the recording of feed intake over a short period prior to sampling. With the concentrations of Ca and P in the feed and ileal digesta, absorbed Ca and P in g/bird/day rather than only the proportions in the diet can be calculated. These might then be scaled to some characteristic of the bird, such as EFFBP or BW (Misiura *et al.*, 2018). This may be particularly important in the case of Ca, where the animal appears to have the ability to absorb what it needs from the digestive tract, even if this requires high levels of active absorption in the large intestine (Hurwitz and Bar, 1969; Beggs, 2021). While Létourneau-Montminy *et al.* (2015) modelled Ca in pigs as being affected by Ca level, P digestion was modelled using total tract digestibilities for different feed ingredients. This might incorporate different control mechanisms for the two minerals into the model: principally at the intestinal wall in the case of Ca and renal control of P. It has been asserted that Ca absorption is controlled to match the needs of the bird for this mineral for growth of bone, with very little required for maintenance and small amounts excreted in the urine. The exception to this metabolic rule occurs when P levels in the diet are very low and bone resorption must exceed accretion to provide P for the maintenance of processes in the soft tissues of the body. In this instance, Ca is released from the bone and must be excreted in the urine to maintain plasma levels at a constant level. While the mode of excretion, whether in the faeces or urine, has not been considered important in the present model, factors that affect the availability of Ca and P must be taken into account.

Due to the passive absorption of Ca, the total amount of Ca absorbed is still higher at higher dietary Ca but the percentage digestible Ca decreases. What is unclear is the rate at which digestibility is modified and the extent to which passive absorption can be limited (most likely by claudin proteins in the tight junctions). A model of Ca and P availability as a response to requirement would require modification by empirical estimates of the extent to which digestibility changes, with parameters introduced to quantify these effects on availability.

The mechanisms through which Ca and P affect one another's absorption and utilisation are complex and varied. In the gut lumen, the interaction is chemical, through the formation and break-down of phosphates and phytate compounds of Ca (Shafey *et al.*, 1990). In the body, the accretion and resorption of bone bind and release these minerals simultaneously. Hence the model must estimate retained Ca and P from growth data (including the Ca/P ratio in bone) as well as digestibility. If there is a constant Ca/P ratio in bone, and a constant P content in soft tissue relative to body protein, then it should be possible to calculate the P requirement for bone growth and soft tissue growth from the Ca and EFFBP growth.

The second aspect of mineral digestibility that requires attention is the effect of phytase on availability (e.g.Pintar *et al.*, 2005; Ravindran *et al.*, 2008; Walk *et al.*, 2012a; Chung *et al.*, 2013). This was not considered in the model evaluation, because no body composition studies were available in which phytase was included in the feeds. The deterministic model developed by Létourneau-Montminy *et al.* (2011) simulated the hydrolysis of phytate in the crop and proventriculus-gizzard following a Michaelis-Menten

law. However, the model was parameterised using data from pig studies, as no broiler data were available at the time. However, several subsequent studies of phytate hydrolysis in broilers have been conducted and a model for broilers could be developed (Shastak *et al.*, 2014; Zeller *et al.*, 2015a; Zeller *et al.*, 2015b; Zeller *et al.*, 2016; Beeson *et al.*, 2017; Perryman *et al.*, 2017a). A novel, non-intrusive approach to monitoring phytate hydrolysis through changes in myo-inositol levels in circulation and in feathers has been proposed (Greene *et al.*, 2020).

While phytase has been included in some models as part of the regression model for digestibility, it is possible that a deterministic model for phytate hydrolysis could be the first step towards a more comprehensive, physico-chemical digestibility model. The release of nPP by phytate hydrolysis and the consequent decrease in PP content of the digesta could provide the inputs to an empirical model of Ca and P availability in the interim.

7.2. Conclusions

The academic purpose of modelling is to provide a framework for the organisation of theory on a particular topic and to guide a program of research that continually and incrementally improves the understanding of the system under consideration.

This thesis describes the formulation of a model based on published literature, and the initial calibration and validation of the model. This can be thought of as the first stage of model development, in which important variables are identified and relationships between them proposed, based on existing theories about the biological phenomena under consideration (Balsa-Canto *et al.*, 2008; Franceschini and Macchietto, 2008). A comprehensive assessment of possible variables and the selection of certain of these for the model does not rule out the possibility of introducing others (e.g. vitamin D) at a later stage. The relationships that are chosen have parameters, such as maintenance coefficients, that have a physical meaning but may be difficult to measure directly. Some estimate of model parameters must be made so that the model can function, however imperfect its initial resemblance to reality. In the absence of direct measurements, e.g. of skeleton mineral mass, some parameters will inevitably have to be estimated by inference from reported experimental measurements, such as whole-body Ca and P masses. Differences between simulated values and experimental measurements was introduced as a result. The absence from the literature of the direct measurements required was an inevitable consequence of research objectives other than modelling, but further experimentation can be conducted with a far more focussed approach.

7.2.1. Research approach

The second step must be to conduct experiments for a more complete calibration of the model. As discussed earlier, modelling of minerals in broilers requires a well-structured series of experiments to estimate the parameters of the model. The experiments on which the model evaluation was based used a classical design approach: apply a grid of treatments, measure the property of interest, fit a statistical model to the

measurements with the treatment levels as the independent variables. The use of a model allows the researcher to take a different approach. Properties of interest are often inferred from measurements of other variables, and these may be chosen so that experimental costs, in terms of time and money, are minimised. For example, the use of Ca and P excreta measurement in order to infer body mineral composition could be implemented within the framework of the model. If a reliable relationship between tibia composition and skeleton composition is established, this could be incorporated into the model to minimise the cost of future calibration exercises.

Optimal Experimental Design, or Model-based Optimal Design of Experiments (Franceschini and Macchietto, 2008) is an iterative approach to the improvement of model parameter estimates that is widely used in industrial engineering, but has also been applied to agriculture, for example in animal breeding experiments (Marsh, 2022). Its role in the modelling process followed in this study is illustrated in Figure 7.1. As its name suggests, it *aims at obtaining the maximum information from an experimental apparatus being modelled by devising experiments that will yield the most informative data* (Franceschini and Macchietto, 2008). Furthermore, it supports the cyclical approach, whereby the model is improved through designs that minimise the error variance of the parameter which is being investigated (Seufert *et al.*, 2021).

The model is used to design experiments, which produce appropriate data for calibration and validation of the model, hence improving the model. This process minimises costs while maximising the impact of experiments on model function. In this study, the cyclical process has been initiated through the proposal and initial calibration of a model based on published literature.

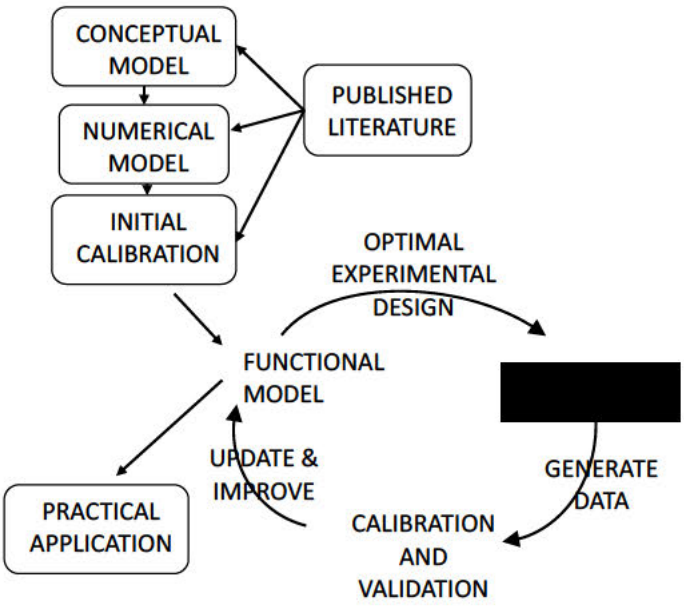

Figure 7.1 Optimal experimental design in model development

Modelling also provides an opportunity for testing the results of experimental work against both the conceptual models in the researchers' heads and a numerical, computer-based model (see Figure 7.2). This

compels the researcher to explore their ideas in a rigorous and quantitative framework. It supports predictions when the system under consideration is complex and influenced by a range of variables, as living organisms are. It can help to identify erroneous experimental data, and also make sense of unexpected but explicable results. *In silico* experimentation can save money and compress weeks of work into minutes of computer time, thereby ensuring that the experiments that are done are carefully designed and deliver useful outcomes.

Modelling does not seek to replace the conceptual models that are developed with years of experience, but to complement them.

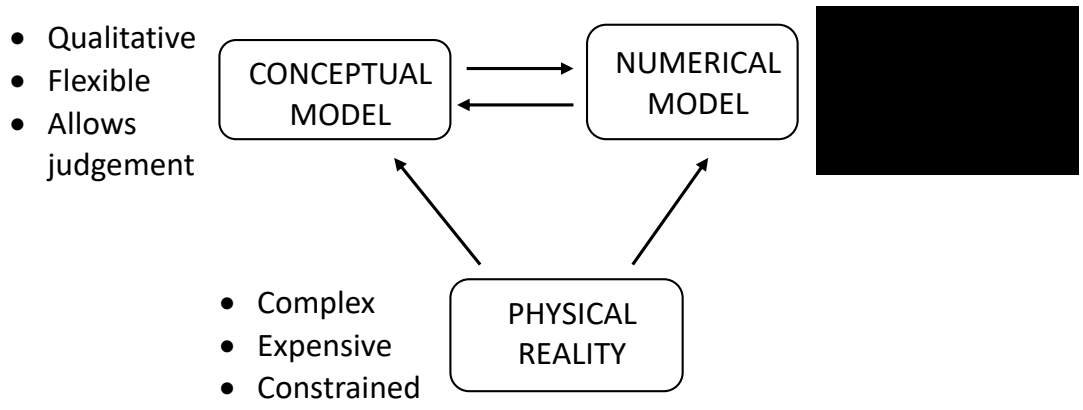

Figure 7.2 A balanced modelling triad

Source: adapted from Brouckaert *et al.* (2022)

This structured approach supports the use of nutritional models in the commercial context.

7.2.2. Practical applications of the model

A model-based approach could be adopted by a poultry producer and integrated with their production system, rather than relying on extensive trials separate from the commercial operation. The model would be used to track their production in parallel. Critical indicator measurements on a sample of birds could be made on a regular planned schedule, or whenever significant production factors change (e.g. ingredient supply). These could be used to adjust the model parameters and keep them up to date. In turn, a broiler modelling system, comprising a feed formulation module, a growth model and an optimisation component, predicts the effects of changes to the production system and suggests adjustments to improve profitability.

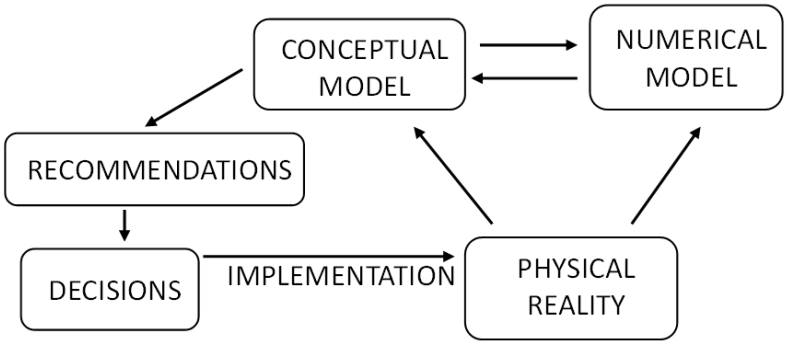

Figure 7.3 A model as a decision support tool

Source: adapted from Brouckaert *et al.* (2022)

The process of developing the model has already suggested a range of experiments (see section 7.1) and many of these are already in the planning, execution or analysis phase through this DSM-sponsored project at UKZN. Unforeseen circumstances, including a global pandemic, have prevented the completion of this process before the end of this study, but the model will be improved still further in an ongoing cycle of optimised experimentation and model development. It is hoped that it will continue to provide ideas for further research in broiler calcium and phosphorus nutrition and serve as a useful tool for producers seeking to increase profit and mitigate environmental concerns.

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APPENDIX 1: TABLE OF MODEL PARAMETERS

Parameters		units	original value	calibrated value
rates of maturing				
B	body rate of maturing (incl. EFFBP)	/d	0.045/ 0.046	0.037
B_f	feather rate of maturing	/d	0.06	0.052
initial weights				
BP_0	EFFBP mass at time 0	g	5	6
FP_0	feather protein at time 0	g	0.1	0.1
mature weights				
BP_m	mature EFFBP	kg	1.48/1.07	1.18
FP_m	mature feather protein	kg	0.32/0.26	0.315
allometric coefficients				
a_L	lipid/EFFBP allometric coefficient a		1.2	1.2
b_L	lipid allometric exponent b		0.29/0.51	0.49
a_w	water allometric coefficient a		3.4	3.4
b_w	water allometric exponent b		0.12	-0.12
a_{ash}	ash/EFFBP isometric coefficient a	g/g BP	0.165	0.19
a_{CaBFB}	Ca/EFFBP isometric coefficient a	g/g BP	0.00042	0.000418
a_{Cabone}	Ca/EFFBP isometric coefficient a	g/g BP	0.042	0.04355
a_{PBFB}	P/EFFBP isometric coefficient a	g/g BP	0.011	0.01026
a_{Pbone}	P/EFFBP isometric coefficient a	g/g BP	0.021	0.02177
maintenance coefficients				
m_{Ca}	Ca for EFFBP maintenance		0.0044	0
m_P	P for EFFBP maintenance		0.1293	0
endogenous losses				
el_{Ca}	Ca losses to faeces	mg/g FI	0.25	0.1
el_P	P losses to faeces	mg/g FI	0.25	0.25
Ca/P ratios in bone				
Ca/P_{min}	minimum allowed ratio for bone growth	g/g	1.8	1.8
Ca/P_{max}	maximum allowed ratio for bone growth	g/g	2.2	2.2

APPENDIX 2: MODEL EQUATIONS

Growth model

Variable	Comment	Inputs	Calculation
PP (g) potential body protein		Calculated from rPPc	$=PP_{t-1}$ + rPPc
rPPc (g) potential growth of protein	Calc from Gomp fn	BPH (kg) 0.005 BPM 1.4 RateB (/d) 0.042	=*RateB* *age (d)*LN(BPM*1000/ age (d))
PA(g) actual body protein limited by P	Calc from rPAc		PA_{t-1} + rPAc
rPAc actual rate of protein growth, g	Modified by ret P: dPP/dt* proportion of normal P retained in BFB		=RateB *age (d)*(bfbphosfactor)* ln(BPM*1000/ age (d))
FPA (g) feather protein	Calc from Gomp fn	FPH (kg) 0.0001 FPM 0.315 rateF (/d) 0.052	=(FPM *EXP(-EXP((LN(-LN(FPH / FPM)))- rateF *age (d)))*1000)/0.9
rFPAc rate of feather growth g	Calc from FPA		FPA_t- FPA_{t-1}
WA (g) body water	Allometry with PA	aw bw 3.4 -0.12	=PA*aw+(PA/1000/ BPM)^bw
LA (g) lipid weight	Allometry with BP	al bl 1.2 0.49	= PA *al+(PA /1000/ BPM)^bl
AA (kg) actual ash	Allometry with BP	aa ba 0.19 0	=PA*aa+(PA/1000/ BPM)^ba
EBW (kg) empty body weight	Sum of chem components		= PA+ WA+ LA + AA – deficit Ca – deficit P_i
Lys req. (mg/d)	Growth of BP & feathers + maint	maintc Grw BP 25 75 FP 18 18 efficiency 0.8	=(rPAc*GrwBP/Eff)+(rFPAc *GrwF/Eff)+(0.008*MntBP/1000* (BPM^-0.27)*(PA/1000))+ (0.01*FPA* MntFP)
FIP_lys Desired food intake	FI to meet Lys req	Lys in feed, mg/g 13.0	=Lys req/Lys in feed

Bone free body (BFB) requirement			
BFBCaP (mg)	Isometry with PA	aBFBCa = 0.000418	= PA* aBFBCa*1000
BFBPhosP (mg)	Isometry with PA	aBFBPhos = 0.01026	= PA* aBFBPhos*1000
rBFBCaPc (mg)	Calc by subtn		$BFBCaP_t - BFBCaP_{t-1}$
rBFBPhosPc (mg)	Calc by subtn		$BFBPhosP_t - BFBPhosP_{t-1}$
Bone (BON)			
BonCaP (mg)	Isometry with PA	aBonCa = 0.041382	= PA* aBonCa*1000
BonPhosP (mg)	Isometry with PA	aBonPhos = 0.02394	= PA*aBonPhos*1000
rBonCaPc (mg)	Calc by subtn		$= BonCaP_t - BonCaP_{t-1}$
rBonPhosPc (mg)	Calc by subtn		$= BonPhosP_t - BonPhosP_{t-1}$
Body			
EFFBCaP Body Ca (g)	BFB + BON		= BFBCaP + BonCaP
EFFBPhosP Body P (g)	BFB + BON		= BFBPhosP + BonPhosP
rEFFBCaPc (mg)	Calc by subtn		$= EFFBCaP_t - EFFBCaP_{t-1}$
rEFFBPhosPc (mg)	Calc by subtn		$= EFFBPhosP_t - EFFBPhosP_{t-1}$
EFFBCaPhosP	Whole body ratio		= EFFBCaP / EFFBPhosP
Endogenous loss			
mCa(.) (mg)	Arbitrary proptn	Camaint = 0.01	= Camaint* rBodCaPc
mphos(.) (mg)	A function of protein growth	Phosmaint = 0.13	=Phosmaint* rBodPhosPc * $BPM^{-0.27}$
Animal requirements			
tCa(.) (mg/d)	dCa/dt + obligatory loss		= rBodCaAc + Camaintc

tphos(.) (mg/d)	dP/dt + obligatory loss		= rBodPhosAc + Phosmaintc
Proposed feed g/kg			
cal (g/kg)	Input according to phases	tCastart 10 tCagrow 9 tCafinish 8	= tCastart (If age ≤ 20) = tCagrow (If 21≤ age< 35) = tCafinish (If age ≥ 35)
phos (g/kg)	Input according to phases	tPstart 4 tPgrow 7 tPfinish 6	= tPstart (If age ≤ 20) = tPgrow (If 21≤ age< 35) = tPfinish (If age ≥ 35)
phytphos (g/kg)	Assumed constant in maize /soya feed	$PP_{maize/soya}$ 1.7	= $PP_{maize/soya}$
nonphytphos(g/kg)	Calculated by subtraction		=tP – PP
DCa (g/kg)	Regression equation provided for but constant propns of Ca available, other coefficients set to 0 to allow adjustment later	*Ca digestibility coefficients* intercept 0 lin coeff Ca 0 6 PP 0 nPP 0 phy 0 $PP \times nPP$ 0 $PP \times phy$ 0	=intercept+cal*Calin+phytphos*PPlin+ nonphytphos*nPPlin+Phytase*phylin+ phytphos * nonphytphos *PPXnPPlin+ phytphos * Phytase *PPXphylin
Dphos (g/kg)	Regression equation using coefficients from the literature (Couture 2020)	*Phos digestibility coefficients* intercept -0 0913 lin coeff sq coeff Ca -0 108 0 013 PP 0 783 0 nPP 0 928 0 phy 1 732 -0 638 $Ca \times PP$ -0 0557 0 $Ca \times nPP$ -0 0318 0 $Ca \times phy$ -0 166 0 $PP \times phy$ -0 364 0 $Ca \times phy \times PP$ 0 088 0	= intercept + cal*Calin+ phytphos*PPlin+ nonphytphos*nPPlin+ Phytase*phylin + cal * phytphos *CaXPPlin+ cal * nonphytphos * CaXnPPlin+ cal * Phytase *CaXphylin+ phytphos * Phytase *PPXphylin+ cal * phytphos * Phytase *CaXphyXPP+(cal ^2)*Casq+(Phytase ^2)*physq

Intake (mg)			
CaInc	Total Ca in feed * FI		=tCa*FIP_lys
PhosInc	Total P in feed * FI		=tP*FIP_lys
DCaInc	Available Ca in feed * FI		= aCa*FIP_lys
DPhosInc	AvailableP in feed * FI		= aP *FIP_lys
ACaInc	Intake available - endogenous loss		=Caav – Camaintc
APhosInc	Intake available - endogenous loss		= Phosavc – Phosmaintc
retained in BFB (mg)			
rBFBCaAc	retained Ca vs constrained Ca BFB		=IF(Caretc > rBFBCaAc, rBFBCaAc, Caretc)
rBFBPhosAc	retained P vs constrained P BFB		=IF(Phosretc > rBonPhosPc, rBonPhosPc, Phosretc)
retainable in bone (mg)			
retBonCac	retainable Ca after BFB vs normal Ca for bone		=IF((Caretc – BFBCaRet)> rBonCaPc, rBonCaPc,(Caretc – BFBCaRet))
retBonPhosc	retainable P after BFB vs normal P for bone		=IF((Phosretc – BFBPhosRet)> rBonPhosPc, rBonPhosPc,(Phosretc – BFBPhosRet))
retained in bone (mg)			
rBonCaAc Output for check/optimiser	Check with possible ratio of Ca:P	BonCa:Phos Ave 2 Max 2.2 Min 1.8	=IF(PRa* CaPhosBon Min≤ CaRa ≤ PRa* CaPhosBon Max),CaRa, PRa * CaPhosBon Ave)

rBonPhosAc Output for check/optimiser	Check with possible ratio of Ca:Phos	BonCaPhos 2 Max 2.2 Min 1.8	=IF(CaRa/ CaPhosBon Max≤ PhosRa ≤ CaRa/ CaPhosBon Min),PhosRa, CaRa / CaPhosBon Ave)
BFB retained/ desired			
bfbcafactor	Retained Ca BFB/ dCa/dt in BFB		= rBFBCaAc / rBFBCaPc
bfbphosfactor	Retained P BFB/ dP/dt in BFB		= rBFBPhosAc / rBFBPhosPc
Bone retained/ desired			
boncafactor	Retained bone Ca / dCa/dt in normal bone		= BonCaRetc / rBonCaPc
bonphosfactor	Retained boneP / dP/dt in normal bone		= BonPhosRetc / rBonPhosPc
total retained			
rBodCaAc Output for check	Retained BFB Ca + Retained bone Ca		= BFBCaRetc + BonCaRetc
rBodPhosAc Output for check	Retained BFB P + Retained bone P		= BFBPhosRetc + BonPhosRetc
BodCaPhos Output for check	Retained Ca/ Retained P		= BodCaRetc / BodPhosRetc
excreted (mg)			
ExCa Output for check/optimisr	Total intake Ca - total retained Ca		= CaInc – BodCaRetc
ExPhos Output for check/optimiser	Total intake P - total retained P		= PhosInc – BodPhosRetc

APPENDIX 3: PAPER PUBLISHED IN THE WORLD'S POULTRY SCIENCE JOURNAL

World's Poultry Science Journal

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/twps20

Constraints on the modelling of calcium and phosphorus growth of broilers: a systematic review

F. Salisbury, A.J. Cowieson & R.M. Gous

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APPENDIX 4: ETHICAL CLEARANCE

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

18 August 2021

Mr Frances Salisbury (822827779)
School of Agriculture, Earth & Environmental Sciences
Pietermaritzburg Campus

Dear Mr Salisbury,

Protocol reference number: AREC/00002326/2021
Project title: A model of calcium and phosphorus in broilers.

Full Approval – Research Application

With regard to your revised application received on 05 May 2021, the Animal Research Ethics Committee has accepted the documents submitted and FULL APPROVAL for the desktop study protocol has been granted.

Please note: There must be adherence to national and institutional COVID-19 regulations and guidelines at all times. Researchers will be personally responsible and liable for non-adherence to national regulations. If in doubt, please contact the Research Ethics Chair and/or the University Dean of Research for advice.

Please note: Any Veterinary and Para-Veterinary procedures must be conducted by a SAVC registered VET or SAVC authorized person.

Any alteration/s to the approved research protocol, i.e Title of Project, Location of the Study, Research Approach and Methods must be reviewed and approved through the amendment/modification prior to its implementation. In case you have further queries, please quote the above reference number.

Please note: Research data should be securely stored in the discipline/department for a period of 5 years.

The ethical clearance certificate is only valid for a period of one year from the date of issue. Renewal for the study must be applied for before 17 August 2022.

Attached to the Approval letter is a template of the Progress Report that is required at the end of the study, or when applying for Renewal (whichever comes first). An Adverse Event Reporting form has also been attached in the event of any unanticipated event involving the animals' health / wellbeing.

I take this opportunity of wishing you everything of the best with your study.

Yours faithfully

[redacted]

Dr Sanil D Singh, BVSc, MS, PhD
Chair: Animal Research Ethics Committee

/kr

cc Supervisor: Prof Rob Gous

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