THE UNIVERSITY of EDINBURGH

Title	Modelling the effects of the infectious environment on pig growth and intake
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Qualification	PhD
Year	2006

Digitisation Notes:

- Pages 95 to 100 missing from original

Modelling the effects of the infectious environment on pig growth and intake

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A thesis submitted towards the degree of Doctor of Philosophy at the University of Edinburgh

2006

Dedication

To my dear Mum and Peter, who throughout have given encouragement and support.

Declaration

I hereby declare that this thesis is of my own composition and that all assistance has been duly acknowledged. The results presented herein have not previously been admitted for any other degree or qualification.

Fredrik Bertil Sandberg

Acknowledgements

A PhD is a very long journey for all parties involved: the student, the student's family and the people providing the mentoring and academic supervision. I have been very fortunate with the support that I have been given throughout my studies at the Animal Nutrition and Health Department. I will now attempt to say thanks to the people involved, including the many unselfish colleagues at SAC, who so often have taken time out of their days to discuss the many different issues that myself and other students have encountered. First and foremost, however, I will try and adequately thank two great teachers. I thank Ilias and Gerry for their support, encouragement and patience. A PhD is a complex combination of individual achievement and team work and together they have helped to me to develop beyond anything that I could have expected.

Gerry provided a hands on approach with everything from using Minitab, Excel and programming logic to writing in English. I am not sure if we ever won the battle between the English and the Svenglish form of this wonderful language. The lunch time 'meetings' over the occasional IPA were also an inspiration for seeing the lighter side of a PhD. The mind is often more perceptive when relaxed. Thanks for all your help and advice and I hope to strive in the future to become a decent nutritionist.

Ilias has a tremendous energy for making progress in science and student development. Your influence in this way was very important for the successful completion of this project. At many times his Sunday was sacrificed to have documents returned to staff and students alike for Monday morning to maintain momentum. Ilias introduced me to many facets of self-management including time management, keeping focused and to not become demoralised when progress was not made. The numerous hours spent discussing issues or documents with the now famous whiteboard at the centre have been invaluable. The fact that the door was always open was equally invaluable. The additional introduction to non-scientific literature and art was equally inspiring and I hope it did not fall on completely deaf ears. I thank you for everything and I hope that one day I will be able to do the same for someone else.

It is impossible, however, to not mention the tag team approach that these two men employ. It was often impossible to know whether I was speaking to the good or the bad

cop. Regardless of the multitude of chapter/paper revisions that came flying back with pen marks all over it, or full of red tracking, or even worse with no comments at all, gave me an insight to writing formal publications and the more intricate details of this process. At many times I wondered why I spent all the extra time turning documents into chapters simply to start again to create publications for submission: I now realise how fortunate I was to be encouraged to do so. The combined encouragement of Ilias and Gerry has seen me produce 4 papers, three of which are in print, in addition to other formal publications. I hope that future students will realise how lucky they are.

The environment at SAC Bush is a friendly one. The three 'schools of thought' made for some interesting lunch time conversations. All staff were extremely approachable, irregardless of 'team' and I have found myself in discussions with many different staff. I would like to give a special mention to some of you. In alphabetical order I would like to acknowledge the help of Andrea Doeschl for help with all issues with programming and mathematics and for moral support, Bert Tolkamp for helping me understand some ruminant nutrition and especially the wonder that is West African Dwarf Goats, Dimitri Vagenas for modelling help and moral support, Ian Wellock for discussing the intricate details of our model and Mike Hutchings for random questions about ecology and for moral support. Thank you.

Last but not least I need to thank those of you who have not been involved with working at Bush but have kept me sane. I want to acknowledge Eleanor, who was a rock, kept me sane and motivated me – I will never forget it. I would like to thank my dear dear Mum and Peter who have motivated me, kicked me when I needed it and having supported me in the last part of my PhD. I owe you so much and the dedication of this thesis to you is one way to say Thank you.

Abstract

Sub-clinical disease can have large effects on animal production with significant economic consequences. Animal health and welfare are increasingly important criteria in animal production, and the removal of antibiotic growth promoters has added pressure on production systems. No general model has yet been proposed for predicting the growth and performance of animals exposed to pathogens. A robust framework for predicting growth during health and disease may assist in the design of nutritional, environmental and genetic management strategies. A core part of any animal growth model is how it predicts the partitioning of dietary protein and energy to protein and lipid retention for different genotypes at different degrees of maturity. Solutions proposed in the literature to the partitioning problem were described in detail and criticised in relation to their scope, generality and economy of parameters (Chapter 1). They all raised the issue, often implicitly, of the factors that affect the net marginal efficiency of using absorbed dietary protein for protein retention. Partitioning rules that withstood qualitative criticisms were then tested against literature data and a general quantitative partitioning rule was concluded for that had two key parameters: the maximum marginal efficiency of protein retention and the energy to protein ratio at which the maximum efficiency is achieved (Chapter 2). A general rule was identified which was able to predict protein retention for both protein and energy limiting diets in healthy animals. In Chapter 3 a general model was developed for predicting effects of sub-clinical pathogen challenges of different doses and virulence on the intake of animals. Pathogen induced anorexia is the major consequence on hosts during the course of infection. The model was for the period from recognition of a pathogen through to acquisition and subsequent expression of immunity. It is crucial to define the pathogen challenge (in terms of dose and virulence) and the degree of resistance of different hosts, when comparing their responses in RFI. There is no general agreement on the consequences of pathogen challenges, other than anorexia, that need to be included in a predictive framework of growth. In Chapter 4 literature data was reviewed for different kinds of pathogen challenges of a range of hosts to identify reductions in growth beyond that caused by anorexia: these were host, dose and time dependent. In only some instances did anorexia fully explain the reductions in growth. Solutions were needed for describing the protein costs of innate and acquired immune responses and repair of damaged tissues. Increased energy requirements depended on immune responses, repair of damage and fever. In Chapter 5 a framework was proposed that predicts the performance of different genotypes (in terms of growth potential and

disease resistance) when challenged by different doses of pathogens and given access to different foods. The model predicts amino acid and energy requirements due to growth and immune responses, and a partitioning rule was developed for partitioning scarce resources between growth and immune responses. Predictions can be made on the performance of different animal genotypes when they are given access to different quality foods and exposed to pathogens. The development of the model and its predictions, together with future testing, may contribute significantly towards our understanding of nutrition and genotype interactions during exposure to pathogens.

Contents

General Introduction

The performance of animals in commercial production environments is often well below what observed in well-maintained experimental or breeding stations (Black *et al.* 1995). A proposed cause for the observed differences in performance has been the presence of stressors, which include physical (ambient temperature, humidity), social (space allowance, group size) and infectious (kind and amount of pathogen). A possible approach for improving our understanding of how stressors affect performance is the use of mathematical modelling techniques. Models attempt to incorporate the underlying biology of the system in terms of deterministic equations, which are solved dynamically to consider the possible interactions between the animal, its food and its environment over time. This may allow for management decisions to be better informed and contribute to the alleviation of the effects of environmental stressors on performance.

Since the initial attempts of modelling pig growth (Whittemore & Fawcett 1974, 1976) there have been several models developed, with often increasing complexity to consider the effects of physical stressors e.g. Black *et al.* (1986), Ferguson *et al.* (1994) and Wellock *et al.* (2003a). More recently Wellock *et al.* (2003b) have considered the effects of social stressors on the performance of pigs. No general framework or model has yet been proposed to predict the effect of infectious stressors, such as pathogen or immune system activation (Williams *et al.* 1997a, b,c), on growth and performance. This is surprising as pathogen challenges have significant effects on growth and production (Beisel 1977; Crompton 1984; Colditz 2002). In pigs in particular, a simple activation of the immune system by maintaining animals in a dirty environment led to a 20-30% reduction in their performance (Williams *et al.* 1997a, b, c). The effects on production, together with the cost of prevention and treatment lead to disease having a significant economic cost. For example, Nieuwhof & Bishop (2005) estimated that gastrointestinal parasitism of sheep in Great Britain cost the producers £84 million per year, while Cutler (2001) estimated that enteric diseases of pigs in Australia incurred a cost of £32 million per year. Recently, Defra (web site) has estimated that sub clinical disease alone is responsible for a £12 million loss per annum in the UK pig industry. These significant financial costs, together with the removal of antibiotic growth promoters (EC Regulations 1831/2003, 1834/2003), increased occurrence of resistance to pharmaceutical interventions (Waller 1999) and increased demands for improved welfare (Kanis *et al.* 2005) highlight the need for an improved understanding of how pathogen challenges, nutrition and different genotypes (genetic resistance, growth potential) interact.

Black *et al.* (1999) when discussing the inclusion of disease in the AUSPIG model (Black *et al.* 1986) suggested that "...increasing maintenance energy requirements by up to 1.3 times the normal predictive value, decreasing the rate of protein deposition by 0.9 times normal and decreasing feed intake down to zero depending on the severity and duration of the disease" would achieve it. This may be seen an initial step towards including the effects of pathogen challenge on performance. Henken *et al.* (1994a, b) proposed a model for predicting the development of coccidiosis in poultry and related the effects of the challenge to the performance of chicks. Their model predicted the growth and transmission of the pathogen in the environment in a comprehensive manner, but the model was unsuitable for predicting interactions between animal, food and environment. There is, therefore a paucity of models that consider the effects of infectious stressors in farm animals.

The manner in which pathogen challenges reduce performance is a contentious issue (Klasing & Barnes 1988; Coop & Kyriazakis 1999; Greiner *et al.* 2000; Escobar *et al.* 2004; Greer *et al.* 2005). A key consequence of pathogen challenges is a temporal reduction in food intake (anorexia) that occurs during challenges of parasites, bacteria and viruses (Crompton 1984; Hart 1988; Kyriazakis *et al.* 1998). However, it is unknown whether this anorexia is the cause of the reduction in performance *per se*, or whether anorexia is the outcome of an overall change in the animal's physiology and metabolism (Wellock *et al.* 2003b). It appears that there are also costs associated with mounting an immune response towards pathogens, both in terms of protein (Houdijk *et al.* 2001), energy (Demas *et al.* 1997), and repairing damaged tissues (Abbott *et al.* 1985). The extent of the reduction in food intake appears to be dependent on the dose of pathogen and the severity of the disease (Kyriazakis *et al.* 1998). It is important to account for these effects when developing a framework to account for the consequences of exposure to pathogens on animal performance. The overall objective of this thesis was to develop a predictive model that would allow for the effects of pathogen challenges on food intake and growth of animals and pigs in particular. This task was achieved through the following steps.

Chapter 1: An animal may be fed *ad libitum* and still be limited in resources due to environmental constraints such as high temperatures (Campbell & Taverner, 1988; Collin *et al.* 2001) or feed factors such as bulk (Kyriazakis & Emmans, 1995) or due to the exposure to pathogens (Kyriazakis *et al.* 1998). A central problem for predicting growth

in animals is to have a sufficient quantitative rule to predict how an animal will allocate scarce resources between its different functions. This applies for both models where food intake is considered as an input and those where food intake is a predicted outcome (Black *et al.* 1986; Emmans & Kyriazakis, 1997; van Milgen & Noblet, 1999). The objective of this chapter, therefore, was to consider the different solutions to this problem that were available in the literature for the healthy animal and examine their suitability to solve the problem qualitatively in the first instance.

Chapter 2: In Chapter 1 several rules of partitioning could be rejected on qualitative grounds, but at least two, those of Black *et al.* (1986) and Emmans & Kyriazakis (1992*a, b*), withstood qualitative testing. In this chapter the quantitative consequences of these solutions were addressed. A comprehensive search of the literature identified several suitable data sets from pigs, to be used for testing the proposed solutions. The objective was to determine whether the marginal response in protein retention to limiting protein or energy intakes was different for hosts of different degrees of maturity, genotype and sex. In addition, it was assessed whether environmental temperature had an effect on the relationship between protein retention and limiting protein or energy intakes. The outcome of this chapter was expected to be a robust rule of partitioning scarce energy and protein resources in healthy animals.

Chapter 3: The effect of pathogen challenges on food intake is a central problem in predicting growth, as discussed above. In this Chapter the phenomenon that is disease induced anorexia was considered in relation to different host and pathogen species. A yet unresolved issue is how the reduction in voluntary food intake occurs in animals challenged by pathogens. Two mechanisms have been put forward: 1) A pathogen challenged reduces an animal's potential for growth, which leads to a reduction in requirements and subsequently food intake (Webel *et al.* 1998a, b). 2) A pathogen challenge directly affects food intake, with subsequent reductions in growth (Kyriazakis *et al.* 1998). Black et al have suggested that a combination of the two mechanisms may account for the reduction in the food intake of pigs exposed to pathogens. The objective of the chapter was to review the literature with a view of proposing a mechanism, and develop a model that would be able to predict the effect of pathogen challenges of different doses on the voluntary food intake of hosts with different degrees of genetic resistance to pathogens. The resulting framework was a generic one as opposed to a certain pathogen specific.

Chapter 4: In Chapter 3 it was assumed that the pathogen challenge caused no other changes to the animal, such as altered requirements or partitioning, than a reduction in food intake. As there is no general agreement on the overall problems that need solving in order to predict growth during pathogen challenges (Black *et al.* 1999; Coop & Kyriazakis, 1999; Powanda & Beisel, 2003) the literature of animal performance during exposure to pathogens was reviewed. The objective was to identify the key consequences of pathogen challenges other than anorexia that would need including in a predictive framework of growth. In addition, it was necessary to consider the effect of nutrition on the key consequences of pathogen challenges.

Chapter 5: The key consequences of pathogen challenges on resource requirements and partitioning were identified in Chapter 4, and these were taken forward as a basis for creating a complete model for predicting food intake and growth in animals challenged by pathogens. To achieve this specific objective it would be necessary to choose suitable functional forms that related the pathogen challenge to changes in resource requirements. In addition, it would be necessary to develop a model that allows for the change in pathogen load of the host to be predicted. It would also be necessary to develop the model in such a way that it would be able to predict the requirements for individual amino acids. Finally a suitable partitioning rule would need to be developed that partitions resources between maintenance, growth and immune responses. The outcome of this chapter was expected to be a framework that was able to predict the performance of different kind of animals, given access to foods of different compositions whilst exposed to pathogens.

General Discussion: Here the achievements of the thesis will be considered together with consequences of the developed framework. Issues raised by the overall framework, including experiments needed for resolving issues such as the underlying mechanism of disease induced anorexia and model parameterisation, will be discussed. The developed framework is discussed in relation to the natural selection of hosts and pathogen, and its consequences for artificial selection. The model was developed for an average animal, which was representative of its genotype. The final part of the general discussion considered whether it would be possible to take the proposed framework for an individual animal and develop it into a population model. Recent work by e.g. Pomar *et al.* (2003)

has shown that the response of the average individual animal may be different from the response of the mean of a population of animals.

Chapter 1

Partitioning of limiting protein and energy in the growing pig.

1. Description of the problem, possible rules and their qualitative evaluation.

1.1 Abstract

A core part of any animal growth model is how it predicts the partitioning of food resources for different genotypes and degrees of maturity. The problem is to predict the rates of protein and lipid retention as responses to different allowances of food of varying concentrations of protein and energy. The range of food compositions, animals and environments over which partitioning rules apply is discussed in this paper together with the problems of description. Rules of partitioning need to be combined with protein and energy systems to make predictions. The animal needs describing in relation to its genotype, live weight and possibly composition. The live weight range of the application of a partitioning rule should extend beyond the "slaughter weight range", and optimistically would include the period from the start of feeding through to maturity. Solutions (or Rules) that have been proposed to solve the partitioning problem are detailed and criticised. They all raise the issue, at least implicitly, of the factors that affect the net marginal efficiency of protein retention. An important question is whether the effects of these animal and food composition variables are independent of each other. Of the rules considered several could be rejected on qualitative grounds. The surviving rules are taken forward for quantitative analysis in the following chapter.

1.2 Introduction

It is useful to see nutrient partitioning as the distribution of resources from an ingested food between protein and lipid retention, once the requirements for maintenance have been met. This is so both for models where food intake is considered as an input and those where food intake is a predicted outcome (Black *et al.* 1986; Emmans & Kyriazakis, 1997; van Milgen & Noblet, 1999). Even an animal fed *ad libitum* may still be limited in resources. Environmental constraints such as high temperatures (Campbell & Taverner, 1988; Collin *et al.* 2001) or feed factors such as bulk (Kyriazakis & Emmans, 1995) may prevent the animal from attaining the food intake needed for achieving potential growth. Emmans & Fisher (1986) recognised that to understand the partitioning of scarce resources, the composition of the food partitioned and the animal within which partitioning occurs, both need sufficient descriptions. In agreement with this view, Black *et al.* (1986) stated that "a full understanding of the animal's response to variations in dietary conditions is required" in order to solve the problem of predicting performance.

The actual intakes of protein and energy will affect the rate of protein retention, which may be below its upper limit, PR_{max}, set by the genotype and the state of the animal (Black *et al.* 1995; Schinckel & de Lange, 1996; Whittemore & Green, 2002; Wellock *et al.* 2004). Inevitably, intakes will also affect the rate of lipid retention. Rules of partitioning allow the prediction of the *actual* rates of both protein and lipid retention given the supply of ingested protein and energy. Combining partitioning rules with protein and energy systems allows the prediction of *actual* rates of protein and lipid retention.

An optimistic assumption is that general rules exist that govern the partitioning of scarce resources (Ferguson *et al.* 1994; Emmans & Kyriazakis, 1997). A different view is that both the kind of pig, and the state that it is in, will affect the partitioning of scarce resources (Fuller & Crofts, 1977; de Greef & Verstegen, 1995; Fuller *et al.* 1995). This is equivalent to saying that there are no general rules. Black *et al.* (1986) and van Milgen & Noblet (1999) propose the intermediate view that there are differences between genotypes and a general systematic effect of live weight.

The problem of nutrient partitioning in growing pigs has been considered in the reviews of Black & de Lange (1995), Susenbeth (1995), de Greef & Verstegen (1995), Schinckel & de Lange (1996), Emmans & Kyriazakis (1997), Whittemore *et al.* (2001), Moughan (2003*a*) and van Milgen & Noblet (2003). In no case, were all of the proposed solutions described, contrasted and criticised both conceptually and against experimental data in the literature. As well as these reviews, the recent partitioning models proposed by Green & Whittemore, (2003), van Milgen & Noblet, (1999) and van Milgen *et al.* (2000) are considered here. There is no general agreement on what the rules of partitioning are. We describe first the solutions in the literature and then their ability to solve the partitioning problem is qualitatively assessed. A solution to the problem of partitioning as conventionally posed is needed before more complex situations, such as disease (Lochmiller & Deerenberg, 2000; Coop & Kyriazakis, 2001; Houdijk *et al.* 2001; Powanda & Beisel, 2003), can be properly considered. In a second accompanying paper we assemble and use a comprehensive and suitable set of data from the literature to evaluate quantitatively the various proposed rules (Chapter 2; Sandberg *et al.* 2005b).

1.3 Partitioning rules

1.3.1 *The scope of the proposed rules*

Any rule of scarce resource partitioning will operate over some range of inputs that may include food, animal and environmental variables. Descriptions of these inputs will therefore be required. These three classes of variables are considered in turn.

1.3.1.1 *Food variables*

The level of feeding can be between zero and *ad libitum*. Any rule should cover as much of the range as is possible. While force-feeding is possible (Dividich & Noblet, 1984; Wieland *et al.* 1993) it is not considered further here. The assumption usually made, for simplicity, is that no components of the food other than its protein and energy contents are affecting growth. The protein and energy dimensions need clear descriptions that are sufficient for the proposed rule to be implemented (Fuller & Crofts, 1977; Kyriazakis & Emmans, 1992*a, b*; de Greef & Verstegen, 1995).

Possible components of the description of the protein content of a food include the crude protein content (*CPC*, kg/kg) and its digestibility that may be apparent (d_a) or ileal (d_i), (Whittemore, 1983; Moughan, 1999; Moughan, 2003*b*). The proportion of protein that is "ideal" (v), in relation to a chosen reference protein is relevant (Fuller *et al.* 1989; Wang & Fuller, 1989). If the material efficiency of retaining the first limiting amino acid differs between amino acids then the first limiting amino acid in the protein also needs to be known.

An energy content of the food (MJ/kg) is needed to turn a food allowance (kg/d) into an energy allowance (MJ/d). There are different solutions to this problem. Whittemore (1983) proposed using digestible energy, *DE*, with correction for the protein content of the food. van Milgen & Noblet (1999) used metabolisable energy, *ME*. Noblet *et al.*, (1994) proposed that net energy, *NE*, could be used. Emmans (1994) proposed a scale called effective energy, *EE*, while Birkett & de Lange (2001*a, b, c*) used the "explicit material flow of ATP".

1.3.1.2 *Animal*

Partitioning rules intended to operate across kinds and states of pig need to include adequate descriptions of the animal. The dimensions of the description will include genotype and current state. A sufficient description of current state could include the

degree of maturity either as live weight (van Milgen & Noblet 1999) or as protein weight (Whittemore & Fawcett, 1976; Whittemore, 1983; Emmans & Fisher, 1986). The description may also include the fatness of the animal and its age. Where live weight is the only state variable used, differences in body protein and lipid proportions cannot be dealt with. Knap *et al.* (2002) proposed that a reasonable body weight range to use would be 10 – 175 kg. Ideally, the range would be from birth to maturity. Pre-natal partitioning of nutrients is not normally considered (Wellock *et al.* 2004).

1.3.1.2 *Environment*

Any partitioning rule would optimistically have adequate conceptual descriptions of how environmental factors may affect rules of partitioning. Partitioning rules often assume that there are either no environmental effects or, in the case of thermoregulation or activity, that the additional energy requirements can be added to maintenance (Whittemore & Fawcett, 1976; Black *et al.* 1986; Wellock *et al.* 2003).

1.3.2 *Proposed rules of nutrient partitioning*

Rules of partitioning found in the literature are presented in roughly chronological order.

1.3.2.1 *Rule 1*

Whittemore & Fawcett (1974) made protein retention a function of the crude protein content of the food (*CPC*, kg/kg) and food intake (*FI*, g/d). They made the gross efficiency with which crude protein intake was retained, *Z*, dependent on live weight (*W*, kg) through the general constants *p*, *k* and *f*:

$$PR = FI.CPC.Z \qquad \text{g/d} \qquad (1)$$

$$Z = p + k.exp(-f.W) \qquad (2)$$

An upper limit to *PR*, here called PR_{max}, was a characteristic of the kind of pig but was independent of *W*. The amount of energy available after meeting maintenance and that needed for *PR* went to *LR*. The model was assumed to apply for dietary crude protein contents between 120 and 280 g CP/kg and $20 < W < 100$ kg. No other limits were stated.

1.3.2.2 *Rule 2a*

Whittemore & Fawcett (1976) proposed an alternative expression for *PR* and set a minimum for the ratio of lipid to protein in the gain. Protein retention was predicted from

the ideal protein supply (*IP*, g/d) as the product of *FI, CPC,* d_a and the biological value of the protein, *v*. Equations (8) and (12) in their paper lead to:

$$PR = IP / [(1 - \phi) + (\phi / (s.(1 - u))] \quad \text{g/d} \quad (3)$$

The value of $u = P/P_m$ is the degree of maturity in protein, where P is the current protein weight and P_m is the mature protein weight. The parameters, $\phi = 0.06$ and $s = 0.23$ were assumed to be constant across genotypes and degrees of maturity. Equation (3) makes the gross efficiency of using *IP* decrease as *u* increases. The gross efficiency is independent of *IP* at a given value of *u*. There are no effects of genotype other than on PR_{max} as in Rule 1. The value of *LR* is calculated on energy grounds as in Rule 1, but now any demand for cold thermogenesis is met first. A further condition is that a minimum is set for the ratio of lipid to protein in the gain $(LR:PR)_{min}$. This condition means that the value of *LR* cannot fall below $(LR:PR)_{min}.PR$.

The rules used by Moughan *et al.* (1987) and de Lange (1995) are essentially the same as those proposed by Whittemore & Fawcett (1976). The scope is the same as that of Rule 1 other than allowing the environment to be cold and food protein to vary in quality.

1.3.2.3 *Rule 2b*

Whittemore (1995) recognized that setting a minimum ratio of lipid to protein in the gain was an unsatisfactory concept. He proposed two other ratios instead. The first was a 'preferred' value for the ratio of the mass of lipid (*L*) to that of protein (*P*) in the body called $(L:P)_{pref}$. The second was a minimum value for this ratio $(L: P)_{min}$. The variable $(L: P)_{min}$ was proposed to "ensure some level of fatness in the body" and "to prevent undue use of *L* for the support of *PR*". A value of 0.5 was proposed for $(L:P)_{min}$. The value for $(L:P)_{pref}$ was believed to be genotype and sex specific. It was not clear whether it varied as the pig grew.

1.3.2.4 *Rule 3*

Fuller & Crofts (1977) presented an equation that related the scaled nitrogen retention (*NR*, $g/kg^{0.73}$ d) to the protein and starch contents of the food. Differentiation of their equation (9) gives (4) below. The response in nitrogen retention (*NR*, $g/kg^{0.73}$ d) per unit starch intake (*S*, $g/kg^{0.73}$ d) was related to the intakes of both starch and nitrogen through:

$$dNR / dS = \varepsilon.exp(-q\ S).(1-A.exp(-r\ N)) \quad (4)$$

Where N is the scaled nitrogen intake (g/kg$^{0.73}$ d) and ε, q, A and r are parameters. The values of the parameters, and hence the efficiency of protein utilisation, were said to depend on animal factors including genotype, sex, age and nutritional history.

1.3.2.5 *Rule 4*

Black *et al.* (1986) proposed that protein retention was a linear function of metabolisable energy intake (*MEI*, MJ/d) when *MEI* was less than that required for PR_{max}. The equations presented were equivalent to:

$$PR = b.(MEI - (c.ME_m)) \quad \text{g/d} \quad (5)$$

$$b = X_{sm}.(v.e^{-y\ W} + z) \quad \text{g/MJ} \quad (6)$$

ME_m is the amount of *ME* needed for maintenance. The rate of response, b, depended on W through (6). The values of the parameters v (0.7), y (-0.0192) and z (0.65) were assumed constant across genotypes, sexes, environments and degrees of maturity. Their biological meaning, if any, is not clear. The parameter 'c' is discussed below. The value of X_{sm} depended only on genotype ranging from 0.68 to 1.2. The value of *LR* was calculated on the grounds of energy balance.

1.3.2.6 *Rule 5*

Kyriazakis & Emmans (1992*a,b*) proposed a model that made protein retention, subject to $PR < PR_{max}$, a function of the ideal protein supply above maintenance, equation (7). The marginal material efficiency of ideal protein retention, e_p, was made a linear function of the ratio of energy to protein in the feed (8) subject to an upper limit of e_p*.

$$PR = e_p.(IP - IP_m) \quad (7)$$

$$e_p = \mu.(MEC/DCPC) \quad (8)$$

MEC and *DCPC* are the contents of metabolisable energy (MJ/kg) and digestible protein (kg/kg) in the food used. The values of μ (0.0112) and e_p* (0.814) were assumed to be constant for all genotypes and degrees of maturity.

1.3.2.7 *Rule 6*

de Greef & Verstegen (1995) proposed that, when energy is in short supply, protein is "adequate" and $PR < PR_{max}$, an increase in energy supply (*DEI*, MJ DE/d) is partitioned between marginal increases in protein and lipid retention using a constant marginal ratio (*MR*). The primary equations are:

$$PR = a + b'.DEI \quad \text{g/d} \quad (9)$$

$$LR = c + d.DEI \quad \text{g/d} \quad (10)$$

From which follows the definition of *MR* as:

$$MR = d / b' \quad (11)$$

The values of the parameters (*a* and *c*, g/d) and (b′ and *d*, g/MJ DE) were seen as varying with genotype and live weight (de Greef & Verstegen, 1995). It is important to note that *MR* is not a parameter in its own right but is *calculated* from the values of b′ and *d*.

1.3.2.8 *Rule 7a*

van Milgen & Noblet (1999) proposed a model where retention of protein and lipid are calculated from the metabolisable energy supply, when protein is assumed to be non-limiting. The data used for the analysis were assumed by the authors (van Milgen, personal communication) to come from experiments where the animals were limited only by energy, and not by protein supply. Each extra MJ of ME above maintenance is partitioned so that X_i MJ/day goes to protein and (1- X_i) MJ/day goes to lipid retention (equations 12 and 13). The value of X_i is made a function of live weight (equation 14) with the values of the parameters c_i and d_i, dependent on genotype.

$$PR = k_p.\ X_i.(ME - MEm) \quad \text{g/d} \quad (12)$$

$$LR = k_f.(1 - X_i).(ME - MEm) \quad \text{g/d} \quad (13)$$

$$X_i = c_i + d_i.(W - 20) \quad (14)$$

The energetic efficiencies with which *ME* is used for protein and lipid retention are k_p and k_f and these may be affected by the nutrient source (van Milgen *et al.* 2000), although it has not been stated how. The values of these two parameters are assumed constant across genotypes and live weights. It is further assumed that at maintenance no protein or lipid is either retained or lost.

1.3.2.9 *Rule 7b*

The model defined by Rule 7a was modified by van Milgen *et al.* (2000) to produce Rule 7b. In this model, the parameters that determine the rates of retention of protein and lipid are k_p, k_f, ME_m, PR_{max} and a new parameter, F. F was defined as the (MEI/ME_m) value at which $PR = PR_{max}$. The full set of equations proposed in the Appendix of van Milgen *et al.* (2000) is complex and the equations are not reproduced here. The authors used a different set of parameters for estimation from an extensive set of data on three kinds of pig. The set included k_p, k_f and ME_m as before. The additional parameters were: PR_{max} at both 100 and 150 days of age, B (the Gompertz rate parameter), F_{100} (MEI as a multiple of ME_m required to attain PR_{max} at 100 kg of body weight) and dF (the change in F due to a change of 1kg in body weight). A consequence of the change from Rule 7a to Rule 7b is that PR and LR are now both curvilinear functions of MEI until $PR = PR_{max}$, rather than linear ones.

1.4. Qualitative assessment of proposed rules

In this section, the rules will be challenged in turn to identify any conceptual weaknesses. The intention is to identify those areas where rejection is not possible on qualitative grounds. Quantitative tests are in the accompanying chapter (Chapter 2; Sandberg *et al.* 2005b).

Rule 1 uses a description of food protein that is now generally seen as inadequate. The parameter Z predicts a reduction in the *gross efficiency* of protein utilisation, without distinguishing between protein requirements for maintenance and protein retention. While an important first step in the modelling of pig growth it is now only of historical interest and is acknowledged as such by its authors (Whittemore *et al.* 2001).

Rule 2a extends Rule 1 to consider the protein supply as ideal protein and makes the efficiency with which it is used a function of the degree of maturity. The efficiency is still the gross rather than the marginal efficiency. The new proposal of a minimum ratio of lipid to protein in the gain does not permit lipid to be lost while there is a gain in protein. There is strong evidence (Dividich *et al.* 1980; Stamataris *et al.* 1991; Kyriazakis & Emmans, 1992*a,b*; Bikker, 1994) that this can occur. Therefore Rule 2a (and by implication that of Moughan *et al.* 1987 and de Lange, 1995) are not considered for quantitative analysis.

Whittemore (1995) rejected Rule 2a. He changed from a ratio of lipid to protein in the gain, to ratios of lipid to protein in the body as a possible constraint to *PR* to produce Rule 2b. Green & Whittemore (2003) applied the revised approach to a model that also had a maximum allowable rate of lipid loss. The implementation of the rule is, however, such that it becomes, at least in part, the same as that of Rule 2a by setting a minimum ratio of lipid to protein in the gain. Green & Whittemore (2003) state that when the actual ratio of *L* to *P* is below that which the pig prefers ($L{:}P_{pref}$) "then the retention of lipid will be given priority over the retention of protein such as to limit *PR* and achieve in the daily gain a ratio of *LR:PR* that is set to the same ratio as is set for $L{:}P_{pref}$". In addition, Green & Whittemore (2003) have a lower limit for the L to P ratio (D.Green, personal communication), which is less than the value of $L{:}P_{pref}$ described by Green & Whittemore (2003) as a limit "below which the animal was not prepared to go". The concept of a desired ratio of lipid to protein in the body that may change with degree of maturity (Emmans, 1988), and the lower limit of the ratio of lipid to protein in the body, are in Figure 1.

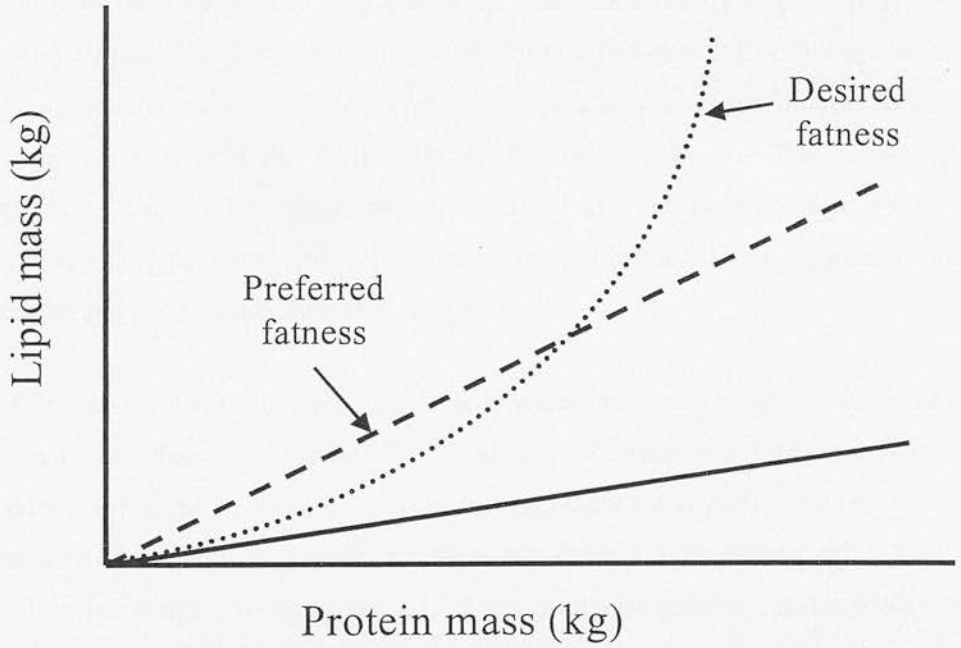

Figure 1 Possible relationships between protein (P) and lipid (L) masses in pigs: Desired fatness (– –) is described by $L_{des} = a\ P^b$ (Emmans, 1988). The preferred fatness (····) is described by $L_{pref} = (L{:}P)_{pref}.P$ and the minimum fatness (———) by $L_{min} = (L{:}P)_{min}.P$ (Whittemore 1995).

Green & Whittemore (2003) raised an important issue: what is the maximum *rate* of lipid loss that an animal could undergo at the expense of growing body protein, before protein retention would become penalised in some way? It is widely recognised (Black, 1974; Emmans & Kyriazakis, 1997; Whittemore, 1995, van Milgen & Noblet, 2003) that pigs may lose lipid, while gaining protein. van Milgen & Noblet (2003) stated that the pig is prepared to lose lipid for only a short period of time but do not state what this is. Wellock *et al.* (2003), and Whittemore (1995), set a minimum value for the ratio of protein to lipid in the body. A necessary consequence is that lipid can be lost only when the ratio of L to P exceeds this minimum. Black *et al.* (1986) also recognised that pigs might not want to lose lipid "indefinitely". Where a model allows lipid to be lost, which is necessary, it should also set a minimum value for the ratio of L to P.

Rule 3 considers the protein and the energy, as starch, supplied by the diet, but it does not clearly account for maintenance requirements for either protein or energy. However, it raises an interesting issue, as was done by Miller & Payne (1961), that the efficiency of using protein for maintenance might be a function of the energy to protein ratio of the food. In addition, Rule 3 implies that the relationship between any additional amount of energy as starch and the efficiency of protein utilisation is curvilinear, rather than rectilinear as suggested by Kyriazakis & Emmans (1992*a,b*). The values of the parameters of Rule 3, and hence the efficiency of utilising protein, were stated to be affected by genotype, state, live weight and nutritional history. These suggestions cannot be rejected and need further quantitative testing.

Rule 4 defines the *ME* needed for a zero rate of energy retention as ME_m. When $ME/ME_m = c$ (equation 6) then PR = 0 while *LR* is negative. No mention is made of any effect of food composition on the value of c, taken as being constant at 0.55. The rule states that the marginal increment in protein retention per extra MJ of ME supplied falls with increasing live weight. To apply the rule to any particular genotype needs evaluation of the parameter X_{sm} (equation 7).

Rule 5 takes into account protein for maintenance and the *CP*, d_i, v and energy values of the diet. The rule allows the prediction of the transition from the 'protein limiting' to the 'energy limiting' phase of protein retention as illustrated by the data of amongst others Campbell *et al.* (1985) and Bikker (1994). The plateau in *PR* predicted to occur when energy is limiting at high protein intakes is below PR_{max}. The rule predicts this effect by

making e_p decline as the (*MEC/DCPC*) value of the diet declines. When the diet is such that lipid retention is predicted to be negative no upper limit is set either for the rate of lipid loss or to the total loss that can occur. If the model is to be made dynamic then certainly the second of these conditions needs to be changed. This was done by Wellock *et al.* (2003) who set a minimum to the ratio of *L* to *P*.

The use of the marginal ratio in Rule 6 has, along with Fuller & Crofts (1977), a high parameter requirement. Its approach to dealing with live weight appears not to be systematic. The rule also only deals with the protein content of the food in a way that is poorly defined (see de Greef & Verstegen, 1995). Figure 2 illustrates the concept of the marginal ratio, and is typical of a linear plateau system.

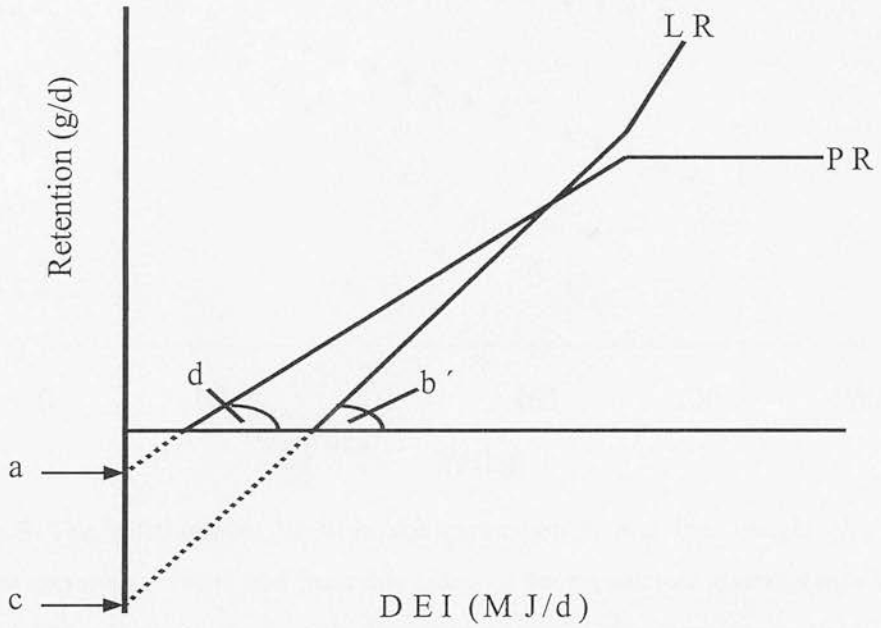

Figure 2 The relationship between the rates of retaining protein (*PR*) and lipid (*LR)* and digestible energy intake *(DEI)* partitioned according to the marginal ratio (MR) rule; MR = d/b′(de Greef & Verstegen, 1995).

It is not possible using Rule 6 to predict whether particular foods or food allowances would be limiting in protein. For this reason, it is not considered further.

Rule 7a predicts that an animal eating an allowance that provides *MEI* that equals its maintenance requirement for energy does not lose or gain any lipid or protein. This is inconsistent with the observation that animals often lose lipid at substantial rates while gaining protein (e.g. ARC, 1981 & Dividich *et al.* 1980). The rule does not consider

protein supply explicitly. The values of the parameters c_i and d_i which depend on genotype and sex, relate W to the value of X_i, which is the partitioning parameter (Equations 12, 13). The relationship between X_i and W is shown in Figure 3 for different genotypes and sexes.

The data used to quantify Rule 7a for seven different kinds of pig came from a single level of feeding, 'close to ad libitum' of a single food except that two of the genotypes had some extra protein.

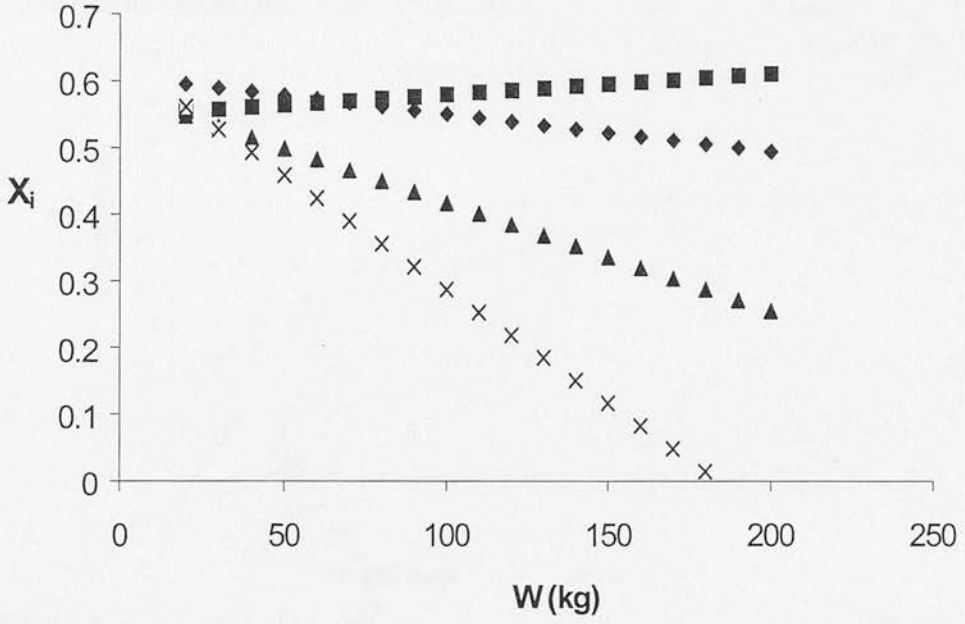

Figure 3 The relationships between the parameter X_i and live weight (W) for four different genotypes, calculated from the values of the parameters given by van Milgen & Noblet (1999): $X_i = c_i + d_i (W - 20)$. The four genotypes are: Synthetic male (♦), Pietrain male (■), Large White male (▲) and Large White female (X).

This must limit the use of the equations in a dynamic model to predict growth for different food allowances with varying concentrations of protein and energy. As the model deals neither with the protein content of the food nor with different levels of feeding (because of the nature of the data used) it will not be discussed further.

The information needed to use the model described by van Milgen *et al.* (2000) here called Rule 7b, is very considerable. The response in *PR* and *LR* to *MEI* is now different to that shown in Figure 2, see Figure 4. To describe a kind of pig it is necessary to feed it at different levels at each of several body weights and to measure the rates of lipid and

protein retention. The data collected, at least in principle, can then be analyzed to yield estimates of the parameters of the model. van Milgen *et al.* (2000) used 145 energy and N balances to estimate the values of the 27 parameters in their full model. The full model was simplified by assuming that the values of three of the parameters were the same for the three genotypes used, leading to a model with 21 parameters. The authors do not discuss the fit of the model nor if there were any patterns in the residuals. The values of some of the important parameters were not well estimated. The standard errors of the maintenance coefficients were 25% of the estimates themselves; the standard errors of the Gompertz rate parameters were 35% of the estimates of the coefficients.

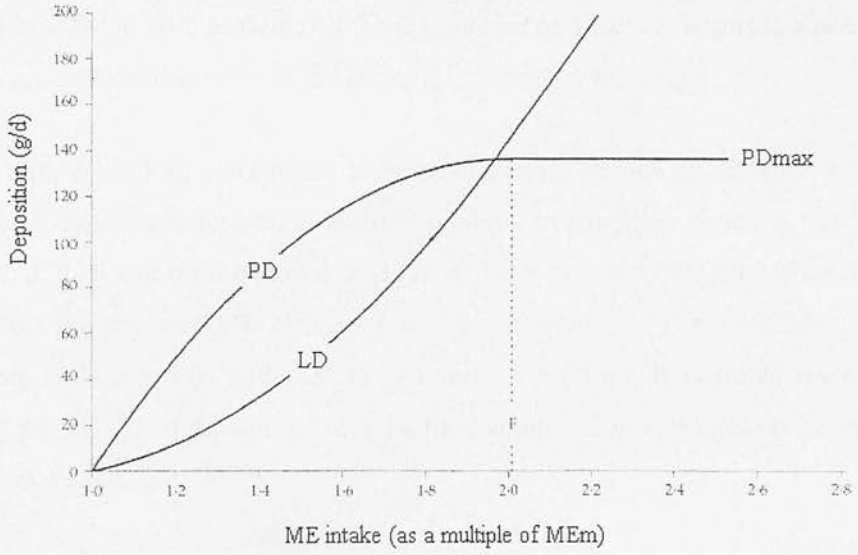

Figure 4 The relationships between protein deposition (*PD*) and lipid deposition (*LD*) and metabolisable energy intake (*ME*) as a multiple of that required for maintenance (*MEm*), as described by van Milgen *et al.* (2000). *F* is the value of *ME/MEm* at which PD_{max} is reached.

The information needed to use Rule 7b for any particular genotype, existing in the future, is unlikely ever to be available. The authors state that the *concepts* of Rule 7b may be employed, and adapted, to deal with more complex situations, including disease. However, it would be difficult to take Rule 7b forward, partly because of the information needed and partly because the supply of protein is not explicitly considered; it is not considered further.

1.5. Conclusions

The rules of partitioning considered here are usually components of more comprehensive pig growth models (Whittemore, 1983; Black *et al.* 1986; Moughan *et al.* 1987; Emmans, 1988; Black *et al.* 1995; Ferguson *et al.* 1997; van Milgen & Noblet, 1999; Knap, 2000; Green & Whittemore, 2003; Lovatto & Sauvant, 2003; Schinckel *et al.* 2003; Wellock *et al.* 2003). Models have increased in size and complexity since those of Whittemore and Fawcett (1974, 1976). Whittemore & Fawcett (1976), Whittemore (1983) and Emmans & Kyriazakis (1997) identified three important problems in predicting actual growth in the pig. These are the genetic upper limit to *PR*, the calculation of the weights of water and ash associated with protein and the application of a partitioning rule when *PR* is less than PR_{max}. Partitioning rules in the literature are now considered.

Rules 1, 2a, 7a and 7b were found to be inadequate, although on different grounds, and will not be considered further. It was not possible to reject the concepts put forward by rules 2a, 3, 4, 5 and 6 on qualitative grounds. They all raise the issue of the factors that may affect the net marginal efficiency of protein retention. These include live weight, genotype, including sex, and the composition of the food. It is important to establish whether the effects of the animal and the food composition variables are independent of each other, or not.

A partitioning rule that has fewer parameters is preferred to one with more, other things being equal. This is a version of the criterion widely used in science called Occam's Razor (Forster, 2000). Rules 3 and 6 can be assessed using this criterion. Rule 3 (Fuller & Crofts, 1977) calls initially for the values of four parameters in order to solve its important equation. Were the values of these to be general then the amount of information needed would be quite small. However, the authors state that their values depend on animal factors including genotype, sex, age and nutritional history. This means that each experiment needs to be carried out across all of these factors in order for the rule to be applied. An enormous amount of information is therefore needed. The position with rule 6 (de Greef & Verstegen, 1995) is the same in that although only four parameters need to be evaluated initially these are also said to depend on genotype and live weight. Again, an enormous amount of information is needed. As there are other rules that do not appear to have such high requirements for information Rules 3 and 6 will not be quantitatively assessed in the next chapter.

Chapter 2

Partitioning of limiting protein and energy in the growing pig.

2. Testing quantitative rules against experimental data.

2.1 Abstract

A companion paper gave descriptions and qualitative assessments of proposed quantitative solutions to the problem of the partitioning of scarce resources in pigs. Those that survived the assessments posed the quantitative problems of the effects of live weight, genotype and food composition on the marginal response in protein retention to protein and energy intakes on protein and energy limiting foods. Protein and energy limiting foods were defined and considered separately. Difficulties with the quantitative description of the ideal protein supply were discussed. It was found that the marginal response in protein retention to protein supply, when protein intake is limiting, is not affected by live weight, genotype or environmental temperature. There is good evidence that live weight does not affect the marginal response in protein retention to energy intake when protein intake is not limiting. The limited data for different genotypes suggested no effects on this response. A general quantitative partitioning rule is proposed that has two key parameters. These are e_p* (the maximum marginal efficiency for retaining the first limiting amino acid) and R* (the energy to protein ratio of the food, MJ ME/kg DCP, when e_p* is achieved). When $R < R$* the material efficiency of using ideal protein is (e_p*/R*). R. The value of e_p* was determined to be 0.763 (SE 0.0130). There was no good experimental evidence that e_p* is different for different amino acids. The best estimate of R* was 67.9 (SE 1.65) MJ ME/kg DCP. Live weight, genotype and temperature did not affect the values of either parameter. A more general understanding of partitioning, including the effects of 'stressors' such as disease, may be achieved by using the preferred rule as a starting point.

2.2 Introduction

Nutrient partitioning in the growing animal is the distribution of ingested protein and energy to protein (*PR*) and lipid (*LR*) retention once the requirements for maintenance have been met. In the previous chapter (Chapter 1; Sandberg *et al.* 2005a) were descriptions of the solutions that have been proposed to the problem of the partitioning of scarce resources and these were criticised qualitatively. Quantitative tests of the rules that withstood qualitative criticisms (Black *et al.* 1986; Kyriazakis & Emmans, 1992*a,b*; Whittemore, 1995) are presented here. Black *et al.* (1986) and Kyriazakis & Emmans (1992*b*) deal with the marginal response in protein retention to both energy and protein intake, whichever is scarce. Black *et al.* (1986) proposed that there are effects of live

weight and genotype on these responses; Kyriazakis & Emmans (1992*a*,*b*) proposed that there were not.

The emphasis in this paper is on the response in protein (amino acid) retention to protein (amino acid) supply. There is disagreement on the form of the response. Whittemore & Fawcett (1974, 1976) proposed the linear-plateau (or broken-stick) model and Whittemore (1983), Black *et al.* (1986, 1995) and Emmans & Kyriazakis (1997) agreed. Fuller & Garthwaite (1993), Bikker (1994) and Gahl *et al.* (1994) proposed a curved response. Whatever the form of the response, it is possible that it is affected by live weight and genotype, including sex. There now appears to be general agreement on the existence of a genetic upper limit to protein retention, which varies with live weight (PR_{max}), and a protein requirement for maintenance (MP).

Even if a linear-plateau model correctly represents the response of any individual at a time, it is still possible that a population of animals will have a response that is curvilinear (Fisher *et al.* 1973) at a time. Curnow (1973) has described the underlying mathematics. Ferguson *et al.* (1997) and Pomar *et al.* (2003) have applied similar arguments to populations of growing pigs. In addition to the variation between individual pigs at a time possibly causing curvilinearity, there is also the effect of each of the individuals changing with time (Pomar *et al.* 2003). Fuller & Garthwaite (1993) attempted to describe the responses of individual pigs experimentally and presented parameter values for linear-plateau and exponential models for individual animals. The authors concluded that the exponential model was to be preferred, but there were problems with the statistical analyses and with the way in which protein retention was scaled to live weight, to take out period effects.

The relationship between PR and energy supply may also be seen as a linear-plateau model (Black *et al.* 1986, 1995; van Milgen & Noblet, 1999; de Greef & Verstegen 1995). van Milgen *et al.* (2000) are alone in proposing a curvilinear response in protein retention to energy intake. The view that protein is produced from 'energy' rather than from protein is not appealing, although de Greef (1992) and van Milgen & Noblet (2003) argue from this point of view.

The material efficiency with which dietary protein supply above maintenance is retained (e_p) is central as shown in Figure 1.

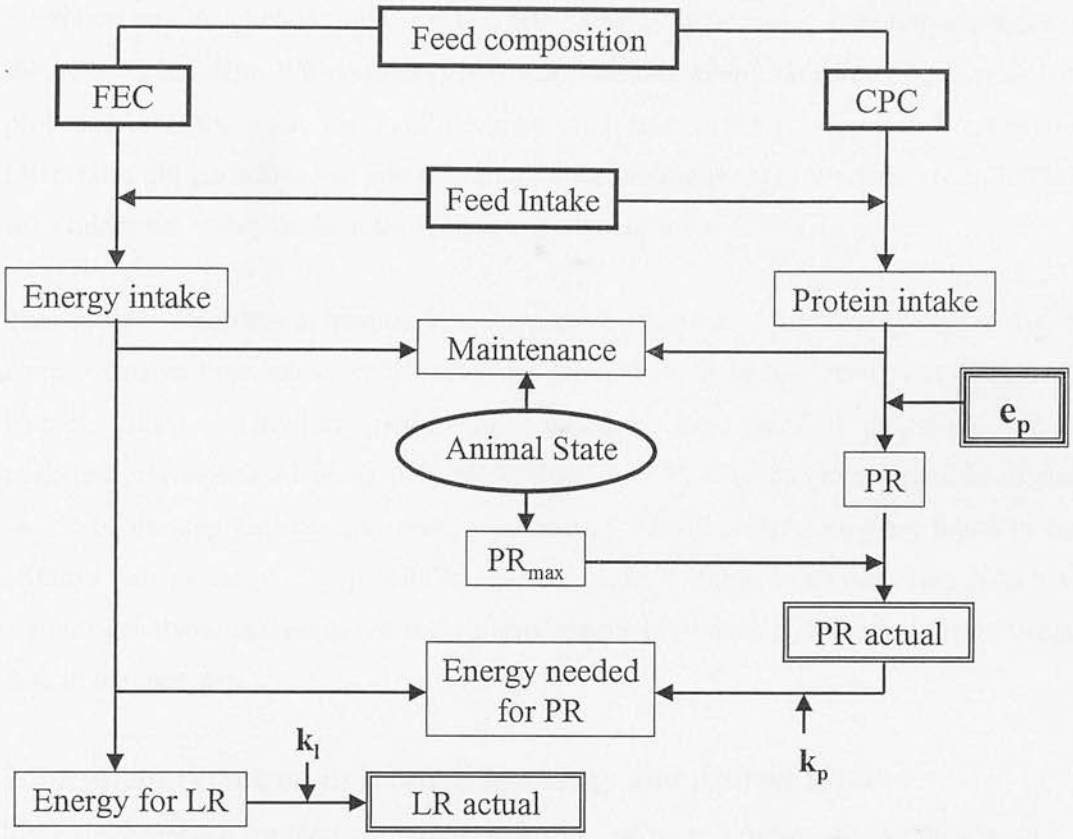

Figure 1 A schematic representation for predicting actual protein (*PR*) and lipid (*LR*) retentions for a pig eating a food containing energy (*FEC*, MJ/kg) and protein (*CPC*, kg/kg). The state of the animal determines its maintenance requirements for energy and protein and its maximum rate of protein retention, PR_{max}.

Whittemore *et al.* (2001) recently concluded that the evidence " ... would point empirically to a value of [the maximum marginal efficiency, e_p*] of 0.75 to 0.85 but gives scant guidance as to how the prevailing value in any given circumstance may be determined". The view proposed here is that the value of e_p and e_p* for particular conditions of food, animal and environment, and estimates of its error, can be determined from suitable experiments.

Models of growth may allow protein gain to be accompanied by lipid loss (Black *et al.* 1986; Wellock *et al.* 2003*a*). It is then necessary to set an upper limit to total lipid loss, which necessarily cannot exceed the total lipid mass. Whittemore (1995) proposed that there was a lower limit to body lipid content, defined as a minimum ratio of body lipid to body protein. Live weight and genotype, including sex, could affect its value. Once the

minimum ratio is reached, lipid retention takes priority over protein retention to maintain this ratio in the gain. Whittemore (1995) was uncertain about the value of this ratio but proposed that "the value may well be lower ... [than] ... 0.5:1." Green & Whittemore (2003) use the same idea but give no value for the minimum ratio. Wellock *et al.* (2003*a*) set a minimum value for the ratio of lipid to protein of 0.1.

This review considers a quantitative perspective of nutrient partitioning that is more comprehensive than others in the literature (Birkett & de Lange, 2001; van Milgen & Noblet, 2003). The key partitioning parameters are identified and their values quantitatively estimated using data in the literature. The quantitative estimates of the values of the key partitioning parameters derived from the literature were found to be within a narrow range. The possibility that the values of these parameters vary with live weight, genotype, including sex and the environment is critically examined, again using data in the literature.

2.3 Protein retention in relation to energy and protein intakes

The rules proposed for the partitioning of energy and protein intakes, above maintenance, to protein and lipid retentions are either from an 'energy perspective' (Black *et al.* 1986) or an 'energy and protein perspective' (Emmans & Kyriazakis, 1992*a,b*). The approaches have similarities that are not immediately obvious. When considering the response in protein retention to protein supply, adequate descriptions of both terms are necessary.

2.3.1 *The response in protein retention to energy intake*

Kyriazakis & Emmans (1992*a,b*) made the marginal efficiency of retaining ideal protein (e_p) a function of the ratio of the metabolisable energy content (*MEC*, MJ/kg) to the digestible crude protein content (*DCPC*, kg/kg) of the food, R (MJ ME/kg digestible protein). The proposed relationship was $e_p = k.R$ with a maximum value for e_p of e_p^* when R was above a specific value, R^*. The value of R^* was calculated as 72.55 (SE 1.53), with $e_p^* = 0.814$ and $k = 0.0112$ (SE 0.00022). The minimum value of e_p was calculated as 0.2, when all of the energy came from protein. In support, Fuller & Crofts (1977) predict a value of 0.17 for the net protein utilisation when all of the energy comes from protein. The strong assumptions made by Emmans & Kyriazakis (1992*a,b*) were that the values of e_p^* and k, and hence R^*, were independent of live weight and genotype, including sex. Black *et al.* (1986, 1995), Fuller *et al.* (1995) and de Greef & Verstegen

(1995), amongst others, disagree with this position and expect effects of live weight and genotype.

The partitioning rule of Black *et al.* (1986), which has a significant following (e.g. Schinckel & de Lange 1996; NRC 1998), can be summarised as the two equations in Sandberg *et al* (2005b):

$$PR = b.(MEI - (c.ME_m)) \quad \text{g/d} \quad (1)$$

$$b = X_{sm}.((v.\ exp(-y.\ W)) + z) \quad \text{g/MJ ME} \quad (2)$$

MEI is metabolisable energy intake and ME_m the amount needed for maintenance, MJ/d. The rate of response, *b*, depends on both live weight, *W*, and genotype through the value of the parameter X_{sm}. The values of the other parameters *v* (0.7), *y* (-0.0192) and *z* (0.65) were assumed constant across live weights, genotypes, including sex and environments. Equation 2 was modified by NRC (1998) to include the effects of different values of the parameter called *MPAR*, the 'mean protein accretion rate over the range of 20 to 120 kg *W*' and ambient temperature (*T*). In addition, the parameters of Equation 2 are now related to digestible energy intake, *DEI* MJ/d, rather than MEI (hence *b'* rather than *b*). The revised equation is:

$$b' = ((17.5\ exp(-0.0192.\ W)) + 16.25).(MPAR/125).(1+(0.015.(20-T))) \quad \text{g/MJ DE} \quad (3)$$

The equation is only for energy limiting foods, but no definition is given of such foods.

Where it is the case that $e_p = k.R$ and $R \leq R^*$, but only then, the two positions described above, one where *PR* is made a function of energy intake and the other where *PR* is made a function of *R*, can be shown to equivalent (Emmans & Kyriazakis, 1992*a,b*). The second position becomes:

$$PR = b.MEI - a \quad (4)$$

The linear coefficient *b* can be shown to be equal to $k\ v$, where *v* is the quality (the proportion of digested protein that is ideal) of the dietary protein. Therefore, when in later sections testing the effects of live weight and genotype, including sex on *b*, the value of of *k* is also being tested given assumptions about *v* (see below). The supply of energy is here

considered as *MEI* as it is consistent with the rules of Black *et al.* (1986) and Kyriazakis & Emmans (1992*a,b*).

2.3.2 *The response in protein retention to protein supply*

The methodology used in analysing the data for protein (or amino acid) supplies and retentions, are described below. Both the independent variable (intake) and the dependent variable (retention) need clear quantitative descriptions, before a value for e_p can be determined. In addition, the different descriptions of protein (or amino acid) retention and protein (or amino acid) supply also need considering.

2.3.2.1 *Protein retained as a function of the ideal protein supply*

The ideal protein supply, *IP* g/d, is that defined by Moughan (2003). It is calculated as:

$$IP = FI.CPC.d_i.v \qquad (5)$$

FI (kg/d) is the food intake, *CPC* (kg/kg) is the crude protein content, d_i is the ileal digestibility and v is the quality of the protein. Ileal digestibility is preferred to faecal digestibility estimates, but may need correction for any endogenous flow of amino acids (Moughan, 2003). The ratio of the first limiting amino acid in the digestible protein, relative to its concentration in the reference protein is v.

There is large variation in the estimates of the amino acid composition of the reference protein, which is used to calculate v (Table 1). Part of this variation may reflect analytical problems. Moughan (2003) pointed out that "Modern amino acid analysis is capable of providing data with a within-laboratory repeatability of 5 % or less and a reproducibility between laboratories of around 10%, but to achieve such results requires careful attention to detail". A further problem is that the amino acid composition of the reference protein can change with increasing supplies of the particular amino acid (Batterham *et al.* 1990).

The marginal material efficiency, e_p, is defined as:

$$e_p = PR / (IP - MP) \qquad (6)$$

In the cases, where e_p was calculated rather than determined by regression of *PR* on *IP* the ideal maintenance requirement (*MP*) was calculated as $0.004P$ where P is the protein weight of the pig calculated as $0.16W$.

2.3.2.2 *The response in inferred amino acid retention to amino acid supply*

For the ideal protein concept to be valid, all amino acids have to be used with the same efficiency, when they are first limiting. Boisen *et al.* (2000), who recently reviewed the definitions and applications of the ideal protein concept, did not mention this necessary assumption. It has been challenged by Heger *et al.* (2002, 2003), who proposed that amino acids were retained with different efficiencies, when first limiting. Whittemore *et al.* (2001) agreed with the above view that "there may be differences in the efficiencies of utilization of amino acids".

Heger *et al.* (2002, 2003) did not measure the retention of each amino acid, but calculated it indirectly from the measured nitrogen retention. It was assumed that all protein retained had the amino acid composition reported by Bikker *et al.* (1994*a*), except for tryptophan where the value was taken from Kyriazakis *et al.* (1993). The amino acid composition of whole body protein has been reported by several authors as shown in Table 1. The variation between authors is substantial. The data of Heger *et al.* (2002, 2003) for amino acid supply and protein retention were combined with the amino acid compositions of body protein shown in Table 1 to estimate a possible range in e_p*. The values for e_p* calculated by Heger *et al.* (2002, 2003) fall within the range presented, and agree on average with the mean (0.83) across the amino acids, Table 1. However, for any one amino acid the uncertainty about its value for e_p* is substantial. Given this uncertainty, which arises from the different estimates of the composition of body protein, it is not justified to use the data of Heger *et al.* (2002, 2003) to conclude that the maximum efficiency of amino acid retention is different for different amino acids. It may therefore be possible to use a single overall efficiency for all amino acids whichever is first limiting, and this is discussed further below.

2.4 Testing the rules with experimental data

The effect of live weight, genotype and environmental temperature on the marginal response in protein retention for protein ($R > R^*$) and energy ($R \leq R^*$) limiting foods are now considered. This definition of protein and energy limiting foods was chosen, as it was the only quantitative one present in the literature. For example, de Greef & Verstegen (1995) recognize the necessity of defining 'protein adequate foods' but do not give a quantitative definition. Preferred tests, would be experiments that consider defined protein and energy supplies across live weights, genotypes and environments. Foods with

a wide range of protein concentrations given at several allowances would be needed. The experiments in the literature that most closely matched these conditions for protein and energy limiting foods are now considered in turn.

Table 1 The amino acid composition of pig body protein from different sources. The consequence of the variation on the calculation of the maximum marginal efficiency of amino acid retention (e_p*) is shown, together with the proposed values of e_p* proposed by Heger *et al.* (2002, 2003).

	Amino acid composition of pig protein (g/kg CP) from different sources													e_p* calculated from the N retention data of Heger *et al.* (2002, 2003)[1]			Values of e_p* proposed by Heger *et al.* (2002, 2003)
	1	2	3	4	5	6	7	8	9	10	Min	Mean	Max	Min	Mean	Max	
Cystine (C)	-	13	12	9	16	-	10	9	-	13	9	11.7	16	-	-	-	-
Histidine	24	35	27	32	27	26	28	28	36	22	22	28.0	36	0.86	**1.09**	1.40	**1.17**
Isoleucine	21	29	37	31	28	32	33	35	37	30	21	31.9	37	0.47	**0.72**	0.86	**0.81**
Leucine	56	76	74	67	68	68	74	65	75	71	56	69.5	76	0.69	**0.86**	0.94	**0.81**
Lysine	55	60	65	64	63	60	70	66	73	60	55	64.2	73	0.70	**0.82**	0.94	**0.91**
Methionine (M)	-	19	19	19	22	-	18	20	-	20	18	19.6	22	-	-	-	-
M + C	-	32	31	28	38	31	28	30	31	33	28	31.5	38	0.78	**0.88**	1.06	**0.85**
Phenylalanine (P)	-	39	38	35	36	-	38	35	-	36	35	36.7	39	-	-	-	-
Tyrosine (T)	-	28	25	25	25	-	26	25	-	25	25	25.6	28	-	-	-	-
P + T	49	67	63	60	61	62	64	59	72	-	49	61.9	72	0.55	**0.70**	0.81	**0.67**
Threonine	30	39	40	38	34	36	38	36	39	34	30	36.9	40	0.67	**0.82**	0.93	**0.83**
Tryptophan	-	7	8	-	-	8	8	-	11	12	7	9.1	12	0.59	**0.77**	1.01	**0.66**

Valine	36	40	51	41	41	44	47	44	50	44	36	44.2	51	0.67	**0.82**	0.95	**0.82**

1 Siebrits *et al.* (1986) 2 Moughan & Smith (1987) 3 Campbell *et al.* (1988) 4 Batterham *et al.* (1990) 5 Kemm *et al.* (1990) 6 Chung & Baker (1992) 7 Kyriazakis *et al.* (1993) 8 Bikker *et al.*, (1994a) 9 Mahan & Shields (1998) 10 Wu *et al.* (1999)

[1] Heger *et al.* (2002, 2003) presented linear regression equations of responses of N retention to increasing amino acid supply. The value of e_p* was calculated as ((slope*6.25)/1000)*proportion of amino acid in pig protein

2.4.1 *The marginal response in protein retention to protein supply in protein limiting foods*

2.4.1.1 *Effects of live weight*

Black & Griffiths (1975) concluded that there was no difference in the marginal response in nitrogen retention to nitrogen intake of liquid fed lambs at live weights between 5 and 25 kg. The response in pigs was estimated from the data of Campbell *et al.* (1984), 45 to 90 kg and Campbell *et al.* (1985*a*), 20 to 45 kg. The regression equations presented by the authors were $PR = 0.382\ CPI - 11.15$, for the 20 to 45 kg pigs, and $PR = 0.425\ CPI - 17.8$, for the 45 to 90 kg pigs. The lower value of the slope for the smaller pigs can be attributed to the fact that only the protein retained in the carcass was analysed. If 0.9 of total protein is in the carcass then it would be expected that the coefficient for the whole body would be 0.382/0.90 = 0.424, which is not different from that found for the 45 to 90 kg pigs. These data thus give no reason to suggest that the marginal response will change with live weight.

Other experiments also suggest that live weight does not have an effect on the marginal response of protein retention to a limiting protein supply. de Lange *et al.* (2001) investigated the marginal response in threonine retention to ileal digestible threonine intake and found no difference at live weights of 40 and 75 kg. The retention of threonine was inferred from the assumed threonine composition of protein retention (determined in a sub-set of the animals on trial). The value of the net threonine efficiency was calculated as 0.734 (SE 0.0111).

Mohn *et al.* (2000) found no effect of live weight (45 and 70 kg) on the marginal response in inferred lysine retention to ileal digestible lysine intake. The efficiency was stated as 0.75 with no estimate of error. The combined data of the two experiments of Bikker *et al.* (1995, 1996) for 20 to 45 kg and 45 to 85 kg pigs do not reject the idea that live weight affected the marginal efficiency. Susenbeth (1995) also concluded that the marginal response in protein retention to limiting protein supply was not affected by live weight.

Campbell & Dunkin (1983*a*) found a high response in nitrogen retention to nitrogen intake ($R > R^*$) for pigs from 1.8 to 6.5 kg, the lowest range possible for post-natal animals. The regression equation of nitrogen retention on nitrogen intake had a slope of 0.880 (SE, 0.05). However, this high estimate is an exception. Campbell & Dunkin (1983*b*) used pigs from 7 to 19 kg, after weaning. Their marginal response was a lot lower at 0.616 as was its standard error of 0.011. It is possible that technical difficulties in measuring nitrogen balance in very small pigs, and the presence of the disease (rotavirus) reported by the authors, lead to the high estimate found by Campbell & Dunkin (1983*a*). It alone is not seen as sufficient to reject the findings of the other experiments that live weight does not affect the marginal response in protein retention when $R > R^*$.

2.4.1.2 *Effects of genotype including sex*

Kyriazakis *et al.* (1995) used entire male Large White x Landrace and pure Chinese Meishan pigs. The estimated values of e_p^* were 0.785 and 0.760 respectively (SED 0.032). Fuller *et al.* (1995) used different breeds including Duroc, purebred Large White and the three sexes of a commercial hybrid, over the 40 to 85 kg weight range. Two foods with either 149.1 or 206.1g CP / kg food were given in three allowances. The data of Fuller *et al.* (1995) where $PR < PR_{max}$ are in Figure 2.

Fuller *et al.* (1995) concluded that "the results indicate that an animal's superiority may result from a greater efficiency of protein utilisation or a higher lean growth potential but that these two characteristics are not simply related". A different interpretation of Figure 2 is possible. There is one data point for the commercial hybrid female that cannot be explained and is clearly indicated on the graph in Figure 2; it was omitted from the analysis here. The slopes for the five genotypes were not significantly different ($P > 0.1$). The common slope was 0.417 (SE 0.025). The conclusion of Fuller *et al.* (1995) may thus be challenged.

In support of no effects of genotype is the experiment of de Greef *et al.* (1992) who gave a commercial (S1) and a sire (S2) strain of pigs the same, one limiting level of protein from a food where $R > R^*$. The pigs retained 42 and 43 g PR / d respectively.

The experiment does not support the idea that pigs with greater PR_{max}, which was determined in the experiment as 187g/d for S1 and 153 g/d for S2, have different values for e_p*.

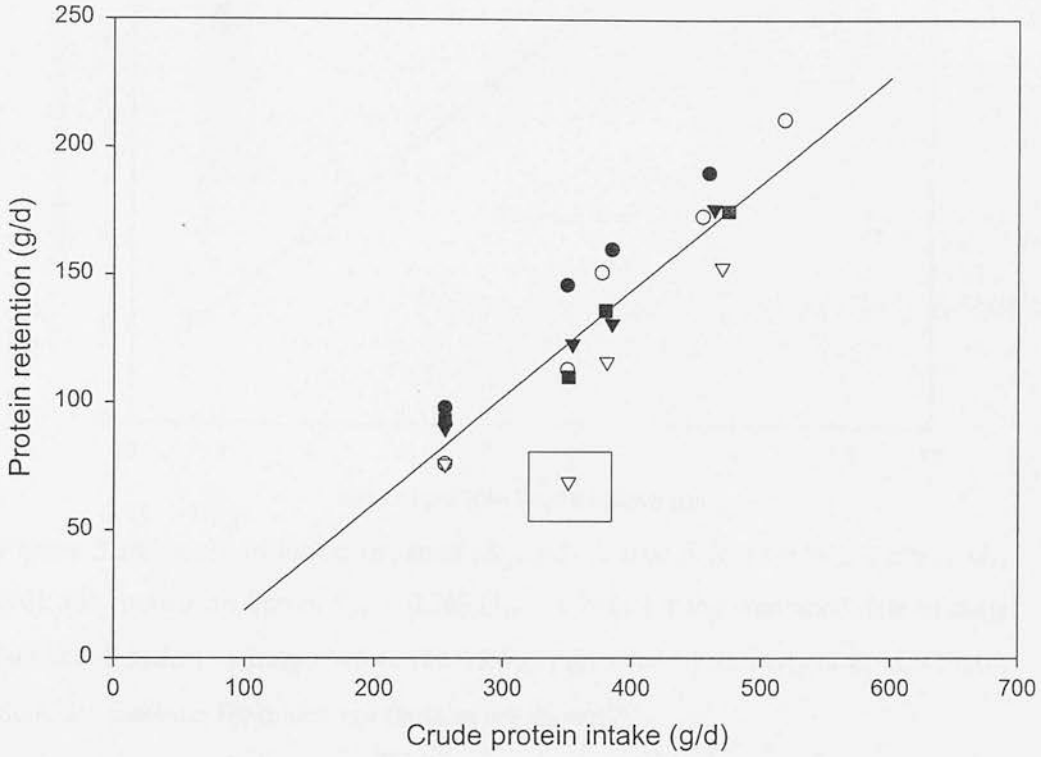

Figure 2 Protein retention (*PR* g/d) is plotted against crude protein intake (*CPI* g/d), for the five kinds of pig: Duroc (•), Large White males (○), commercial hybrid males (▼), commercial hybrid females (▽) and commercial hybrid castrates (▪) used by Fuller *et al.* (1995). Data points that were clearly part of a plateau were omitted; an outlier is shown in the square box. The regression line is $PR = 0.417\ (CPI - 55.4)$, which has the common slope and the average intercept.

There is evidence that different sexes of pigs use a limiting protein supply with similar efficiency. Campbell *et al.* (1984) and Campbell *et al.* (1985*b*) found no difference in e_p* between entire males and females. The genetic maximum for protein retention was greater for males in agreement with Black *et al.* (1995). The data of Batterham *et al.* (1990) are shown in Figure 3; they lend strong support to this conclusion. Kyriazakis & Emmans (1992*a,b*) did not find any effect of sex on e_p*.

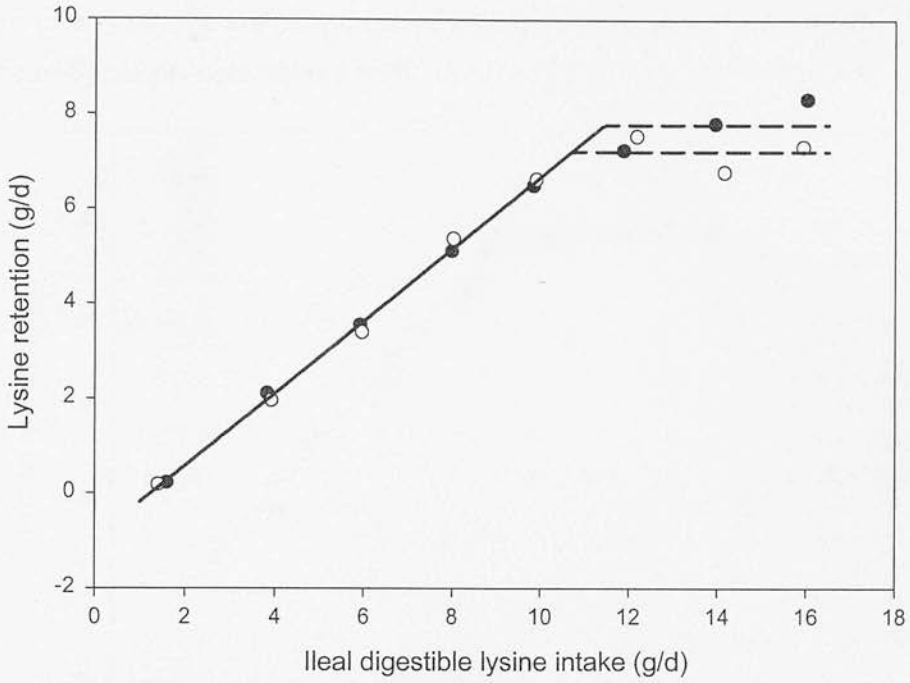

Figure 3 Response in lysine retention (R_{lys} g/d) to ileal digestible lysine intake (I_{lys} g/d): the regression line is $R_{lys} = 0.763.(I_{lys} - 1.245)$ for the combined data of male (•) and female (○) Large White (20-45 kg) pigs used by Batterham *et al.* (1990). Separate plateaux for males and females are shown.

It would appear safe to conclude from the above experiments that different genotypes (including sex) do not use a limiting protein supply, with different marginal efficiencies when $R > R^*$.

2.4.1.3 *Effect of temperature*

Models of growth that consider environmental temperature e.g. Black *et al.* (1986) and Wellock *et al.* (2003*a*), assume that temperature does not have an effect on the marginal response in protein retention on protein limiting foods. No evidence is presented in support of this assumption. Campbell & Taverner (1988) grew pigs from 9 to 20 kg at 14 and 32 °C. The slopes of protein retention on crude protein intake were 0.524 (SE 0.013) and 0.485 (SE 0.013), respectively, which are not significantly different ($P > 0.2$). Ferguson & Gous (1997) grew ad libitum fed pigs from 13 to 30 kg on foods with 93 - 230 g CP / kg at 18, 22, 26 and 30 °C. The data (Figure 4)

show clearly that the marginal response in protein retention 0.525 (SE 0.015) was not affected by temperature when $R > R^*$.

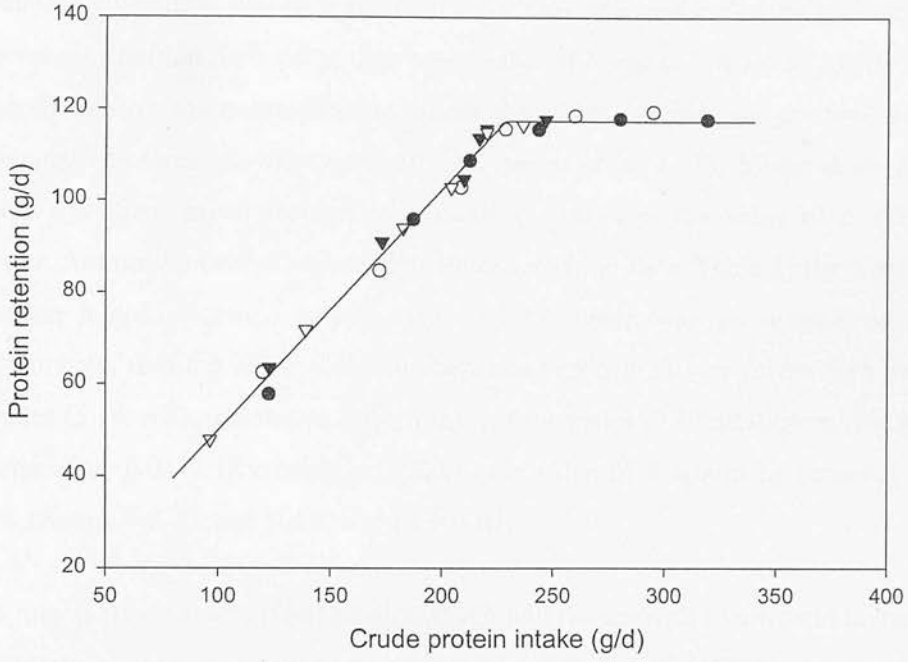

Figure 4 The response in protein retention to crude protein intake for pigs fed ad libitum foods that were limiting in protein at four different temperatures: 18°C (●) 22°C (○) 26°C (▼) and 30°C (▽) as found by Ferguson & Gous (1997). The solid line is described by $PR = 0.525\ (CPI - 4.92)$ until the plateau of 117.4 g/d is reached.

In support of these experiments is that of Berschauer *et al.* (1983) who kept young pigs at 22 and then 10°C and did not find an effect on the marginal response in nitrogen retention to nitrogen intake. Hence, it would appear safe to conclude that temperature does not affect the marginal response in protein retention when the protein supply is limiting.

2.4.2 *The marginal response in protein retention on energy limiting foods*

The following sections consider the effects of genotype, live weight and temperature on the response in protein retention to energy intake for foods where $R \leq R^*$. The conclusions of Mohn & de Lange (1998) are considered first.

Mohn & de Lange (1998) presented a table (their Table 5) with 20 estimates of b (where $PR = b.MEI - a$) across different genotypes and live weights. Although it cannot be guaranteed that all experiments that they analysed had values of $R \leq R^*$, a necessary condition for $b = k\,v$, their mean value of b was 6.2 (SD 2.3) g PR/MJ ME. In none of the three experiments where the effect of live weight was directly estimated (de Greef & Verstegen, 1993; Quiniou *et al.* 1995; Mohn & de Lange, 1998) was there any indication of systematic change in the value of b with live weight. Across the twelve experiments on males (from their Table 5) the correlation between b and W was $r = 0.23$ (P = 0.465). There was an indication, across experiments, that the mean value for castrates (3.97, n 3) was lower than that for females (5.16, n 5), which was lower than that for males (7.17 SE 0.64, n 12). With k estimated as 0.0112 (Kyriazakis, 1992*a,b*) the value of b would be expected to be 7.84, when $v = 0.70$, and 10.08 when $v = 0.90$.

The rule of Black *et al.* (1986) predicts that b will reduce with live weight towards an asymptotic value that is dependent on genotype. Black *et al.* (1986) presented values for the genetic partitioning parameter X_s, which is defined in Equation 2: "X_s has a value of 1.2, 1.0 and 0.78 for entire males, females and castrates of the fast growing genotype and corresponding values of 1.0, 0.85 and 0.65 for a slow growing genotype". When the value of the genetic parameter $Xs = 1.2$ the value of b falls from 10.0 (W = 1kg) to 5.0 (W = 250kg) g PR/MJ ME. With $Xs = 0.65$ the equivalent change in b is from 5.4 to 2.7 g PR/MJ ME.

NRC (1998) adapted the approach of Black *et al.* (1986) and made the response of PR to energy intake depend on genotype, through the value of $MPAR$' and W, Equation 3. Across four genotypes ($MPAR$ = 100, 125, 150, 175 g/d), and W varying between 20 to 120 kg, the predicted mean estimate of b was 5.94 g PR/MJ ME. The experiments that are relevant tests of the effects of live weight, genotype and temperature on the value of b are now considered.

2.4.2.1 *Effect of live weight*

Quiniou *et al.* (1995) performed nitrogen balances at 45, 65, 80 and 94 kg. The pigs were given four levels of feeding, all of which provided a constant high supply of

protein (420-450 g CP/d). The treatments may be viewed either as measuring the response in *PR* to *MEI* or as a means of seeing how e_p varies with *R*. The common slope *b* across live weights was 8.85 (SE 0.63). Fitting a model that allowed different slopes (7.39, 8.88, 10.23 and 8.23 at 45, 65, 80 and 90 kg respectively) at each weight gave no statistical improvement (P > 0.5). In a similar, experiment (48, 64, 79, 94 kg) on three kinds of pigs, Quiniou *et al.* (1996) concluded, "the stage of growth had no significant effect on [*b*]".

Mohn *et al.* (2000) gave pigs six levels of energy intake at two levels of protein at both 45 and 75 kg. Protein retention is plotted against *MEI* in Figure 5 with the two highest levels of *MEI* excluded in all four cases because *PR* could have reached PR_{max} on these treatments.

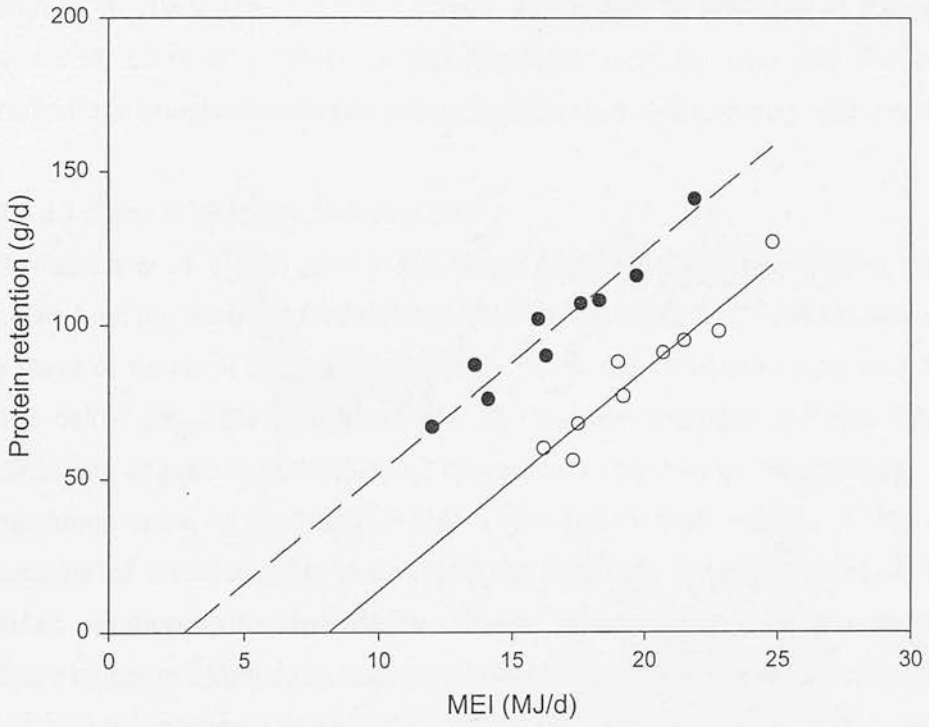

Figure 5 The response in protein retention (*PR* g/d) to metabolisable energy intake (*MEI* MJ/d) at two live weights (• 45 and ○ 75 kg) as found by Mohn *et al.* (2001). The regression lines were fitted with a common slope and different intercepts: *PR* = 7.18 (*MEI* – 2.77) for the 45kg pigs (‾ ‾) and *PR* = 7.18 (*MEI* – 8.12) for the 75 kg pigs
(—).

The common slope for the two weights was 7.18 (SE 0.53) g PR / MJ ME. Allowing different slopes did not improve the fit of the model ($P > 0.5$).

Dunkin & Black (1985) presented estimates of b, for pigs of a range of live weights (30, 46, 74 and 90 kg) that were fed eight levels of an energy limiting food. The values of b were presented as g NR/MJ ME without estimates of error. Expressed as g PR/MJ ME the slopes were 8.25, 6.44, 5.75 and 6.75 respectively. These estimates do not support a systematic change in the value of b with increasing live weight as is suggested by Black *et al.* (1986) and NRC (1998). Campbell & Dunkin (1983*a*) performed an experiment with very small pigs (1.8 – 6.5 kg). The food can be seen as energy limiting with $R = 56.3$ MJ ME/kg DCP. The value of b was determined here by regression as 7.61 (0.31), which is not different to the value for the 90 kg pigs of Dunkin & Black (1985) or that for the 75 kg pigs of Mohn *et al.* (2001). The evidence, taken as a whole, is thus consistent with the view that the marginal response in protein retention on energy limiting foods does not vary with live weight.

2.4.2.2 *Effect of genotype including sex*

Kyriazakis *et al.* (1994) gave pure Chinese Meishans and Large White x Landrace pigs a high protein basal food that was diluted with starch to different extents to give a range of values of R (33-119 MJ ME/kg DCP). The allowances were such that PR was below PR_{max} for both genotypes. The authors concluded that "the calculated efficiency of protein utilization, e_p, was found to be directly proportional, up to a maximum value, to the ME:DCP ratio of the diet for both breeds …". The overall constant of proportionality was 0.0108 (SE 0.00024), the value of which did not differ significantly between the two breeds. The values of b, determined here by linear regression from three treatments where a linear response to additions of starch (energy) was observed, were 9.93 (SE 2.55) and 9.65 (SE 0.16) for the Large Whites and Chinese Meishans. These did not differ significantly ($P > 0.4$).

The experiment of Quiniou *et al.* (1996) used three genotypes, boars (bPPx) and castrates (cPPx) of a Large White x Pietrain breed and castrates of a Large White breed (cLW). The response in PR (determined by nitrogen balance) to four levels of

energy intake at a constant high protein intake was considered at four live weights. Quiniou *et al.* (1996) concluded that the response in protein retention to increasing supplies of metabolisable energy intake above maintenance, ME_p MJ/d, was independent of live weight, but did depend on genotype. The values of b for the bPPx, cPPx and cLW pigs were 6.0, 4.0 and 3.4 (g/MJ) respectively with no standard errors given.

Table 2 The response in protein retention, *PR* g/d, to the metabolisable energy available for production, ME_p MJ/d. The regression is $PR = a + b.ME_p$. Two different models were fitted to the data of Quiniou *et al.* (1996), for three pig genotypes (cPPx, bPPx and cLW). Model I, used by Quiniou et al. (1996), fitted a common intercept but allowed different slopes for each genotype. Model II used a common slope that allowed different intercepts for each genotype. The differences in the slopes (M I), and intercepts (M II) compared to cPPx are shown.

Ml	df[1]	RSS[1]	RMS[1]	b (SE)		a (SE)	
I	141	48125	341	cPPx	3.81(0.38)	85.5 (6.05)	
				(cPPx – bPPx)	1.61(0.24)		
				(cPPx – cLW)	–1.07(0.22)		
II	141	48030	341	4.06 (0.38)		cPPx	80.90(6.83)
						(cPPx – bPPx)	24.36(3.80)
						(cPPx – cLW)	–17.67(3.80)

[1] df = residual degrees of freedom, RSS = residual sums of squares, RMS = residual mean square.

However, in the regressions of Quiniou *et al.* (1996) of *PR* on *MEp* the intercept was fixed by the authors at 64 g/d for all three genotypes. Here a model with a common slope that allowed different intercepts for the three genotypes was also tested. It is not possible to distinguish between the two models from a formal statistical point of view as is shown in Table 2. The variation seen in this data set may partly be attributed to different foods being used for each genotype. When considering the data where *PR* is plotted against ME_p it is difficult to decide whether a particular point belongs to a positive response or to the plateau. This problem is present in the data of

Quiniou *et al.* (1996) and J. van Milgen (personal communication). The data for the individual Pietrain castrates are in Figure 6, in which the linear regression is shown.

The value of the regression co-efficient, estimated assuming that there was no plateau, was 4.223 (SE 0.648). Using the method described below the same data were used to estimate the values of the parameters of the linear plateau model that is also shown in Figure 6. The linear plateau model gave a better fit, but not significantly so. The value of the linear coefficient was 4.815 (SE 0.862), which was 1.14 times as great as the estimate where no plateau was allowed.

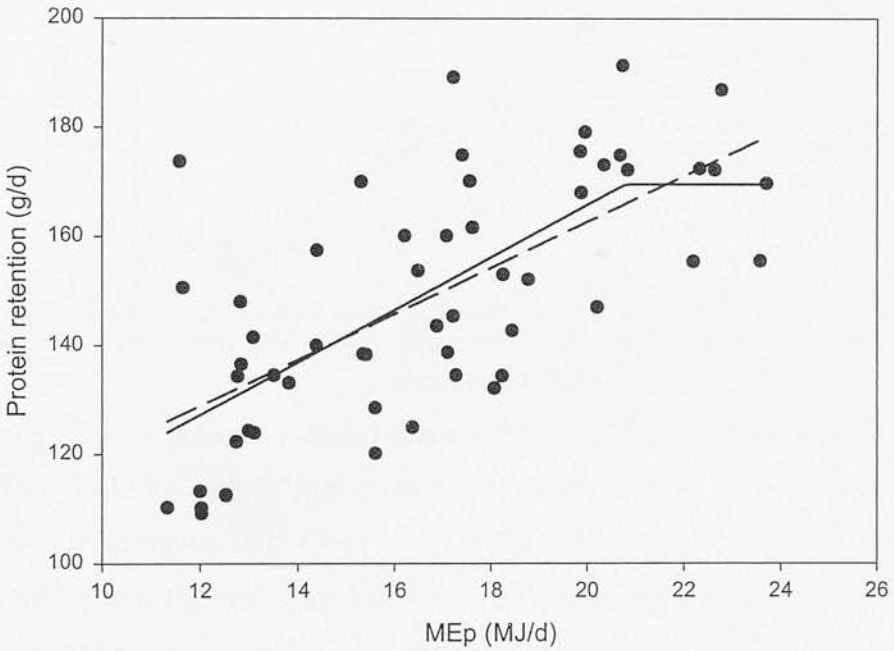

Figure 6 The response in protein retention (*PR* g/d) to metabolisable energy intake above maintenance (MEp, MJ/d) for the individual Pietrain castrate pigs (Quiniou *et al.* 1996 and personal communication). The linear regression (– –) and the linear plateau model (——) are both shown.

2.4.2.3 *Effects of temperature*

Neither the model of Black *et al.* (1986) nor that of Wellock *et al.* (2003*a*), who consider the effects of temperature, assume that temperature has any effect on the marginal response in protein retention to energy intake on protein adequate foods.

However, NRC (1998) states that the marginal response to energy intake on protein adequate foods, falls as temperature increases (Equation 3). The argument follows from the experiment of Close *et al.* (1978). There are two problems. The first is that the food used was probably limiting in protein (R = 86.6 MJ ME/kg DCP). The second is that, even when interpreted as an energy experiment, the data are far from persuasive that the response varied with temperature (Figure 7).

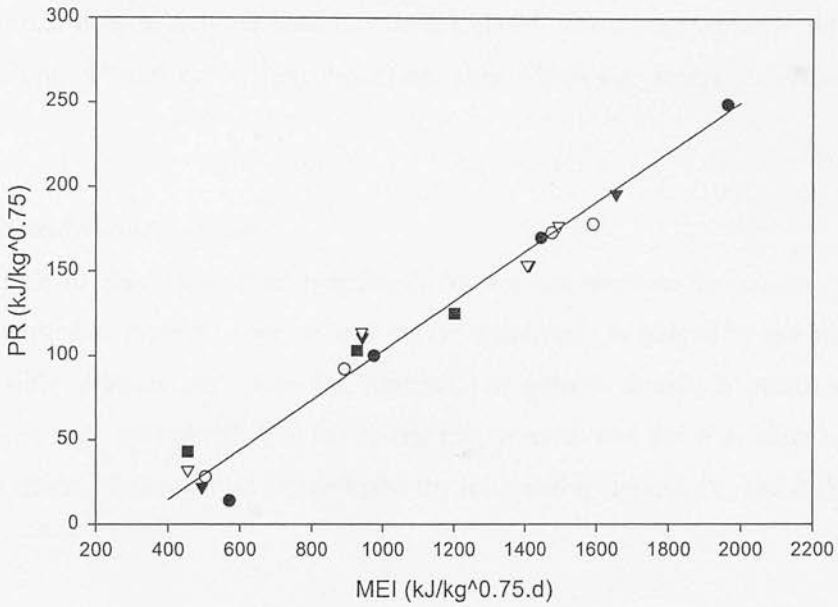

Figure 7 The response in protein retention (PR kJ/kg$^{0.75}$.d) to metabolisable energy intake (MEI kJ/kg$^{0.75}$.d) for pigs given different allowances of the same food at five different temperatures from Close *et al.* (1978); 10°C (●) 15°C (○) 20°C (▼) 25°C (▽) 30°C (■). The regression line for all the data is PR = 0.147 (0.0062).MEI – 44.07 (7.58).

There appears to be no experiment where MEI has been varied at different temperatures, using energy limiting foods ($R < R^*$). In the absence of any evidence to the contrary it is provisionally concluded that there are no effects of temperature on the marginal response in protein retention to energy limiting foods. An experiment to test this conclusion is warranted.

The testing performed here supports the view that there are general partitioning rules where the marginal response in protein retention to protein or energy limiting foods

is independent of live weight, genotype and temperature. The questions that remain from the testing are considered in the discussion. Attempts are now made to determine the values of the key partitioning parameters.

2.5 The estimation of the values of the parameters e_p*, k and R*

The general rule $e_p = k.R$ requires two of its three partitioning parameters to be determined, from which the third can be calculated. The statistical methodology for determining R* and e_p* is first described, after which the parameter estimates are given.

2.5.1 *Statistical methodology*

Two kinds of regression were performed. Where the response in protein or amino acid retention to protein or amino acid, intake was linear, as judged by eye and below any visible plateau set either by energetic or genetic limits, a standard linear regression was performed. The other regression used was for a continuous linear plateau model. This was used to describe the relationship between e_p and R (Equation 8).

A linear plateau model for e_p has a constant derivative for $e_p < e_p$* and 0 for $e_p > e_p$*. Fitting such a function in a statistical software package is not straightforward. However, the important parameter R* needs determining with estimates of its error. For this reason the derivative of the plateau model was approximated by the continuous function:

$$A\ (e_p) = 0.5\ k.(1 + tanh.[w.(R - R^*)]) \qquad (7)$$

For large values of w, $A\ (e_p)$ converges to k for $R < R$* and to 0 for $R > R$*. This equation can be integrated to yield Equation 8, which is a good approximation of the linear plateau model.

$$e_p = e_p^* + 0.5\ k.R - 0.5/w.k.(\ln(cosh(w.R^*)) + \ln(cosh(w.(R-R^*)))) \qquad (8)$$

The continuous approximation of a linear plateau model (Equation 8) was fitted in the statistical software Sigmaplot version 7.0 (SPSS Inc, Chicago, Illinois, USA). This permitted estimation of the values of the slope (k), the break point (R^*) and the maximum marginal efficiency (e_p^*) with their errors. Sigmaplot uses the Marquardt-Levenberg algorithm for least squares estimation of the parameters (Marquardt, 1963). The parameters are assigned initial estimates from which the best estimates are determined by the least squares method from 100 iterations. The larger the value of w the more abrupt the transition between the linear part and the plateau part of the relationship. This approach is preferred to an alternative approach, piece-wise, linear regression, where again the slope of the second phase is constrained to zero (Hudson, 1966).

2.5.2 *Parameter values*

The determination of the value for e_p^* in some experiments has relied by necessity on assumptions about digestibility, protein quality and protein requirements for maintenance. The approach to determine e_p^* with a minimum amount of assumptions is done by regressing determined ileal digestible amino acid intake on determined amino acid retention when $R > R^*$. This assumes that the ileal digestibility is representative of the 'truly' available amino acid.

Surprisingly few experiments were found in pigs that considered the response in ileal digestible amino acid intake to a large range of amino acid supplies, where above mentioned dimensions were measured. The experiments that are used here to estimate the value of e_p^* are those of Batterham *et al.* (1990), Bikker *et al.* (1994*b*) and Chung & Baker (1992). The response of Chung & Baker (1992) was at three levels of methionine intake, while Batterham *et al.* (1990) used eight levels of lysine and Bikker *et al.* (1994*b*) used 15 levels of lysine. The results of the regressions of amino acid retention on ileal digestible amino acid intake are presented in Table 3.

The determined values for e_p^* in Table 3 are overall lower than that proposed for e_p^* by Kyriazakis & Emmans (1992*a,b*) of 0.814. The slightly lower value for methionine may be because Chung & Baker (1992) took the ileal digestibility of methionine to equal unity from a companion experiment and the authors did not give

any estimate of error of the parameter estimates. The residual of Bikker *et al.* (1994*b*) is much higher than that of Batterham *et al.* (1990).

Table 3 The regression of amino acid retention (g/d) on ileal digestible amino acid supply (g/d) from three sources. All diets had more than 83 MJ metabolisable energy per kg digestible CP.

Source[1]	slope (s.e)	Intercept (s.e)	res sd (g/d)
A	0.763 (0.0130)	-0.950 (0.0850)	0.121
B	0.699 (0.0384)	2.536 (6.438)	3.55
C	0.717	-41	NA

[1] A is Batterham *et al.* (1990) for lysine, B is Bikker *et al.* (1994*a*) for lysine and C is for Chung & Baker (1992) for methionine.

The estimate of Batterham *et al.* (1990) is taken as the best with a value of e_p* of 0.763 (SE 0.0130). The lower estimate of Bikker *et al.* (1994*b*) of 0.699 (0.0384) was not as well estimated and is not significantly different. The experiments of de Lange *et al.* (2001) considering threonine (e_p*, 0.73 SE 0.011) and Mohn *et al.* (2000) considering lysine (e_p*, 0.75) are in support of this estimate. In addition, the value for e_p* determined by the continuous linear-plateau regression for the combined data of Kyriazakis & Emmans (1992*a,b*) and Kyriazakis *et al.* (1994) for two genotypes and two sexes was 0.783 (SE 0.0112), not significantly different to the value of Batterham *et al.* (1990). The values of k, R* and e_p* for these experiments are shown in Table 4.

The value of R* from the continuous linear plateau regression of the combined data (Table 4) is similar to the above values and was equal to 67.9 (SE 1.65). Then, taking the best estimate of e_p* from the experiment of Batterham *et al.* (1990), permits calculation of the slope k as 0.763/67.9 = 0.0112. Therefore, the best parameter estimates are: e_p* = 0.763 (SE 0.0130) and R* = 67.9 (SE 1.65) with the subsidiary parameter k estimated as 0.0112. These estimates are seen as applying across live weight, genotype, including sex and temperature. Partitioning rules, which permit

loss of lipid, require another parameter that sets the lower limit to the pigs lipid content.

Table 4 The response in the efficiency of retaining ideal protein, e_p, to the ratio of metabolisable energy to digestible CP (R, MJ ME / kg DCP) in the food, estimated by the continuous linear plateau model (see text).

Source	Genotype	n	R	k (s.e)[1]	e_p* (s.e)[2]	R* (s.e)[3]	res sd
Kyriazakis & Emmans (1992a, b)	Large White x Landrace	40, 44	43 – 125	0.0138 (0.0015)	0.795 (0.0151)	67.8 (2.10)	0.0566
Kyriazakis *et al.* (1994)	Large White x Landrace	23	33 – 119	0.0103 (0.0014)	0.709 (0.0185)	64.8 (3.30)	0.0452
Kyriazakis *et al.* (1994)	Chinese Meishan	25	33 – 119	0.0145 (0.0013)	0.822 (0.0168)	70.2 (2.65)	0.0443
All	Both	132	33 – 125	0.0131 (0.0009)	0.783 (0.0112)	67.9 (1.65)	0.0582

[1] Regression co-efficient of e_p on R [2] Plateau value for e_p [3] The value of R at which e_p reaches e_p*

2.6 The lower limit to body fatness in pigs

It is a necessary condition for a partitioning rule that permits loss of lipid when protein retention is positive to have some lower limit, for the amount of lipid, that can be lost (Sandberg *et al.* 2005a). Whittemore (1995) stated that "there may be postulated a value for $(Lt:Pt)_{min}$, a crisis value below which the metabolic system may experience trauma". A value for $(Lt:Pt)_{min}$ of 0.5 was proposed. However, it was recognised that the value could be lower (Whittemore, 1995). Green & Whittemore (2003) state that the value of $(Lt:Pt)_{min}$ is likely to be dependent on genotype, but did not give the values. Green & Whittemore (2003) also have an absolute minimum fatness defined as $0.05W$. Wellock *et al.* (2003*a*) stated "that an animal cannot lose lipid that is not present, and must have some minimum lipid content (L_{min}) necessary for survival". They proposed that the minimum value was $0.1P$, where P is the

protein mass in the body, which is conceptually equivalent to $0.05W$ as proposed by Green & Whittemore (2003).

The lower limit to a pig's lipid content, of relevance to a partitioning rule, is the ratio of lipid to protein at which the pig reduces its rate of protein retention to maintain its lipid content. This may be due to the limit for the biological structures of the pig (Wellock *et al.* 2003*a*: $0.1.P$) or some higher limit (Whittemore, 1995: $0.5.P$). It is not clear whether these ratios differ with either live weight or genotype. Experimental data where the rates of retention of protein and lipid were measured at low body lipid to protein ratios would help to decide this point. The food would need to be sufficient in protein to ensure that the animal was only limited in energy.

Campbell & Dunkin (1983*a*) performed a similar experiment to that preferred. Pigs between 1.8 – 6.5 kg were given four allowances of a food that contained 359 g CP / kg DM and 25.4 MJ GE. At the lowest allowance of food, the pigs were retaining 24 g/d of protein, and the marginal response was linear in relation to the protein supply. The ratio of lipid to protein in the body was $0.29P$. This suggests that the estimate of Whittemore (1995) to be too high as an estimate of the minimum ratio of lipid to protein in the body.

Close & Stanier (1984) determined that newborn pigs (1.34 kg) had a ratio of $0.23P$ and stated that these pigs grew normally up to weaning. It is therefore not possible to reject the ratio of $0.1P$ proposed by Wellock *et al.* (2003*a*). The above values suggest that the minimum ratio may be less than $0.23P$ to $0.29P$. The question that remains is whether this ratio is different for genotype and/or live weight.

2.7 Discussion

The rules of partitioning proposed by Black *et al.* (1986), Kyriazakis & Emmans (1992*a,b*) and Whittemore (1995) withstood the qualitative criticisms in the previous chapter (Chapter 1; Sandberg *et al.* 2005a). The problem raised is the marginal response in protein retention to limiting protein or energy supplies for different genotypes at different live weights (Black *et al.* 1986; Kyriazakis & Emmans, 1992*a,b*). In models which predict lipid loss coupled with protein gain it is necessary

to define the ratio of lipid to protein in the body at which the pig reduce its rate of protein retention to maintain the lipid content (Whittemore, 1995).

It has been discussed here and elsewhere that b, the slope of PR on MEI, is equal to $k.v$, given that $R < R^*$. The distinction between the approach where $e_p = k.R$ and the approach where $PR = b.MEI$, is made by R^*. This was not recognised by van Milgen & Noblet (2003) who stated in relation to $e_p = k.R$ "...as this function intersects the origin, the approach is essentially similar to a linear-plateau function between energy intake and PD". The view that there is considerable real variation in the value of b (e.g. de Greef & Verstegen, 1995; van Milgen & Noblet, 1999; Schinckel & de Lange, 1996) can be explained, at least in part, by the variation in the energy to protein ratios of the food. In addition, some of the variation in b may be attributed to variation in calculating v from variation in amino acid compositions of food and pig proteins, which may either actually exist, or, may arise from differences in analytical methods (Moughan, 2003).

There is a significant amount of evidence suggesting that live weight does not have an effect on the marginal response in protein retention to protein or energy limiting foods. The evidence for no effect of genotype was not as abundant. The data reviewed here suggest that it is safe to assume that different genotypes have the same marginal responses to protein and energy limiting foods. Temperature does not appear to affect the marginal responses in protein retention to protein limiting foods. In the absence of evidence no conclusions could be drawn for energy limiting foods. The questions that remain unresolved and some possible experiments that could address these questions are considered below.

The overall conclusion of the analysis done here is that the rule of Black *et al.* (1986) and its modified form (NRC, 1998) does not agree as well with literature data as the general rule of Kyriazakis & Emmans (1992*a,b*). The parameters of the rule were determined as e_p^* (0.763 SE 0.013), R^* (67.9 SE 1.65) and k (0.0112). For modelling purposes and future experiments, it is useful to identify general rules. The partitioning rule in this case is general as its parameters are independent of genotype, live weight and temperature, which increases the flexibility of using a rule within

larger models. In addition, as the rule is seen as general it may be useful for investigating the partitioning of scarce resources during times of disease and/or social stress (as discussed below).

It may be useful to consider experimentally the marginal responses of different genotypes, at both very low and high live weights, to help to solve the problem raised by one set of data (Campbell & Dunkin, 1983*a*) on very small pigs. To determine the effect of live weight on the maximum efficiency of protein retention, the energy to protein ratio of the food would need to be greater than 67.9 MJ ME/kg DCP and it would be necessary to ensure that $PR<PR_{max}$ on all treatments. To asses the effects of live weight on R^* and k a range of energy to protein ratios above and below 67.9 MJ ME/kg DCP would be needed.

There may be effects of temperature on the partitioning rule used by pigs for energy limiting foods. This could not be assessed here, as suitable data were not identified. The effect of temperature should be assessed for pigs kept in environments that are cold, thermoneutral or hot for a pig of a particular genotype and state (Wellock *et al.* 2003*a*). The estimation of e_p^*, R^* and k, and quantification of the parameters of the energy system used, would strengthen the test of any partitioning rule in such a situation.

The value of the maximum material efficiency of protein (amino acid) utilisation is a debated area (Whittemore *et al.* 2001). The assumption that has been made here is for the same value to be used for the material efficiency of using all amino acids that constitute 'ideal' protein. There appears to be a lack of evidence of sufficient detail (such as Batterham *et al.* 1990) for the estimation of e_p^* for different amino acids in pigs. A large range of values for the maximum material efficiency of protein (amino acid) utilisation has been proposed for pigs ranging from 0.55 Susenbeth (1995) to 0.94 Leibholz (1985). However, in both of these cases e_p^* was calculated using a number of assumptions. The experimental data from the poultry literature are in support of a much narrower range. Baker (1991) concluded from poultry and murine literature that different amino acids are used with different efficiencies. However, he also concluded that "utilisation efficiency of individual dietary amino acids varies

around the 76% figure [taken from Velu *et al.* (1971)], with slow-turnover amino acids such as lysine being used more efficiently (80%) than fast-turnover amino acids such as isoleucine (61%)".

Recent experimental data in poultry suggest smaller differences. The values for e_p* were lysine (0.76) Edwards *et al.* (1999), valine (0.73) Baker *et al.* (1996), threonine (0.82) Edwards *et al.* (1997) and methionine (0.68) Edwards & Baker (1999). The methodology used was similar to that of Batterham *et al.* (1991), although the true digestibility of the crystalline amino acids used was assumed as 100% from another experiment. The mean of the above estimates is 0.75, which is not different from our proposed best estimate, 0.763, and the central value of 0.76 proposed by Baker (1991).

There is not complete agreement for the estimates of e_p* for the different amino acids from the pig and poultry literature. In pigs the estimate for threonine of 0.734 (de Lange *et al.* 2001) is very similar to that for methionine of 0.717 (Chung & Baker, 1992). In poultry the estimate for threonine of 0.82 (Edwards *et al.* 1997) was very much greater than that of 0.68 for methionine (Edwards & Baker 1999). Rather than the difference between amino acids truly varying between species it is likely that all of these four estimates do not differ from each other. Their mean does not differ from of 0.763 taken here for e_p*. It may be useful to experimentally determine the maximum efficiency of amino acid utilisation in pigs (and poultry) using a standardised procedure. The standardised procedure would include attempts to minimise the variation of amino acid analysis (Moughan, 2003), ensure that $R > R$* and to implement a range of amino acid supplies that is similar to that of Batterham *et al.* (1990).

It is necessary for the chosen partitioning rule to have a complementary constraint to the loss of lipid to maintain positive protein retention. Wellock *et al.* (2003*a*) proposed a value of $0.1P$ calculated on biological grounds of what the lowest amount of lipid in a biological structure may be. This is taken forward, but it is possible that this would be to low an estimate. The selection for lean genotypes at higher weights may have reduced the "desired ratio" of lipid to protein in the body at a protein

weight (Emmans & Kyriazakis 2000). This may increase the likelihood that pigs of an improved genotype will meet the minimum ratio at a faster rate than those of an unimproved genotype. Experiments may be warranted to explore the value of the minimum ratio across different genotypes.

The general rule of Kyriazakis & Emmans (1992*a,b*) which considers both available protein and energy supplies is preferred to other rules of partitioning considered here and in Chapter 1. The partitioning of scare resources is of current interest in relation to stressors such as disease (Lochmiller & Deerenberg, 2000; Coop & Kyriazakis, 2001; Houdijk *et al.* 2001; Powanda & Beisel, 2003) and social stress (Wellock *et al.* 2003*b*). It is useful to have a general rule as a starting point to consider any effects of disease or social stress. The values of the key parameters e_p* and R* may be explored experimentally in relation to such stressors to further explore scarce resource partitioning. The particularly relevant stressor is that of pathogen challenges as there is a current lack of such information. Therefore, it may be useful to determine the value of e_p* as done by Batterham *et al.* (1990) and R* in relation to the exposure of pigs to different pathogens to further our understanding of resource partitioning in such circumstances. The pathogens considered should include bacteria, viruses and parasites of a range of challenge doses to permit comparisons to be made of these. Challenge with pathogens may increase body temperature, stimulate the immune response and require resources to be directed towards the repair of damaged tissues. In addition, challenge with a pathogen may reduce the voluntary food intake (Kyriazakis *et al.* 1998).

The purpose of this and the previous chapter (Chapter 1; Sandberg *et al.* 2005a) was to describe the partitioning problem, identify solutions and then criticise these qualitatively and quantitatively. It has been possible to conclude for a general rule which when combined with an energy system, permits prediction of protein and lipid retention from protein and energy supplies. General rules are useful for models of growth as they permit for models to expand and consider new areas of research. The work done here will contribute to future considerations of scarce resource

partitioning and growth modelling in the pig. Future work will include quantitative descriptions of the infectious environment on scarce resource partitioning.

Chapter 3

A model for predicting feed intake of growing animals during exposure to pathogens.

3.1 Abstract

A general model is proposed for predicting effects of sub-clinical pathogen challenges of different doses and virulence on the relative feed intake (RFI) of animals. The RFI is defined as the feed intake of the animal challenged by a pathogen divided by its feed intake, FI, in the same state had it not been challenged. Actual FI can be predicted from the RFI and animal state. The RFI was assumed to be affected only when animals were naïve to a particular pathogen (i.e. had no prior experience of it), and when the challenge dose was above a predetermined threshold. The model is for the period from recognition of a pathogen through to acquisition and subsequent expression of immunity. The way in which RFI changes with time is described by 5 main parameters and is based on data for RFI during different pathogen challenges of a range of hosts. Lag time (L d) is the delay from a pathogen challenge until any effects on RFI are seen. Reduction time (R d) describes the time it takes for the lowest value of RFI (λ) to be achieved. The duration time (D d) describes the time that λ is maintained for and ρ /d describes the rate of recovery of RFI until RFI = 1. There is no compensatory intake and RFI is always ≤ 1. The effects of host resistance on the values of model parameters are proposed. Attempts were made to parameterize the model; when data were scarce, initial parameter values were derived on conceptual grounds. Predictions of the effects of pathogen dose, virulence and host resistance are described and discussed. It is crucial to define the pathogen challenge (in terms of dose and virulence) and the degree of resistance of different hosts, when comparing the responses in RFI for different genotypes. Possible interactions between dose, virulence and resistance were explored. Feed intake of healthy and challenged animals, at a time, may be different once the challenged animal has recovered (RFI = 1). The issue of reductions in FI during pathogen challenges is important for nutritionist and animal breeders. The large variation that has been observed for reductions in FI during pathogen challenges may be a viable point of selection. The points highlighted will aid selection strategies by quantifying effects of pathogen dose and virulence, and time, on the FI of challenged animals. The proposed model may be integrated with other models of growth to predict animal performance during exposure to pathogens.

3.2 Introduction

The desired feed intake is defined as the amount of a given feed that an animal of a given genotype and state needs to attain its potential growth (Emmans and Fisher, 1986). The actual feed intake (FI kg/d) may differ from the desired due to feed composition or constraints in the physical, social (Wellock *et al.* 2003a,b) or infectious environments (Black *et al.* 1999). An understanding of how these constraints affect intake will allow management, feeding, and genetic selection strategies to be better informed.

Pathogen challenges that overcome the innate immune defenses may cause an acquired immune response and can lead to disease that is either sub-clinical, with no visible symptoms, or clinical. Pathogen challenges leading to sub-clinical disease cause a reduction in FI of immunologically naïve animals (Steel *et al.* 1980, 1982; Kyriazakis *et al.* 1996). A greater reduction in intake is seen during clinical disease (Crompton, 1984; Kyriazakis *et al.* 1998; Black *et al.* 1999).

Reduced performance during sub-clinical and clinical infections has been reviewed extensively (Parkins & Holmes, 1989; van Houtert & Sykes, 1996; Coop & Kyriazakis, 1999, 2001), but no general quantitative framework exists for predicting feed intake and performance during infection. Kyriazakis *et al.* (1998) described a qualitative relationship between feed intake and the size (dose) of a pathogen challenge, and that model is developed further here. The quantitative effects of exposure to a pathogen that leads to sub-clinical disease on intake of animals with no prior experience of it are considered. The model proposed is integrated with an existing model of growth (Wellock *et al.* 2003a) to allow prediction of the FI of animals (pigs are used as an example) exposed to infectious stressors.

3.1 Materials and Methods

3.1.1 Feed Intake and Growth in Healthy Animals

The desired feed intake (DFI kg/d) in healthy animals is predicted using a previously published model of growth of pigs (Wellock *et al.* 2003a). Related models exist for poultry (Emmans, 1989), sheep (Jones *et al.* 2004) and cattle (Amer & Emmans, 1998). An animal is predicted to have requirements for energy (ERQ MJ/d) and protein (PRQ kg/d) for potential growth (ADG_p kg/d) which is a function only of animal genotype and state, to be attained. The DFI that an animal requires for achieving its ADG_p is then an

outcome of requirements and the contents of energy (EC MJ/kg) and protein (IPC kg/kg) of the feed. The requirements and contents need to be described on consistent scales. In the model of Wellock et al. (2003a) the scales used are effective energy (Emmans, 1994) and ideal digestible CP (Moughan, 2003).

$DFI_E = ERQ/EC$ kg/d (1)

$DFI_P = PRQ/IPC$ kg/d (2)

The DFI is the greater of DFI_E and DFI_P, i.e. the animal eats for the more limiting of energy or protein. Constraints on intake in the model include feed bulk and those arising from the environment. The predicted FI of the healthy animal, once the climatic environment and feed composition have been taken into account, is the starting point for predicting FI during exposure to pathogens.

3.1.2 Feed Intake and Growth during Pathogen Challenges

Sub-clinical disease is the consequence of a pathogen challenge that leads to no visible symptoms, but where there are measurable reductions in performance (Kyriazakis *et al.* 1998; Henryon *et al.* 2001; Vercruysse & Claerebout, 2001).

The model may be able to be extended to deal with reductions in FI due to clinical disease by changing the values of its parameters, but this is not done here.

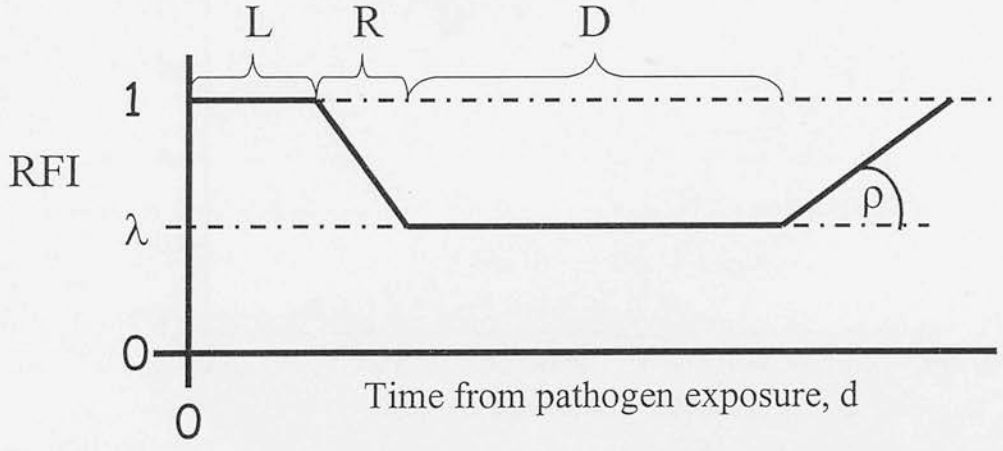

Figure 1a The response in relative feed intake (*RFI*) to time of pathogen exposure, through five parameters: lag time (*L*, d), reduction time (*R*, d), reduction in relative feed intake (λ), duration of the reduction (*D*, d) and the rate of recovery of relative feed intake (ρ, RFI/d).

Feed intake is initially expressed as the intake relative to that of a similar animal at the same weight when not challenged by a pathogen, termed relative feed intake, RFI. The general pattern of RFI during a pathogen challenge can be summarized by the main parameters of the proposed model, shown in Figure 1a.

The lag time (L d) is the delay from the pathogen challenging the host until RFI falls below unity where $0 \leq L \leq L_{max}$. The RFI then falls over the reduction time (R d) where $0 \leq R$, to a level λ. The lowest intake, $0 \leq \lambda \leq 1$, is maintained for the duration time (D d) where $D \geq 0$. It is assumed that for a given case there is no further reduction in RFI, which then starts to recover at a rate (ρ /d), where $0 \leq \rho$, until RFI = 1.

Data from an experiment with sheep that were sub-clinically challenged with the gastrointestinal parasite *Trichostrongylus colubriformis* (Kyriazakis *et al.* 1996) are used to illustrate the model in Figure 1b. This data set is particularly useful as it covers the period of 160 d from the beginning of the daily challenge with the pathogen to full recovery, when RFI = 1.

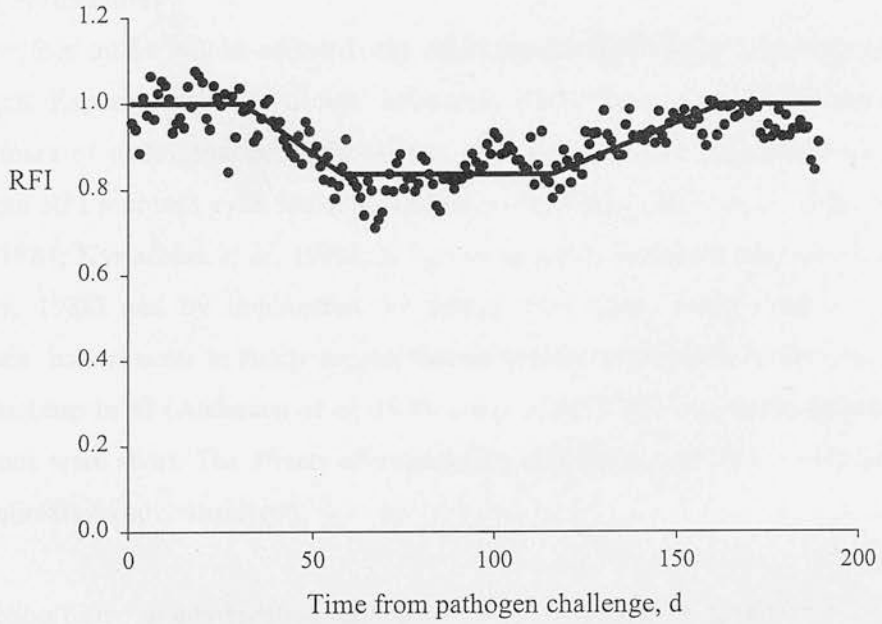

Figure 1b Proposed model for RFI shown with data (•) from (Kyriazakis et al., 1996) with the model parameterised for these data.

Responses in RFI as described in Figure 1a have been seen in experiments with different pathogens: gastrointestinal macroparasites (*Ostertagia circumcincta*, Coop *et al.* 1982;

Haemonchus contortus, Knox & Steel, 1999); other parasites (*Plasmodium vivax*, Fern *et al.* 1985); bacteria (*Salmonella typhimurium*, Balaji *et al.* 2000; Turner *et al.* 2002a, b; *Escherichia coli*, Houdijk *et al.* 2005; *Actinobacillus pleuropneumoniae*, Balaji *et al.* 2002; Kerr *et al.* 2003); viruses (*influenza virus*, Conn *et al.* 1995; *PRRS virus*, Greiner *et al.* 2000; *Sialodacryoadenitis virus*, Sato *et al.* 2001). On the basis of such evidence, it is proposed that the model in Figure 1a is general across pathogens and hosts.

Actual growth (ADG kg/d) is predicted from the actual feed intake and a partitioning rule, when reductions in FI make resources scarce. The partitioning rule used here is that proposed for healthy pigs by Kyriazakis & Emmans (1992a,b), which has been supported by Sandberg et al. (2005a,b). It is recognized that pathogen challenges leading to sub-clinical disease may affect maintenance requirements (Black *et al.* 1999; Houdijk *et al.* 2001), or the efficiency with which resources are used (Steel *et al.* 1980, 1982), or both. In this model, for simplicity, this evidence is initially ignored and it is assumed that FI can be predicted relative to that of a healthy animal, and that there are no additional requirements associated with the pathogen challenge and subsequent immune responses.

3.1.2.1 Assumptions

Relative feed intake will be affected only when animals have had no prior experience of a pathogen. Experiments with 'trickle' infections, where animals are challenged by small daily doses of pathogens, have shown that once animals have acquired immunity to a pathogen RFI recovers even under a continuous challenge (Steel *et al.* 1980; Wagland, 1982, 1984; Kyriazakis *et al.* 1996). A loss of acquired immunity may occur over time (Barger, 1988) and by implication, an animal may again become naïve to a given pathogen. Experiments in sheep suggest that on secondary pathogen challenges, there are no reductions in FI (Anderson *et al.* 1976; Greer *et al.* 2005), but the times between re-infections were short. The effects of a secondary challenge of the same pathogen on the FI of animals, is not considered.

Production traits, in combination with descriptions of disease symptoms have been used as an indication of the 'level' of disease (Kyriazakis *et al.* 1998; Henryon *et al.* 2001; Vercruysse & Claerebout, 2001). Kyriazakis *et al.* (1998) proposed a qualitative model, which related FI to pathogen challenge, while making a distinction between sub-clinical and clinical reductions in FI. The lower threshold (T_d n/d) may indicate either the amount of pathogen that the innate immune system can cope with, or doses for which the

pathogen is not recognized, and therefore the host does not develop an acquired immune response (Symons *et al.* 1981; Windon *et al.* 1984). The upper dose (T_c n/d) is the amount of pathogen challenge above which clinical disease and severe depressions in FI occurs (Black *et al.* 1999). The model of Kyriazakis *et al.* (1998) has been modified as it appears that λ, the greatest reduction in RFI, is affected by different levels of pathogen challenges that are sub-clinical, as is shown in Figure 2.

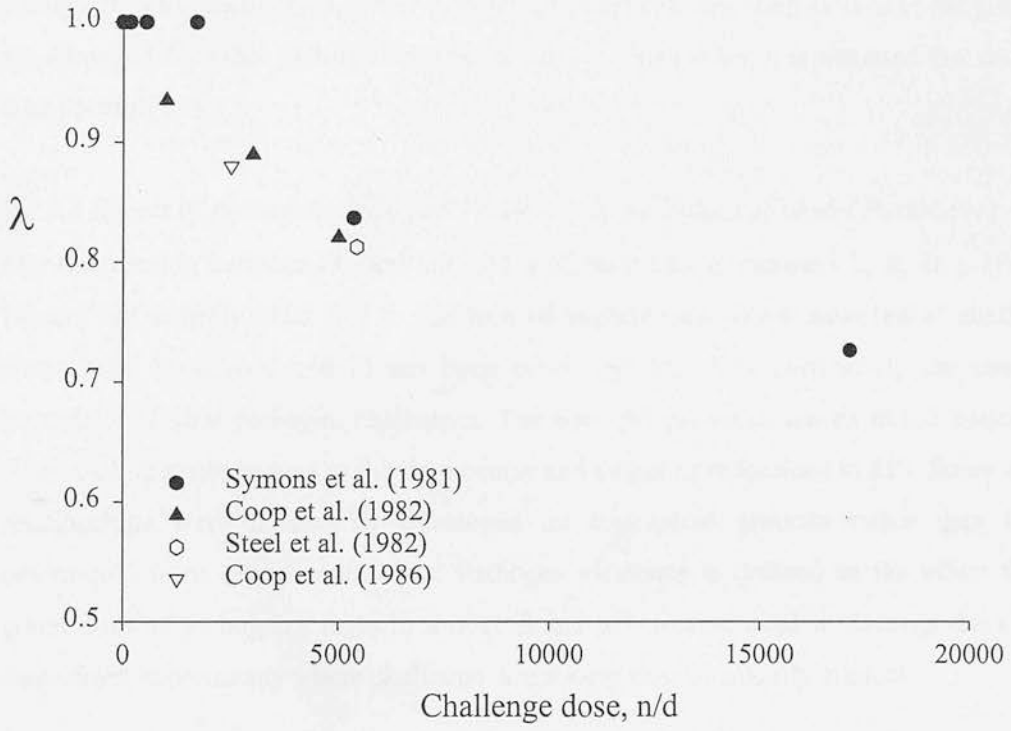

Figure 2 Calculations of the lowest value of RFI (λ) for different sub-clinical challenge doses from data of RFI from Coop *et al.* (1982, 1986), Symons *et al.* (1981) and Steel *et al.* (1982) for sheep challenged with different doses of the gastrointestinal parasite *Ostertagia circumcincta*.

Initial estimates of T_d and T_c are made for parasitic and bacterial pathogens, and initial suggestions for a viral pathogen. The model predicts FI of growing animals during sub-clinical disease. It is necessary to recognise and hence quantify pathogen doses that lead to clinical disease.

The model input is the number of pathogens challenging the animal per day (PC n/d) chosen due to constraints of available data. The rate is assumed applicable to different kinds of pathogen challenge. Challenges may occur as a single or as a cumulative event.

Either the host copes by innate immune responses with no effects on RFI, or an acquired immune response occurs with subsequent effects on RFI. The model is applicable for trickle infections given the value of the parameter T_c that defines doses at which the host becomes clinically diseased for a given set of circumstances of genotype, feed quality and environment.

There may be circumstances where small doses ($PC < T_d$) lead to reductions in RFI due to pathogens being recognized, when 'normal' physical barriers, such as skin or the gut wall are damaged, by other factors affecting the host. In this model, it is assumed that no such event occurs.

3.1.2.2 Effects of Pathogen, Dose and Virulence on the Values of Model Parameters

The relationship between PC and the values of the model parameters L, R, D, ρ (Figure 1a) are not entirely clear due to the lack of experiments where sub-clinical challenge doses have been used and FI has been measured. This was particularly the case for bacterial and viral pathogen challenges. The concepts proposed are an initial attempt to relate pathogen challenges to the time course and extent of reductions in RFI. Some of the relationships were necessarily developed on conceptual grounds rather than being determined from experimental data. Pathogen virulence is defined as the effect that a given dose of pathogen causes to a host. Some information used to develop the model came from experiments where challenge doses were near or actually clinical.

3.1.2.2.1 Lag Time. The lag time parameter (L d) describes the time it takes for a pathogen challenge dose to affect RFI. The value of L is specific to the kind of pathogen. Bacterial and viral challenges can have a lag time of a few hours (Greiner *et al.* 2000; Balaji *et al.* 2002) while parasites may take several weeks (Kyriazakis *et al.* 1994, 1996) to have an effect. A possible explanation may be that greater amounts of antigen are presented from bacterial and viral pathogens, due to greater rates of proliferation, than are presented by non-proliferating parasites accumulating in the host at a slower rate (May & Nowak, 1995). On the other hand, it may be due to recognition being different by parasites avoiding detection. The value of the parameter L is proposed to depend on PC, with larger doses having a greater effect on L because a larger dose of pathogen is more likely to overcome innate immune responses and for the pathogen to become recognized by the host more quickly. The relationship between L and PC is:

$$L = (L_{max} - (\exp^{(\sigma.(PC - T_d))})) + 1 \quad (3)$$

L_{max} is the maximum lag time, achieved when $PC > T_d$; σ (d/n) describes the rate of change in L with increasing PC. The relationship between L and PC is shown in Figure 3. A more virulent pathogen is expected to have a greater value of σ and a lower threshold T_d with lower lag times, as it may be more able to overcome the immune defenses (Beveridge *et al.* 1989; Lipsitch *et al.* 1995).

3.1.2.2.2 The Lowest Relative Feed Intake. The value of the parameter λ describes the lowest relative FI during a pathogen challenge. It is seen as pathogen independent, but as affected by PC (Symons *et al.* 1981; Coop *et al.* 1982; Houdijk *et al.* 2005). The value of λ is proposed to reduce gradually as PC increases until it reaches its lowest value for

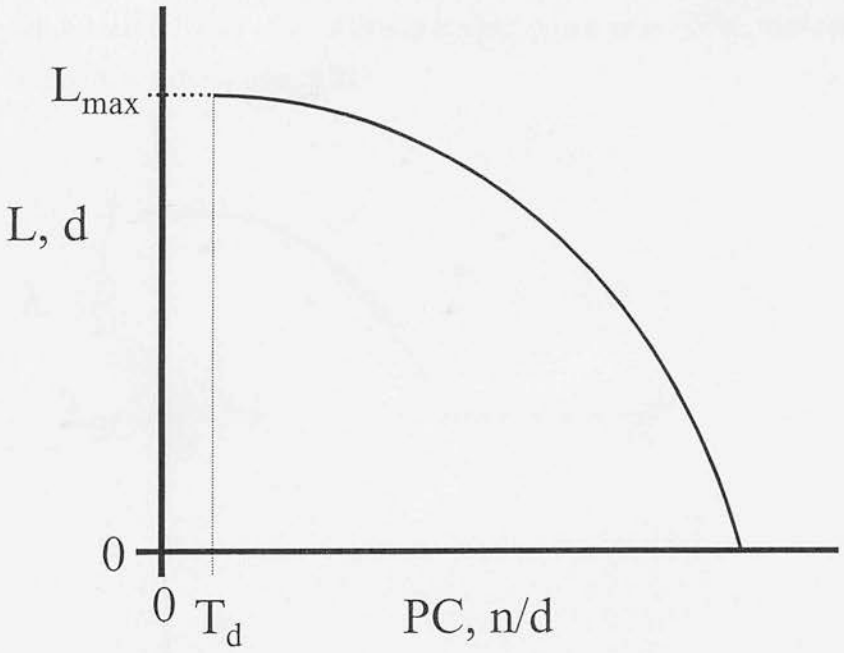

Figure 3 The proposed relationship between lag time (L d) and the level of pathogen challenge (PC n/d).

sub-clinical pathogen challenges (λ_{sc}). The value of λ is then dose independent and equal to λ_{sc} while $PC < T_c$. The relationship between PC and λ, when $\lambda > \lambda_{sc}$ is proposed to be:

$$\lambda = (\lambda_{max} - (\exp^{(\phi.(PC - T_d))})) + 1 \quad (4)$$

The chosen form is based on how an animal's immune system may be responding during different levels of exposure to a pathogen (Schwartz, 2002). The value of λ_{max} is always equal to unity and ϕ, describes the rate of change in λ with increasing PC, when $\lambda > \lambda_{sc}$. The parameter ϕ may represent the level of communication from the immune system that the host perceives from the presence of pathogens (Schwartz, 2002), which reaches saturation at a certain level of dose and then $\lambda = \lambda_{sc}$ as is shown in Figure 4. A more virulent pathogen would not affect the values of ϕ and λ_{sc}, except through its possible effects on T_d.

3.1.2.2.3 Reduction Time. The parameter (R d) describes the time it takes for λ to be achieved. The value of R depends on the type of pathogen and is independent of PC. The reduction is of much longer duration with gastrointestinal parasites (Steel *et al.* 1980, 1982), than with bacteria (Balaji *et al.* 2000) or viruses (Conn *et al.* 1995; Greiner *et al.* 2000). The value of R is independent of PC.

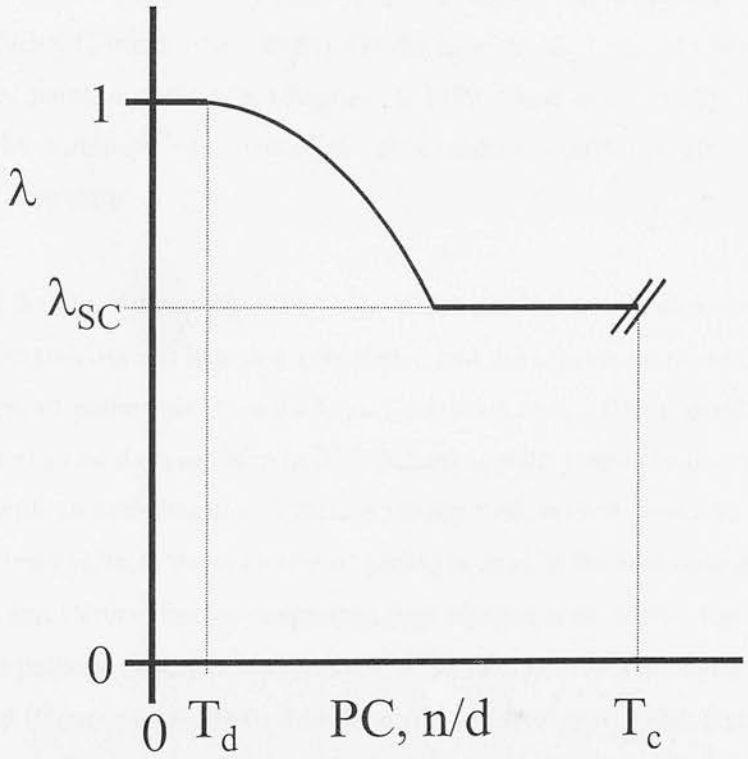

Figure 4 The proposed relationship between the lowest relative reduction in feed intake, λ and the level of pathogen challenge (PC n/d) with λ_{sc} being the lowest possible value of λ, affected while PC is greater than the threshold, T_d, and, while the animal is sub-clinical ($T_d < PC < T_c$).

While the value of λ decreases slightly with increasing dose the rate of reduction in RFI also increases so that R remains constant (Valles *et al.* 2000). It is possible that that the value of R will be lower for a more virulent pathogen, as the host may have evolved to respond at a faster rate to such a pathogen (Lipsitch *et al.* 1995). There may be instances when $R = 0$.

3.1.2.2.4 Duration Time. Duration time (D d) may reflect the time taken by the immune system to start controlling and subsequently expelling the pathogens. It is thus a reflection of the rate of acquisition of immunity and needs to be seen specific to the type of pathogen challenge. There is a degree of overlap between the phase of acquisition of immunity and the expression of immunity (Bachmann & Kopf, 2002; Tuma & Pamer, 2002). It is assumed that the value of D is independent of PC, as is suggested by both bacterial and parasitic challenges (Steel *et al.* 1980; Sykes *et al.* 1980; Houdijk *et al.* 2005). Duration time can be very short (e.g., *Salmonella typhimurium* (Turner *et al.* 2002a,b and PRRS (Greiner *et al.* 2000)). On the other hand, it can take several days for gastrointestinal parasite challenges (Steel *et al.* 1980; Coop *et al.* 1982). It may not be affected by the virulence of a pathogen, as virulence would not affect the rate of acquisition of immunity.

3.1.2.2.5 Rate of Recovery. The value of the parameter ρ /d describes the rate at which RFI increases once D has been completed, and the expression of immunity results in the expulsion of pathogens from the host. Kyriazakis *et al.* (1996) found that the RFI recovered within a few days in sheep trickle challenged with gastrointestinal parasites that were treated with an anthelminthic. Untreated sheep took several weeks to recover their RFI.. The recovery reflects the reduction of pathogen load in the host once immunity has been acquired and starts to become expressed (van Houtert *et al.* 1995). The value of ρ is proposed to be pathogen specific as the recovery is much faster for bacterial (Balaji *et al.* 2000) and viral (Greiner *et al.* 2000) than for parasitic (Steel *et al.* 1980; Kyriazakis *et al.* 1996) challenges. This may reflect the rate of expression of immunity, once acquired, with the removal of worms being slower than the removal of bacteria and viruses (Wakelin, 2000). The value of ρ is proposed to be independent of PC: parasitic (Coop *et al.* 1982) and bacterial (Houdijk *et al.* 2005) pathogen challenges. A more virulent pathogen may be more difficult for the host to expel and may result in a lower value of ρ.

The model does not permit compensatory FI: the animal is said to have recovered once RFI = 1 and RFI is always ≤ 1. Therefore, the RFI of the animal continues to recover at the rate ρ until it has reached the FI appropriate to its state when RFI = 1. Experimental supports for this assumption are the findings of Chapman *et al.* (1982) and Kyriazakis *et al.* (1996).

3.1.2.3 Effect of Host Genotype and Feed Composition on the Values of Model Parameters

The values of some of the model parameters were proposed to be affected by pathogen virulence and may be affected by host genotype and feed composition, through possible differences in rates of pathogen recognition, acquisition and subsequent expression of immunity. A genetically resistant genotype is defined as one that is more able to cope with a pathogen challenge than a genotype that is susceptible. The values of the model parameters as affected by pathogen virulence, host genotype or feed composition could not be well assessed from literature data. Effects of virulence, host resistance and feed composition on the values of model parameters are described.

A genotype that is genetically more resistant may have a higher threshold dose, as the innate immune system may be more capable of dealing with a pathogen challenge (Wakelin, 2000). The value of L_{max} is the maximum lag time achieved when PC > T_d. Its value is unaffected by genotype. In a more resistant genotype, the value of σ may be greater, reflecting a greater ability to recognise and respond to pathogens. The main effect on the predicted value of L is likely to be through an increase in the value of T_d, and consequently, a more 'resistant' genotype would have a longer lag time. The value of L and its associated parameters are proposed to be unaffected by feed composition (Brailsford & Mapes, 1987; Kyriazakis *et al.* 1994, 1996).

The value of R may be less for a more resistant genotype as it recognises and starts to acquire immunity at a faster rate, but it is proposed to be unaffected by feed composition (Kyriazakis *et al.* 1996; van Dam *et al.* 1997, 1998). The values of ϕ and λ_{sc} are unaffected by host resistance. The values of the parameter λ and λ_{sc} are assumed to be independent of the kind of feed (Brailsford & Mapes, 1987; Kyriazakis *et al.* 1996, 1994; van Dam *et al.* 1998).

The value of the parameter D depends on genotype (Coop & Kyriazakis, 2001). Resistant genotypes would be likely to have a shorter duration by acquiring immunity at a greater rate. Analysis of FI data for individual sheep exposed to a sub-clinical *Trichostrongylus colubriformis* challenge from Kyriazakis *et al.* (1996) suggest that the feed protein content significantly affected D. It was not possible to propose a general relationship between D and the feed protein content, and as such, it is not included in the current model, but experiments to determine such effects are warranted.

The parameter ρ is linked with the expression of immunity. Similarly, as for the arguments of the other model parameters, a resistant genotype would be able to express immunity at a greater rate and expel the pathogens resulting in greater rates of recovery in RFI. It would be expected that ρ would be affected by the composition of the feed, as expression of immunity has been found to be affected by additional supplies of metabolizable protein (van Houtert *et al.* 1995; Coop & Kyriazakis, 1999) during this phase of immunity. An analysis of FI data from Kyriazakis *et al.* (1996, unpublished) is in support. Sheep challenged with *Trichostrongylus colubriformis* and given a poorer quality feed recovered more slowly (0.0063 RFI/d) than sheep given the same challenge and a better quality feed (0.0094 RFI/d). As with the duration parameter, it was not possible to propose a relationship between ρ and the feed composition, and as such, it was not included in the current model.

The proposed effects of genotype on the values of model parameters may be correlated. For example, a resistant genotype that is proposed to have a shorter reduction time, shorter duration and a faster recovery may be described by a small number of additional parameters. Similarly, such correlation may also exist for the effects of virulence on parameter values. Initial relationships between the degree of resistance and the degree of virulence are proposed here, to provide a starting point for modelling RFI, e.g. Henken *et al.* (1994a,b), Wellock *et al.* (2003b). The proposed relationships between resistance, virulence and parameter values are summarized in Table 1. The values of model parameters as affected by host resistance and pathogen virulence were calculated as the proposed values of model parameters (see below) multiplied by the respective scaling multipliers shown in Table 1.

Table 1 The proposed relationships between scaling multipliers and model parameters as affected by the degree of host resistance (R) and the degree of pathogen virulence (V) with a hypothetical scale of R and V of 0 to 0.5.

Parameter	Proposed relationship
T_d	$RV_{Td} = 1 + (R - V)$
σ	$RV_{\sigma} = 1 + (R + V)$
R	$RV_R = 1 - (R + V)$
D	$R_D = 1 - R$
ρ	$RV_{\rho} = 1 + (R - V)$

The proposed relationships between degree of resistance and virulence and the scaling multipliers assume that over a certain amount of change in resistance and virulence the effect would be linear. The correlation for the change in the values of model parameters was assumed to equal 1, as this model describes the response of a single animal, rather than a population of animals. The scale of resistance and virulence used in predictions was set from 0 to 0.5.

3.1.2.4 Parameter Values

The model requires the values of 9 parameters to be determined to predict changes in RFI with the time course of different pathogen challenges of different doses and initial estimates are summarized in Table 2. Estimating the values of the parameters in Table 2 is made difficult because of sparse experimental data. Attempts are made here to assign values to model parameters whenever possible from experimental data. Where experimental data were not available, initial estimates are suggested on conceptual grounds.

Table 2 A summary of the parameters that are used to predict relative feed intake (RFI) during exposure to pathogen challenges.

Parameter	Description	Proposed constants	Effect of genotype[b]	Effect of virulence[b]	Effect of FPC[a,b]
T_d	Dose for an effect on RFI to occur	-	√	√	X
T_c	Dose for clinical reductions in RFI	-	√	√	√

L	Lag time for an effect on RFI to start	L_{max}, σ	√	√	X
λ	RFI at the end of the reduction	λ_{SC}, ϕ	X	X	X
R	Time for λ RFI to be reached	-	√	√	X
D	The duration of RFI = λ	-	√	X	√
ρ	Rate of recovery of RFI from λ to 1.0	-	√	√	√

[a]Effects of feed protein content (FPC) are described, but were not included in this model

[b]√ = Effect, X = No effect

3.1.2.4.1 Thresholds of Sub-clinical and Clinical Reductions in Relative Feed Intake. To predict the effects of sub-clinical pathogen challenges on RFI it is necessary to define pathogen challenge doses that lead to sub-clinical and clinical disease. Initial estimates of threshold pathogen doses for sub-clinical, T_d, and clinical, T_c, disease were determined for a range of pathogens (antigen) challenges and these are presented in Table 3.

Table 3 Estimation of the threshold pathogen challenge doses for sub-clinical (T_d) or clinical (T_c) responses in relative feed intake as described by the model of Kyriazakis *et al.* (1998).

Pathogen	**Host**	**T_d, n/d**	**T_c, n/d**	**Source**
T.colubriformis (larvae)[a]	Sheep	1000 – 3000	≈ 5000	Coop *et al.* (1976)
T.colubriformis (larvae)[a]	Sheep	≈ 2000	< 30 000	Steel *et al.* (1977)
F.hepatica (metacercariae)[a]	Sheep	≤ 3	> 14	Sykes *et al.* (1980)

O. circumcincta (larvae)[a]	Sheep	1000 – 2000	> 5000	Coop *et al.* (1982)
E.coli (cfu's)[b]	Pigs	$< 1 \times 10^6$	$\approx 1 \times 10^8$	Houdijk *et al.* (2005)
S.dentatus (larvae)[a]	Pigs	< 72	> 850	Hale & Marti (1983)
Lipopolysaccharide (μg LPS/kg W)[b]	Pigs	< 0.5	> 5 < 30	Sakumoto *et al.* (2003)
Lipopolysaccharide (mg LPS/kg W)[b]	Chickens	< 0.5	> 5	Sell *et al.* (2003)
Lipopolysaccharide (μg LPS/kg W)[b]	Mice	< 0.1	≈ 3.0	Swiergiel *et al.* (1997)

[a]Continuous challenge [b]Single challenge, cfu = coliforming unit

Experiments suitable for estimating T_d and making suggestions for T_c are those of Steel *et al.* (1980, 1982), Symons *et al.* (1981) and Coop *et al.* (1982). The pathogens used were the gastrointestinal parasites *Ostertagia circumcincta* and *Trichostrongylus colubriformis*. The value of T_d for *Ostertagia circumcincta* was found to be within the range of 1,000 to 2,000 n/d (Figure 2) and for *Trichostrongylus colubriformis*, the range was found to be 430 to 1,500 n/d (Steel *et al.* 1980). T_c may be greater than 17,000 (Symons *et al.* 1981) for *Ostertagia circumcincta* and less than 4,300 for *Trichostrongylus colubriformis* (Steel *et al.* 1980). Data from the experiments of Houdijk *et al.* (2005) using pathogen challenges with *Escherichia coli* were used to estimate the threshold values for a bacterial pathogen challenge. T_d for a bacterial pathogen was estimated as $< 1 \times 10^6$ with T_c being estimated as $\approx 1 \times 10^8$.

Experiments where FI has been measured appear to rarely focus on the range of doses that lead to sub-clinical disease; this range may be very narrow for viruses and bacteria. Therefore, the proposed values for a viral challenge are suggestions, based on the kind of doses that have been used in experiments (Greiner *et al.* 2000). However, these initial estimates provide a starting point for defining the kind of challenge doses that lead to sub-clinical and clinical disease with subsequent reductions in RFI.

3.1.2.4.2 Lag Time and Associated Parameters. The value of L has been found to vary from <0.01 d in the case of some bacterial (Houdijk *et al.* 2005) and viral (Greiner *et al.* 2000) pathogen challenges to 32 d for a parasitic challenge (*T.colubriformis*; Kyriazakis *et al.* 1996). In the case of another gastrointestinal parasite, *Ostertagia circumcincta*, L appeared shorter at approximately 21 d (Coop *et al.* 1982). The lag for a protozoan pathogen was estimated as 7 d (Verstegen *et al.* 1991) and found to be consistently around this value for other challenges with this kind of pathogen (Zwart *et al.* 1991; van Dam *et al.* 1997, 1998). The value of σ could not be determined from literature data as no experiment was found that included a sufficient range of challenge doses. Therefore, values of σ were calculated to reproduce lag times consistent with experiments (Steel *et al.* 1980; Kyriazakis *et al.* 1996) and the proposed estimate of L_{max}. The value of L_{max} is proposed for model bacterial and viral pathogens to be 5 d and for the model parasite challenges to be 40 d. Consequently, the values of σ for parasitic, bacterial and viral pathogens were calculated as $1.4x10^{-3}$, $3.1x10^{-8}$ and $3.4x10^{-3}$, respectively, which for an initial attempt of modelling RFI during pathogen challenges may be acceptable (see Henken *et al.* 1994a,b).

3.1.2.4.3 Reduction Time. The value of R has been estimated to be 1 to 2 d for bacterial and viral pathogen challenges (Greiner *et al.* 2000; Houdijk *et al.* 2005). For a parasite challenge, the value of R was estimated as 40 d from the experiment of Kyriazakis *et al.* (1996).

3.1.2.4.4 The Lowest Value of RFI and Associated Parameters. The value of λ_{max} is always equal to unity. The value of ϕ for a parasitic pathogen challenge was estimated as $4.5x10^{-5}$ from experiments with *Ostertagia circumcincta*, with λ_{sc} being estimated as 0.72 (Symons *et al.* 1981; Coop *et al.* 1982; Steel *et al.* 1982). The estimate of λ_{sc} is assumed to be general. The values of ϕ for a bacterial ($2.5x10^{-7}$) and viral ($5.0x10^{-5}$) pathogen challenges were calculated on conceptual grounds to agree with their respective pathogen dose scales.

3.1.2.4.5 Duration Time. The value of D was estimated as 1 d for the model bacterial (Houdijk *et al.* 2005) and viral (Greiner *et al.* 2000) pathogen challenges. These are the lowest values that a model with a time step of 1 d can include. Literature data suggest that the onsets of sub-clinical reductions in FI be very fast for viral and bacterial

pathogens. The value of D for a parasitic pathogen was estimated as 50 d from (Kyriazakis *et al.* 1996).

3.1.2.4.6 Rate of Recovery. The value of the recovery parameter, ρ, was determined as 0.0046 (SE 0.0006) from Symons *et al.* (1981) for a pathogen challenge with *Ostertagia circumcincta*. The value of ρ was determined as 0.0022 (SE 0.0004) from Kyriazakis *et al.* (1996) for a pathogen challenge with *Trichostrongylus colubriformis*, demonstrating different rates of recovery for these two pathogens. The rate of recovery for bacterial challenges was estimated as 0.3 from Balaji et al. (2000, 20002) and Turner et al. (2002a,b). This value of 0.3 is also used for other host replicating pathogens such as the viral pathogen challenges. Parameter values that are used in the model are summarized in Table 4 for three different pathogens.

Table 4 Initial estimates of parameters for a parasite (based on Coop *et al.* 1976; Kyriazakis *et al.* 1996), a bacteria (based on the experiments of Houdijk *et al.* 2005) and a virus (based on the experiment of Greiner *et al.* 2000 and estimates for a bacterial pathogen)[a].

Parameter	Parasite (*T.colubriformis*)	Bacterium (*E.coli*)	Virus (*PRRS*)
T_d	1×10^3	*1×10^3*	*1×10^2*
T_c	5×10^3	1×10^8	*1×10^4*
L_{max}	*40*	*5*	*5*
σ	*1.4×10^{-3}*	*3.1×10^{-8}*	*3.4×10^{-3}*
λ_{SC}	0.72	0.72	0.72
ϕ	*5.0×10^{-5}*	*2.5×10^{-7}*	*5.0×10^{-5}*
R	40	2	2
D	50	1	1
ρ	0.004	0.3	0.3

[a]Due to the lack of data for viral challenge estimates are made the same as another host replicating pathogen (bacteria), parameter values that were created on conceptual grounds are in italics.

3.2 Results

The model was used to predict the effects of host resistance, pathogen dose and virulence on RFI, and hence relative or actual feed lost. The default model inputs (described in Appendix A) were used, unless stated otherwise. The total feed lost (FL kg) due to pathogen challenge was calculated as the difference between the cumulative feed intake of the unchallenged less that of the challenged animal. The total relative feed intake lost, RFL was calculated as the sum of the daily differences (1 - RFI) during the period when RFI < 1.

3.2.1 Predicting the Sensitivity of the Values of Model Parameters on Total Feed Lost

The model was used to predict the effects of varying the values of model parameters on the predicted FL for a model parasitic pathogen. The value of parameter L was not included in the sensitivity analysis, as it does not affect FL. It is worth noting, however, that in instances where L was predicted to be greater than the experimental (simulation) period the overall FL was underestimated. The base values of model parameters are in Table 4. For each set of predictions, the value of one parameter was varied at 0.1 intervals between 0.5 to 1.5 times the base value, while keeping the other parameters at their base values. The change in FL predicted for each alteration of the parameter values was determined as a percentage of the FL when all parameters were at their base values. The pathogen challenge dose was set to 2,000 macro parasites and the results are in Table 5.

The model parameters whose change in value affected FL during a pathogen challenge to the greatest extent were found to be T_d, ϕ, and D. The FL changed in an essentially linear manner over this range of parameter values. The value of the parameter ρ did not have a large effect on FL, within the range of values considered. The value of FL was not linearly related to ρ, and as its value becomes small, the effects on FL were predicted to be much greater. The reduction in FL caused by increasing the threshold T_d was due to its proposed effects on λ the lowest value of RFI. The large increase in FL that was observed when increasing the value of ϕ was also through its effect on the predicted value of λ. The large effect of D on FL was through the time it maintains the lowest reduction in RFI, λ. The value of λ and the parameters leading to it (T_d, ϕ), and the value of D are identified as the key parameters of the model.

Table 5 The effect of changing the values of model parameters on predictions of the total amount of feed intake lost (FL kg) relative to that for base parameters values (Table 4).

	FL relative to base parameter values				
Change in parameter[a]	**T_d**	**R**	**ϕ**	**D**	**ρ**
0.5	157.0	82.5	47.1	64.5	109.2
0.6	145.3	86.0	57.2	71.5	106.1
0.7	133.9	89.5	67.5	78.7	104.0
0.8	122.9	93.0	78.1	85.7	102.3
0.9	111.6	96.5	89.0	93.2	101.2
1	100	100	100	100	100
1.1	89.3	103.2	111.6	107.2	99.2
1.2	78.7	106.9	122.9	114.2	98.5
1.3	67.9	110.3	134.8	121.6	97.9
1.4	57.6	113.6	146.9	128.8	97.4
1.5	47.4	116.9	159.2	136.0	96.9

[a]The value of each parameter was changed by X*base value

3.2.2 Predicting the Effects of Pathogen Dose on Feed Intake at Either a Weight or Age

The model was used to predict the actual FI of a pig (initial BW 10kg). The feed was balanced in terms of the ratio of energy to protein at the beginning of the simulation. The predicted FI prior, during and after the pathogen challenge was considered in relation to body protein weight, BW and age. The pig genotype was defined in the model as a typical fast growing genotype (Knap, 2000), and comparisons were made between a healthy and pathogen challenged animal. The effect of a bacterial challenge on the FI of a 10-kg pig in relation to its healthy control is shown in Figure 5.

The intake of the challenged pig did not recover to the same level as that of the healthy control, when the effects of the pathogen were over. At the same body protein weight, however, there was no difference in intake between the healthy and challenged animals, when the effects of the pathogen were over (data not shown). In this model, the level of dose affects the extent of the reduction in FI and therefore the extent of the difference in protein weight at a time.

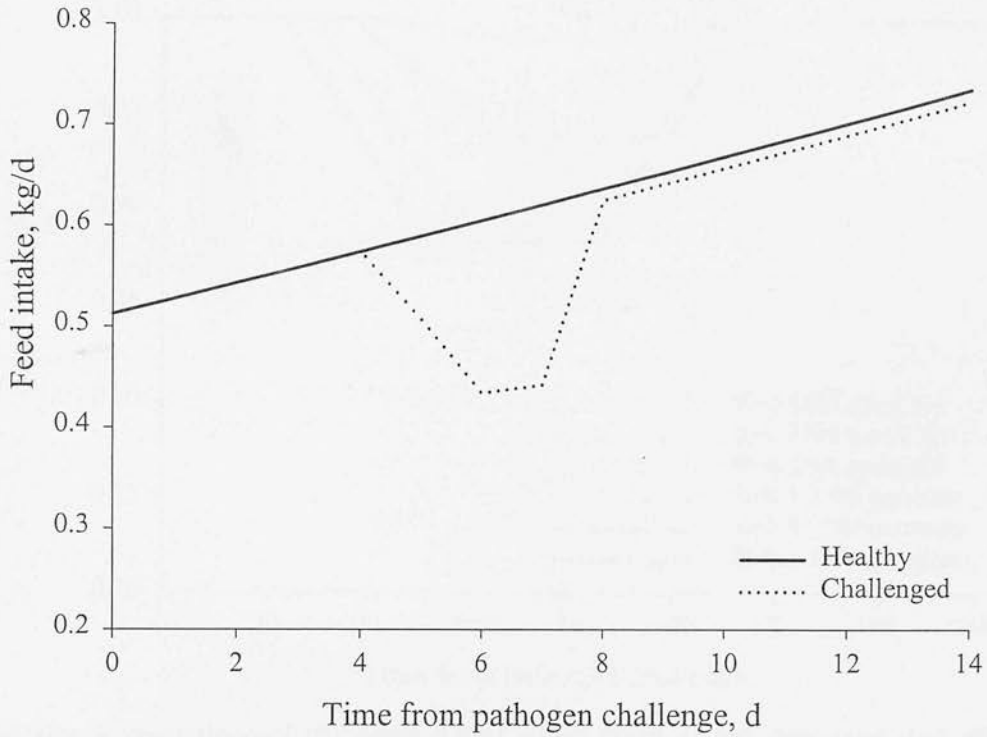

Figure 5 The predicted feed intake of a 10 kg pig challenged with a bacterial pathogen in relation to a healthy control.

3.2.3 Predicting the Effects of Host Resistance and Pathogen Dose on RFI and RFL

The model was used to predict RFI and RFL of a host that was resistant (R) to different extents on a hypothetical scale, during a parasitic pathogen challenge of different doses. Predictions of RFI for a genotype that was made 'susceptible' having base parameter values, and for a genotype that was resistant and assigned a value of 0.3 for resistance are shown in Figure 6.

The 'resistant' genotype performed better. However, the predictions in Figure 6 show differences in the time scale of the reductions in RFI. The resistant animal may already have achieved its lowest reduction in RFI, while the susceptible animal was still reducing its RFI. This may lead to unsuitable comparisons of RFI, at a time, for different genotypes. The resistant genotype that was challenged with the medium dose of 3,500 parasites was predicted to have achieved the lowest reduction in RFI, while the susceptible counterpart was still reducing its RFI.

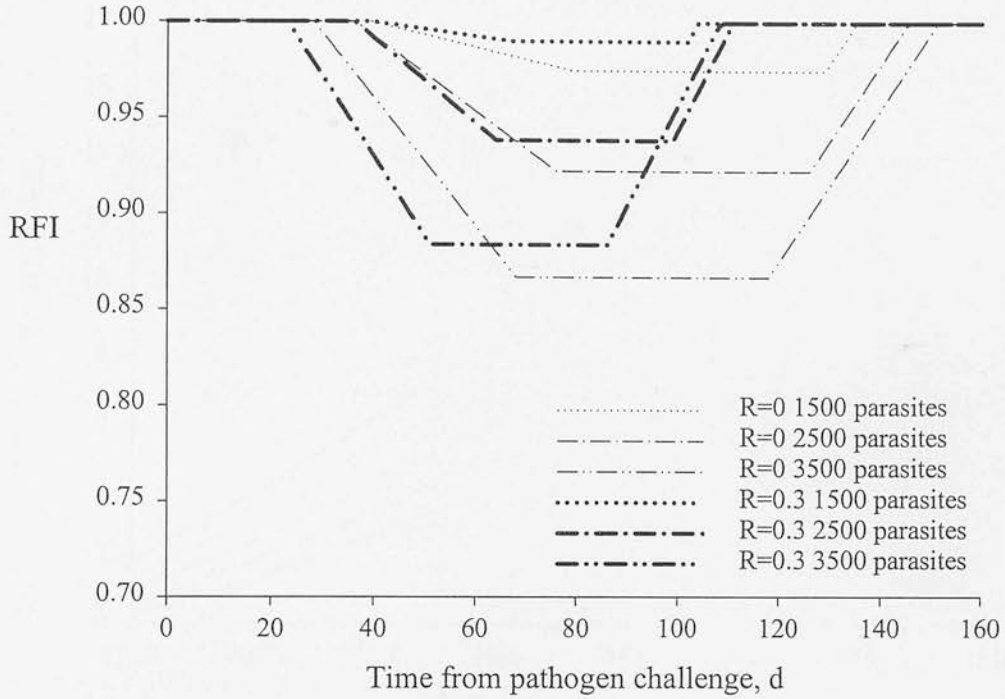

Figure 6 Predictions of the relative feed intake (RFI) of two genotypes with different degrees of resistance (R) that were challenged with three doses of a parasitic pathogen.

Simulations for relative feed intake lost (RFL) were performed for four different genotypes with degrees of resistance on a hypothetical scale of 0 to 0.4 and for 7 different challenge doses of a parasitic pathogen, Figure 7. The predictions show that a more resistant genotype always performed better by having lower amounts of RFL. However, the absolute difference between the different genotypes in RFL was more pronounced at larger doses. This effect was observed due to the proposed effects of resistance on the threshold T_d and the duration D of the lowest reduction in RFI. Predicted effects of resistance and pathogen dose demonstrated that by using an insufficient or unsuitable range of pathogen doses, or an in-sufficient time scale, could lead to misperceptions of the effects of genotype on RFI and RFL.

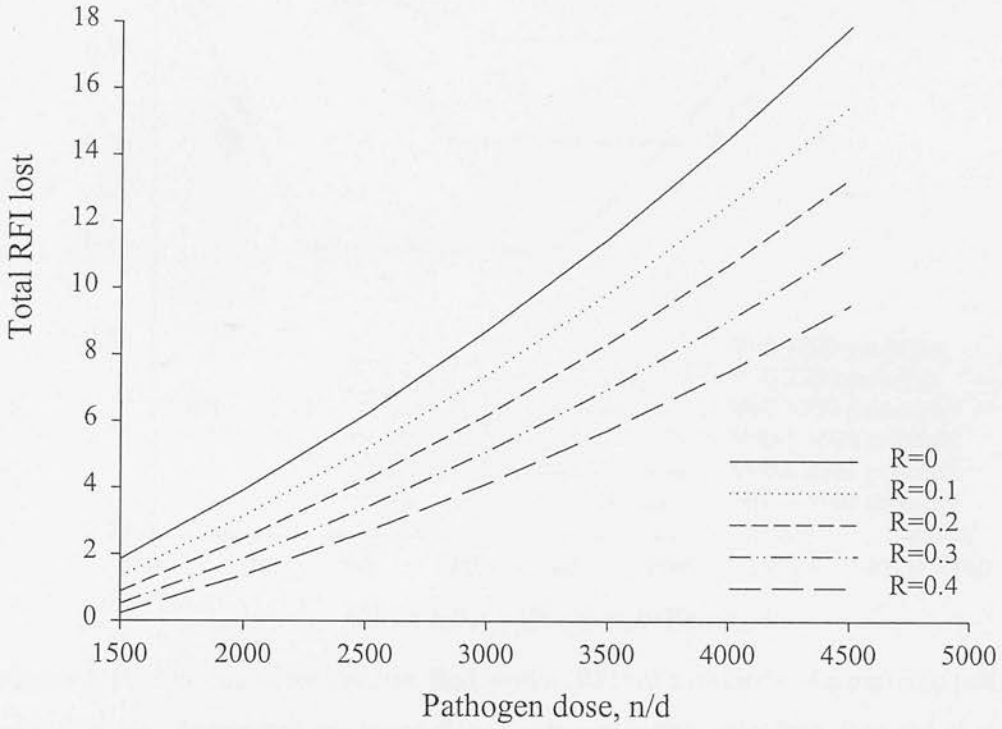

Figure 7 The total relative feed intake (RFI) lost during pathogen challenges of different doses of a parasitic pathogen for different host genotypes that had different degrees of resistance (R) expressed as a hypothetical scale of 0 – 0.5.

3.2.4 Predicting the Effects of Pathogen Dose and Virulence on RFI and RFL

The model was run for a range of pathogen dose and virulence. The predictions of RFI against time are shown in Figure 8 for a pathogen of 'normal' virulence (0) and a pathogen with medium level of virulence (0.3). Pathogen virulence was predicted to have large effects on RFI, with the animal exposed to the more virulent pathogen having greater reductions in RFI. However, as was the case for resistance, the effect of virulence on the overall time scale of reductions in RFI through to full recovery of RFI showed quite large differences for the different doses. In particular, as virulence was proposed to both reduce the threshold T_d and increase the value of σ, the lag time was predicted to be much shorter for the largest dose used. The model was also used to predict the responses in RFL for 7 pathogen doses at 4 levels of virulence. The effect of virulence on absolute RFL was also more pronounced at greater pathogen doses. Pathogens that were more virulent caused greater RFL to be predicted at all doses.

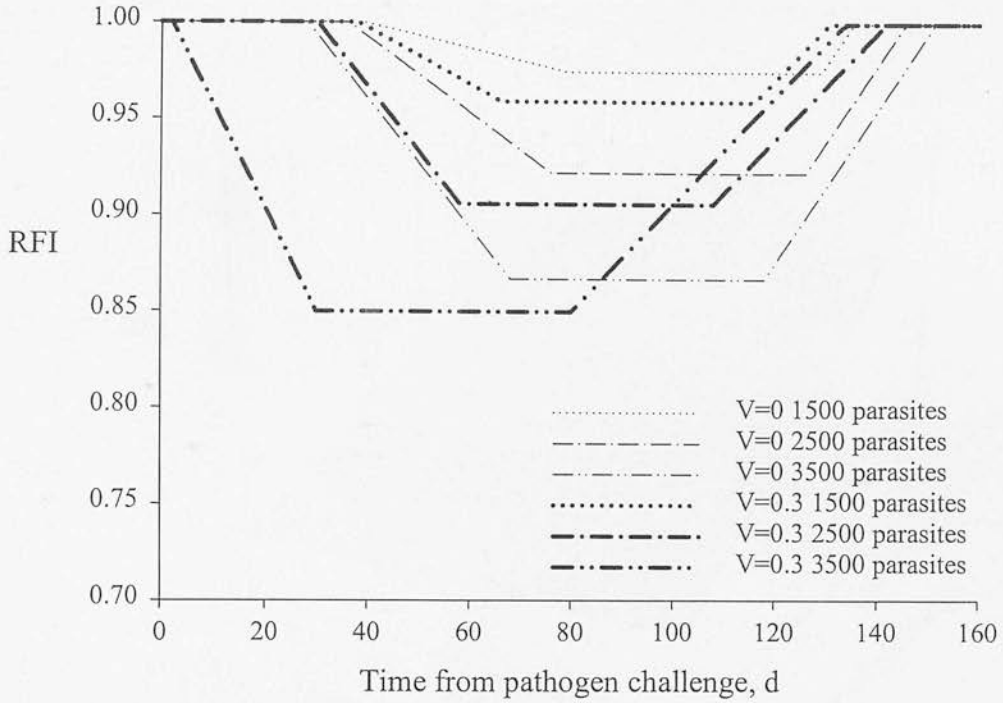

Figure 8 Predictions of the relative feed intake (RFI) of two kinds of a parasitic pathogen with different degrees of virulence (V) as a hypothetical scale from 0 to 0.5 that were challenged with three doses of a parasitic pathogen.

3.2.5 Predicting the Effects of Host Resistance, Pathogen Dose and Virulence on RFL

Predictions were made of the RFL for different combinations of host resistance, pathogen dose and virulence. The results of predictions of RFL, at a dose, for different amounts of resistance and virulence are shown in Figure 9. At higher resistance, virulence was not predicted to have a large effect on RFL. However, at low resistance the effect of increasing virulence was more pronounced. At a high virulence, it was predicted that RFL would be affected to a smaller extent as resistance was reduced from 0.5 to 0.3. A larger increase in RFL was predicted at a high virulence when resistance changed from 0.3 to 0. The interactions arose from the relative effects that resistance and virulence were proposed to have on the values of the key parameters of the model (T_d and D). The balance between effects of host resistance, pathogen dose and virulence seems to be a key component when considering reductions of RFI and RFL during pathogen challenges of different host genotypes.

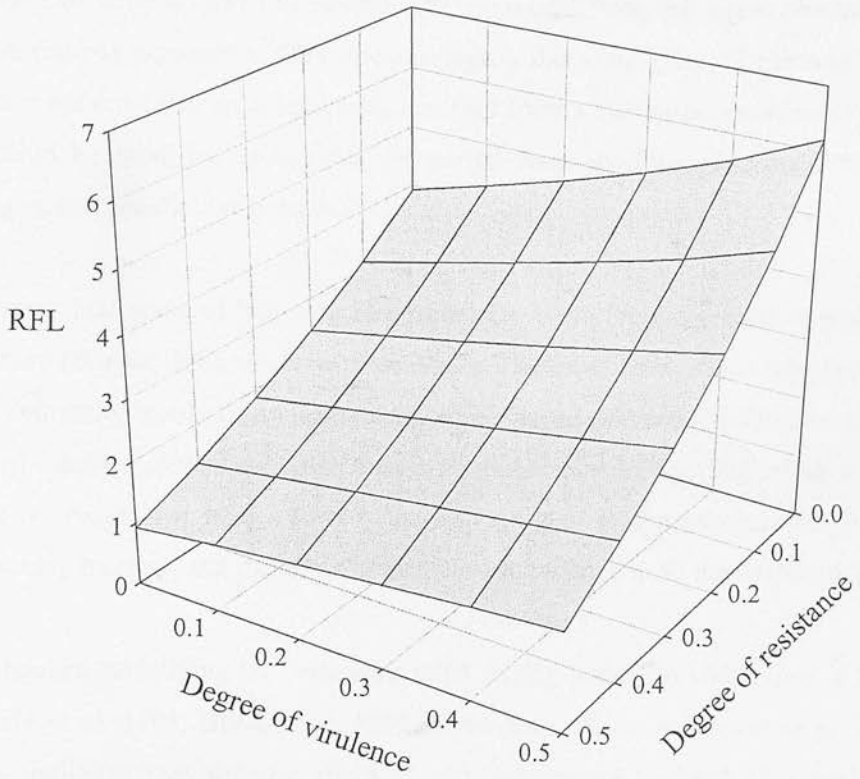

Figure 9 The relationship between the total amount of relative feed intake lost (RFL) and degree of pathogen virulence and host resistance that were both on a hypothetical scale from 0 to 0.5 at a pathogen dose of 2,000 parasites.

3.3 Discussion

Although the effects of sub-clinical pathogen challenges on FI have long been recognized, no general model has been proposed. That proposed here is a starting point for predicting the relative and actual FI of animals challenged by pathogens. The model is a more general and comprehensive than previously published attempts (Henken *et al.* 1994a, b; Kyriazakis *et al.* 1998; Black *et al.* 1999). The phenomenon of reduced FI during challenge has been considered explicitly, and generally, for different kinds of pathogens. The model may also contribute to our understanding of the mechanisms by which different kinds of pathogen challenges (species, dose, and virulence) affect the FI of hosts that have different levels of genetic resistance.

An assumption is that only animals that are naïve to a particular pathogen show a reduced FI. For animals that are continuously exposed to a pathogen this assumption would seem to hold (Kyriazakis *et al.* 1996; Greer *et al.* 2005). Feed intake appears not to be reduced

in animals that have acquired immunity, and recovered from the initial challenge, even during continuous exposure to the pathogen (Kyriazakis *et al.* 1996; Greer *et al.* 2005). It was further assumed that an animal fully recovers from a challenge, in terms of RFI. The model could be modified to account for partial recovery through a reduction in the animals genetic potential for growth.

Animals may lose some of their acquired immunity when their exposure to pathogens is discontinued (Barger, 1988; Jackson *et al.* 2004). The question raised is whether a loss of acquired immunity would lead to reductions in FI during re-infection. Greer *et al.* (2005) challenged immune ewes 2 wk after drenching and removal of pathogen exposure; they observed no reductions in FI. Hence, the rate of loss of the expression of acquired immunity may be slow, and its value in relation to reductions in FI needs determining.

The mechanism underlying the reduction in FI during pathogen challenges is not clear (Kyriazakis *et al.* 1998; Black *et al.* 1999; Kyriazakis, 2003; Schinckel *et al.* 2003). A pathogen challenge may directly affect FI and consequently growth (Kyriazakis *et al.* 1998). Alternatively the potential for growth may be reduced, which leads to reduced requirements and consequently a reduced FI (Schinckel *et al.* 2003). Some authors argue that both mechanisms act simultaneously (Black *et al.* 1999; Broussard *et al.* 2001). The experiments of Bown *et al.* (1991) and Kyriazakis *et al.* (1994) are in support of a direct effect of a pathogen challenge on FI. Bown *et al.* (1991) challenged sheep with *Trichostrongylus colubriformis* that were given a basal feed with or without an abomasal infusion of either energy or protein. Extra protein, but not energy produced a rate of N retention equal to that of the uninfected controls. Treatment did not affect FI. These results suggest that the reduced performance was due to a direct effect on appetite, rather than to the potential for growth being reduced.

Kyriazakis *et al.* (1994) showed that sheep challenged by pathogens and given a choice of feeds with different crude protein contents favoured the high protein feed. This would be expected if the animals had a reduction in FI, but not if the animals had a reduction in their potential for growth. The reduction in protein requirements due to a reduced potential would likely be greater than the increased requirements for protein associated with parasitism.

On the other hand, Williams *et al.* (1997a,b,c) fed pigs feeds of different protein contents in environments intended to have either high or low effects on the immune system (IS). Williams *et al.* (1997a) found that intake in the high IS environment was 0.919 of that in the low IS environment, but this effect was not formally significant. In further experiments (Williams *et al.* 1997b,c) the reduction in FI in the high IS environment was similar in magnitude and in some cases formally significant. There was no significant interaction of the level of immune system activation and the feed crude protein content in any of the above three experiments. The upper limit to protein retention of pigs kept in the high IS environment was estimated as 0.74 of those in the low IS environment (Williams *et al.* 1997a,b,c). These findings as a whole support a direct effect on appetite, and suggest in addition an effect on the upper limit for protein retention, in agreement with the results of Webel *et al.* (1998a,b). Black *et al.* (1999) have also proposed this dual effect.

Reductions in FI in naïve animals acquiring immunity may be beneficial for the animal (Kyriazakis *et al.* 1998; Bazar *et al.* 2005). Reductions in FI may also occur due to the pathogen manipulating the host. Greer *et al.* (2005), however, found that immunosuppression prevented immunologically naïve sheep challenged with a gastrointestinal parasite developing any reductions in FI. This would suggest that a reduction in FI during pathogen challenges is a host response, rather than being caused by the pathogen manipulating the host.

Several factors have been proposed as the specific cause of the reduction in FI during pathogen challenges (see reviews by Johnson, 1998; Broussard *et al.* 2001). These include members of the cytokine family (Langhans, 2000) and leptin (Grunfeld *et al.* 1996). Faggioni *et al.* (1997) using mice that were genetically unable to produce leptin found that a challenge with LPS still produced anorexia. Cytokines have multiple effects on the host (Miyajima *et al.* 1992; Sanchez-Cuenca *et al.* 1999). Therefore, such kinds of evidence would unlikely provide an answer to whether either of the above two mechanisms causes reductions in FI during pathogen challenges.

The model presented is for a host that is challenged by one pathogen at a time; it is possible that the challenge is by more than one pathogen. The assumptions made in such an event are that the effects on the host will either be additive or multiplicative (Sykes & Greer, 2003). Taylor *et al.* (1989) and Parkins *et al.* (1990) challenged calves with two

pathogens (*Ostertagia ostertagia* and *Cooperia oncophora*) either singly or in combination. The combined challenge caused a reduction in FI that was no greater than that caused by the single challenge that gave the greater effect. Effects of multiple challenges may also be expected to depend on the dose at which an animal becomes clinically diseased (Sykes & Greer, 2003).

The proposed model can be developed further to account for the effects of pathogen challenges on energy and protein requirements as affected by pathogen kind and dose. This may include the effects of fever on energy requirements (Akinbamijo *et al.* 1997; Escobar *et al.* 2004) and the effects of acquired and innate immune functions on resource requirements (van Houtert & Sykes, 1996; Coop & Kyriazakis, 1999, 2001). The extended model will need to account for apparent differences, or not as might be the case, of resource partitioning during disease. It is important that the description of the 'level of challenge' that a particular animal is exposed to is sufficient. In the current model, as was dictated by literature data, no scaling rule was used for the pathogen challenge doses. A scaling rule needs to predict the dose that would be equivalent for animals of different mature and current sizes (Greer *et al.* 2005).

Even after recovery an animal that had been challenged was predicted by the model to have reduced FI at a time compared to its healthy counterparts. Pathogen challenges may affect only a proportion of animals in a group (Yu *et al.* 2000), and to different extents depending on the level of challenge, resulting in the variation in FI at a time to increase. Predictions of RFI of hosts that were made to have different resistance and challenged by different levels of pathogen showed that it is very important to consider the entire time course of reductions in RFI. Otherwise, the effects of a pathogen challenge may be greatly misinterpreted.

Parameterization by using the average RFI of animals challenged by pathogens, when averaged over a period of time, may lead to an underestimation of one of the essential parameters of the model: the lowest possible value of RFI during sub-clinical disease, λ_{sc}. In the model the lowest value of RFI that is allowed during sub-clinical pathogen challenges was 0.72. Yu *et al.* (2000), when discussing sheep challenged by *Trichostrongylus colubriformis*, stated that 'daily intakes were reduced by up to 30% during wk 5 to 7 of dosing and up to 50% during wk 11 to 13 of dosing in individual animals, yet some did not experience any inappetence.' This large variation in reductions

in FI has also been observed when analysing data of individual sheep from the experiment of Kyriazakis *et al.* (1996). The proposed value of λ_{sc} (0.72) that is used in the model may therefore be an underestimate. It may be better to determine the values of the model parameters from the RFI of individual animals, from which average values of parameter values could be obtained. Animals that do not show reductions in RFI should not be included as it can not be certain that they have exceeded their threshold for reductions in FI to occur. The parameterization of the model, in this way, may become very important when considering different host genotypes.

An animal that copes better in terms of FI at a given pathogen load is by definition more resistant. This definition of resistance has been used by Akinbamijo *et al.* (1997) for the case of trypanosomes and by Bishop & Stear (2003) for other pathogens. This definition is useful for production animals exposed to a variety of pathogens. A description of host resistance requires more information as input to the model. A simplifying assumption would be that resistance is a general property of the host that had similar proportional effects on the value of all of the parameters of the model.

The issue of predicting reductions in FI during pathogen challenges is important not only for nutritionist, but also for breeders. The large variation that has been observed for reductions in FI during pathogen challenges could be a viable point of selection. The points highlighted in this paper may need to be taken into account during genetic selection: the effects of dose and time on the FI of animals challenged by pathogens, during and after their FI having been affected.

Chapter 4

The effects of pathogen challenges on the performance of naïve and immune animals: The problem of prediction.

4.1 Abstract

Predictive frameworks for performance under both physical and social stressors are available, but yet no general framework exists for predicting the performance of animals exposed to pathogens. The aim of this paper was to identify the key problems that would need to be solved to achieve this. Challenges of a range of hosts by a range of pathogens were reviewed to consider reductions in growth beyond those associated with reductions in voluntary food intake, *VFI*. Pair-feeding and marginal response studies identified the extent, and mechanisms of how further reductions in growth occur beyond those caused by reduced *VFI*. Further reductions in growth were pathogen, host, dose and time dependent; in some instances reductions in *VFI* fully explained reductions in growth. Marginal response experiments showed increased maintenance requirements during exposure to pathogens, but these were different for specific amino acids, with no clear effects on marginal efficiency. Innate immune functions, repair of damaged tissue and expression of acquired immunity caused significant but variable increases in protein (amino acid) requirements. More resistant genotypes had greater requirements for mounting immune responses. The partitioning of protein (amino acids) was found to be different during pathogen challenges. Prediction of the requirements and partitioning of amino acids between growth and immune functions appears to be a crucial problem to solve when predicting performance during pathogen challenges of different kinds and doses. The problems of accounting for reductions in performance during pathogen challenges that are described here may provide a useful starting point for future modelling and experimental solutions.

4.2 Introduction

Predicting the effects of physical (Black *et al.* 1986; Wellock *et al.* 2003*a*), social (Wellock *et al.* 2003*b*) and infectious (Black *et al.* 1999) stressors on performance is important for guiding future management, genetic selection and experimental strategies. No general model exists that predicts growth and performance of different host genotypes, when given access to different kinds of foods and challenged by pathogens. The aim of this paper was to characterize reductions in growth during exposure to different kinds and amounts of pathogen that were not caused by a reduction in voluntary food intake (Sandberg *et al.* 2006). This allowed for the main consequences of pathogen challenges that would need addressing in a predictive framework of growth to be identified.

There is no general agreement on the overall problems that need solving in order to predict growth during pathogen challenges (Black *et al.* 1999; Coop & Kyriazakis, 1999; Powanda & Beisel, 2003). A general but comprehensive model may provide a clearer background for the design of future experiments, and help improve our current understanding of pathogen challenge – host – nutrition interactions. To achieve this, three kinds of problems were considered in this review. The extent of reductions in growth independent of those caused by reduced voluntary food intake was assessed using pair-feeding and marginal response experiments. Data that permitted further characterisation of changes in requirements during pathogen challenges were then considered. The final part problem considered was the partitioning of scarce protein and energy between maintenance, growth and immune functions.

Sandberg *et al.* (2005*a,b*) described solutions to the partitioning of scarce resources in healthy animals. A solution which predicted the marginal response in protein retention, *PR* g/d, to ideal protein intake from the energy to protein ratio of the food (Kyriazakis & Emmans, 1992ab) agreed well with literature data. This solution coupled with protein (Moughan, 2003) and an energy (Emmans, 1994) system allows the prediction of actual growth as rates of *PR* and lipid retention, *LR* g/d. To predict growth during health, sub-clinical and clinical disease it is necessary to test for consistency with the components of the above framework, and identify any additional components, which apply during pathogen challenges.

Previous reviewers have considered one or several effects of pathogen challenges on voluntary food intake, resource requirements or partitioning in relation to the ability of an animal to cope with a challenge. Reviews have been from the perspectives of animal production (van Houtert & Sykes, 1996; Coop & Kyriazakis, 1999, 2001; Koutsos & Klasing, 2001), human medicine (Powanda, 1977; Powanda & Beisel, 2003) and evolutionary ecology (Sheldon & Verhulst, 1996; Lochmiller & Deerenberg, 2000). The overall problem considered in all of these fields was how pathogen challenges (kind, dose and virulence) affect the level of disease and rate of growth of an animal, when given a certain kind of food. The approach taken here is different, in that it considers the consequences of pathogen challenges that need including in a predictive framework. The requirements and partitioning of both protein (amino acids) and energy were considered for different pathogens and hosts. The critical analysis of literature evidence combines to

provide a description of the overall problem of reduced performance during pathogen challenges, and may provide a starting point for predictive and experimental solutions.

4.3 Accounting for reductions in growth during pathogen challenges

The following conceptual equation (e.g. Parks 1982) provides a starting point for accounting for reductions in rates of growth, *dW/dt,* during different pathogen challenges, which is the problem considered here:

$$dW/dt = e_w \cdot (VFI - m.W) \quad (1)$$

where e_w is the net efficiency of using a food for live weight gain, *VFI* is the voluntary food intake and *m* represents the multiplier of live weight, *W*, to determine requirements for maintenance. Reductions in *VFI* (Sandberg *et al.* 2006) or efficiency of resource use (Sykes, 2000), or, increased maintenance requirements (Black *et al.* 1999; Houdijk *et al.* 2001) could lead to reductions of *dW/dt*. Reductions in growth could also occur due to a combination of these factors. Further 'marginal response' relationships have also been proposed, which relate to specific resources such as protein or energy intakes to *PR* and *LR* e.g. Kyriazakis & Emmans (1992ab) and Black *et al.* (1986). The problem was to identify which components of these relationships may be affected during pathogen challenges.

To assess the effect of pathogen challenges on components other than *VFI* in Equation 1 (and equivalent parameters for other marginal responses) data from pair feeding and marginal response experiments were first considered. The aim was to identify the extent, and the mechanism(s), by which growth was reduced during pathogen challenges. When considering marginal response experiments it seemed necessary to consider also the effects of pathogen challenges on resource digestibility and protein quality (biological value). Other kinds of evidence for quantifying the causes of reductions in growth beyond those caused by a reduction in food intake such as repair of damage, cost of mounting an immune response and fever were then considered.

4.3.1 The characteristics of reductions in growth during pathogen challenges

4.3.1.1 Responses in growth during pair-feeding

To compare rates of growth of an uninfected and a challenged animal a pair-feeding method can be used to eliminate differences in growth due to differences in *VFI*. One of the main effects of exposure to pathogens is a reduction in VFI (pathogen induced anorexia, Kyriazakis *et al.* 1998). The healthy control is given the same amount of food as the challenged animal, at a time, but for practical reasons often with at least one day's delay. In principle, their rates of growth can then be compared to quantify reductions in growth that are not associated with reduced *VFI* i.e. changes in digestibility or biological value, requirements or partitioning.

4.3.1.1.1 Parasitic challenges. Experiments with abomasal parasites such as *Teladorsagia (Ostertagia) circumcincta* and *Ostertagia ostertagi* have shown that challenged animals grew slower than their pair fed controls (Sykes & Coop, 1977; Fox *et al.* 1989). The average live weight gains of sheep challenged with *Teladorsagia circumcincta* were 0.79 of their pair fed controls (Sykes & Coop, 1977). Nitrogen balances performed at wks 2-3, 7-8 and 12-13 post infection demonstrated time dependent effects on the nitrogen balance: live weight gains for the challenged sheep for weeks 2-8 were 0.66 of the pair-fed controls. Reductions in growth in naïve hosts are related to the time course of an infection, and may reflect the acquisition and expression of immunity (Coop & Kyriazakis, 1999).

Mansour *et al.* (1991, 1992) challenged calves with either a single, or a trickle dose of the abomasal parasite *Ostertagia ostertagi* and found no difference in average growth rate between challenged calves and their pair-fed controls. The observation may have been due to the size of the challenge doses: the single dose was 609 larvae/kg BW and the trickle dose was 7 larvae/kg BW/d. Fox *et al.* (1989) using the same type of parasite, but a larger trickle dose (98 larvae/kg BW/d) found differences in growth rate between challenged calves and pair-fed controls. The effects of abomasal parasites on growth, beyond effects on *VFI*, depended on both the level of challenge and the stage of infection.

The immunological state of an animal i.e. whether it is naïve to a pathogen, or whether it has acquired immunity needs to be accounted for when comparing rates of growth of challenged and healthy animals. Takhar & Farrell (1979b) found no differences in growth rate between immune chicks (data indicated increased *FI*) and healthy pair-fed controls when re-challenged with *Eimeria acervulina* or *Eimeria tenella*. There were, however, clear differences in growth between pair-fed controls and immunologically naïve chicks.

Immune animals may cope with larger challenge doses compared to immunologically naïve animals before effects on growth occur.

Kimambo *et al.* (1988) challenged sheep with *Trichostrongylus colubriformis* (a parasite of the proximal small intestine) and found the average *dW/dt* of the challenged sheep to be 0.11 and 0.83 of the pair-fed controls between weeks 6-13 and 13-20, respectively. As indicated by faecal egg counts and blood eosinophil counts the sheep were starting to express immunity somewhere during weeks 6-13. During weeks 13-20 the sheep were fully immune with faecal egg counts reduced to zero, but as this was a continuous challenge (2500 larvae per day) blood eosinophil remained elevated. The 17% reduction in growth (wks 13-20) suggested a cost of expressing immunity. The findings of MacRae *et al.* (1979) are in quantitative agreement, and those of Symons & Jones (1975), Poppi *et al.* (1986) and Datta *et al.* (1998) agree qualitatively.

Pair-feeding experiments with parasites other than those affecting the stomach and small intestine have also shown reductions in growth beyond those caused by reductions in food intake. The liver parasite *Fasciola hepatica* given at a low dose (3 *metacercariae* per host) did not cause any significant differences in the rate of growth or body composition between parasitised sheep and their pair-fed controls (Sykes *et al.* 1980). At higher doses (8 and 14 *metacercariae*), the challenge did have effects on growth compared to pair-fed controls. These findings are in support of those for abomasal parasites where reductions in growth, when compared to pair-fed controls, depended on the level of challenge.

Akinbamijo *et al.* (1997) found that calves of two breeds (N'Dama and Gobra zebu bulls) challenged with a blood borne parasite (*Trypanosoma congolense*) grew slower than their pair-fed controls, but the two breeds were not affected to the same extent. Abbott *et al.* (1985) also found effects of genotype (Finn Dorset vs Scottish Blackface sheep) and nutrition (high or low protein) on differences in growth rate between sheep challenged with *Haemonchus contortus* and their pair-fed controls. In the case of the effect of nutrition, the lower reduction in growth seen for the higher protein diet would have been associated with the extent of damage. The effect of host genotype is more difficult to explain. It may be the outcome of greater genetic resistance, an outcome of damage affecting a genotype to a lesser extent than others, or the outcome of genotypes used (growth potential and resistance) and the level of nutrition. A framework of growth during

pathogen challenges would need to distinguish between these factors causing reductions in growth.

4.3.1.1.2 Bacterial challenges. Experiments with intravenously administered bacteria (Ruot *et al.* 2000; Papet *et al.* 2002), lipopolysaccharide, *LPS*, (Steiger *et al.* 1999), and local (nasal or oral) bacterial (Wannemacher *et al.* 1971; Powanda *et al.* 1971; Wannemacher *et al.* 1974), or, bacterial antigen challenges (Klasing *et al.* 1987; Arnold *et al.* 1989) have shown reductions in growth that were not associated with reduced *VFI*. Challenges with *LPS*, while not a 'true' bacterial challenge, stimulate a similar kind of response, but of smaller and shorter magnitude, to that of bacterial challenges. As for macro-parasites these effects were dose dependent since Hunter & Grimble (1997) and Raina *et al.* (2001) failed to demonstrate any reductions in growth that were not associated with reduced *VFI* in *LPS* challenged rats.

Klasing *et al.* (1987) challenged growing chicks with non-pathogenic antigens (sheep red blood cells or sephadex), bacterial antigens (*Escherichia coli* or *Salmonella typhimurium LPS*) or heat killed *Staphylococcus aureus*. Pair feeding was done four times per day to account for rapid effects of bacterial antigens on *VFI*. The relative growth rates (challenged growth rate/control growth rate) of chicks challenged with non-pathogenic antigens (0.90) and pathogenic antigens (0.83) were different. This may suggest that a host may respond in relation to the kind of pathogen challenging it (for a review see Baxter & Hodgkin, 2002). The more virulent pathogen may have stimulated a greater immune response, rather than causing more damage to the host and through a larger resource requirement of the greater immune responses had greater reductions in growth (see below).

4.3.1.1.3 Viral challenges. A smaller number of experiments were identified that considered the effects of viral challenges on growth in pair-fed animals compared with parasitic and bacterial models. Zijlstra *et al.* (1997) found that pigs (starting weight ≈ 1.5kg) infected with *rotavirus* grew slower than their pair-fed controls. The findings of Koyama *et al.* (1997) agree as rats challenged with *adenovirus* also grew slower than their pair-fed controls. Roberts & Almond (2003) found no difference between pigs challenged with *porcine respiratory reproductive syndrome* (*PRRS*) virus and *Mycoplasma hyopneumoniae* and their pair fed controls. This may have been due to the pathogen or dose used, or due to the nature in which the data was analyzed (average growth rate

calculated over several weeks). Several experiments have shown that reductions in growth beyond those caused by reduced *VFI* were variable over the time course of pathogen challenges.

The experiments considered here included a variety of pathogens and hosts. Naïve and immune animals responded differently, with little or no reductions in growth in immune animals. The extent of unaccounted reductions in growth in immunologically naïve animals during pathogen challenges were, as expected, both pathogen (kind and dose) and time dependent. The literature data suggested, however, that over a certain range of doses, reductions in *VFI* fully explained reductions in growth. Effects on growth, beyond reduced *VFI*, depended on both host genotype and nutrition. To assign mechanisms for these effects on growth, marginal responses are now considered.

4.3.1.2 Responses in growth to resource intake

Marginal response experiments determine the amount of food (or resource) that is required for an animal to maintain component weight; thereafter rates of growth increase along the margin until a maximum response is achieved. In Equation 1 weight gain is made a function of *VFI*. It may be better to consider the response to a specific resource (either protein or energy), as it may allow for a better accounting of the effects of pathogen challenges. The relationship between protein retention, *PR* g/d, and ideal protein intake, *IP* kg/d is a typical example of a marginal response. *IP* is the product of *VFI*, the crude protein content of the food *CPC* (kg/kg), its true ileal digestibility, d_{CP}, and the biological value of the protein (see below), v:

$$PR = e_p.((VFI.CPC.d_{CP}.v) - MP) \qquad (2)$$

where e_p is the marginal response of *PR* to *IP*, above maintenance, *MP* kg/d, and the relationship applies when $PR < PR_{max}$ g/d, the animals' maximum genetic potential for *PR*. Both energy supplies in relation to protein intakes (reviewed by Sandberg *et al.* 2005b) and stressors (Wellock *et al.* 2003*b*) may prevent the attainment of PR_{max}. To try to identify how reductions in growth occur during pathogen challenges, food digestibility, biological value and marginal responses (including PR_{max}, *MP* and e_p) are now considered in turn.

4.3.1.2.1 The digestibility of food during pathogen challenges. The amount of protein or energy that is available to an animal is the outcome of the amount of organic matter entering the gastrointestinal tract and its true digestibility. A pathogen challenge may affect the ability of a host to break down and absorb organic matter causing a reduction in available protein and energy (Turk, 1972). The literature is not consistent, however, on whether there are effects on true or apparent digestibility (Sykes & Greer, 2002). For this purpose it is important to consider the main site of infection of a particular pathogen. A pathogen that causes damage to the gastrointestinal tract could affect the ability of the host to digest organic matter components of a food. On the other hand, pathogens, which do not affect the gastrointestinal tract, would not be expected to affect either digestion or absorption (Klasing & Barnes, 1988).

The apparent digestibility of protein was reduced during pathogen challenges with parasitic worms of the stomach, small and large intestine in pigs (Hale, 1986). The reduction in apparent digestibility was smaller for pathogens that affected mainly the stomach, compared to parasites of the small and large intestine (Hale, 1986). The additional nitrogen excreted in the faeces, however, may have been associated with nitrogen leakage due to tissue damage and other endogenous nitrogen containing secretions e.g. mucin or plasma, and not due to a reduction of organic matter digestibility. Pathogen challenges affecting the small intestine or parts further down of the gastrointestinal tract may result in damaged (or leaked) tissue not being digested and reabsorbed, while endogenous nitrogen leaving the stomach may be fully or partly re-absorbed in the small intestine. The observation that parasites of the small intestine (*Trichostrongylus colubriformis*: Kimambo *et al.* 1988) causing greater effects on growth, beyond those associated with reduced *VFI* when compared with parasites of the stomach (*Teladorsagia circumcincta:* Sykes & Coop, 1977) is in support of this.

The true digestibility of organic matter during pathogen challenges has also been estimated. Symons & Jones (1970) estimated the true digestibility of protein using radioactively labelled protein in monogastrics (mouse and rat) and ruminants (sheep). They found no effect of large single doses with either one of three different gastrointestinal nematodes (*Nematospiroides dubius*, *Nippostrongylus brasiliensis* and *Trichostrongylus colubriformis*) on the true digestibility of protein. Poppi *et al.* (1986) using duodenal and ileal cannulas in sheep continuously infected with a sub-clinical trickle dose of *Trichostrongylus colubriformis* found a reduction in apparent digestibility,

but no effect on the true digestibility of nitrogen. Sykes & Greer (2003) considering further evidence for sheep are in agreement.

At low to moderate level challenges of the gastrointestinal tract, it appears that the true digestibility of food resources is not affected. It is likely that any effects on the ability of the host to digest organic matter will depend on the extent of damage caused by the pathogen. Damage incurred by a host increases with increasing levels (doses) of a challenge (Symons *et al.* 1981) and the virulence (Turk, 1972) of a pathogen. It may be necessary to identify the doses and virulence at which true digestibility and absorption could be affected. This would allow the important distinction to be made between pathogen challenges that cause either only damage, or, damage and reductions in true organic matter digestibility.

4.3.1.2.2 The ideal amino acid composition of food protein during pathogen challenges. Once reductions in *VFI* and any effects on the digestibility of food resources have been taken into account, the biological value (or idealness) is the final measure of protein (amino acid) supply. The biological value (denoted by v in Equation 2) is calculated from the ratio of the first limiting amino acid (*AA*) in the food to that in a reference protein e.g. pigs whole body protein. The aim of this section was to determine how immune proteins differed in their amino acid contents, *AAC* gAA/kg CP, to that of a 'reference protein' used for healthy animals, as v is an important measure of protein supply. The ratio of essential to non-essential *AA's*, becomes particularly relevant for low protein foods (Deschepper & de Groote, 1995), as the total amount of non-essential *AA's* may become limiting.

Animals may produce significant amounts of certain nitrogenous compounds such as cytokines, antibodies, acute phase proteins or specific immune cells during pathogen challenges. Beisel (1977) and Wannemacher (1977), amongst others, have proposed that such immune responses lead to specific requirements for certain amino acids. Immune proteins and cells have been found to contain high proportions of certain *AA's* (Reeds *et al.* 1996). Immune cells may also have high requirements for certain essential e.g. sulphur *AA* (Grimble & Grimble, 1998) and non-essential e.g. glutamine and arginine (Newsholme, 2001; Field *et al.* 2002) *AA's*. Table 1 summarises the *AAC* of acute phase proteins (Reeds *et al.* 1996), other immune proteins (Houdijk & Athanasiadou, 2003),

colostrum and milk (Csapo'-Kiss *et al.* 1995) and a reference protein (whole body protein of pigs; Kyriazakis *et al.* 1993).

The comparison of colostrum and milk is included as colostrum contains a large proportion of maternal antibodies. There are some marked differences between the different kinds of proteins in terms of their *AAC*. The proteins considered in Table 1 included proteins part of the innate immune responses (acute phase proteins) and proteins normally associated with expression of acquired immunity (immunoglobulins).

Table 1 A summary of the amino acid composition of different proteins that are associated with the immune response, in relation to the reference protein that is normally used for calculating the biological value of food protein. The amino acid composition of colostrum (a source rich in immune proteins), milk and reference protein (whole body protein of pigs) are also shown for comparison.

	Amino acid composition (g/kg protein)[1]												
	Pig protein	Milk	Colostrum	APP1[3]	APP2[3]	APP3[3]	APP4[3]	APP5[3]	APP6[3]	IgA	IgE	Sheep MCP	Mucin
Phenylalanine	38	18	24	105	46	64	83	30	103	26	30	29	15
Tyrosine	25.8	9	22	50	56	74	27	70	67	24	43	29	13
Tryptophan	7.9	-	-	42	35	30	11	32	45	26	20	8	-
Leucine	74.4	11	59	91	62	101	124	82	29	120	87	90	31
Isoleucine	35.0	5	29	54	32	48	49	47	29	13	39	69	15
Valine	46.9	54	145	77	48	46	59	84	18	96	80	73	222
Lysine	70.5	28	99	71	77	75	92	92	33	39	57	53	18
Histidine	28.1	21	100	16	27	17	37	38	35	11	18	29	-
Metionine	17.5	1	6	16	32	11	28	16	22	6	7	33	-
Cysteine	10.3	2	8	13	15	18	6	24	0	39	28	24	85
Threonine	38.2	115	46	58	60	74	66	54	30	75	103	45	224
Arginine	67.0	6	25	36	84	52	23	28	116	32	39	57	16
Proline	71.0	52	39	44	48	34	41	44	34	75	67	49	83
Glycine	91.4	32	63	46	59	19	33	44	61	69	52	86	49

Serine	40.1	288	88	84	91	31	49	40	47	126	103	86	65
Alanine	64.5	21	58	31	29	36	43	54	106	56	46	69	42
ASX[2]	86.2	19	46	82	113	102	106	113	128	75	82	57	56
GLX[2]	136.5	318	145	112	119	173	136	115	87	94	93	90	64

[1]Amino acid composition of whole body pig protein from Kyriazakis *et al.* (1993), acute phase proteins from Reeds *et al.* (1994) and for IgA, IgE, sheep mast cell proteases (MCP) and mucin were taken from Houdijk & Athanasiadou (2003)

[2]ASX – Asparagine + Aspartate

[2]GLX – Glutamine + glutamate

[3]APP(X) represents six different acute phase proteins: (1) C-reactive protein, (2) Fibrinogen, (3) alpha-1-glycoprotein, (4) alpha-1-antitrypsin, (5) Haptoglobin and (6) Amyloid A

The *AAC* of acute phase proteins, *APP's*, was calculated as the average of 6 acute phase proteins (C-reactive protein, fibrinogen, alpha-1-glycoprotein, alpha-1-antitrypsin, haptoglobin and Amyloid A) and compared to the reference protein. Large differences were noted (*AAC* of *APP's* vs *AAC* reference protein) for phenylalanine (72 vs 38g aa/kg CP), tyrosine (57 vs 25g aa/kgCP), tryptophan (32 vs 8g aa/kgCP) and threonine (57 vs 38g aa/kgCP). The largest differences between the average of the two immunoglobulins and the reference protein were tryptophan (23 vs 8g aa/kgCP), valine (88 vs 47g aa/kgCP), cysteine (34 vs 10g aa/kgCP) and threonine (89 vs 38g aa/kgCP). Serine, a non-essential *AA*, was present at greater concentrations in both kinds of immune proteins, and especially in immunoglobulins (115 vs 40g aa/kgCP), and McRae (1993) has discussed its potential importance during pathogen challenges.

There were very large differences for the amino acid contents of immune proteins to that taken to be the normal reference amino acid content. The important issue, however, is whether the total requirement for 'ideal protein' for potential growth, PR_{max}/e_p, and that for making immune proteins, IMP/e_p g/d, and their amino contents lead to a continuously changing reference protein in challenged animals. It is assumed that PR_{max} is the animal's genetic maximum for protein retention and that *IMP* is the amount of immune protein produced at a particular point in time. It is also assumed that e_p is the same for both challenged and healthy animals. During health, v is taken as a constant for a given kind of food and reference protein. As the value of *IMP* changes over the time course of an infection, it may be necessary to calculate v by weighting the requirements and *AAC* of the protein requirements for growth and immune responses:

$$v = ((((PR_{max}/e_p).AAC_{growth}) + (IMP/e_p).AAC_{im})) / ((PR_{max} + IMP)/e_p)) / AAC_{food} \qquad (3)$$

where AAC_{growth} is the normal reference protein, AAC_{im} is the *AAC* of the immune proteins and the content of the food protein is AAC_{food}. The potential protein retention (PR_{max}) is used in Equation 3 as it is necessary to estimate v from potential protein requirements before predicting the partitioning of ideal protein towards actual *PR*. As PR_{max} (due to host state) and *IMP* (due to stage of infection and level of challenge) change over time it

would be necessary to perform the above calculation on a time step basis. Modelling simulations would provide a more robust test of whether the ideal protein concept needs replacing (or revising) during pathogen challenges by taking into account changes in requirements for individual amino acids for both growth and immune functions.

4.3.1.2.3 Effects of pathogen challenges on the upper limit for growth. It is reasonable to assume that an animal has an upper limit for growth, which is defined by its genotype and state e.g. Emmans & Fisher (1986). In marginal response experiments, it is assumed that this limit is achieved when no further response is observed to additional levels of resource intake: the linear-plateau concept. During pathogen challenges it would appear that challenged animals have not achieved the same upper limit for growth as their healthy controls (Willis & Baker, 1981*a,b*; Williams *et al.* 1997*a,b,c*; Webel *et al.* 1998a). The authors' conclusion was that the 'potential upper limit' for growth was reduced.

Webel *et al.* (1998a) found a lower plateau (maximum) for *PR* for *LPS* challenged chicks than their healthy controls, while Willis & Baker (1981ab) found that chicks challenged with *Eimeria acervulina* had a lower plateau for average daily body weight gain. The reduction in PR_{max} was estimated roughly to be 25% from Williams *et al.* (1997*a,b,c*) for pigs in a high immune stimulation environment, while Webel *et al.* (1998*a,b*) found a reduction of 11% for the response to lysine, but not for threonine or arginine in chicks. A plateau, however, may also arise due to insufficient amounts of energy in relation to protein, or due to exposure to a non-infectious stressor (Kyriazakis & Emmans, 1992b; Wellock *et al.* 2003*b*).

Kyriazakis & Emmans (1992b) showed that a reduction in the marginal efficiency for growth produces a plateau response, and that the marginal efficiency depended on the energy to protein ratio of the food. Williams *et al.* (1997*a,b,c*) used foods with different energy to protein ratios, and the highest level of protein, which was the only level that yielded a plateau response, had an energy to protein ratio close to the critical value for a reduction in efficiency to occur (Sandberg *et al.* 2005b). The findings of Webel *et al.* (1998*a,b*) and Williams *et al.* (1997abc) do not, as some authors have assumed e.g.

Escobar *et al.* (2004), necessarily show that an animal's upper limit for growth is reduced during pathogen challenges.

A reduction in appetite (anorexia) could also result in the kind of responses that have been observed for marginal responses. The experiments of Willis & Baker (1981*a,b*), Williams *et al.* (1997*a,b,c*) and Webel *et al.* (1998a) were performed over a defined time, rather than weight range. Effects of pathogen challenges on growth are often transient, with an initial drop in growth rate, after which the animals recover as shown in the example from Hein (1968) in Figure 1.

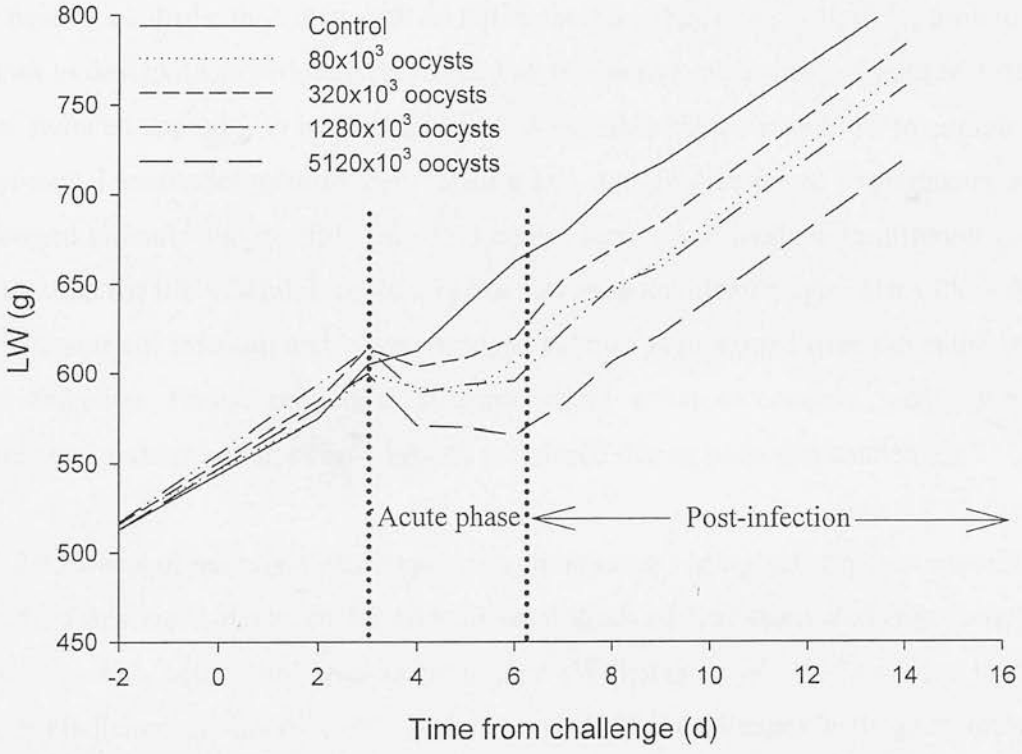

Figure 1 The effect of different single challenge doses of *Eimeria acervulina* on the live weight, *LW* g, of chicks over the time course of an experiment, including the acute- and post-infection phase (Hein, 1968).

The transient drop (2 – 3 days long) in growth rate shown in Figure 1 dictated the extent to which the healthy and challenged animals differed in size after they had overcome the

infection (post-infection). The extent of the difference depended on the dose of oocysts of *Eimeria acervulina*. As the challenged animals were smaller post infection they would also have a smaller upper limit for growth at a time, compared with healthy controls. In the marginal response experiments that were mentioned earlier, calculations of average growth rates included post-infection growth rates. This would bias the average growth rate of challenged animals downwards, but the difference would be detectable at only higher protein intakes.

Bown *et al.* (1991) found that sheep given abomasal infusions with nitrogen, while challenged with a gastrointestinal parasite achieved the same level of nitrogen retention as their healthy controls; thus their data do not support a reduced upper limit for growth. It is difficult to design an experiment that would allow the two mechanisms (reduced potential versus reduced appetite) to be distinguished. A possible solution could be to combine the experimental methodologies of pair feeding and marginal response experiments where challenged animals and healthy (pair fed) controls are given foods with different protein contents. On the other hand, it could be possible to use an infusion approach with different levels of nutrient infusion and where nitrogen balance is measured over the entire period of an infection. Future modelling attempts, using either mechanism, may contribute towards our understanding of how growth is reduced during pathogen challenges.

4.3.1.2.4 Effects of pathogen challenges on maintenance. Marginal response experiments to address this were identified for five different kinds of 'pathogen challenges'. A 'high immune system activation' treatment of pigs (Williams *et al.* 1997*a,b,c*), a bacterial antigen challenge of chicks (*LPS*, Webel *et al.* 1998), challenges with gastrointestinal parasites of chicks (*Eimeria acervulina*, Willis & Baker 1981*a,b*) and sheep (*Trichostrongylus colubriformis*, Poppi *et al.* 1986, Datta *et al.* 1998) and a challenge of pigs with blood borne parasites (*Trypanosoma vivax*, Fagbemi *et al.* 1990; Otesile *et al.* 1991). Data were analyzed using either linear or continuous linear plateau regressions.

The experiments of Williams *et al.* (1997*a,b,c*) and Webel *et al.* (1998*a,b*) were the only experiments found in the literature, which have measured *PR* in relation to different levels

of protein or amino acid intake during a 'challenge'. In the experiments of Williams *et al.* (1997*a,b,c*), foods with different crude protein contents were used (designed with lysine as the first limiting amino acid). Webel *et al.* (1998*a,b*) formulated diets to provide different levels of lysine, threonine or arginine. The response in *PR* to lysine intake is shown in Figure 2, for chicks challenged with *LPS*, with a continuous linear plateau model (described in Sandberg *et al.* 2005b) fitted to the data.

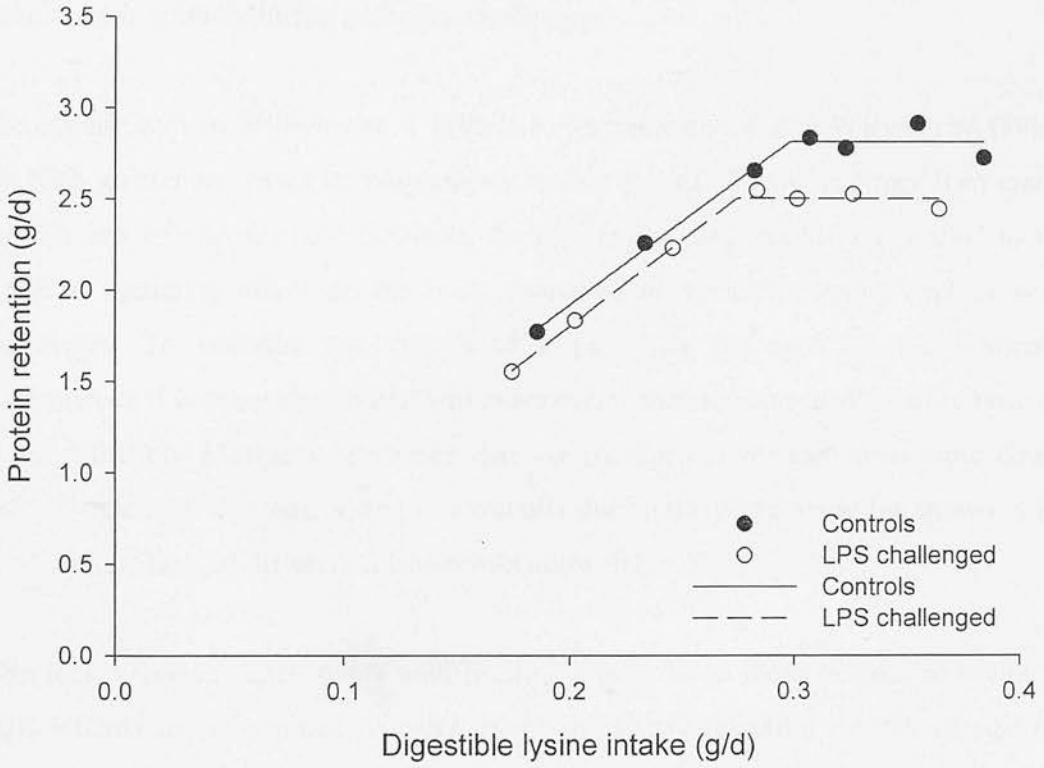

Figure 2 The response in protein retention (g/d) to digestible lysine intake (g/d) of chicks challenged with LPS on every second day over an 11 day period, in relation to their unchallenged controls (data from Webel *et al.* 1998a). A continuous-linear-plateau model (described in Sandberg *et al.* 2005b) was fitted to the data.

The marginal response in Figure 2 was not different, while maintenance increased slightly, and the challenged animals did not achieve the same maximum rate of *PR*, as discussed earlier. The response varied with different amino acids ranging from a 1 to 1.3 times increase of healthy maintenance. Webel *et al.* (1998*a,b*) found that chicks supplemented with lysine had an increased maintenance, while those with threonine

tended towards an increase, with no effect for arginine. Different effects on maintenance may suggest that amino acid requirements were affected to different extents. LPS stimulates an acute phase response, including the production of acute phase proteins, which have different amino acid contents to body protein retained in the healthy animal (see Table 1). The experiments of Webel *et al.* (1998*a,b*) also suggest that it may be necessary to consider the requirements of individual amino acids to fully account for reductions in growth during pathogen challenges.

The experiments of Williams *et al.* (1997*a,b,c*) agree with those of Webel *et al.* (1998*a,b*), but with greater increases in maintenance in two cases (2.9 and 1.6 times their controls). In both sets of experiments, however, the challenges used would be expected to have a small to moderate effect on the host, compared to actual bacterial, viral or parasitic challenges. To describe the effects of a particular pathogen on requirements for maintenance it is necessary to perform experiments that account for the entire time course of an infection. Marginal responses that are measured over the entire time course of defined pathogen challenges, and in particular during the acute phase (as shown in Figure 1) of an infection, at different doses would allow this.

Marginal response experiments with limiting energy have shown increased maintenance requirements as determined by regressions of energy retention against energy intake, Figure 3. West African Dwarf goats challenged by *Trypanosoma vivax* (van Dam, 1996) had approximately 1.2 times greater maintenance requirements than their healthy counterparts.

Experiments with pigs are in further support of an increase in maintenance requirements for energy (Fagbemi *et al.* 1990; Otesile *et al.* 1991). In these experiments the pigs were challenged with the same dose of *Trypanosoma brucei* at two different live weights. The 10kg pigs (Fagbemi *et al.* 1991) had a greater increase in energy maintenance requirements (2.2 times healthy controls) than 100kg pigs (Otesile *et al.* 1991), which had maintenance requirements 1.7 times of healthy controls. This suggests that animals of

different sizes may have different changes in requirements at a challenge dose, but the issue that remains is how to relate pathogen challenges to size.

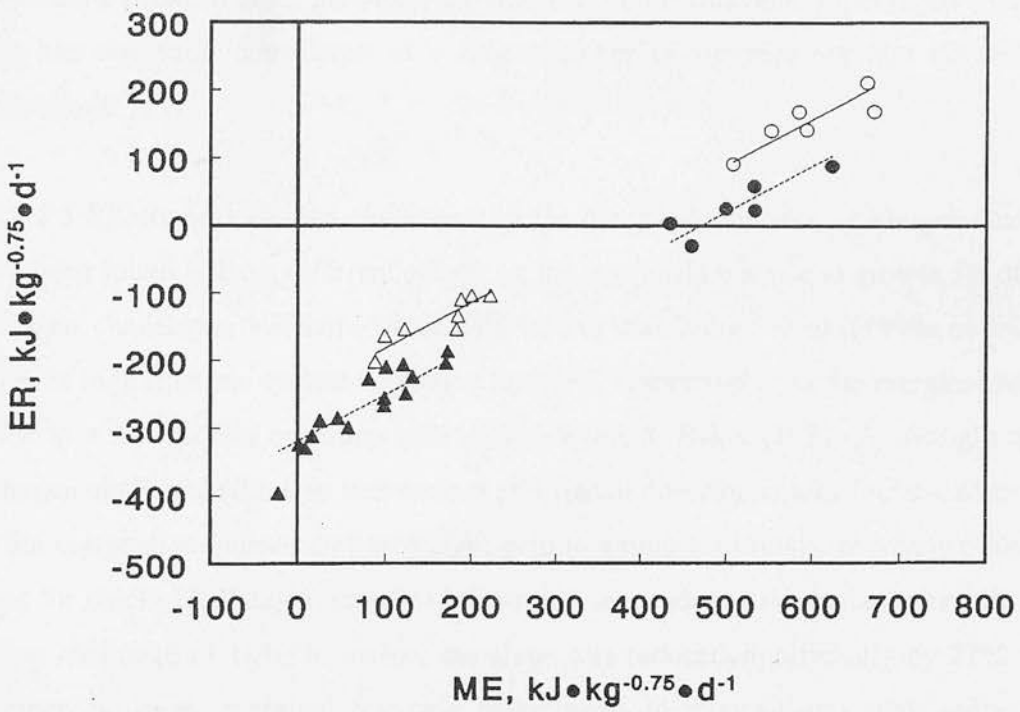

Figure 3 The marginal response in energy retention (ER) to metabolisable energy intake (ME) for West African Dwarf goats challenged with *Trypanosoma vivax*; figure reproduced from van Dam (1996) with permission. Regressions fitted with a common slope; open symbols are the non-infected, and closed symbols are the infected goats: the circles and triangles make the distinction between two different quality (low and high resource content) foods.

The effects of different challenge doses on hosts of different sizes need to be accounted for in both predictive frameworks and in the design of experiments. The issue is in two parts: 1) what is the relevant measure of animal size when quantifying the effects of pathogen challenges; 2) is it necessary to take into account, not only animal size, but also an animal's degree of maturity (or age) through some scaling rule? No clear data exists which allows for a choice to be made between body weight, protein weight and protein weight combined with a measure of fatness, as a measure of size. The second issue is even

more difficult, and experiments that provide sufficient data to test it are needed. Satisfactory quantification of a pathogen challenge becomes crucial when considering interactions between dose, genotype and nutrition on the outcome of pathogen challenges. This has not been considered in a large number of experiments that address these interactions.

4.3.1.2.5 Effects of pathogen challenges on the marginal response. Pathogen challenges have been found to have different effects on the marginal response in growth for different pathogen challenges. Williams *et al.* (1997*a,b,c*) and Webel *et al.* (1998*a,b*) found no effect of high immune system activation and *LPS*, respectively, on the marginal response in *PR* to either protein or amino acid intake. Willis & Baker (1981*a,b*), using a specific pathogen challenge (*Eimeria acervulina*) at different doses on chicks found a slight effect on the marginal responses in live weight gain to amino acid intake at a larger dose. The slope for chicks challenged with 2x10^5 oocytes was reduced numerically by 10%, while with a challenge of 1x10^6 oocytes the slope was reduced significantly by 22%. Taken together, however, marginal response experiments in monogastrics may suggest little effect on the marginal material efficiency of growth.

Experiments with sheep where either live weight gain or nitrogen retention was regressed against *FI* suggested an effect on the marginal response (Poppi *et al.* 1986; Datta *et al.* 1998). The marginal response of the sheep was not affected during the initial weeks of a continuous challenge. Around the time when the sheep likely had the greatest worm burdens (pathogen load) and just prior to starting to express immunity, the slope was reduced and the intercept displaced, indicating a reduced marginal response and an increase in maintenance. The marginal response was no different to that of their controls once the animals had acquired immunity, and, overcame the challenge (Poppi *et al.* 1986; Datta *et al.* 1998).

The time dependent effect on the slope as suggested by Poppi et al. (1986) appears to coincide with the accumulation of the worm burden for that pathogen e.g. van Houtert *et al.* (1995). These time (pathogen load) dependent effects on the marginal response may

explain why Webel *et al.* (1998*a,b*) and Williams *et al.* (1997*a,b,c*) did not find any effects on the slope. *LPS* challenges have short-term effects, and Williams *et al.* (1997*a,b,c*) used an undefined 'high immune system activation'. To predict the effects of pathogen challenges on marginal responses in growth to resource intake it may be necessary to account for the kind and dose of pathogen together with the development of a challenge over time. The effect of pathogen challenges on the marginal response in growth, however, would appear to be small in magnitude.

4.3.2 The causes of increased requirements during pathogen challenges

Pathogen challenges cause increased requirements for both protein and energy, and these appear to be variable for different kinds and doses of pathogens. In addition to marginal response studies, it may be possible to quantify the increased requirements by considering other kinds of evidence. Such evidence provides estimates of the protein and energy costs of specific consequences of pathogen challenges. The quantitatively more important consequences of pathogen challenges include mounting an immune response, repairing damaged tissues and replacing lost fluids, and mounting a fever. In principle, such evidence may be combined with that considered in earlier sections to provide a clearer description of the nutritional costs that are associated with pathogen challenges.

4.3.2.1 The protein cost of an immune response

Attempts have been made to quantify the specific protein cost of mounting an immune response either by trying to sum up the masses of immune proteins (Klasing & Calvert, 1999; Houdijk *et al.* 2001), or through direct experimentation (Pilorz *et al.* 2005). It is difficult to derive such a cost, as the immune response consists of several different components, many of which are expressed differently over the time course of a pathogen challenge. Hosts may respond with innate or acquired immunity, or a combination of these, depending on the level of challenge and the stage of a particular infection (Heegard *et al.* 1998; Klasing, 1998; Taylor-Robinson, 2000). An approximation of the protein cost of mounting an immune response, however, could provide an initial estimate that could be included in a predictive framework of resource requirements and partitioning during pathogen challenges.

Klasing & Calvert (1999) attempted to sum up the amounts of different immune proteins of innate and acquired immune functions to estimate the cost (in terms of lysine) of mounting an immune response. The lysine cost for the immune response was equivalent to a reduction in growth of 0.031g ADG/kgBW/d: this would approximate to 0.0015g of protein (0.031*0.3*0.16) for a 0.3kg chick. Klasing *et al.* (1987) challenged chicks with *LPS* and found a difference in body growth rate of 31.5 – 25.8 g/chick/d = 5.7 g/d compared to pair-fed controls. In part, some of this reduction in growth rate would have been associated with the cost of mounting a fever as reported by the authors. Their finding does suggest a larger cost for producing immune proteins (0.16*5.7 = 0.9 g/d) than that predicted by Klasing & Calvert (1999). The kind of host examined may also be important. Kreukniet & van der Zijpp (1989) found that chicks selected for a higher peak antibody response to sheep red blood cells also produced greater amounts of antibody at any dose compared to non selected chicks.

The marginal response experiment of Webel *et al.* (1998ab) suggested that chicks challenged by *LPS* had an increase in maintenance requirements for certain amino acids. Their challenge would have mainly stimulated an innate immune response e.g. the production of acute phase proteins. *LPS* challenges would not result in a cost of damage to the host, as may be the case for 'real' bacterial challenges (Wannemacher *et al.* 1971; Powanda *et al.* 1971; Wannemacher *et al.* 1974). Thus, the increase of 1.3 times the maintenance requirement of the healthy animal (Webel *et al.* 1998b) is indicative of the cost of an innate immune response. The ideal protein requirement for maintenance, *MP* g/d, for a healthy 0.3kg chick could be estimated as 0.26 g/d from the equations of Emmans & Fisher (1986). A 1.3 times increase in maintenance would then lead to the average cost for mounting an innate immune response to be estimated as 0.08 g/d of ideal protein (or 0.27g ideal protein/kg BW), assuming an efficiency of 1. This cost is sufficiently large to warrant inclusion in a framework of protein (amino acid) requirements and partitioning.

Houdijk *et al.* (2001) attempted to estimate the metabolisable protein cost for sheep expressing immunity to a gastrointestinal parasite. The sum of increased cell flow in lymph, flow of IgA, production of mucosal mast cells and sheep mast cell proteases was 52.8 mg/kgBW$^{0.75}$/d: this would be 0.02 g of metabolisable protein for a 0.3kg animal, or 0.07gMP/kg. Scrimshaw (1991) based on nitrogen balance experiments of humans proposed much greater requirements for protein which included all consequences of a pathogen challenge. An average estimate for infections ranging in severity was proposed as 0.57CP/kg/d, which could rise to 1.2gCP/kg/d for more severe infections. The approach taken by Houdijk *et al.* (2001) and Klasing & Calvert (1999) were restricted by it effectively being impossible to sum up the production of all immune proteins during a pathogen challenge. The underestimation may occur by not accounting for all immune proteins, not accounting for greater requirements for certain amino acids than others, through an effect on protein (amino acid) partitioning, or a combination of these.

There is other evidence in favour of significant requirements for mounting an immune response. A series of experiments were performed at the United States Army Medical Research Institute of Infectious diseases e.g. Powanda *et al.* (1972), Thompson *et al.* (1973), Wannemacher *et al.* (1971, 1974) and Berendt *et al.* (1977) using a rodent model. In all of their experiments, which mainly included bacterial challenges, large increases occurred in the uptake of certain radioactively labelled amino acids by the liver; this lead to a reduced uptake of amino acids by muscle. Assuming that the increased uptake is due to the production of immune proteins, Thompson *et al.* (1973) estimated that the amino acid uptake of the liver had increased by 385/258 = 1.38 times that of pair-fed controls. The increases coincided with drops of certain free amino acids in blood, which also appeared to coincide with the growth and decay of the pathogen load in the host (*Klebsiella pneumoniae*, Berendt *et al.* 1977). In further support of a host mounting an immune response that is proportional to its pathogen load is the experiment by Taylor-Robinson (2000) for *Plasmodium vivax*.

Experiments are warranted where the marginal response is measured using a suitable methodology (Batterham *et al.* 1990; Chung & Baker, 1992) at different time points of a

pathogen challenge for different amino acids. Such experiments may enhance our current estimates of the amino acid requirements during expression of both innate and acquired immune responses. It would also be necessary to measure the development of the pathogen challenge in the host, together with indicators of immune responses. While such experiments are complex and expensive, they may significantly contribute to the lacking evidence base for amino acid requirements during pathogen challenges.

4.3.2.2 Repair and replacement of damaged tissues and body fluids

Pathogens may cause damage to a host's tissues (e.g. gut wall) or specific cells (e.g. red blood cells) and cause body fluids to leave their natural compartments such as plasma leaking into the gastrointestinal tract (Abbott *et al.* 1985; Yu *et al.* 2000). The animal would need to repair such damage or replace lost fluids to maintain normal function, which is thus a direct cost to the animal (Berendt *et al.* 1977). It would be expected that such costs are larger than those associated with expression of immunity. The factors that may need describing fully to account for this cost include the type of pathogen, level of challenge and ability of the host to withstand a particular challenge.

Parasitic worms used in the experiments summarised by Hale (1985) affected the stomach (*Hyostrongylus rubidus*), small intestine (*Strongyloides ransomi*), large intestine (*Oesophagostomum. spp* and *Trichuris suis*) and the kidneys (*Stephanus dentatus*). In a number of cases, the reduction in nitrogen retention was explained by reduced nitrogen intake (due to anorexia) combined with increased nitrogen in the faeces. The additional nitrogen in the faeces would likely be associated with damaged tissues and endogenous secretions. These effects were noted to the greatest extent for challenges of the small and large intestine. The kidney parasite, at the dose used, caused a reduction in nitrogen retention that was only associated with the reduction in *FI*. Findings for gastrointestinal parasites in sheep (Sykes & Coop, 1977; Kimambo *et al.* 1988) agree that level of cost associated with damage depends on the location of the gut that is affected.

Literature data shows that the extent of the costs associated with damage is not only dependent on the pathogen species but also on level of challenge. Le Jambre (1995)

considered the relationship between blood loss and estimated worm numbers (pathogen load) of *Haemonchus contortus* in the abomasum of sheep. The pathogen causes blood loss by direct sucking of blood and from a short period of leaking once the parasite has detached. Le Jambre (1995) found that the relationship between blood loss and worm numbers was of an exponential relationship. This may indicate that a host which has already suffered a certain amount of damage may be less capable to deal with the pathogen challenge. Beer *et al.* (1974) also found that erythrocyte losses into the gastrointestinal tract of pigs challenged by three different levels of *Trichuris suis* were directly related to the level of challenge.

Powanda *et al.* (1975) measured the pathogen load of *Francisella tularensis* and the extent of liver damage in rats: the form of the relationship was the same as that found for *Haemonchus contortus* by Le Jambre (1995). Thus, the relationship between damage and pathogen load may be general for different pathogens, but quantitatively different. A challenge with *Actinobacillus pleuropneumoniae,* which causes lesions of the lungs, has also shown that during the early stages of infections lesions develop and once the animals reach slaughter these are much less abundant or absent (Magnusson *et al.* 1997; Black *et al.* 1999).

The extent of plasma loss has been estimated in a number of experiments over the time course of challenges of sheep with different doses of gastrointestinal parasites. Data of plasma loss from Steel *et al.* (1980) were used to estimate protein requirements associated with plasma loss for different doses of *Trichostrongylus colubriformis*. It was assumed that plasma contains 75g protein/litre (Houdijk *et al.* 2001). The protein requirement due to plasma loss was also directly related to the level of challenge, and the form of the response appears to be similar to how the pathogen load (or worm burden) develops in the host. Symons *et al.* (1981) estimated the plasma loss due to a challenge of sheep with *Teladorsagia circumcincta* was an approximation, as it is difficult to the estimate the extent to which plasma is reabsorbed. While the relationship between the different doses were not as clear as those observed for *Trichostrongylus colubriformis* by Steel *et al.* (1980), the total quantity of protein lost was quite similar. The average protein

requirement due to plasma loss was calculated as 9.6, 12.4 and 10.4 g protein/d for challenge doses of 12000, 37500 and 120000 of *Teladorsagia circumcincta* larvae, respectively. These are very significant costs to the animal.

Data from challenges with gastrointestinal parasites and bacteria show that the repair and replacement of damaged tissues and lost fluids may be a significant cost to the animal. Pathogen induced damage appears to be of a general kind for different kinds of pathogens, but clearly the extent of the damage, at a pathogen load, would depend on pathogen species and virulence (Willis & Baker, 1981a). Therefore, to predict the extent of damage it would be necessary to account for pathogen kind, dose and virulence. The ability of the host to overcome the infection and prevent damage to be caused would also need to be considered. The interactions between pathogen kind, host genetic resistance and nutrition (see below) on the extent of damage could then be explored.

4.3.2.3 Requirements for energy during pathogen challenges

It has been proposed that a pathogen challenge may cause increased requirements for energy due to the production of immune proteins (Demas *et al.* 1997), additional nitrogen processing (Sykes & Coop, 1977) and especially expression of fever (Baracos *et al.* 1984). In the framework considered here any energy costs associated with the production of immune proteins could be incorporated within the overall cost of protein production, while other costs would need accounting separately. The current quantitative evidence of these costs is now considered.

4.3.2.3.1 Non-pathogenic antigen challenges. It is difficult to estimate the energy cost that is associated with the production of only immune proteins. The model used by several authors e.g. Demas *et al.* (1997), Mashaly *et al.* (2000), Pilorz *et al.* (2005) is challenges with non-pathogenic antigens such as sheep red blood cells or keyhole limpet hemocyanin (an immune stimulant glucoprotein). In principle, any effects on energy metabolism would be the consequence of the expression of immune proteins and not due to repair of damage or fever.

Several experiments were identified in which total energy expenditure was measured in-directly through measurements of respiration (oxygen consumption rate) during challenges with non-pathogenic antigens. Any difference in energy expenditure is then estimated roughly from the rate of oxygen consumption (20.1 kJ/litre O_2; Eraud *et al.* 2005) between challenged and non-challenged animals, which are shown in Table 2.

Table 2 The relative change in energy expenditure (RCE, challenged/controls) and energetic cost ($E_{antibody}$, challenged energy expenditure – control energy expenditure) due to antibody production, as measured by changes in oxygen consumption of mammals and birds challenged with non-pathogenic antigens.

Host	Body weight (g)	RCE	$E_{antibody}$ (kJ/kgBW/d)	RCE proportional to antibody production	Source
Mice[1]	36.0	1.55	230	Yes	Demas *et al.* (1997)
Blue tits	10.0[2]	1.10	275	Yes	Svensson *et al.* (1998)
Great tits	18.6	1.09	148	-	Ots *et al.* (2001)
House sparrows	28.4	1.29	148	-	Martin *et al.* (2002)
Collared doves	130	1.09	36	Yes	Eraud *et al.* (2005)

[1] The antigen used was keyhole limpet hemocyanin (an immune stimulant glucoprotein) in all other cases it was sheep red blood cells. [2]Estimated from age.

The data in Table 2 show that a significant energy requirement is associated with the production of immune proteins, given that any changes in energy expenditure are due to

antibody production. The relative changes in energy expenditure appear to be within a similar range, except of that of Demas *et al.* (1997): this may be explained by the use of a different antigen, which induced an increase (>1°C) in colonic temperature. Demas *et al.* (1997), Svensson *et al.* (1998) and Eraud *et al.* (2005) all found that the largest increase in energy expenditure coincided with peak antibody production. It would appear, therefore, that the energy cost of mounting an immune response is strongly related to the level of the immune response. This in turn may explain why some authors have not found any effects on overall energy expenditure or growth (Henken & Brandsma, 1982; Pilorz *et al.* 2005).

Difficulties may also arise from other components of an animal's energy budget being affected. Even a non-pathogenic antigen challenge (such as sheep red blood cells) may reduce the energy expenditure associated with activity (Henken & Brandsma, 1982) as has been observed for pathogenic challenges (van Diemen *et al.* 1995; van Dam, 1996). Energy requirements due to production of immune proteins may therefore be partially 'hidden' within the overall energy budget of the animal. Experiments that have tried to consider the individual components of an animal's energy budget are now considered to further characterise the effects of pathogen challenges on energy requirements.

4.3.2.3.2 Energy balances, marginal responses and fever. Experiments with both pathogenic antigen challenges and challenges with live pathogens have shown increases in maintenance requirements that ranged from 1.05 – 1.35. There appears to be highly specific pathogen differences, with the greatest energetic cost occurring for pathogen or antigen challenges that lead to a fever e.g. Verstegen *et al.* (1991), Zwart *et al.* (1991) and Demas *et al.* (1997). Local pathogen challenges may cause less of an effect as was demonstrated by van Diemen *et al.* (1995) who considered the effect of atrophic rhinitis on heat production in pigs.

Gastrointestinal parasites may cause an increase in energy requirements through the repair of damaged tissues in the gut, or through the reprocessing of nitrogen leaked into the gastrointestinal tract that appears to later be partly reabsorbed (Poppi *et al.* 1986). MacRae *et al.* (1979) performed both energy and nitrogen balances of sheep, at different time

points of a challenge with *Trichostrongylus colubriformis*. No differences were observed prior to week 4 between challenged sheep and their pair-fed controls, after which the metabolisability (ME/GE) was reduced compared with pair-fed controls, as is shown in Figure 4 together with their faecal egg counts.

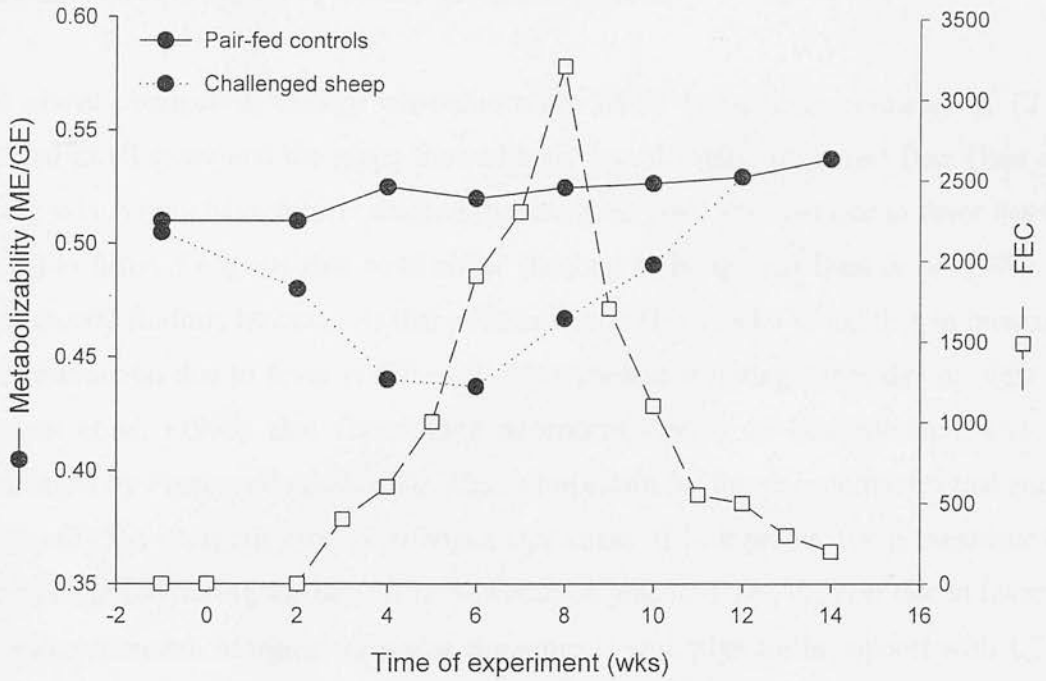

Figure 4 The relationship between food metabolizability (ME/GE), fecal egg counts (FEC) and time of a sub-clinical trickle infection of sheep with *Trichostrongylus colubriformis* (McRae *et al.* 1982)

This suggests a significant increase in excretion of energy either in the urine or faeces, which is associated with the peak of the pathogen challenge; as in later balance periods there was no difference in metabolisability. As was the case for the nitrogen cost of damage, it appears necessary to predict the energetic cost of repairing damaged tissues, which appears to be related to the level of challenge of the host.

A significant body of evidence exists from experiments with *Trypanosoma vivax* infection in West African goats (Verstegen *et al.* 1991; Zwart *et al.* 1991; van Dam *et al.* 1996, 1997, 1998) on both energy and nitrogen requirements. *Trypanosoma vivax* in these

experiments caused an increase in body temperature (consistently + 0.8 to 1°C) and damage to red blood cells (van Dam, 1996). The total increase in energy requirements for maintenance was 1.21 (van Dam *et al.* 1996), 1.27 (van Dam *et al.* 1997), 1.15 (Verstegen *et al.* 1991) and 1.16 (Zwart *et al.* 1991) times healthy control animals. The findings of van Dam *et al.* (1996, 1997) are also shown in Figure 3.

The above changes in energy expenditure are likely to be underestimates as *FI* was reduced in all cases and the goats showed behavioural coping responses (van Dam *et al.* 1996), which may have further masked the energetic cost. The cost due to fever has been shown to differ for goats that were either standing or lying (van Dam *et al.* 1996). The most crucial finding, however, is that of Zwart *et al.* (1991) who found that an increase in heat production due to fever is different, when measured during either day or night. van Diemen et al. (1995) also found time dependent effects on heat production in pigs challenged by *Pasteurella multocida*. This is important for future experiments that attempt to quantify the energetic cost of pathogen challenges. If heat production is measured over a sample period during the day (as is the common practice) then the cost due to fever will be underestimated. Marginal response experiments with pigs are in support with 1.7 and 2.2 times greater maintenance requirements in pigs challenged with a *Trypanosome* (Fagbemi *et al.* 1991; Otesile *et al.* 1991).

Experiments with other pathogens appear consistent with those using the Dwarf goat-*Trypanosoma vivax* model in terms of the energetic cost of fever. Baracos *et al.* (1984) reviewed the energy requirements caused by fever and concluded that a 1°C increase in body temperature due to fever, increased energy requirements on average by 1.1. It was discussed, however, that the effect of fever on metabolic rate was time dependent, which is likely explained by its close relationship to the pathogen load in a host. Different types of antigens (either *LPS* or heat killed *Staphylococcus areus*), however, may cause different energy requirements (Benson *et al.* 1993), as chicks challenged with *LPS* grew much less lipid than controls or chickens challenged with heat killed *S. aureus*. A predictive framework would need to consider the relationship between fever and pathogen challenge (kind, dose and virulence) and have clear definitions of how to include other

energetic costs such as production of immune proteins or repair of damage within the overall energy budget of a host.

4.3.3 Partitioning of scarce protein and energy resources during pathogen challenges

The food intake of an animal may be less than its desired intake (which would permit maximal growth) due to the nature of the food, the environment (i.e. the animal is hot) or pathogen induced anorexia. They key to predicting growth in such circumstances is to have a suitable partitioning rule. Sandberg *et al.* (2005*ab*) argued that e_p (the marginal material efficiency of protein retention) is central to the overall system of (protein and energy) partitioning as once protein retention has been predicted, lipid retention can accounted for on energy grounds e.g. Emmans (1994). The problem is whether pathogen challenges lead to a different overall system of partitioning. This has been considered qualitatively by Beisel (1966), van Houtert & Sykes (1996), Coop & Kyriazakis (1999), Klasing & Calvert (1999) and Lochmiller & Deerenberg (2001) amongst others. The focus here is to bring together suitable quantitative evidence of both protein (amino acid) and energy partitioning to assess the largely qualitative partitioning rules that have been proposed during disease.

4.3.3.1 Rules of partitioning during pathogen challenges

Partitioning rules that have been proposed during pathogen challenges have either considered resource supply in terms of protein (Coop & Kyriazakis, 1999), amino acids (Koutsos & Klasing 2001; Le Floc'h, 2004) or energy (Schrama *et al.* 1997). With the exception of Coop & Kyriazakis (1999) partitioning of resources between different arms (innate or acquired) of immune responses has not been considered. It may be necessary, or not, to distinguish between different immune responses: the most attractive solution would be where all consequences of a pathogen challenge could be considered as one source for allocation. Two types of partitioning rules are considered here. The classical view is where requirements for maintenance are first met (including costs associated with pathogen challenges), after which resources are partitioned to growth (Klasing *et al.* 1991; Schrama *et al.* 1997; Elsasser *et al.* 2000; Lochmiller & Deerenberg, 2001; Colditz 2002). Alternatively it has been suggested that there is a degree of competition between growth

and immune functions once the requirements for maintenance (which may still include some consequences of disease) have been met (Coop & Kyriazakis, 1999), when nutrient and energy resources are scarce.

The consequence of classical partitioning is that for above maintenance intakes no further improvements in immune responses would be observed. There are several experiments to contradict this, including those of Bhargava *et al.* (1970), Datta *et al.* (1998) and Lee *et al.* (2002). Some authors propose that pathogen challenges always lead to a release of amino acids from body protein stores such as muscle e.g. Klasing *et al.* (1991) and Elsasser *et al.* (2000). This would be likely to be the case for sub-maintenance intakes, (i.e. the animal could still maintain some small degree of immunity). It is unlikely, however, that an animal would preferentially utilise body protein when dietary resources are available. This would lead to a fitness penalty in the long term (Coop & Kyriazakis, 1999). Thus, it is necessary to consider the quantitative evidence of partial protein (amino acid) partitioning between growth, consequences of pathogen challenges and immune functions.

During pathogen challenges, energy requirements for maintenance are increased, with no further effect on energy partitioning e.g. Benson *et al.* (1993) and van Dam (1996). It has been observed that during pathogen challenges heat production of an animal was increased, while food intake and energy retention were reduced e.g. Verstegen *et al.* (1991) and Zwart *et al.* (1991). This effect, however, appears to be both pathogen and dose specific. Takhar & Farrell (1979a) found that a challenge with *Eimeria acervulina* did not affect the heat production of chicks, compared to their pair-fed controls. In a predictive framework energy requirements during pathogen challenges could therefore be accounted for as part of maintenance and the cost of protein retention, and any shortfalls would be met by using energy stores in the form of lipid. This would have the consequence that only protein partitioning during health would need to be revised in a predictive framework of growth during disease. In support are the several experiments that show no improvement in immune responses when additional levels of energy are supplied e.g. van Heugten *et al.* (1996) and Spurlock *et al.* (1997). Therefore, only protein (amino acid) partitioning during pathogen challenges are now considered.

4.3.3.2 Quantitative assessment of protein partitioning during pathogen challenges

The experiments of Williams *et al.* (1997*a,b,c*) and Webel *et al.* (1998*a,b*), described earlier, found no effect on the marginal responses in protein retention of growing pigs and chicks, respectively, to protein and amino acid supplies. A possible interpretation is that as marginal response in protein (amino acid) retention to protein (amino acid supplies) was not different, this leads to the rejection of partial prioritisation of protein between growth and immune responses. Another interpretation is that the partitioning of protein between growth and immune responses could not be detected in these experiments when measuring overall protein retention. Protein that is partitioned towards growth and immune responses are both retained in the body. Any net benefit that is observed in terms of overall growth rate, may only be due to an alleviation of damage caused by the pathogen e.g. Abbott *et al.* (1985) and in particular Datta *et al.* (1998). The relationships between level of nutrition, innate or acquired immune responses and damage are now considered.

4.3.3.2.1 Innate immune responses. The immune proteins that are produced during the acute phase of an infection, such as the acute phase proteins and complement are here defined as innate immune proteins e.g. Murata *et al.* (2004). It is recognised that the acute phase proteins have multiple functions, which include supporting the acquired arm of the immune response and assisting in the repair of damaged tissue (Murata *et al.* 2004). The question is whether these immune functions are affected by protein (amino acid) availability, in animals that are growing.

Dritz *et al.* (1996) did not find any effect of three different foods on the expression of the acute phase protein haptoglobin ($P = 0.17$) in pigs that were gaining weight. Sakamoto *et al.* (1998) reviewed evidence of how nutrition may affect another set of innate immune proteins (complement) and did not find any effects of nutrition in 'normal' animals. In malnourished animals, however, it would appear that an additional supply of resources may affect the expression of the complement system, but this was only the case for severely malnourished humans. Fleck (1989) stated that "Prolonged protein-energy

depletion in man sufficient to yield a decrease in body weight of 25% led to only a 7% decrease in concentration of albumin...When the increase in plasma volume is taken into account there was no change in the amount of albumin in the circulation". It would appear, therefore, that costs relating to innate immune responses may need including as part of the requirements for maintenance. The marginal response studies of Webel *et al.* (1998*a,b*) are in support as they used *LPS* as an antigen, which largely stimulates an acute phase response.

4.3.3.2.2 Acquired immunity. Coop & Kyriazakis (1999, 2001) reviewed information in the literature on the effects of protein supplementation on immune responses and growth in sheep challenged with gastrointestinal parasites. They concluded that the expression of immunity was affected by nutrition from experiments with sheep (Bown *et al.* 1991b; Kambara *et al.* 1993; Coop *et al.* 1995), cattle (Mansour *et al.* 1991, 1992) and goats (Singh *et al.* 1995). There is also similar evidence in pigs (van Heugten *et al.* 1995; Johansen *et al.* 1997) and mice e.g. Ing *et al.* (2000). Other kinds of pathogen challenges were considered here to determine whether the improvement of the expression of acquired immunity is a general response across different types of pathogens.

Tsiagbe *et al.* (1987) found that growth and immune responses (antibody expression) of chicks were increased with increasing levels of methionine supplementation. Antibody expression was only improved once a plateau had been achieved in growth rate, thus their data suggest that growth was prioritised over immune responses. Bhargava *et al.* (1970b), on the other hand, found that chicks challenged with the Newcastle virus had improved growth rates and geometric mean antibody production when given foods that had increasing proportions of L-threonine. This suggests a partial prioritisation of amino acid between growth and immune responses, shown in Figure 5. Their data suggest that as the animals reached a plateau in growth there was no distinct improvement in antibody titer, which would not be expected if protein was being partitioned between these two functions.

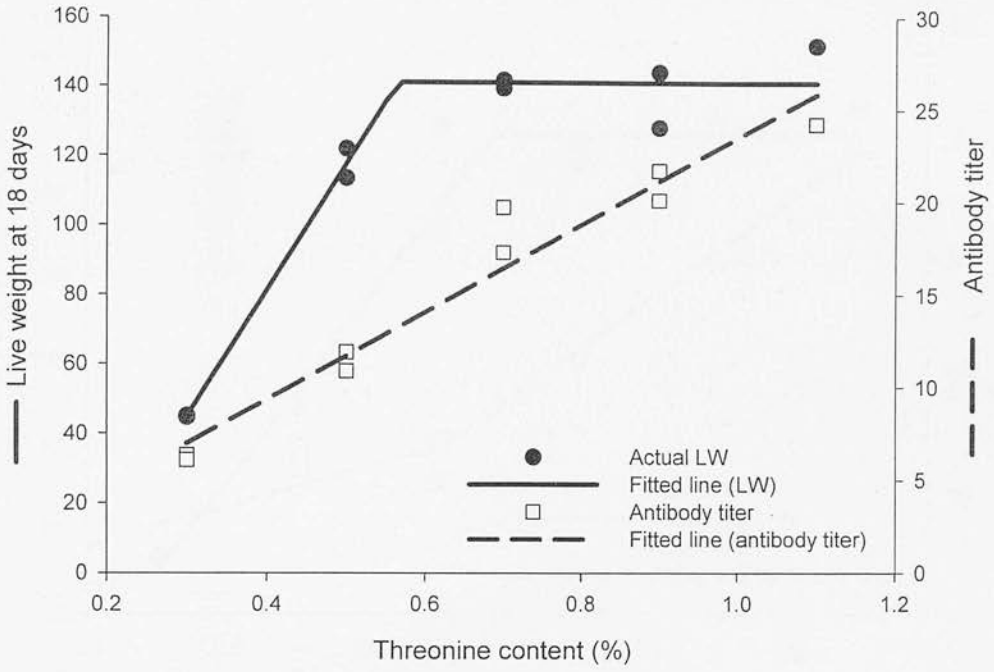

Figure 5 The live weight at 18 days (LW) and geometric mean antibody titers (AT) for chicks challenged with a Newcastle virus and given foods that had different contents of L-threonine from Bhargava *et al.* (1970b). Linear plateau response was approximated as a linear function of threonine content for live weight gain (LWG = 364.T – 64.4) until the plateau of 141.1 was reached. The linear regression of antibody titer against valine content was equal to AT = 23.63T – 0.143.

Bhargava *et al.* (1970a), however, found a different response for chicks challenged with Newcastle virus and given food of different amino acid (valine) contents, shown in Figure 6. In this case, the response in antibody titer did further increase when the animal reached a plateau in terms of growth, and thus providing strong evidence that valine was being partitioned between growth and immune functions. The data in Figure 6 are in support of a partial prioritisation of additional levels of amino acid supply between growth and immune responses. Furthermore, these two experiments indicate that the requirements of specific amino acids and their partitioning need accounting for predicting growth and immune responses. The level of challenge may affect the extent to which protein (amino acid) is partitioned between growing 'body' protein and immune proteins (Wannemacher *et al.* 1974).

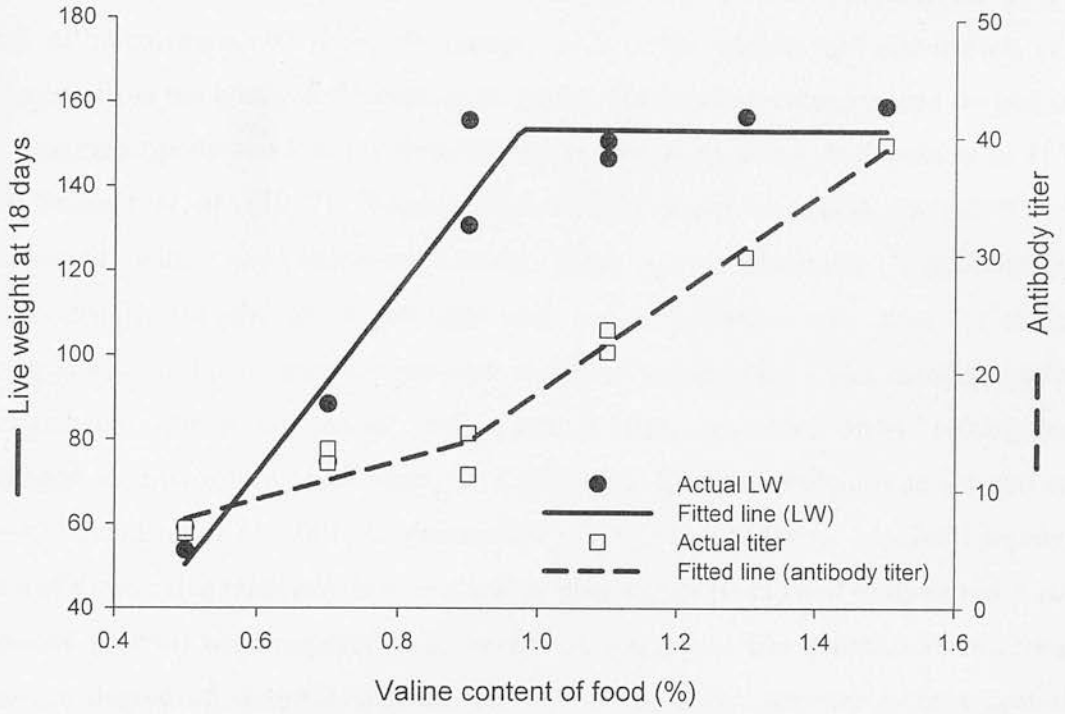

Figure 6 The responses in live weight over 18 days (LW) and antibody titers (AT) to increasing valine contents (V) of a food for chicks challenged with a Newcastle virus from Bhargava *et al.* (1970a). Linear plateau response was approximated as a linear function of valine content (LW = 217.V – 58.2) until the plateau of 153.6 was reached. The linear regressions of antibody titer against valine content until maximum LW was reached was AT = 16.37.V – 0.6325 whereas for the subsequent phase was AT = 41.35.V – 23.04.

There are several publications in favour of additional levels of protein (amino acid) supplementation having a beneficial effect on the host coping with several different kinds of pathogen challenges. Lee *et al.* (2002), for example, found that arginine supplementation (a limiting amino acid in birds) improved immune responses in chicks challenged with the infectious bronchitis virus. Low *et al.* (2003) found that mice given a whey protein based diet, which is rich in certain amino acids found in large amounts in immune proteins, had increased antibody responses to several bacterial and viral antigens.

The likely explanation why hosts cope better with pathogen challenges when provided with sufficient amounts of protein (amino acid) is the control and elimination of the pathogen from the host (van Houtert *et al.* 1995). The level of pathogen load (in particular the maximum pathogen load) is directly related with survival e.g. Powanda *et al.* (1975) and Berendt *et al.* (1977). Suzuki *et al.* (1993) found increased survival in mice challenged with *Staphylococcus aureus* when given glutamine supplementation. Unfortunately, the change in pathogen load and performance over time for different bacterial and viral pathogens has not been measured extensively, whilst nutrition has been manipulated. Sheep challenged with gastrointestinal parasites show reductions in pathogen load (worm burden) when given additional levels of protein (van Houtert *et al.* 1995; Donaldson *et al.* 2001) or amino acid (methionine: Miller *et al.* 2000) supply. In most of these cases relatively low levels of protein supply (compared to those required for maximal growth) were required to achieve such responses. The quantitative relationship between degree of resource scarcity and rate of pathogen removal is thus central to predicting performance during pathogen challenges.

The problem of predicting resource partitioning during pathogen challenges appears to consist of three sub-problems. Firstly, it would be necessary to predict the partitioning of amino acids between growing 'body' and immune proteins during exposure to different levels and kinds of pathogens. Secondly, it would be necessary to be able to account for the net benefit an animal would gain from investing in an immune response. Finally, a predictive framework would need to adequately describe how different genotypes partition their resources between growth and immune functions. This would allow consequences of genetic selection strategies (i.e. increased potential for growth or resistance to pathogens) to be explored, and for management (nutrition) strategies to be better informed.

4.4 Discussion

Improving current predictive growth models will help genetic selection and management strategies intended to combat the effects of disease on animal performance and improve their health and welfare. This has become increasingly important, as pathogens have

evolved resistance to pharmaceutical interventions (Waller 1999), antimicrobial growth promoters have been banned (EC Regulations 1831/2003, 1834/2003) and animal welfare (including health) has become more central to animal production (Kanis *et al.* 2005). No comprehensive attempt has been made to model the effects of disease on growth and performance. Black *et al.* (1999) when discussing the inclusion of disease in the AUSPIG model (Black *et al.* 1986) suggested that "...increasing maintenance energy requirements by up to 1.3 times the normal predictive value, decreasing the rate of protein deposition by 0.9 times normal and decreasing feed intake down to zero depending on the severity and duration of the disease" would achieve this. This quasi-quantitative suggestion might be a good first step. The aim here was to extend the description of the problem of predicting growth during pathogen challenges; issues that have not yet been considered are now discussed.

It appears from literature evidence that energy partitioning during pathogen challenges might remain the same as that during health, with any effects of pathogen challenges on energy requirements being included as part of maintenance. During bacterial, viral and some parasitic pathogen challenges it is common for an animal to mount a fever (Hart 1988; Blatteis, 2003). A febrile response (within a certain range) may be seen as a beneficial host reaction, rather than being a detrimental and unavoidable consequence of pathogen challenges e.g. Hart 1988, Mackowiak *et al.* (1997), Blatteis (2003). Consequences of pathogen challenges such as fever, anorexia and other acute phase responses have been viewed as part of an overall coping mechanism by hosts challenged by pathogens (Hart 1988; Kyriazakis *et al.* 1998). Fever may be beneficial to the host as bacterial and viral growth rates are sensitive to changes in their ambient temperature, or, through the increased body temperature having positive effect on the host immune responses. Jiang *et al.* (2000) demonstrated that the latter of these two routes was the most significant, and Blatteis (2003) have reviewed further evidence in favour of fever having a beneficial effect on host immune responses during exposure to certain kinds of pathogens.

It is, therefore, important to try and relate the ability of an animal to mount fever to the effectiveness of its immune response. Furthermore, Bradley & Kauffman (1988) found

that protein malnutrition may reduce the extent of fever. This provides a further link between the immune response, which amongst other things depends on resources, and fever. Future experiments are warranted for investigating any interactions between protein partitioning, fever and the ability of an animal to cope with infection. This would be crucial for prediction by models that attempt to incorporate the effects of the physical (i.e. thermal) environment alongside those of the infectious environment on performance.

Existing rules of protein partitioning during disease were found to be qualitative, and testing their qualitative predictions against literature data showed that there is a competition for amino acids between growth and immune functions. This is contrary to the prevailing view that immune responses are prioritised over other body functions, when food resources are scarce (Klasing *et al.* 1991; Schrama *et al.* 1997; Lochmiller & Deerenberg, 2001). This competition for resources (and its fitness benefits) raises some interesting questions in terms of how an animal has evolved to cope with infection (Sheldon & Verhulst, 1996; Rauw *et al.* 1998). Pathogen challenges clearly have a significant fitness cost on a host as it can lead to mortality or severe morbidity (Rigby *et al.* 2002). In some cases, however, the consequences of a pathogen challenge may be rather small (Coop *et al.* 1985). This could have lead to animals being able to mount an immune response and partition resources for this that is proportional to the extent of a challenge, including pathogen virulence. It is likely that natural selection in nature would have occurred during high levels of infection and when resources were scarce. A more resistant genotype is here seen as more able to deal with a pathogen challenge with lower pathogen loads and a lesser consequence on food intake (Sandberg *et al.* 2006). The remaining issue is how genetic selection for growth and/or resistance has affected the ability of animals to cope with pathogen challenges.

Attempts have been made to select animals that cope better with disease by different approaches in pigs (Wilkie *et al.* 1998), chickens (Siegel & Gross 1980) and sheep (Bisset *et al.* 1996; Douch *et al.* 1996) amongst others. In relation to the framework discussed here the important issues include the relationship between degree of resistance (when defined in a particular way) and (i) reductions in food intake, (ii) changes to requirements

for mounting an immune response and (iii) the extent of partitioning towards either growth or immune functions by the host. Different methods have been used to attempt to select for more resistant genotypes. Wilkie *et al.* (1998) selected for high and low responder pigs in terms of general immunity by using an index for several immune traits when challenged by several different types of antigen. Siegel & Gross (1980) and Yunis *et al.* (2000) selected chickens for a single immune trait (either high or low antibody responses to sheep red blood cells). Genetic selection in sheep against gastrointestinal parasitism uses a proxy measure of the level of challenge (faecal egg counts), which has been found to have a strong relationship with reduced worm burdens (Bisset *et al.* 1996). Unfortunately, there is an absence of evidence of controlled challenge trials of strains of different resistance, where food intake, performance and measures of immunity have been recorded. This may have allowed for the consequences of genetic selection for resistance to be better understood.

Wilkie *et al.* (1998) selected for responses in both cellular and humoral immune components to a range of antigens in attempt to achieve *general* resistance (Magnusson *et al.* 1997, 1998 & 1999; Reddy *et al.* 2000). Magnusson *et al.* (1997) showed that pigs selected for high immune responses were more responsive to vaccination (indicating a greater ability to recognize antigens) and *LPS* challenge. Antibody responses were greater, and peak (maximum antibody level) responses occurred faster than in pigs selected for low immune responses. This would suggest that more resistant animals would have a higher requirement for immune proteins at lower pathogen loads. The high response lines, however, achieved 90kg over a shorter time than the low response lines (Wilkie *et al.* 1998) when challenged by other pathogens. This may suggest that while more resistant genotypes invest more resources in immune responses, there is also a net benefit on growth. Gross *et al.* (2002) also found that chickens selected for resistance as by the method of Siegel & Gross (1980) had less damage (air sac lesions) when challenged with *Mycoplasma gallisepticum*.

Selection for high immune responses may not always be beneficial as in a challenge experiment with *Mycoplasma hyorhinis* in pigs the high response lines had a greater

incidence of arthritis (Magnusson *et al.* 1998), attributed to higher activation of the immune response. An inevitable consequence of immune system activation is a non-specific immunopathology that does not confer any obvious advantage to the host, except for the overall positive outcome of a challenge. An improved general understanding of resistance and its effects on growth and performance is needed as different pathogens are dealt with by immune responses with different relative influences of innate, cellular and humoral immune responses and higher immune responses may not always necessarily lead (or indicate) to improved resistance (Adamo, 2004). There is some weak evidence in the literature, which suggests that animals selected for faster growth were affected to a greater extent when challenged by pathogens (Praharaj *et al.* 1995; Yunis *et al.* 2000; Huff *et al.* 2005). This has been attributed to the prioritisation of resources towards growth rather than immune functions (Rauw *et al.* 1998). It is essential, therefore, for future experiments to consider well defined genotypes (differing both in terms of genetic growth potential and resistance) when challenged by pathogens.

In future work we will present some possible quantitative solutions to the problems that have been raised in this review. It will be necessary to have an adequate scaling rule for describing pathogen challenges, which accounts for how animals of different sizes and degrees of maturity respond to a pathogen challenge. Suitable functional forms need to be chosen that relate the level of a pathogen challenge to amino acid and energy partitioning and requirements. The effects of host resistance on the parameters of these functional forms would also need to be taken into account to be able to predict the performance of hosts with different levels of resistance. The model could then be used to assess how different genotypes (both growth potential and degree of resistance) cope during pathogen challenges when given access to different kinds of food. The model, and its future parameterisation and testing through relevant experiments may improve our understanding of the relationship between host performance, and pathogen exposure in different environments.

Chapter 5

Predicting the effects of pathogen challenges on the performance of naïve and immune animals: towards a possible solution

5.2 Introduction

Pathogen challenges can lead to significant reductions in animal performance and have large economic consequences (Coop *et al.* 1982; Black *et al.* 1999; Nieuwhof & Bishop 2005). Animal health and welfare have also become increasingly important criteria in animal production, and the removal of antibiotic growth promoters has added pressure on production systems (Kanis *et al.* 2005). No general framework has been proposed for predicting the effects of pathogen challenges on growth and performance of different host genotypes (different genetic potential for growth and disease resistance) when given access to different kinds of food. Previous attempts have either been to simplistic (Black *et al.* 1999) or lacking in the scope of their predictions of growth (Henken *et al.* 1994b). In this chapter, an initial attempt is made to develop a framework, which in principle could be used to predict the effect of pathogen challenges on growth and performance of different genotypes of farm animals, and such a framework may have both a heuristic and predictive value.

In the previous chapter, literature evidence on growth and performance during pathogen challenges was reviewed, with the aim of identifying how pathogen challenges affect resource (energy and amino acid) requirements and partitioning. The key problems to solve were the relationships between level and kind of challenge, and requirements for amino acids and energy to mount an immune response (including production of immune proteins and fever) and repairing damaged tissues (Wannemacher *et al.* 1971; Powanda *et al.* 1971; Wannemacher *et al.* 1974; Abbott *et al.* 1985; Poppi *et al.* 1986; Reeds et al. 1994; Klasing & Calvert 1999; Taylor-Robinson 2000). Literature evidence in Chapter 4 showed that different kinds and levels of sub-clinical pathogen challenges lead to reductions in growth to different extents (Steel *et al.* 1980; Coop *et al.* 1982). In this initial attempt, it was therefore necessary to choose a model pathogen (a replicating micro-parasite) to develop the framework and attempt parameterisation. The proposed framework may be applicable to different kinds of pathogen challenges (parasites, bacteria and viruses), but further evidence would be needed.

The framework considers the transition between an animal being immunologically naïve to a particular pathogen (i.e. having had no prior experience of it) and becoming immune. The model predicts the balance between pathogen growth and host elimination of the

pathogen, leading to the actual pathogen load in the host. The traditional 'turn over' period of deterministic growth models has been a day (Emmans 1981; Black *et al.* 1986; Wellock *et al.* 2003a). In the instance of pathogen replicating in the host it may be insufficient to use a day as a time step as it introduces unacceptably large steps in for example pathogen load, and as a result non-sensical predictions of performance may be made. The model that is presented here uses an hour as its time step to overcome this issue.

The proposed solutions were solved dynamically, allowing for predictions of voluntary food intake, growth, and protein and lipid retention prior to, during, and after a pathogen challenge. This allows for interactions between level of pathogen challenge, nutrition, host genetic resistance and potential for growth to be explored over a range of combinations. Model parameterisation has identified several areas where data needs to be obtained by suitable experiments. The proposed framework may be integrated with other general models of growth to predict the effects of pathogen challenges on growth and performance.

5.3 Model description

The model proposed here builds on the concepts for predicting growth and performance as developed by Emmans (1981), Emmans & Fisher (1986) and Wellock *et al.* (2003a). The main components during health, and any modifications or additions for times of pathogen challenge, are described here. Parameter values were derived where possible from literature data that came from experiments with controlled pathogen challenges. Where this was not possible, as was the case for several model parameters, their values were derived on conceptual grounds. As an initial attempt, this may be acceptable to allow quasi-quantitative predictions of the model to be explored. The underlying framework for the healthy animal is described first, and thereafter, alterations and enrichments of the framework to include the effects of pathogen challenges are developed.

5.3.1 *Predicting food intake and growth during health*

Emmans (1981) and Emmans & Fisher (1986) proposed a framework where food intake is predicted from the requirements for potential growth for protein and energy and the composition of a particular food. In the case where constraints limit the intake of an

animal, effects on growth can be predicted using a rule(s) for partitioning protein and energy to protein, *PR* kg/hr, and lipid, *LR* kg/hr, retention.

5.3.1.1 *Potential growth and intake*

The chemical composition of the animal at the start of the simulation period can be calculated from the initial body weight, assuming that the animal has its desired chemical composition. The lipid (*L,* kg), ash (*A,* kg), water (*Wa,* kg) weights are calculated from protein weight *P* (kg) using allometric equations (Emmans and Fisher, 1986; Emmans and Kyriazakis, 1997). Once having calculated the initial chemical composition of the body it is possible to predict the potential growth rates of protein, PR_{max} (kg/hr), ash, AR_{max} (kg/hr), and water, WR_{max} (kg/hr):

$$PR_{max} = P.B.log_e(P_m/P) \quad \text{kg/hr} \quad (1)$$

$$AR_{max} = a.PR_{max} \quad \text{kg/hr} \quad (2)$$

$$WR_{max} = PR_{max}.(W_m/P_m).w.(P/P_m)^{w-1} \quad \text{kg/hr} \quad (3)$$

B (/d) is the Gompertz rate parameter, which in this model is dependent only on animal genotype. The value of *B* is in the model converted to a rate /hr. The desired lipid retention, LR_{des} (kg/hr), may be related to the animals' desired fatness at a given degree of maturity, which is predicted from the animals potential protein retention and mature lipid to protein mass ratio (Wellock *et al.* 2003a).

In this chapter, it is not the requirement for ideal protein that is considered, but the requirement for the individual essential amino acids and the amount of nitrogen required in the form of non-essential amino acids. From the value of PR_{max} and an estimate of its amino acid composition, it is possible to predict the amount of each essential amino acid, Eaa_i g/hr, and the remainder as the sum of the non-essential amino acids, *NEaa* g/hr that is retained in potential growth. The subscript aa_i refers to an equation being representative of each of the essential amino acids and *Neaa* (g/hr) refers to the sum of the non-essential amino acids:

$$Eaa_i = PR_{max}.AA_iC_B \quad \text{g/d} \quad (4)$$

$$Neaa = PR_{max} - \sum Eaa_i \quad \text{g/d} \quad (5)$$

Where AA_iC_B (g/kg P) is the essential amino acid content of the whole body of a pig, which is assumed constant over different degrees of maturity. The findings of Mahan & Shields (1998) who measured the whole body amino acid content of pigs are in support of this assumption. Their range in live weights was between 1.5 to 146 kg live weight, which corresponds to a range in protein weight of 0.16 to 18.7 kg protein. The requirement for non-essential amino acids may be calculated from the difference of total protein minus the total requirement for essential amino acids, as the proportions of Eaa_i and $NEaa$ in the whole body protein of pigs have been found to consistently be around 0.43: 0.57 (Kyriazakis *et al.* 1993; Mahan & Shields, 1998).

The model uses the effective energy system of Emmans (1994) to calculate how energy from the food is used by the animal, through taking into account losses of energy in urine, faeces and as heat for a given food. Estimating the effective energy content of a food (see below) allows the effective energy requirements of the animal to be put on the same scale as energy supply with the constant efficiencies of using effective energy for growth and maintenance. The requirements for maintenance of effective energy, E_m MJ EE/hr, and protein, MP kg/hr, are predicted in relation to the scaled protein weight following Taylor (1981) and Emmans & Fisher (1986):

$$E_m = M_e.(P/P_m^{0.27}) \quad \text{MJ/hr} \quad (6)$$

$$MP = M_p.(P/P_m^{0.27}) \quad \text{kg/hr} \quad (7)$$

The values of M_e (1.63) and M_p (0.004) are assumed constants which are in units of MJ/d and kg/d, and are in the model converted to MJ/hr and kg/hr.

It is necessary to have an estimate of the amino acid composition of protein required for maintenance to estimate the amino acid requirements for maintenance. Wang & Fuller (1989) and Fuller *et al.* (1989) estimated the amino acid composition that is required for maintenance in pigs. The estimate of Fuller *et al.* (1989) of the amino acid content of protein required for maintenance and growth are shown in Table 1 together with an average amino acid content of pig whole body protein from Sandberg *et al.* (2005b, Chapter 2). The amino acid content of maintenance protein is taken as that of Fuller *et al.* (1989) and the amino acid content of protein retained is taken to be that calculated by Sandberg *et al.* (2005b).

Table 1 The amino acid contents of protein required for growth and maintenance.

	Amino acid content (g aa / kg P)		
Amino acid	Maintenance[1]	Growth[1]	Growth[2]
Histidine	-	-	28
Isoleucine	10	43	32
Leucine	16	78	70
Lysine	23	68	64
Methionine	7	19	20
Methionine + Cysteine	33	36	32
Phenylalanine	14	41	37
Phenylalanine + Tyrosine	28	84	62
Threonine	32	47	37
Tryptophan	7	12	9
Valine	12	53	44
Total	182	481	435

[1]Fuller et al. (1989) who determined these values using nitrogen balance

[2]Sandberg *et al.* (2005b) who calculated an average amino content of whole body pig protein from several amino acid analyses.

The requirement for individual essential ($MPEaa_i$ g/hr) and the sum of non-essential ($MPNEaa$ g/hr) amino acids for maintenance, assuming a material efficiency of using amino acids for maintenance of 1 (Fuller *et al.* 1989) are predicted as:

$$MPEaa_i = MP.AAC_B \quad \text{g/hr} \quad (8)$$

$$MPNEaa = MP - \sum MPEaa_i \quad \text{g/hr} \quad (9)$$

To predict the requirement for essential Eaa_iRQG (g/d) and non-essential $NEaaRQG$ (g/d) amino acids for growth it is necessary to predict the marginal material efficiency, e_p, with which amino acids are used for growth. This is done here using the rule of Kyriazakis & Emmans (1992ab), which has recently been supported by Sandberg *et al.* (2005b). Their rule makes e_p a function of the energy to protein ratio of the food only, which allows the important distinction to be made between energy and protein limiting foods:

$$e_p = k.(MEC/DCPC) \quad (10)$$

Where k (0.0112) is a constant (Sandberg *et al.* 2005b) and *MEC* (MJ/kg) is the metabolisable energy content (corrected for the crude protein content of the food) and *DCPC* (kg/kg) is the digestible crude protein content of the food. The value of e_p reaches a maximum value ($e_p^* = 0.76$) when the ratio of energy to protein in the food reaches 67.9 MJ ME/kg DCPC (Sandberg *et al.* 2005b). The value of e_p^* is assumed constant for different amino acids, genotypes and degrees of maturity (Sandberg *et al.* 2005b). Thus, for a food with a given energy to protein ratio it is possible to predict the amino acid requirements for growth, and together with requirements for maintenance, the total individual essential amino acid requirements, $TEaa_iRQ$ (g/hr):

$$Eaa_iRQG = Eaa_i / e_p \quad \text{g/hr} \quad (11)$$

$$NEaaRQG = NEaa / e_p \quad \text{g/hr} \quad (12)$$

$$TEaa_iRQ = MPEaa_i + Eaa_iRQG \quad \text{g/hr} \quad (13)$$

Once having predicted the requirements for essential amino acids and non-essential amino acid nitrogen it is necessary to predict requirements of energy for growth. The energy system of Emmans (1994) is used here as it allows for constant efficiencies of using effective energy for positive protein and lipid retention to be used, once losses associated with eating and processing a particular kind of food have been taken into account. The requirement for effective energy for growth, *EERQ* MJ EE/hr, is therefore:

$$EERQ = b_p.PR_{max} + b_l.LR_{des} \quad \text{MJ/hr} \quad (14)$$

The values of b_p (50 MJ EE/kg *PR*) and b_l (56 MJ EE/kg *LR*) are constants from the effective energy system of Emmans (1994). In the case where lipid retention is negative, its heat of combustion is used to calculate the amount of lipid that is required to satisfy any energetic deficit. Negative energy retention is permitted in the model until the minimum lipid to protein ratio in the body (0.1P) is reached. In the case where the minimum lipid to protein ratio is reached, protein retention is reduced to maintain the necessary minimum amount of lipid in the body.

By relating the above requirements with the effective energy content, *EEC* MJ EE/kg, and essential amino acid contents, Faa_iC (g/kg CP) of food protein it is possible to predict the

animals desired food intake, *DFI* kg/hr, as by energy, DFI_E kg/hr, or each essential amino acid, DFI_{aai} kg/hr:

$$DFI_E = (E_m + EERQ)/EEC \quad \text{kg/hr} \quad (15)$$

$$DFI_{aai} = (MPEaa_i + EaaRQG_i)/Faa_iC.DCPC \quad \text{kg/hr} \quad (16)$$

The actual *DFI* of the animal is the largest out of DFI_E and DFI_{aai}. The model is not set up to deal with situations where total nitrogen required as non-essential amino acids become first limiting, and in the instance where this happens the model stops. There is evidence in both rodents (Hrupka *et al.* 1997, 1999; Markison *et al.* 1999) and pigs (Owen *et al.* 1994) that animals are capable of eating for either energy or protein, and for a balanced amino acid composition (Summers *et al.* 1992) or individual amino acids. It may therefore be reasonable to predict a desired food intake as by the first limiting resource for growth, including individual amino acids.

5.3.1.2 *Actual growth and intake*

When no infectious stressors are operating, there are only two constraints to intake and performance that may apply: the capacity for bulk and the ability to lose the heat produced. The animals capacity for bulk is predicted using equations from Whittemore *et al.* (2003), which relate the water holding capacity of a food, *WHC* (kg water / kg dry food), with an animals ability for water holding capacity C_{WHC} kg/d, which is a function of its live weight, *W* (kg):

$$C_{WHC} = a.W - b.W^2 \quad \text{kg/d} \quad (17)$$

The value of *a* (0.192) and *b* (0.000299) are assumed to be constants. From the estimate of C_{WHC} and the *WHC* of the food, it is possible to predict the amount of food that an animal is able to eat according to bulk converted to a rate per hour VFI_B (kg DM/hr):

$$VFI_B = C_{WHC} / WHC \quad \text{kg/hr} \quad (18)$$

The value of VFI_B is compared to *DFI* to determine whether bulk constrains the animals voluntary food intake (*VFI* kg/hr), and hence growth (see below).

Pomar *et al.* (2003) proposed that for a thermoneutral environment, the capacity of an animal to lose heat on an unbalanced food is set to that predicted for a balanced food. A balanced food was stated to contain "12.6 MJ EE / kg, a protein: energy ratio that meets requirement and a protein balance and chemical composition similar to the one suggested by NRC (1998) for 20 – 50kg pigs". This constraint reproduces the findings that on low protein foods pigs do not retain very large amounts of lipid with greatly increased levels of heat production e.g. Emmans & Kyriazakis (1992b). In this model, the energy value of 12.6 MJ EE /kg is used for a balanced food, while the balanced amino acid composition is that predicted for potential requirements for growth and maintenance.

To predict the heat production for a balanced food it is necessary to predict the balanced ideal crude protein content, *BICPC* kg /kg, calculated from the ratio of total protein requirement and energy requirement:

$$BICPC = 12.6.((MP + PR_{max}/e_p)/(E_m + EERQ)) \quad \text{kg/kg} \quad (19)$$

Having predicted the *BICPC* it is possible to estimate the digestible energy content, *DEC* MJ /kg, of the food, using constants from the effective energy system (Emmans 1994):

$$DEC = EEC + (h_{iom}.\ IOM_c) + ((N_{ue} + h_{Nex}).BICPC) - (DF_a.z.DF_c) \quad \text{MJ/kg} \quad (20)$$

Where h_{iom} (3.8, MJ/kg) and h_{Nex} (4.67, MJ/kg) are the heat productions associated with inorganic matter (IOM_c, 0.12 kg/kg) and protein excretion, respectively. The energy contained in nitrogenous compounds in the urine is N_{ue} (5.63 MJ/kg). It was assumed that 0.9 of digestible fat content, DF_c (0.03 kg/kg) can be retained in the pigs body as lipid (DF_a), and z (12) is an adjustment to account for the difference in heat increment of forming lipid from lipid and non-lipid dietary sources (Emmans, 1994). The metabolisable energy content corrected for zero nitrogen retention, MEC_n MJ /kg, can then be estimated:

$$MEC_n = DEC - (N_{ue}.BICPC) \quad \text{MJ/kg} \quad (21)$$

The maximum amount of heat an animal can produce and hence lose, HL_{max} MJ/d, is calculated from the energy balance of an animal growing at its potential and eating a perfectly balanced food:

$$HL_{max} = (VFI.MEC_n) - ((h_p - N_{ue}).PR_{max}) - (h_l.LR_{des}) \quad \text{MJ/hr} \quad (22)$$

The parameters h_p (23.8 MJ /kg) and h_l (39.6 MJ /kg) are the heats of combustion of retained protein and lipid, respectively. Actual heat production, *HP* MJ/d, is calculated as the sum of maintenance heat, heat associated with food intake (heat increment of feeding, w_f MJ/kg, times food intake) and heat production associated with actual positive protein (w_p 36.5, MJ/kg) and lipid (w_l 16.4, MJ/kg) retention:

$$HP = E_m + h_f.VFI + (w_p - N_{ue}).PR + w_l.LR \quad \text{MJ/hr} \quad (23)$$

The model compares *HP* with HL_{max} and where $HP > HL_{max}$ *VFI* is reduced in 0.1% steps and actual protein retention is re-calculated in relation to each of the essential amino acids, $PRaa_i$:

$$PRaa_i = (e_p.(Faa_iC.d.DCPC.VFI - MPEaa_i))/AA_iC_B \quad \text{kg/hr} \quad (24)$$

The parameter *d* represents the standardised ileal digestibility of amino acids in food. The model re-calculates energy balance, predicting revised values of *PR* and *LR* until the revised *HP* is equal to HL_{max}.

The protein, lipid, water and ash retained are added to the previous days *P*, *L*, *Wa* and *A* weights from which live weight is predicted assuming 5% gut fill:

$$W_{i+1} = 1.05((P_i + PR) + (L_i + LR) + (Wa_i + WR) + (A_i + AR)) \quad \text{kg} \quad (25)$$

The model continues to predict the above system on a daily basis until the pre-defined growth period has ended. Model predictions during health were explored and tested throughout its development.

5.3.2 Predicting food intake and growth during pathogen challenges

The above framework is now modified to allow for the prediction of growth and performance during both health and disease. The framework is proposed for a pig that is initially immunologically naïve to a pathogen and follows it through the progression of

acquiring and expressing immunity. Sandberg *et al.* (2006) described a model for predicting the relative food intake, *RFI*, of a naïve host challenged by pathogens. This model is integrated here with solutions to the problems of predicting resource partitioning and requirements as discussed in Chapter 4. The model that is proposed here focuses on sub-clinical disease, as did that of Sandberg *et al.* (2006) in Chapter 3.

5.3.2.1 Predicting the development of a disease

The model uses both the challenge dose of a pathogen, *PC* n/d, and the pathogen load, *PL* (number of pathogens, n), of the host to describe a challenge. Sandberg *et al.* (2006) predicted the effect of *PC* on *RFI*, and this description is maintained here for predicting the lowest reduction in *RFI*. The remainder of the model is driven by how *PL* changes in the host over time. How well a host recovers from an infection is reflected by the degree of expression of immunity leading to the elimination of the pathogen load. The *PL* (described as number of pathogens, n) in a host is the outcome of the challenge dose, the growth of the pathogen population and the rate of killing and removal of pathogens by the host's immune functions (Austin & Anderson 1996; Hoshen *et al.* 2000):

$$PL_{t+1} = r.PL_t - PLR_t \qquad \text{n} \qquad (26)$$

Where PL_{t+1} is the pathogen load (number of pathogens in a host) in relation to its previous pathogen load PL_t and r (/hr) describes pathogen replication. The initial *PL* (PL_0) is equal to *PC* when *PC* is above a certain threshold. In the model, r is set arbitrarily to an hourly replication rate of 1.03, which is in approximate agreement with proposed doubling rates for replicating micro-parasitic bacteria (Lin-Chao & Bremer, 1986). The value of PLR_t (n/hr) describes the rate at which the immune system disables and removes pathogens from the host. The value of PLR_t depends on the degree of expression of immunity of the host, which in turn depends on the extent to which the host has acquired immunity, its level of nutrition and its genotype (degree of resistance). The prediction of protein (amino acid) and energy requirements during pathogen challenges are then described, followed by a solution to predicting growth during pathogen challenges when resources are scarce. Parameter estimates and the effects of host genotype (degree of genetic resistance) on their values are discussed.

5.3.2.2 Predicting the requirements for amino acids during pathogen challenges

Amino acid requirements during pathogen challenges appear to be dependent on three main consequences of pathogen challenges including innate immune responses, repair and replacement of damage and the cost of expressing acquired immunity. Solutions to predicting these consequences of a pathogen challenge are now described.

5.3.2.2.1 *Amino acid cost of innate immunity*

The innate immune system is a first line of defence to pathogen challenges and it plays important roles in both recognising and actively protecting a host from pathogens (Beutler, 2004). The innate immune system represents physical barriers such as skin and cilia, and chemical barriers such as lysozyme, complement and acute phase proteins. Cellular components of the innate immune system include white blood cells such as phagocytes (including neutrophils, monocytes and macrophages) and natural killer cells. Several of the aforementioned components of the innate immune system may contain large amounts of protein, and their production may contribute towards an additional requirement for protein during pathogen challenges of different kinds and doses. It is clear from literature data that the increases in maintenance due to innate immune functions are related directly to the host's pathogen load e.g. Taylor-Robinson (2000).

Marginal response experiments have suggested 1-3 fold increases in maintenance, but these estimates are from average marginal responses, and may underestimate the maximum requirement. The protein cost of innate immunity is as an initial attempt predicted as a multiple of the maintenance requirement of the healthy animal, which thereby relates it to the size of the animal. The amino acid content of the protein required for the innate immune responses, aa_iIIC (g/kgP), was taken as the average of six acute phase proteins (Reeds *et al.* 1998). This allows the amino acid requirement for innate immunity, $IIaa_i$ g/hr, to be predicted as a multiple of requirements for maintenance during health through the value of α and $\alpha \geq 1$, which is a function of PL through:

$$\alpha = 1 + (R.\alpha_a).exp.(-exp(-(PL - (PL_\alpha/R))/\Phi)) \quad (27)$$

$$IIaa_i = (((\alpha.MP) - MP)/ep).\ aa_iIIC \quad \text{g/hr} \quad (28)$$

At low doses, there is little effect on α while the dose is still less than that required to cause a reduction in RFI (Sandberg *et al.* 2006). Thereafter the requirement increases, at a

rate, Φ /n which is always ≥ 0, with increasing pathogen loads towards the genetic maximum, α_{max}. The value of PL_{α} (n) describes the inflection point of the curve. This relationship is preferred to a broken stick function because of the economy of parameters: as it is a continuous function, it may better reflect the underlying biology. The form of the relationship is seen as specific to the degree of resistance (R) of the host and the value of R has a hypothetical range of 0.1 – 0.5, to coincide with the effects of resistance on *RFI* (Sandberg *et al.* 2006). A more resistant genotype is proposed to recognise a pathogen earlier and thus mount an immune response at a lower *PL*, and, it is able to achieve a greater maximum response e.g. Kreukniet *et al.* (1994).

5.3.2.2.2 Amino acid cost of expressing acquired immunity

The innate immune response provides fast protection against pathogens, but after a certain level of challenge has been exceeded, the animal recognises the pathogen and mounts an acquired immune response. An animal needs to acquire the ability to mount an immune response before it can express it. The degree of acquired immunity, EIR_p, can have a value between 0 (completely naïve to a pathogen) and 1 (fully immune to the pathogen). The rate at which an animal acquires immunity is a function of the degree of resistance and its previous experience of a particular pathogen which is described here as the cumulative pathogen load, *CPL* n. The animal acquires immunity above a certain minimum threshold for an acquired immune response to occur, CPL_A n, through:

$$EIR_p = exp.(-exp(-((CPL - CPL_A) - (CPL_0/R))/\Psi)) \quad (29)$$

The parameter Ψ *(/n)* describes the rate at which the host acquires immunity and is kept constant in the model, while through the effect of R on the inflection point (CPL_0, n*)* a more genetically resistant genotype recognises the pathogen at lower *CPL* and acquires immunity at a greater rate.

The actual expression of immunity is proposed to be related to the *PL* of a host as it would appear that animals invest proportionate amounts of resources towards a given pathogen load e.g. Taylor-Robinson (2000), Johnson *et al.* (2004) and Kringel & Roepstorff (2006). The maximum expression of immunity (EIR_{max}, kg/hr) and amino acids (aa_iEIR_{req}, g/hr) for expression of immunity may be predicted as:

$$EIR_{max} = (R.\epsilon.MP.EIR_p).\ exp.(-exp(-((PL - PL_A) - (PL_{EIR}/R))/\delta)) \quad \text{kg/hr} \qquad (30)$$

$$aa_iEIR_{req} = (EIR_{max}/ep).aa_iEIRC \quad \text{g/hr} \qquad (31)$$

Where EIR_p is the extent of acquired immunity, which was described above. The value of $\epsilon.MP$ sets the maximum amount of protein produced for an acquired response to be a multiple of its requirement for maintenance, and as a starting point $\epsilon = 0.5$. The scaled maintenance requirement is used as it has the consequence of a larger animal being able to produce more immune proteins and cope better with a given level of challenge than a smaller animal (Fagbemi *et al.* 1991; Otesile *et al.* 1991). The values of PL_{EIR} (n) and δ (kg.n/hr) together describe the rate of the response in expression of immunity to increasing levels of *PL*. The requirement for amino acids is calculated from an assumed amino acid content of proteins in acquired immunity, aa_iEIRC g/kg EIR, which is the average amino acid content of two immunoglobulins (Chapter 4). As was described for innate immune responses, a more resistant genotype is proposed to produce more immune proteins for expression of immunity at a given *PL* than a less resistant one. This is achieved in the model by reducing the inflection point and increasing the asymptote with increasing degrees of resistance.

5.3.2.2.3 Amino acid cost of repair and replacement of damaged tissues and body fluids

A pathogen may cause damage to host tissues such as gut wall or blood cells and cause losses of body fluids, which need to be repaired and replaced by the animal. In Chapter 4 evidence was considered for the relationship between level and kind of pathogen challenge and the extent of damage caused to the host. Both intracellular bacteria and viruses will cause damage during their replication phase, and other kinds of bacteria may cause further damage due to the secretion of toxins (Hasday *et al.* 2003; Graham *et al.* 2005). The model predicts that the damage caused on a particular day is a direct cost to the animal on that day. The protein cost associated with replacing damaged tissues and lost fluids, *DR* kg/hr, is related to *PL* through:

$$DR = ((\phi.MP).\ exp.(-exp(-((PL - PL_A) - (PL_{DR}))/\omega)))/ep \quad \text{kg/hr} \qquad (32)$$

Where $\phi.MP$ sets the maximum amount of damage that a pathogen can cause to a host on a particular day, and as an initial attempt $\phi = 1.4$ as it has been observed that the cost of repairing damage is a significant cost to the animal (Chapter 4). The inflection point (PL_{DR}, n) and rate parameter ω (kg.n/hr) describe the extent of damage caused by a

particular pathogen at a given *PL*. It is assumed that the material efficiency of replacing damaged body protein is the same as that predicted for *ep* during health. The amino acid content of protein for replacement and repair of damaged tissues is assumed as that of the amino acid content of whole body protein (AA_iC_B) which allows the prediction of the amino acid requirement for repair of damaged tissues aa_iDR g/hr:

$$aa_iDR = DR.AA_iC_B \quad \text{g/hr} \quad (33)$$

It is unlikely, that for the chosen model pathogen that maximum damage rate is ever achieved as it would likely be associated with severe clinical disease or death. The cost due to damage at a time once the animal is expressing immunity may be affected by the extent at which immunity is expressed. Thus by being able to express immunity fully, at a time, the rate of the reduction in *PL* (and hence cost due to damage) would be faster than in the instance where the animal is able to express immunity to a lesser extent e.g. because of being restricted in terms of resources, or due to not having fully acquired immunity.

5.3.2.3 Predicting the requirement for energy during pathogen challenges

A pathogen challenge may cause increased requirements for energy for the production of immune proteins (i.e. the energetic cost of expressing immunity) and the cost of mounting a fever for the model bacteria considered here. The cost of making immune proteins is incorporated in the energetic cost of protein retention and is predicted as was described earlier. It is proposed here that the main cost associated with a pathogen challenges such as bacteria and viruses is that associated with fever. In the case of gastrointestinal parasites, the most significant cost appears to be associated with damaged tissues resulting in additional amounts of nitrogen appearing in the urine (McRae *et al.* 1982), but this is not considered here.

To predict the energy requirement associated with fever it is necessary to account for the relationship between fever and *PL* and the energetic cost of each degree of fever. The increase in body temperature due to fever, E_F °C, is predicted as:

$$E_F = s.(PL\text{-}PL_F) \quad (34)$$

The value of s describes the rate of increase in body temperature per unit increase in *PL*. Fever is not predicted to occur below a certain threshold (PL_F). The increase in energy requirement for each degree of fever θ (MJ/°C) appear to be consistently around 1.15 times maintenance / deg C of fever e.g. van Dam *et al.* (1998). Thus the energetic cost of fever, F_{EE} MJ/d, is predicted as a multiple of maintenance, which means that a larger animal will have a greater energetic cost for expressing fever:

$$F_{EE} = E_F.\theta.E_m \qquad (35)$$

The above system allows the prediction of the requirements for amino acids and energy during pathogen challenges, which are added to the requirements during health to predict the overall amino acid and energy requirements. To predict the actual food intake and growth during pathogen challenges it is necessary to integrate the above framework with the framework for predicting anorexia (Sandberg *et al.* 2006) and rules for the partitioning of amino acids and energy between growth, damage and immune functions. Solutions to these problems are now presented and predictions of the model are then explored.

5.3.2.4 Predicting actual growth and food intake during pathogen challenges

Chapter 4 considered literature data of resource partitioning during disease, and found that it may be necessary to consider the partitioning of amino acids between growth and immune functions. In this model, the requirement for innate immune proteins is included as part of maintenance as these appear insensitive to nutrient supply. Amino acids are partitioned first to maintenance, which includes the traditional maintenance requirement, requirement for innate immune proteins and repair and replacement of damaged tissues. The remaining amino acid supply above maintenance is then partitioned towards growing 'body' protein and immune proteins. It is assumed that the marginal material efficiency of growing body protein (or immune proteins) from food amino acids remains the same as that during health and that its value applies for all amino acids. The marginal material efficiency of amino acid retention is predicted as was described earlier.

The model for predicting the reduction in voluntary food intake that occurs during pathogen challenges of immunologically naïve animals was described in Sandberg *et al.* (2006), and it is only briefly mentioned here, together with some enrichment to join it with the framework for predicting *PL*. Their model predicted the relative food intake

(RFI), which was defined as the voluntary food intake of a diseased animal divided by the voluntary food intake of that animal in the same state if not challenged by a pathogen. The general pattern of *RFI* during a pathogen challenge can be summarized by five main parameters. The lag time (L d) is the delay from the pathogen challenging the host until *RFI* falls below unity where $0 \leq L \leq L_{max}$. The *RFI* then falls over the reduction time (R d) where $0 \leq R$, to a level λ. The lowest intake, $0 \leq \lambda \leq 1$, is maintained for the duration time (D d) where $D \geq 0$. It is assumed that for a given case there is no further reduction in *RFI*, which then starts to recover at a rate (ρ /d), where $0 \leq \rho$, until $RFI = 1$. In this model, the value of λ_{min} (the lowest reduction in *RFI*) is predicted as a function of the challenge dose and host genetic resistance. The actual *RFI* is predicted using a relationship that relates *PL* with *RFI* and $RFI \geq \lambda_{min}$. This model reproduces that described in Sandberg *et al.* (2006), while allowing for D and ρ to become dependent on the extent to which the host is able to express immunity.

To predict the partitioning of amino acids towards growing body protein and immune proteins a partitioning parameter (γ) is used which is made a function of *PL*, and it is assumed constant for different amino acids:

$$\gamma = (R.\gamma_{max}).\ exp.(-exp(-((PL - PL_A) - (PL_{EIR}/R))/\kappa)) \quad (36)$$

Where the value of γ_{max} is the largest proportion of resources that are partitioned towards immunity, which in the model is greater for a more resistant genotype. The value of PL_{EIR} and κ describe the rate of change in partitioning for increasing pathogen loads. At low pathogen loads, it would be expected for a host to partition small amounts of amino acids towards immune responses, but as the level of challenge and risk to the host increases, the host would prioritize resource allocation towards expression of immunity. Thus actual non-immune protein retention (PR', kg/hr) and actual expression of immunity (*EIR*, kg/hr):

$$PR' = (e_p.(1- \gamma\,(Faa_iC.d.DCPC.VFI.RFI)) - (MPEaa_i + IIaa_i + aa_iDR))/\,AA_iC_B \text{ kg/hr} \quad (37)$$

$$EIR = (e_p.(1- \gamma\,(Faa_iC.d.DCPC.VFI.RFI)) - (MPEaa_i + IIaa_i + aa_iDR))/\,aa_iEIRC \text{ kg/hr} \quad (38)$$

Equations (37, 38) apply while $EIR < EIR_{max}$ and $PR < PR_{max}$. The above partitioning rule has the consequence that while an animal is facing a situation of scarce resources it is still possible for the animal to mount a significant immune response. At a high degree of resource scarcity, the animal is penalised in terms of immunity to a greater extent. Once the actual rate of expression of immunity has been predicted, it may be possible to predict the extent at which the host prevents the pathogen from replicating, and, killing off a proportion of the pathogen population. The model predicts that the overall rate of removal and killing of pathogens from the host expression of innate and acquired immunity:

$$PLR_t = EIR.\tau + IIR.\sigma \qquad \text{n/hr} \qquad (39)$$

The parameters τ (n/kg) and σ (n/kg) describe the amount of PL that a unit of innate or acquired immunity can kill and remove from the host. This approach of linking the amount of immune protein to the extent of pathogen killing has been used elsewhere e.g. Takumi *et al.* (2005). The condition of $\tau > \sigma$ applies as the acquired immune response is more efficient at removing and killing pathogens from the host. A given pathogen has a maximum growth rate (r_{max}), which in the case where the animal is not expressing immunity (i.e. it is naïve to the pathogen) is the actual growth rate of the pathogen load. This growth rate is reduced as the host starts to express immunity. In the instance where the animal is not able to express the amount of immunity that requires (i.e. resources are scarce) the pathogen growth is not reduced to the same extent through:

$$r = r_{max} - g.(EIR/EIR_{max}) \qquad \text{/hr} \qquad (40)$$

Where g (/hr) is a constant relating the proportional expression of immunity to the pathogen growth rate and as an initial attempt g = 0.0125. The above system is solved on an hourly basis predicting the change in pathogen load as a balance between pathogen growth and host killing through immune responses.

The total PR on a particular day ($PR = PR' + EIR$) can then be used to predict the revised maximum heat loss in Equation (22). The actual heat production is also recalculated to include the effect of fever:

$$HP = (E_m + F_{EE}) + h_f.VFI.RFI + (h_p - N_{ue}).PR + h_l.LR \qquad \text{MJ/hr} \qquad (41)$$

Due to fever increasing *HP* and anorexia reducing heat production associated with the amount of food eaten and growth, the overall *HP* would not always be greater than that of a healthy animal. In the case where *HP* is greater than HP_{max} it is assumed that in this instance the animal will prioritise *PR* and reduce *LR* to ensure that $HP = HP_{max}$. *LR* is then predicted on energy balance grounds given *VFI*, *MEC*, and *PR*.

5.3.2.5 Parameterisation

In order to simulate the above framework it was necessary to choose the parameters values for each of the functional forms. Due to a lack of data, it was not possible to parameterise the model in a formal manner. The model was therefore set up for a hypothetical challenge dose range of 10 000 – 150 000 n/d which together with the pathogen growth rate lead to a range in *PL*. In order to choose values for the rate and inflection point parameters it was necessary to approximate the maximum response i.e. maximum increase due to innate, acquired or damage. There is an indication in the literature of what these values may be as was reviewed in Chapter 4, and these are shown together with the other parameter values in Table 2.

Table 2 A summary of the model parameters used to predict amino acid and energy requirements together with their proposed values for a chosen model pathogen. The main parameters are shown in bold, followed by their sub-parameters in italics.

Parameter	Description	Value or range
R	Scaling parameter for the degree of genetic resistance of a host	0.1 – 0.5
IIaa$_i$	Amino acid requirement for innate immunity	Assumed
α	*Multiplier of maintenance for innate immunity requirement*	Assumed
α_a	*Maximum value of* α	0.2
PL_0	*Inflection point for calculating* α	$1x10^5$
Φ	*Rate parameter for calculating* α	$3x10^4$
aa$_i$IIC	*Amino acid content of innate immunity proteins*	1
aa$_i$EIR$_{req}$	Amino acid requirement for acquired immunity	Assumed
EIRp	Potential level of acquired immunity (0-1)	Assumed
CPL_0	*Inflection point for calculating EIRp*	$6x10^5$
Ψ	*Rate parameter for calculating EIRp*	$9x10^4$
EIR$_{max}$	Rate of expression of immunity at a particular time	Assumed

ϵ	*Constant for predicting EIR_{max}*	0.5
PL_{EIR}	*Inflection point for calculating EIR_{max}*	$2x10^6$
δ	*Rate parameter for calculating EIR_{max}*	$8x10^5$
aa_iEIRC	*Amino acid content of proteins part of acquired immunity*	[1]
$\mathbf{aa_iDR}$	Amino acid requirement for repair of damage	
ψ	*Constant for predicting maximum damage*	1.4
PL_{DR}	*Inflection point for calculating damage*	$6.0x10^6$
ω	*Rate parameter for calculating damage*	$5x10^6$
$\mathbf{F_{EE}}$	Energy requirement due to fever	
F_{max}	*Maximum increase in body temp due to fever*	1.5
PL_F	*Inflection point for calculating fever*	$1x10^7$
s	*Rate parameter for calculating fever*	$8x10^6$
θ	*Increase in energy requirement per degree of fever*	1.15
γ	Partitioning parameter	Assumed
γ_{max}	*Maximum value of γ*	0.15
PL_{EIR}	*Inflection point for calculating γ (same as that for EIR_{max})*	$1x10^5$
κ	*Rate parameter for predicting γ*	$3x10^4$
$\mathbf{r}$	Maximum growth rate of bacteria (/d)	1.03
PLRt	Rate of removal and killing of pathogens	Assumed
τ	*Number of pathogens removed per unit acquired immunity*	$1.7x10^5$
σ	*Number of pathogens removed per unit innate immunity*	$1x10^3$
λ	Reduction in RFI	Assumed
λ_{min}	*Lowest reduction in RFI during sub-clinical disease*	0.72
λ_{rate}	*Rate parameter for reduction in RFI with increasing PL*	$3x10^{-7}$
T_d	*Threshold PL for pathogen to be recognised*	$1x10^4$
T_c	*Threshold PL at which host becomes clinical*	$3x10^8$

[1] See Chapter 4

The assumed maximum value for each of the forms was taken from indications in the literature. These were then combined with the range of *PL* considered and thereby the values of the intermediate parameters (rate and inflection point parameters) were derived. As an initial attempt to explore the system this may be seen as acceptable e.g. Austin & Anderson (1996) and Hoshen *et al.* (2000). The overall model has now been described and in the following sections, model predictions and their consequences were explored.

5.4 Results

The proposed framework was developed to predict the effects of pathogen challenges of different doses on pigs with different degrees of resistance and/or growth characteristics, when given access to different kinds of foods. The model was used to make predictions across the resulting experimental space. The first scenario considered was the effect of dose on performance at two live weights. Then the outcome of challenges with different doses on a host that was given different foods was explored. The experimental space was increased to consider the outcome of different doses on a host of different degrees of resistance, when given different kinds of food. The final set of simulations considered the effect of different doses on a host with different potentials for growth and degrees of resistance, when challenged by different doses, and given access to different kinds of food.

5.4.1 The effects of pathogen challenges of different doses on performance at different live weights

Pathogen challenges of three different doses (20 000, 40 000 and 60 000) were simulated for a pig of either 20 or 50kg live weight at the point of the challenge.

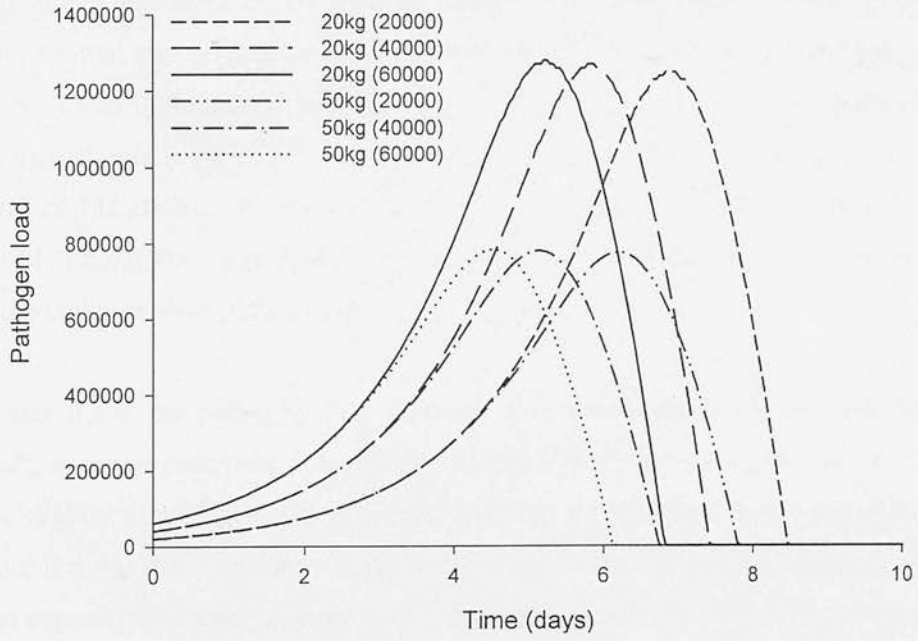

Figure 1a Predictions of pathogen load over time, of either a 20 or 50 kg BW pig when challenged with one of three different doses of a pathogen, and given *ad libitum access* to a high quality food.

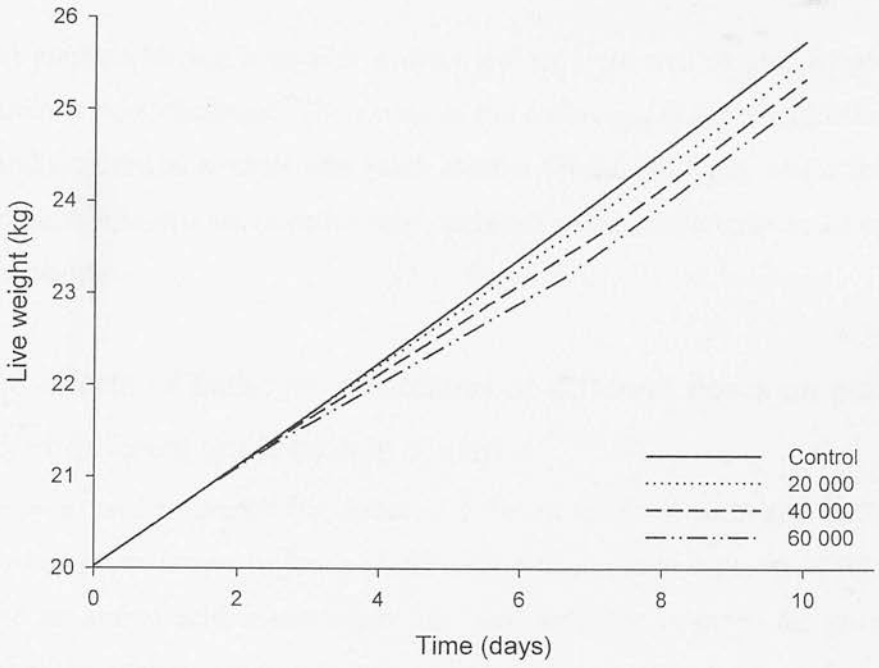

Figure 1b The predicted live weights of an animal given one of three doses of a pathogen challenge when given access to a good quality food that was balanced (for the 20kg pig) in terms of protein (amino acids) and energy.

Default model parameters were used for amino acid composition of food protein and growth potential and genetic resistance of the animal. Two foods were used that ensured that protein was first limiting for both weights and that the food was balanced in protein and energy (Pomar *et al.* 2003). The 20kg pig were given a food containing 0.2 kg CP/kg DM and 15 MJ DE/kg DM and the 50kg pig was given a food with 0.18kg CP/kg DM and 16 MJ DE/kg DM. The change in pathogen load over time is shown in Figure 1a for the two weights at three pathogen doses.

At greater doses the pathogen load develops at a faster rate, while the host acquires immunity to it at a faster rate, and therefore at larger doses the pathogen loads both peaks (with a slightly greater pathogen load) and starts to decline at an earlier time point. The 50kg pig is more able to reduce the pathogen load (and a lower peak pathogen load) as it has the capacity to mount a greater immune response. The performance of the animal follows closely the extent of the pathogen load in the animal, as a non-limiting food was used here (Figure 1b). The predicted reduction in protein retention follows the reduction in food intake (data not shown). Once the animal has recovered from the challenge, the protein retention was less than its controls, at a time. The difference occurs from the

challenged animals having a smaller protein weight (reflected in live weight) than the healthy controls post challenge. The extent of the difference in protein retention between controls and challenged animals was much smaller for the 50kg pig, as for this genotype the rate of change in protein retention with increasing size was greater at 20 compared to 50kg body weight.

5.4.2 The effects of pathogen challenges of different doses on performance for foods of different crude protein contents

The model was used to predict the effect of different levels of pathogen challenges on a 20kg pig when given access to foods of different crude protein contents (CPC). The food protein had an amino acid composition that was perfectly balanced for growth. It was assumed that the animal was in a thermoneutral environment throughout. In Figure 2 are the predicted responses in protein retention for two foods and two doses used (40 000 and 80 000). On a low protein food (0.12 kg CP/kg DM) it would be expected that the healthy animal has a constrained intake due to the production of heat (Pomar *et al.* 2003).

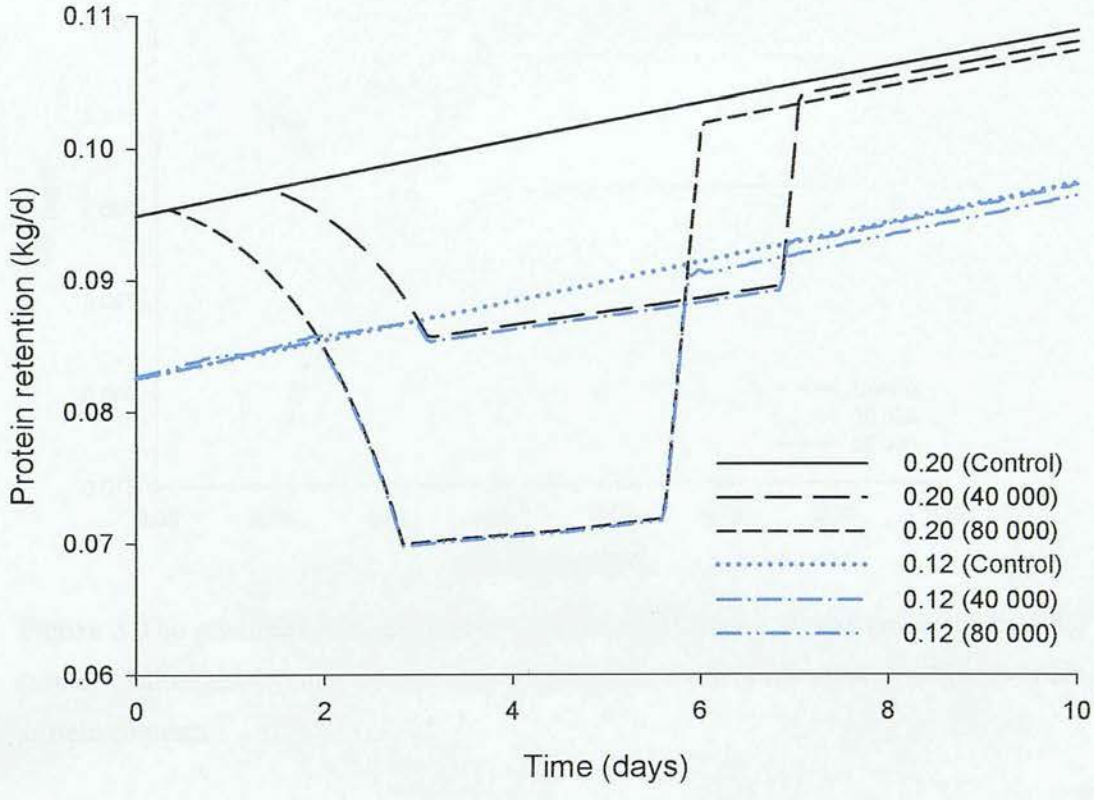

Figure 2 The predicted rates of protein retention for a 20kg pig when given a food containing 16MJ DE/kg DM and either 0.12 (blue lines) or 0.20 (black lines) kg CP/kg DM and challenged by two different doses of pathogen.

The predicted rates of protein retention, show an interaction between dose and crude protein content on the extent of the reduction in protein retention in relation to controls on the same diet. This effect was more prominent at a dose of 40 000 compared to the higher dose of 80 000, as expected. The interaction arose from the pig on the low protein food reaching its heat loss constraint, and thus the extent of the reduction in food intake being smaller compared to its healthy control, which was also constrained by its heat production.

The effects of different crude protein contents on the outcome of a pathogen challenge was investigated further by performing an marginal response experiments as done normally (Williams *et al.* 1997a,bc,). The model was set up to run for defined period of time (10 days) and the model calculates the average protein retention over this period and the average protein intake over this period, as done in 'real' time rather than weight experiments. The 20kg pig was given *ad libitum* access to a food with different crude protein contents where the amino acid composition was the default of the model and constant digestible energy content (16 MJ DE/kg DM).

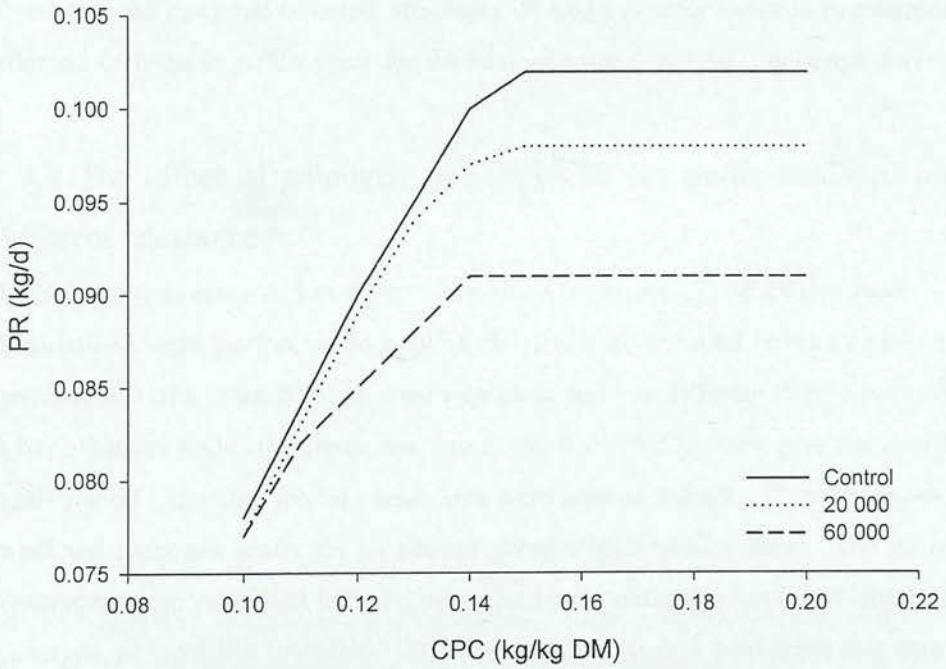

Figure 3 The predicted average protein retention (PR) over a 10 day period of an 20kg animal challenged by one of two doses and given foods with several different crude protein contents.

A dose of either 20 000 or 60 000 was used and the model was run for foods ranging from 0.1 to 0.28kg CP/kg DM in 0.01 steps. For the *ad libitum* high quality food the predictions were as expected, and these are shown in Figure 3.

The predictions in Figure 3 show the response in protein retention to increasing levels of crude protein in the food for a 20kg animal. The challenge doses caused a slight displacement due to an increase in maintenance, which was slightly greater at the higher dose. The response of the low dose is similar to that of control, once having taken into account the displacement, but as the crude protein content increases, the challenged animal reaches a lower plateau than the healthy control. This effect was greater at the higher dose. This occurs due to the challenged animal, at a time, being smaller than the healthy control and therefore having lower maximum protein retention, at a time. At a degree of maturity, there was no difference in the maximum protein retention. At the higher dose, the slope of the line was affected. This effect occurred due to a larger dose having a greater effect on food intake and growth with greater effects on the size of the animal. The relationship between protein retention and protein intake (data not shown) show that the marginal material efficiency of using protein for protein retention was not affected with the slope the same for the healthy control and two challenge doses.

5.4.3 The effect of pathogen challenges on the performance of hosts with different resistance

5.4.3.1 The resistance of genotypes when given access to a good quality food.

Simulations were performed to explore the effect of different doses of pathogen on the performance of a 20kg BW host that was made to have different degrees of resistance on a hypothetical scale of genetic resistance (R: 0.1 – 0.5), when given access to a good quality food. All other model parameters were kept at default values. In Figure 4 are the predicted pathogen loads for an animal given a high quality food. The more resistant genotypes were predicted to have overall a lower pathogen load than the less resistant genotype, as would be expected. The responses in pathogen load show that care is needed for the dosing regimen used and the time point for measuring pathogen loads, when trying to assess the degree of resistance of different genotypes.

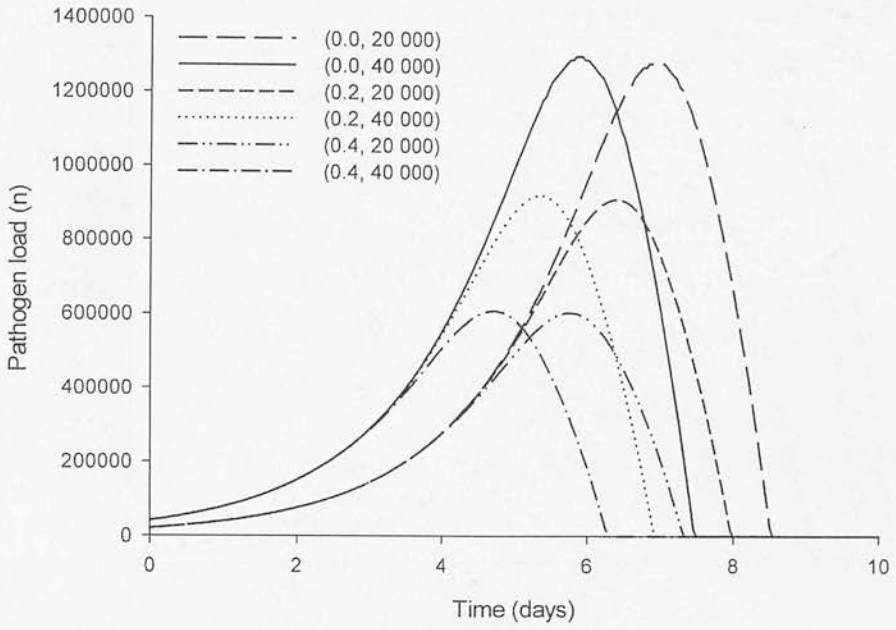

Figure 4 The predicted pathogen load of an animal at three different degrees of resistance (0.0, 0.2, 0.4) and two doses (20 000, 40 000) when given a good quality food.

5.4.3.2 The extent of resistance when given either a high or low quality food and restricted by bulk

The model was set up to run simulations for the same sets of resistance and dose as were shown in Figure 4, but on this occasion the foods were of two kinds (0.1kg CP/kg DM or 0.2kg CP/kg DM). In addition, the animals were restricted through the bulkiness of the food. The predicted pathogen loads for R (0.0) and R (0.2) are shown in Figure 5a and 5b, respectively.

This experiment was performed to assess whether a more resistant genotype, as defined by this framework, would still appear more resistant in a situation where resources are either very scarce or sufficient. The predicted pathogen loads in Figure 5a show that a genetically susceptible animal that is restricted and given a poor quality food has large penalties on immunity, with subsequently greater pathogen loads, and it took longer for the animal to eliminate its load. The model predicted an interaction between dose and crude protein content in that the increase in the maximum pathogen load for the low protein food was greater at the dose of 40 000 compared to the dose of 20 000.

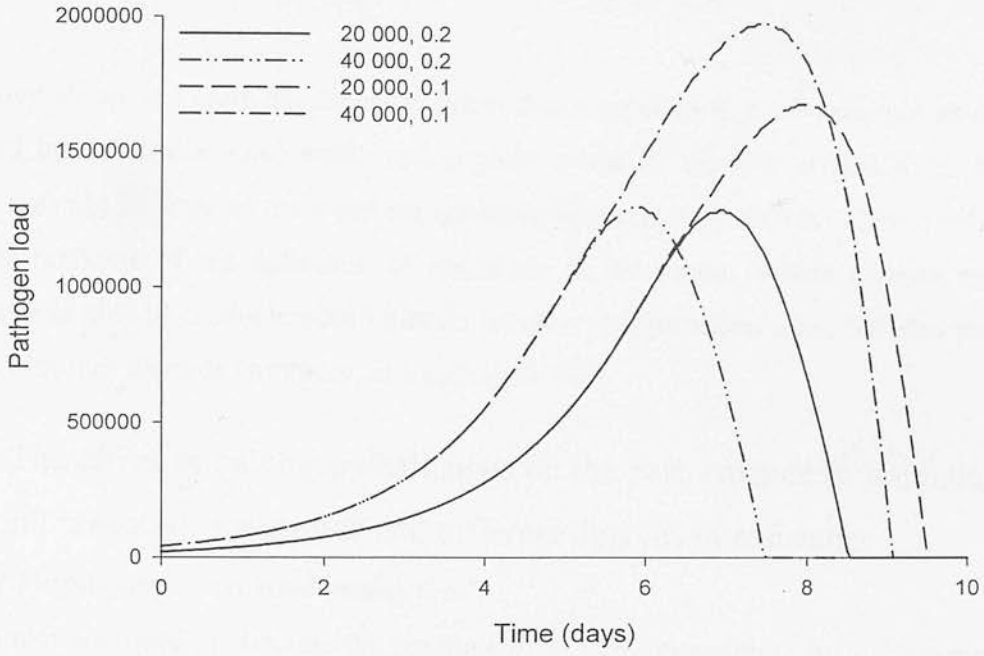

Figure 5a The predicted pathogen loads of a genotype with genetic resistance of 0 (the default genotype in the model), when challenged by either 20 000 or 40 000 pathogens and given a food containing either 0.1 or 0.2 kg CP/kg DM. For details of feeding regimen see text.

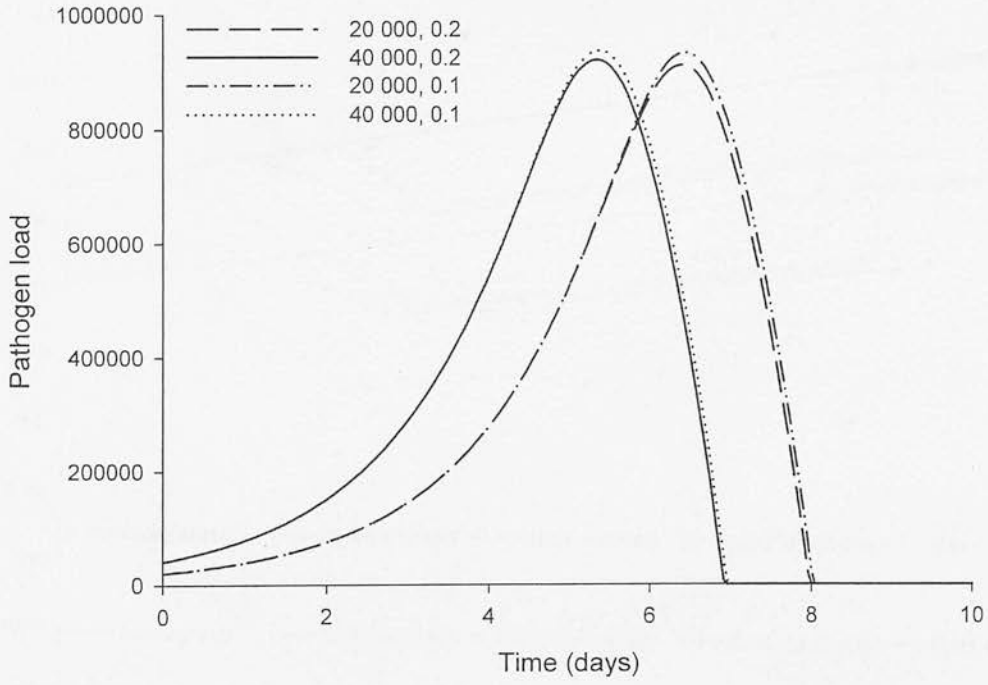

Figure 5b The predicted pathogen loads of a genotype with genetic resistance of 0.2, when challenged by either 20 000 or 40 000 pathogens and given a food containing either 0.1 or 0.2 kg CP/kg DM. For details of feeding regimen see text.

The predictions in Figure 5b, however, show that a genetically more resistant animal (as defined by this framework) performed slightly worse on the low protein food, but the effect was not as large as observed for the more susceptible genotype. These predictions are the outcome of the definition of resistance in the model, where a more resistant genotype is able to produce more immune proteins at a pathogen load, but also partition more resources towards immunity, at a pathogen load.

5.4.5 The effect of pathogen challenges on the performance of animals with different potentials for growth and different degrees of resistance

5.4.5.1 Performance on a good quality food

The model was used to simulate the outcome of a 2x2x2 experiment for a 20kg pig with two levels of resistance (Default and 0.2), two levels of potential for growth (B = 0.011 or 0.012/d) and two pathogen doses (20 000 and 40 000/d). The food was such that both genotypes were eating for amino acids, but were able to achieve their potential when healthy.

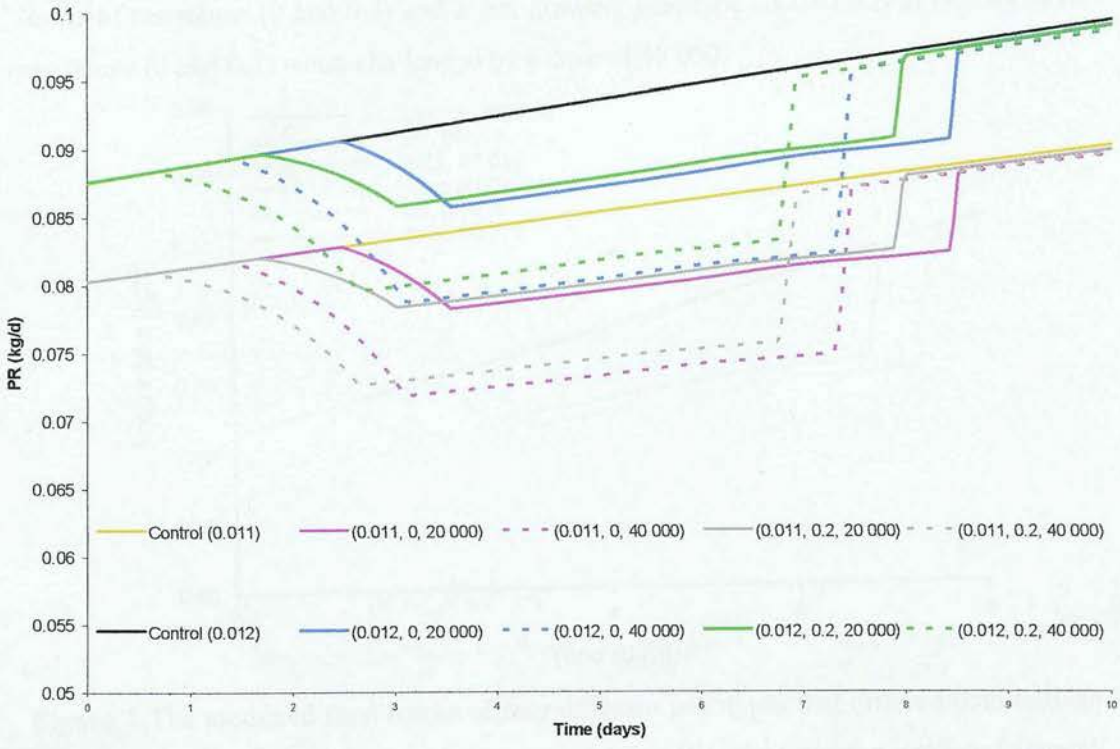

Figure 6 The predicted protein retention of four genotypes with different combinations of growth potential and resistance (Potential, Resistance) when challenged by a dose of 40 000 pathogen and given a good quality food.

The food contained 0.2kg CP/kg DM and had a DE content of 16 MJ/kg DM. The predicted rates of protein retention are shown in Figure 6 for one level of dose (40 000). The predicted rates of protein retention in Figure 6 (and those for the dose of 20 000, data not shown) indicated an interaction between dose, degree of resistance and growth potential for the extent of the reduction in protein retention at a time.

The potentially faster growing genotype was predicted to grow less than the healthy slow growing control, and for a brief period the faster growing genotype with R = 0.0 grew slower than the slower growing genotype with a R=0.2. This interaction did not occur for the lower dose (data not shown), where the genotype with the higher potential for growth had faster rates of protein retention in all cases. This experiment was repeated for a range of combinations of R and B, and for higher degrees of resistance the above interaction did not occur, and the faster growing genotype performed better at all times. Similarly, a fast growing genotype (B = 0.015/d) also had greater rates of protein retention at all times, compared the slow growing genotype (B = 0.011/d).

5.4.5.2 Performance when given either a high or low quality food and restricted by bulk

The model was set up to run simulations for a slow growing genotype (B 0.013/d) at two levels of resistance (0 and 0.2) and a fast growing genotype (B 0.015/d) at two levels of resistance (0 and 0.2) when challenged by a dose of 40 000.

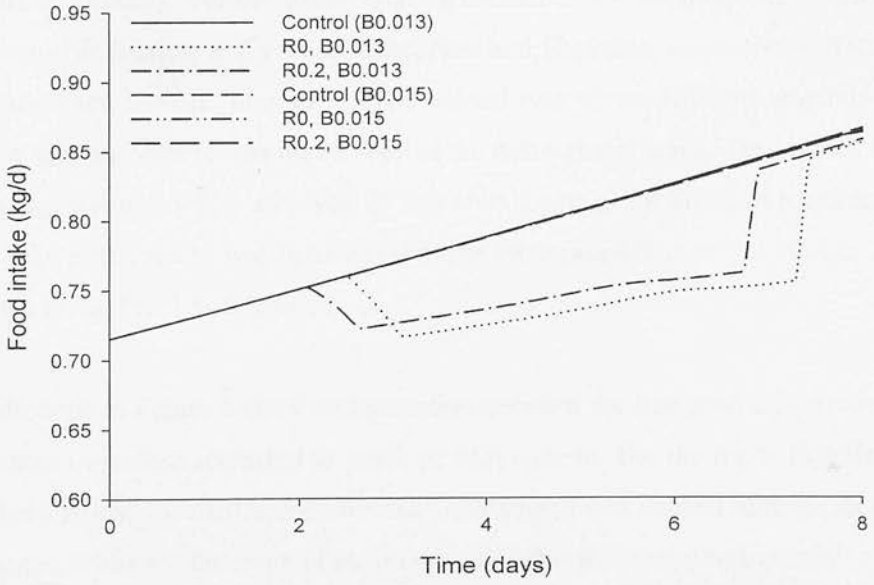

Figure 7 The predicted food intake of four different genotypes that differed both in their growth potential (B) and degree of genetic resistance (R) when given a restricted amount of a food containing 0.2kg CP/kg DM.

The simulations were performed for a 20kg pig that was given two kinds of food: a low protein food (0.1kg CP/kg DM) or a high protein food (0.2kg CP/kg DM). As was described earlier the animals were restricted through the bulkiness of the food to the intake of the slow growing genotype on a balanced food. There was an interaction between crude protein content and growth potential on the predicted food intake: the predictions for the 0.2kg CP/kg DM food are shown in Figure 7. The predictions in Figure 6 show that the slow growing genotype exhibited anorexia at both degrees of resistance, while the faster growing genotype did not. This occurred because of the bulk restricted intake being less than the voluntary food intake predicted for the fast growing genotype.

5.4.6 The effect of pathogen challenges on the performance of different genotypes when given foods with different first limiting amino acids

The model was used to explore the effects of food proteins with different amino acid contents to the default in the model (ideal composition for growth). The model was run for a challenge dose of 40 000 to identify the ranking of the first, second, third and fourth limiting amino acids during the peak requirement for immune responses, when given a food protein with the default model amino acid composition. The first, second, third and fourth most limiting amino acids were determined by running the model to be Tryptophan, Methionine + Cysteine, Threonine and Histidine, respectively. Two sets of simulations were then performed: 1) The animal was given different amounts of food protein, where its content was maintained at the same proportion of the protein, but with lysine being first limiting in all cases. 2) The animal was given different amounts of food protein, where its content was maintained at the same proportion of the protein, but with tryptophan being first limiting in all cases.

The predictions in Figure 8 show an interaction between the first limiting amino acid and the response in protein retention to crude protein content. For the foods first limiting in lysine there is less of a difference between challenged and control animals in terms of maintenance, while for the tryptophan limiting food this difference was greater.

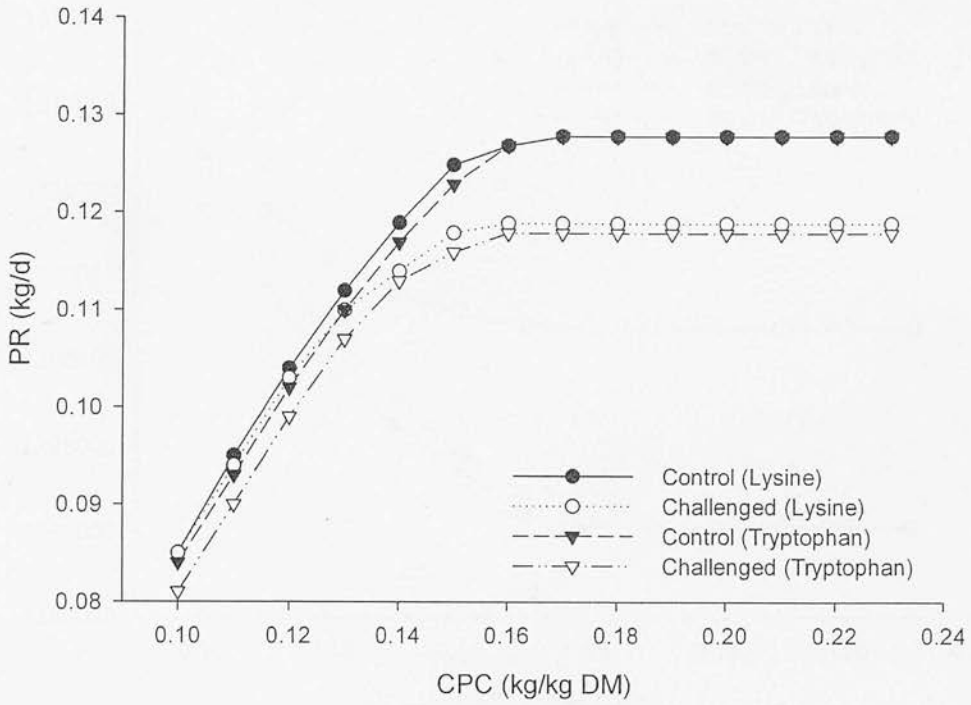

Figure 8 The predicted protein retentions (PR, kg/d) of a fast growing genotype (B = 0.016/d) when given foods with different crude protein contents (CPC, kg/d) which were either first limiting in lysine or tryptophan.

This would imply that when trying to identify the nutritional cost of pathogen challenges, it is essential that one amino acid is first limiting throughout, and that the amino acid is one which is required more for the immune response (Tryptophan in this case). As was observed earlier the challenged animals did not achieve the same plateau as their healthy controls due to a difference in size at a time.

The same experiment as that described above was performed for four genotypes: these were B=0.013 and 0.016/d that either had a degree of resistance of 0 or 0.2. The model estimated the maximum pathogen loads and these were plotted against the crude protein content of the food. These are shown in Figure 9 for the fast and slow growing genotype with a resistance of 0.

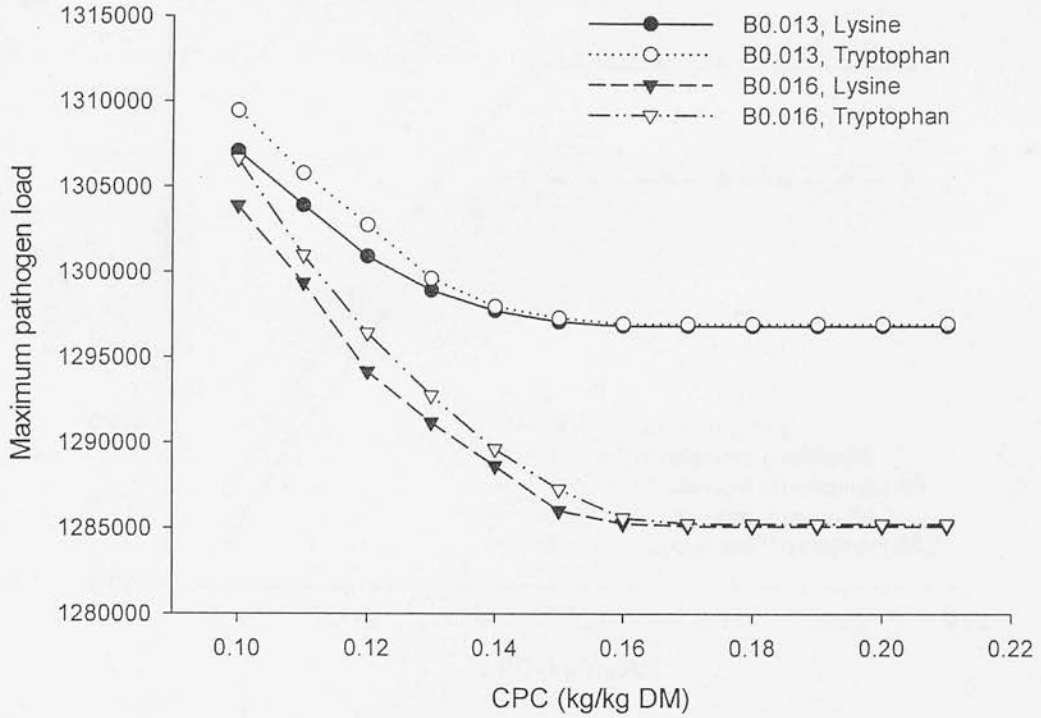

Figure 9 The predicted maximum pathogen loads of a slow and a fast growing genotypes (B = 0.013 or 0.016/d) when given foods with different crude protein contents (CPC, kg/d) which were either first limiting in lysine or tryptophan.

The predictions in Figure 9 show an interaction between crude protein content and growth potential on the ability of the animal to cope with a challenge, measured here as the maximum pathogen load. At high crude protein contents (0.18 – 0.21 kg/kg DM) the fast growing genotype copes better with the challenge. Thereafter, as the crude protein content is reduced, the increase in the maximum pathogen load is greater than for the slower growing genotype. This was particularly the case for the food first limiting in Tryptophan, which is present in large quantities in immune proteins. The crude protein content at which the maximum pathogen load starts to become affected is also higher in the fast growing genotype. These results show that it is also essential to adequately design experimental diets when comparing the performance of different genotypes. This is shown further in Figure 10, where the predicted protein retentions are plotted against crude protein intake for two genotypes that differed in their degree of resistance.

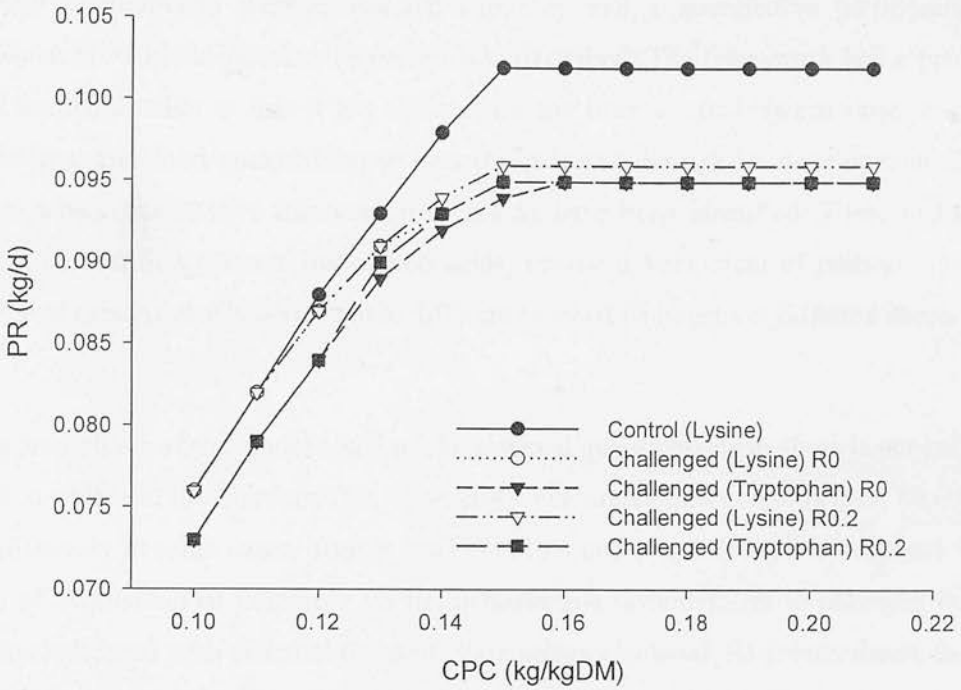

Figure 10 The predicted protein retention (PR, kg/d) of two genotypes differing in their degree of resistance when given foods with different crude protein contents (CPC, kg/kg DM) which were either first limiting in lysine or tryptophan.

At low crude protein contents, the model predicted that there was no difference in performance between the two resistance genotypes when given either a lysine or tryptophan limiting protein. At slightly higher crude protein, the difference in resistance could be observed for the more resistant genotype eating the tryptophan limiting protein, but not for the lysine limiting food protein. Overall, the predictions of the model have demonstrated several difficulties in comparing the performance of different genotypes when given different foods and challenged by different doses.

Discussion

The effects of sub-clinical pathogen challenges on growth and performance have long been recognized, but no general model has been previously proposed. The framework that has been proposed here is a starting point for predicting the effects of pathogen challenges of different doses on growth and performance of genotypes with different degrees of resistance and potentials for growth when given access to different kinds of food. The model is a more general and comprehensive than previously published attempts (Henken *et al.* 1994a,b; Black *et al.* 1999). The effect of pathogen challenges on amino acid and

energy requirements were considered explicitly and a quantitative partitioning rule between growth and immune responses was described. The framework has a predictive and heuristic value in that it has allowed for the interactions between dose, degree of resistance and food composition to be explored, and through its development, several areas where quantitative information is lacking have been identified. These include the effect of nutrition (protein and amino acids) on the development of pathogen loads in different genotypes when exposed to different types of pathogens at different doses.

The predictions of the model could not be assessed quantitatively as there is not sufficient data available in the literature (see Chapter 4). The predictions can, however, be assessed qualitatively in some cases. Timms *et al.* (2001) found a similar kind of response for the rate of acquisition of immunity for the parasitaemia (synonymous to pathogen load) of mice challenged with different doses of *Plasmodium chabaudi*. At greater doses, the peak pathogen load occurred earlier and was slightly greater. Thus, the assumption in the model that an animal that is challenged by a larger dose, acquires immunity at a faster rate appears to be reasonable. The effect of dose on live weight is a typical response seen in several experiments (Hein 1964). The effect of nutrition on pathogen load is not clear in the literature for replicating micro-parasites, but the predictions agree with those observed in sheep challenged by gastrointestinal macro-parasites (van Houtert *et al.* 1995) when given foods of high and low protein contents. van Houtert *et al.* (1995) observed that during the initial period of the challenge, there was no difference in worm burden, but once the sheep had acquired immunity the animals given the high protein diets had a faster reduction in worm burden. The predicted pathogen loads for susceptible and resistant genotypes also agree with the findings of for example Paling *et al.* (1991) who found that cattle that are more resistant to *Trypansomes* had at a time lower pathogen loads compared with a more susceptible genotype. In this case, the more resistant genotypes also grew better, as would be predicted by this framework.

Marginal response experiments give animals foods of different crude protein contents, and the average protein intake and retention is measured over the experimental period. Experiments where marginal responses have been measured for animals kept in 'high immune system activation environments (Williams *et al.* 1997a,b,c), during challenges with lipopolysaccharide (Webel *et al.* 1998a,b) and *Eimeria acervulina* (Willis & Baker 1981ab) have found that challenged animals at high crude protein contents had a lower

plateau response (i.e. lower maximum protein retention). In turn, these findings have been used widely as support for pathogen challenges directly affecting an animals potential for growth e.g. Escobar *et al.* (2002, 2004) and Humphrey & Klasing (2004). It has been shown here that a model where the mechanism for reducing food intake is a direct effect on food intake also produces such a response (Figure 3, 7 and 9). This effect was greater at larger doses (Figure 3). The lower maximum protein retentions were predicted as an outcome of an animal being smaller at a time, compared with its healthy controls, rather than its potential protein retention at a degree of maturity being affected.

In this framework, pathogen challenges in a naïve host directly affect food intake, rather than reducing the growth potential of the animal. A central question is what benefit this reduction in intake has to the animal, which has been subject of much debate (Hart 1988; Kyriazakis *et al.* 1998). Anorexia may be part of an immunoregulatory response (Bazar *et al.* 2005), while it may also be *part* of a general beneficial response to cope with a pathogen challenge (Hart 1988; Kyriazakis *et al.* 1998). Elucidating the mechanism (and its purpose) by which anorexia occurs is, and remains, a key problem to solve. An interesting question that this and the work of Wellock *et al.* (2003b) raise is whether infectious stressors and social stressors affect performance by the same mechanism: the model of Wellock *et al.* (2003b) proposes that an animals potential for growth is reduced during exposure to social stressors. Experiments are warranted which address the possible interactions between social and infectious stressors on performance, and the mechanism by which these stressors reduce performance.

A central issue to this framework is how more resistant genotypes, and genotypes with greater degree of resistance partition their resources (Coop & Kyriazakis 1999). In this framework, it was proposed that a more resistant genotype partitions more resources towards its immune responses, as well as producing more immune proteins. This has the consequence that a more resistant genotype will also perform better than a more susceptible on poor quality low protein foods. Simulations performed but not shown have demonstrated that if a more resistant genotype was only to produce more immune proteins at a pathogen load, and have the same value for the partitioning parameter; it would perform worse at low levels of resource supply. It is likely that natural selection for genetic resistance has occurred at times when resources were scarce and pathogen challenge doses were high. Thus, the assumption of the model that a more resistant genotype produces more immune proteins, and partitions more food protein towards

immune functions at a pathogen load may be a useful definition of a resistant host for purposes of artificial selection. The predictions of the model for a resistant and susceptible genotype agree with the scarce data available in the literature for food intake (Akinbamijo *et al.* 1998) and pathogen load (Paling *et al.* 1991).

The model, however, predicted contrasting effects of an animals growth potential on its ability to cope with a pathogen challenge (as measured by maximum pathogen load) on either good or poor quality foods. The negative effect of growth potential was more pronounced when a specific amino acid, which the immune system requires large amounts of, was used. It has been proposed that faster growing genotypes partition more resources towards growth over immune responses and therefore immune responses are less in faster growing genotypes (Bayyarietal *et al.* 1997; Rauw *et al.* 1998; Ask *et al.* 2006). It is not often recognised, however, that the evidence on this issue are not consistent. Ask *et al.* (2006) compared a number of turkey genotypes (on a standard experimental diet) after a challenge with *Escherichia coli*, and the extreme slow growing genotype (99g/7days) had more lesions than the fast growing genotype (295g/7days), and the reduction in live weight gain was greater in the slow growing genotype. Further evidence from turkeys selected for 16-week body weight showed that faster growing genotypes might produce more immune proteins during challenge (Li *et al.* 2000). Coltman *et al.* (2001) found that feral Soay sheep that were larger were also parasitized to a lesser extent, although this may be due to several reasons. Evidence from sheep selected for fast wool growth, suggested slight greater faecal egg counts (which are a proxy of pathogen load) in the sheep selected that grew more wool when challenged by gastrointestinal parasites (Williamson *et al.* 1995ab).

The possible effect in sheep selected for fast wool growth, however, may be due to the very high requirement for sulphur amino acids for wool growth (Williamson *et al.* 1995ab). Sulphur amino acids as predicted by this model are for a food protein balanced for amino acids for growth the 4th most limiting amino acid during pathogen challenges. Miller *et al.* (1998) tested this idea by giving abomasal infusion with cysteine to the sheep genotypes developed by Williamson *et al.* (1995ab). The relative reduction in worm burden of *Trichostrongylus colubriformis* due to cysteine infusion was greater for the genotype selected for wool growth. This framework could explain their findings by including a requirement for amino acids for wool growth. The above experiments, overall

do not demonstrate clearly whether faster growing genotypes are penalised differently to slow growing genotypes, when exposed to pathogens.

A key issue raised by the model is the performance of different genotypes (growth potential and degree of resistance) when given foods of different amino acid compositions. The specific requirements of certain amino acids for the immune response lead to several interactions between the predicted maximum pathogen load and protein retention at a crude protein content. There is further support in the literature (see Chapter 4) that low protein supplies do not always lead to immunity being penalised to a great extent: this may be the outcome of using food proteins with different amino acids compositions. This model has for the first time shown that by accounting for the amino acid requirements for maintenance, growth and immune responses it may be possible to account for the effects of pathogen challenges on growth and performance of different genotypes. A key model assumption that needs testing, however, is whether all amino acids are partitioned with the same ratio, or whether certain amino acids have a higher partitioning ratio. It is possible that at time of resource scarcity for a certain amino acid that animals have evolved to partition greater amounts of those amino acids for which the immune response has a high requirement. This is, however, unattractive proposition as it would greatly increase the parameter requirement of the model.

The proposed framework is an initial attempt at modelling the effects of pathogen challenges on growth and performance. The model will need testing with suitable experiments and the framework refined. The future development of this framework may also include the integration with models for predicting the effects of physical (Black *et al.* 1986; Wellock *et al.* 2003a) and social stressors (Wellock *et al.* 2003b) on performance. There is evidence that social stressors affect the immune responses of a host (Gross & Siegel 1965; Gross 1972). As discussed in Chapter 4 there may also be a relationship between the extent of fever and the ability of the host to mount an immune response (Bradley & Kauffman 1988; Jiang *et al.* 2001). The poorer performance of animals in commercial production environments compared to well maintained experimental stations (Black *et al.* 1995) will be the outcome of all of these stressors acting individually or simultaneously. Models now exist to describe the effects of all three stressors on performance, and a complete model, which includes all of these stressors, would be a key step towards managing the performance and health of production animals.

General Discussion

Introduction

The effect of pathogen challenges on animal performance is a central problem to current and future livestock industries (Black *et al.* 1995; Kanis *et al.* 2005). The relationships between pathogen challenges, performance and health are also central to several other branches of biology, including animal science (van Houtert & Sykes 1995; Black *et al.* 1999; Coop & Kyriazakis, 1999), evolutionary ecology (Sheldon & Verhulst, 1996; Lochmiller & Deerenberg, 2000) and human medicine (Powanda, 1977; Powanda & Beisel, 2003). In this thesis, mathematical modelling techniques were combined with critical analysis of the available literature to produce a framework for predicting the effects of pathogen challenges on animal performance and health. The framework presented in this thesis has attempted to incorporate the underlying biology of the system in terms of deterministic equations, which were then solved dynamically to consider the possible interactions between the animal, its food and its environment over time (Emmans & Fisher, 1986; Emmans, 1988). In principle, such a framework may guide us towards future experiments that are needed to improve our understanding of the system, and point towards genetic selection and management procedures that may alleviate, or completely remove the effects of disease on performance (Black *et al.* 1995; Bishop & Stear, 2003).

The first step when developing the framework, was to consider proposed partitioning rules for the healthy animal, as this is a key issue for predicting growth and an area of growth modelling where several (apparently) different solutions have been put forward (Chapter 1; Sandberg *et al.* 2005a). The second step was to test proposed partitioning rules that withstood qualitative testing, against suitable data, and identify the values of the key partitioning parameters (Chapter 2; Sandberg *et al.* 2005b). The preferred partitioning rule was that proposed by Kyriazakis & Emmans (1992a, b), which makes the marginal efficiency of material use of protein a function of food composition. The third step was to identify the nature of the reduction in voluntary food intake (anorexia) that occurs during pathogen challenges and develop a model for its prediction (Chapter 3; Sandberg *et al.* 2006). A framework has been put forward that accounts for the consequences of exposure to pathogens on the reduction in food intake in farm animals. The fourth step was to consider reductions in growth during pathogen challenges, and attempt to explain those which were not caused by anorexia, and how these were affected by nutrition (Chapter 4). In the final chapter (Chapter 5) a framework was presented which can be used as a basis for the prediction of growth and performance of different genotypes

(potential for growth, genetic resistance) when given access to different kinds of food. The above steps combined to produce a general solution to the question posed by the overall objective of the thesis. This was the prediction of performance of different genotypes when challenged by different doses and given different kinds of food. The proposed model has both a predictive and heuristic value. In this the general discussion the issues raised by the overall framework, and future directions in this field are considered.

The remaining issues

The development of a modelling framework is an iterative process: no model can ever be completed, but only be improved or rejected e.g. Emmans & Fisher (1986). The overall objective of this thesis was achieved in that a model which predicts growth and performance of different genotypes during exposure to pathogens was developed and its predictions explored. This model is an initial attempt to solve this important problem and suitable experiments are needed to not only test the predictions of this framework, but also to improve its assumptions. The model was not parameterised adequately, and it was often necessary to choose the values of parameters on conceptual grounds. This was due to a lack of suitable data describing the effects of different doses of pathogens on the performance of pigs, and animals in general. This can be seen as an acceptable exercise since several other modelling attempts have used a similar approach e.g. Austin & Anderson (1996) and Hoshen *et al.* (2000).

The model was able to predict the qualitative outcomes of several experiments in the literature. For example the model is able to predict the transient reduction in voluntary food intake and the manner in which the pathogen load changes in a host (Berendt *et al.* 1977; van Houtert *et al.* 1995; Balaji *et al.* 2000, 2002; Greiner *et al.* 2001). In addition, the model predicts the kind of marginal responses observed during exposure to high immune activation environments (Williams *et al.* 1997a, b, c) and pathogens (Willis & Baker, 1981a, b). This would suggest that if suitable data was obtained, the model could be parameterised for a particular pathogen.

For the model to predict large effects on the pathogen load of a host it was necessary to both restrict the animal through bulk and give it access to low protein foods (Figure 5a, Chapter 5). This may be the outcome of the requirements for immunity as predicted by model to have been underestimated, or the partitioning parameter to have been

overestimated. In the latter case too much food resources may have been directed towards the immune response. Experiments which determine food intake, growth (including both protein and lipid retention) and pathogen loads of different host genotypes that are given foods of different protein contents are needed to further test this consequence of the model. In addition, it would be necessary to measure the change in immune responses (and the rate of removing the pathogen load) when animals are supplemented with different nutrients e.g. amino acids e.g. Bhargava *et al.* (1970a).

To parameterise the model it is necessary to account for the transient nature of pathogen challenges in immunologically naïve animals, e.g. Akinbamijo *et al.* (1995) and van Houtert *et al.* (1995). Methods of estimating nutrients requirements during pathogen challenges will need to deal with both the requirement and pathogen load changing over time and with dose at a time (Adamo, 2004). The functional forms used in this model were static mechanistic equations, which predict the requirement of, for example the expression of immunity, at a pathogen load. To resolve this issue suitable indicators of requirements for immune responses will need to be identified (Adamo, 2004) and measured at several time points during a pathogen challenge. Alternatively, it could be possible to use the rate of change in pathogen load as a proxy indication whether the requirement for mounting an immune response for a particular nutrient (amino acid) has been met. An animal would be given increasing levels of a particular amino acid and the rate at which the animal eliminates its pathogen load should be measured. At the point at which the rate of removing the pathogen load is no longer improved, it can be assumed that the requirement for a particular nutrient for the expression of immunity has been met and that the host has achieved its genetic maximum for this function. This situation, however, creates a high information requirement, as it would be necessary to consider all of the individual amino acids to get complete information of the requirement of the animal.

A key issue that the model and available literature data has not been able to resolve was whether the reduction in voluntary food intake occurs as an outcome of a reduced growth potential, or through a direct effect on appetite as is proposed in this framework. Model predictions showed that even when the model invokes the mechanism of a direct effect on appetite, it is able to predict the findings of Willis & Baker (1981a, b), Williams *et al.* (1997*a, b, c*) and Webel *et al.* (1998*a, b*). These experiments suggest a reduction in animal performance, even when resource intake is increased and have often been used as

evidence for a direct effect on an animal's potential for growth (Greiner *et al.* 2000; Escobar *et al.* 2002, 2004). A possible approach to determining which of these two mechanisms is acting during pathogen challenges, could be the use of direct infusion of individual amino acids, together with measures of nitrogen balance. This would allow for the findings of Bown *et al.* (1991) where sheep given abomasal infusions of nitrogen and challenged with gastrointestinal parasites retained the same amount of nitrogen as their healthy controls to be assessed in monogastrics. The findings of Bown *et al.* (1991) have been interpreted as evidence that pathogen induced anorexia is a consequence of a direct effect on appetite. Alternatively, it could be possible to measure the marginal response of animals challenged by pathogens and given several different levels of a first limiting amino acid throughout an infection. For example, an automated system that would allow for nitrogen balance to be calculated continuously over time may do this. The marginal response, at a particular time point, could then be considered in light of the pathogen load in the host and its transient reduction in food intake. In the case where the nitrogen retention of the challenged animal was the same as that of the healthy controls, at a time, and food intake at this time was lower in the challenged animals, it could be concluded that pathogen challenges have had a direct effect on appetite. This is different from the traditional approach to marginal response experiments where protein retained is calculated as the difference between start and end protein weights. There is an alternative view: that pathogen challenges affect both food intake directly, and the animal's potential for growth (Black *et al.* 1999). This 'sitting on the fence' assumption could also be tested by the above experiments.

The application of the framework to different kinds of pathogens

The proposed framework may be applicable for different pathogens: i.e. the functional forms may be generally applicable but their parameter values may be different for micro and macro parasites (Bhargava *et al.* 1970a, b; Beer *et al.* 1974; Berendt *et al.* 1977; Chapman *et al.* 1982; Greiner *et al.* 2001). Challenges with micro parasites lead to a pathogen load in the host, which grows quickly until the point where the host starts to control it and eventually eliminate it through its immune responses (Berendt *et al.* 1977). On the other hand most macro parasites do not replicate within a host and as a consequence host pathogen load is dependent on incoming parasites (van Houtert *et al.* 1995). The question that this difference raises is whether in terms of the rates of the immune response animals have evolved different responses to different pathogens which lead to different functional forms relating pathogen load to requirements. The scant

evidence that exists suggest that functional forms may be similar across different pathogens. For example, Beer *et al.* (1974) and Berendt *et al.* (1977) found similar forms of the responses between damage caused to the host and pathogen load when challenged by *Trichuris suis* and *Klebsiella pneumoniae*, respectively. The framework that has been proposed in this thesis provides a good basis for making critical comparisons of the effects of bacterial, viral and macro-parasitic challenges on the performance and health of animals. It would be an attractive proposition if it could be shown that one framework relating pathogen load to resource requirements and partitioning could be used for different pathogens. To achieve this, however, it may be necessary to account for possible difference in how different pathogens grow and develop in a host and the immune response they invoke for this purpose (Chapter 5, Henken *et al.* 1994a).

Selecting for genetic resistance: the balance between host, pathogen and nutrition

There is significant interest in breeding animals that are genetically more resistant to either one type of pathogen, or several types of pathogens (Douch *et al.* 1996; Magnusson *et al.* 1998; Yu *et al.* 2000; Bishop & Stear, 2003). The predictions of the model showed that it is necessary to have several measures of host performance (food intake, growth rate, pathogen load) to obtain a good assessment of the degree of resistance of a particular genotype. For example, if considering only food intake as a measure of resistance it would not be possible to distinguish between different genotypes when given access to low protein foods, as due to the constraint of heat production, and challenged animals, including their healthy controls would be performing similarly (Figure 2 in Chapter 5). Understanding and being able to predict such interactions between the genotype and its environment is crucial for interpreting and applying the resistance of different genotypes.

Attempts have been made to select animals that cope better with disease by different approaches in pigs (Wilkie *et al.* 1998), chickens (Siegel & Gross 1980) and sheep (Bisset *et al.* 1996; Douch *et al.* 1996) amongst others. Wilkie *et al.* (1998) selected for high and low responder pigs in terms of general immunity by using an index for several immune traits when challenged by several different types of antigen. Siegel & Gross (1980) and Yunis *et al.* (2000) selected chickens for a single immune trait (either high or low antibody responses to sheep red blood cells). Genetic selection in sheep against gastrointestinal parasitism has often used a proxy measure of parasite fitness, i.e. faecal egg counts, which have been found to have a strong relationship with reduced worm burdens (Bisset *et al.* 1996). It may not be suitable, however, to select genotypes on the

basis of the extent to which they produce a particular immune response (Siegel & Gross, 1980), or cope with pathogen challenges (e.g. worm burden) when they are given access only to good quality foods. It would be logical, that in natural selection animals have evolved towards increased resistance at times when resources were scarce and when the infectious pressure from the environment was high. This has the consequence, however, that such animals would not evolve to only produce greater amounts of immune response to control challenges of higher doses, but also to counter resource scarcity, through a greater partitioning of resources towards immune responses. Artificial selection, however, may be performed in well maintained breeding stations, where often high quality diets are given (Black *et al.* 1995). This could lead to animals which appear more resistant in such circumstances, but when constrained and facing scarce resources they would suffer greater penalties on immunity, and thereby appear more susceptible. This appears to have been the outcome of selection experiments in sheep bred for resistance to gastrointestinal parasites.

A possible and perhaps more suitable selection criteria would be a combination of pathogen load (or maximum pathogen load) as an indication of the arm of the immune responses responsible for killing and removing the pathogen and a reduction in the extent of anorexia to reduce impacts on performance: this is essentially the definition of a more resistant genotype that is used in this framework. Measuring immune proteins and the extent of their expression during different nutritional regimens may also be informative, but great caution is needed as the expression of immune proteins is not always positively correlated with resistance (Adamo, 2004). It may also be useful to record the extent to which a host's immune response causes non-specific immunopathology (Graham *et al.* 2005). This includes damage to the host by the hosts own immune response in the process of eliminating the pathogen. It is difficult however to distinguish between damage caused by the pathogen *per se* and damage caused by the host's immune responses. Measuring the extent of damage to different doses of pathogens in immuno-supressed animals (Greer *et al.* 2005) may in part solve this problem. There is already an indication that selection for resistance may increase non-specific immunopathology. Magnusson *et al.* (1998) found that pigs selected for high immune responses were also prone to a greater degree of arthritis (inflammation) in the knee joints. A possible solution would be to select animals during times of artificially imposed resource scarcity, as this may improve the chances that such genotypes would cope better in commercial production environments, where other stressors (physical, social) may be present and constraining their intake.

The proposed framework as a basis for a population model

The model presented here is for the average animal of a population, without taking into account any genetic variation that may exist in growth and resistance parameters. Previous deterministic growth models e.g. Ferguson *et al.* (1994) and Wellock *et al.* (2003a, b) have been turned into population models (Ferguson *et al.* 1997; Pomar *et al.* 2003). In principle such models can be used to interpret the performance of populations of animals, by accounting for genetic and phenotypic variation in the population response (Knap 2000; Pomar *et al.* 2003). The predicted response of an individual may be different to that of the population: a typical example is the linear-plateau model used in this model for relating protein retention to protein intake becoming increasingly curvilinear (Ferguson *et al.* 1997; Pomar *et al.* 2003) as the variation of the population increases. To convert a model such as the one proposed for the healthy animal, information is needed for the genetic variation of the core growth parameters. These include variation in the Gompertz growth parameter, the mature protein weight and the mature protein to lipid ratio to create variation in the potential growth rate of the animal. To overcome the negative correlation between the Gompertz growth parameter and the mature protein weight (Emmans, 1988; Knap 2000; Lewis et al. 2002) the Gompertz parameter needs to be scaled to the mature protein weight (Pomar *et al.* 2003). The model can then be explored to assess the effects of genetic variation and for example different feeding regimens on the actual phenotypic population response in the manner done by Ferguson *et al.* (1997) and Pomar *et al.* (2003).

For the framework proposed here it may also be possible to introduce genetic variation in the parameters describing resistance, R. It was assumed for the sake of simplicity that this trait can be described by a single parameter. Introducing genetic variation in this trait would not be straight forward as there has been very large variation observed in measures of pathogen load (Sykes & Coop, 1977; Steel *et al.* 1980, 1982) and especially immune traits (Hulten *et al.* 2003; Adamo 2004). Standardisation of measurement techniques and the manner in which data are presented e.g. per kg BW or better expressed in relation to degree of maturity may in part reduce the effect of experimental error on measured variation. A further issue is to obtain estimates of co-variation between genotypic parameters describing growth potential and resistance. Bishop & Stear (1997) assumed that there was no such co-variation between growth characteristics and the ability of

sheep to cope with gastrointestinal parasites. This leads to a simpler model, which, however, may not have been a good representation of the system.

There are several resistance parameters in this framework that have been proposed as being dependent on host genotype. These included the rate parameters for describing the response in immune requirements with increasing pathogen load, and the extent of anorexia. To estimate genetic co-variation it would be important to first test whether all genotype dependent parameters in the model actually are dependent on the genotype. This may reduce the number of co-variations that would need to be described. Again Bishop & Stear (1997) assumed that the three traits that are assumed to characterise host resistance to gastrointestinal parasites (i.e. parasites establishment, fecundity and mortality) were related to each other. It is highly likely, however, that there is a high degree of co-variation between such traits.

The resulting population model would then need to be integrated with a model which describes the contribution of each animal to the infectious load in the environment, as the response of the population will depend on how animals in the population excretes pathogens into the environment whilst state changes from being susceptible to resistant. This would be equivalent to an epidemiological component of the infection being integrated into the model (Austin & Anderson, 1996). While developing such a model would prove to be difficult, it could allow for a greatly improved understanding of the genotypic and phenotypic correlations between growth and resistance traits. This is an area which is much debated with no real common ground (Bishop & Stear 2003).

The way forward

The proposed framework is an initial attempt towards modelling the effects of pathogen challenges on growth and performance. It will now be possible to integrate the proposed framework with other frameworks that relate physical and social stressors to performance (Wellock *et al.* 2003b, c). This may allow for a more complete account of how an animal interacts with its environment. The 'complete' model could in turn be used as the basis for a population model. Such a comprehensive model would be useful for interpreting and managing performance of animals in different production environments.

References

Abbott EM, Parkins JJ & Holmes PH (1985) Influence of dietary protein on the pathophysiology of ovine haemonchosis in Finn Dorset and Scottish Blackface lambs given a single moderate infection. *Research in Veterinary Science* **38,** 54 – 60.

Adamo SA (2004) How should behavioural ecologists interpret measurements of immunity? *Animal Behaviour* **68,** 1443 – 1449.

Akinbamijo OO, Bennison JJ, Romney DL, Wassink GJ, Jaitner J, Clifford DJ & Dempfle L (1997) An evaluation of food intake, digestive physiology and live weight changes in N'dama and Gobra zebu bulls following experimental Trypanosoma congolense infection. *Animal Science* **65,** 151-158.

Amer P & Emmans GC (1998) Predicting changes in feed energy requirements due to genetic changes in growth and body composition of growing ruminants. *Animal Science* **66,** 143-153.

Anderson N, Blake R & Titchen DA (1976) Effect of a series of infections of *Ostertagia circumcincta* on gastric secretions of sheep. *Parasitology* **72,** 1-12.

Austin DJ & Anderson RM (1996) Immunodominance, competition and evolution in immunological responses to helminth parasite antigens. *Parasitology* **113,** 157 – 172.

ARC (1981) The nutrient requirements of pigs. Agricultural Research Council, Commonwealth, Agricultural Bureaux, Slough, Berkshire, UK.

Arnold J, Little RA & Rothwell NJ (1989) Energy balance and brown adipose tissue thermogenesis during chronic endotoxemia in rats. *Journal of Applied Physiology* **66,** 1970 – 1975.

Ask B, van der Waaij EH, Stegeman JA & van Arendonk JAM (2006) Genetic variation among broiler genotypes in susceptibility to colibacillosis. *Poultry Science* **85,** 415 – 421.

Bachmann MF & Kopf M (2002) Balancing protective immunity and immunopathology. *Current Opinions in Immunology* **14,** 413 – 419.

Baker DH (1991) Partitioning of nutrients for growth and other metabolic functions: Efficiency and priority considerations. *Poultry Science* **70,** 1797 – 1805.

Baker DH, Fernandez SR, Parsons CM, Edwards HM, Emmert JL & Webel DM (1996) Maintenance requirement for valine and efficiency of its use above maintenance for accretion of whole body valine and protein in young chicks. *Journal of Nutrition* **7**, 1844 – 1851.

Balaji R, Wright KJ, Hill CM, Dritz SS, Knoppel EL, & Minton JE (2000) Acute phase responses of pigs challenged orally with *Salmonella typhimurium*. *Journal of Animal Science* **78,** 1885 – 1981.

Balaji R, Wright KJ, Turner JL, Hill CM, Dritz SS, Fenwick B, Carroll JA, Zannelli ME, Beausang LA, & Minton JE (2002) Circulating cortisol, tumour necrosis factor-α interleukin-1β, and interferon-γ in pigs infected with *Actinobacillus pleuropneumoniae*. *Journal of Animal Science* **80,** 202 – 207.

Baracos VE, Whitmore WT & Gale R (1987) The metabolic cost of fever. *Canadian Journal of Physiology and Pharmacology* **65,** 1248 – 1254.

Barger IA (1988) Resistance of young lambs to *Haemonchus contortus* infection, and its loss following anthelmintic treatment. *International Journal for Parasitology* **18,** 1107-1109

Barnes DM, Song Z, Klasing KC & Bottje W (2002) Protein metabolism during an acute phase response in chickens. *Amino Acids* **22,** 15 – 26.

Batterham ES, Andersen LM, Braigent DR & White E (1990) Utilisation of ileal digestible amino acids by growing pigs: effect of dietary lysine concentration on efficiency of lysine retention. *British Journal of Nutrition* **64**, 81-94.

Bayyari GR, Huff WE, Rath NC, Balog JM, Newberry LE, Villines JK, Skeeles JK, Anthony NB & Nestor KE (1997) Effect of the genetic selection of turkeys for increased body weight and egg production on immune and physiological responses. *Poultry Science* **76,** 289 – 296.

Baxter AG & Hodgkin PD (2002) Activation rules: the two signal theories of immune activation. *Nature Reviews Immunology* **2,** 439 – 446.

Bazar, K. A., Yun, A. J. and P. Y. Lee. 2005. "Starve a fever and feed a cold": feeding and anorexia may be adaptive behavioural modulators of autonomic and T helper balance. *Medical Hypotheses* **64,** 1080 – 1084.

Beer RJ, Sansom BF & Taylor PJ (1974) Erythrocyte losses from pigs with experimental Trichuris suis infections measured with a whole body counter. *Journal of Comparative Pathology* 84, 331 – 345.

Beisel WR (1977) Magnitude of the host nutritional responses to infection. *American Journal of Clinical Nutrition* **30,** 1236 – 1247.

Benson BN, Calvert CC, Roura E & Klasing KC (1993) Dietary energy source and density modulate the expression of immunologic stress in chicks. *Journal of Nutrition* **123,** 1714 – 1723.

Berendt RF, Long GG, Abeles FB, Canonico PG, Elwell MR & Powanda MC (1977) Pathogenesis of respiratory Klebsiella pneumoniae infection in rats: bacterial and histological findings and metabolic alterations. *Infection & Immunity* **15,** 586 – 593.

Berschauer F, Close WH & Stephens DB (1983) The influence of protein: energy value of the ration and level of feed intake on the energy and nitrogen metabolism of the growing pig. 2. N metabolism at two environmental temperatures. *British Journal of Nutrition* **49**, 271-283.

Beveridge I, Pullman AL, Phillips PH, Martin RR, Barelds A, & Grimson R (1989) Comparison of the effects of infection with *Trichostrongylus colubriformis*, T.vitrinus and T.rugatus in Merino lambs. *Veterinary Parasitology* **32,** 229 – 245.

Bhargava KK, Hanson RP and Sunde ML (1970a) Effects of methionine and valine on antibody production in chicks infected with Newcastle disease virus. *Journal of Nutrition* **100,** 241 – 248.

Bhargava KK, Hanson RP and Sunde ML (1970b) Effects of threonine on growth and antibody production in chicks infected with Newcastle virus. *Poultry Science* **50,** 710 – 713.

Bikker P (1994) Protein and lipid accretion in body components of growing pigs: effects of body weight and nutrient intake. *PhD Thesis*. Department of Animal Nutrition, Wagening Agricultural University.

Bikker P, Verstegen MWA & Bosch MW (1994a) Amino acid composition of growing pigs is affected by protein and energy intake. *Journal of Nutrition* **124**, 1961 – 1969.

Bikker P, Verstegen MWA, Campbell RG & Kemp B (1994b) Digestible lysine requirement of gilt's with high genetic potential for lean gain, in relation to the level of energy intake. *Journal of Animal Science* **72**, 1744 – 1753.

Bikker P, Karabinas V, Verstegen MWA & Campbell RG (1995). Protein and lipid accretion in body components of growing gilts (20 to 45 kilograms) as affected by energy intake. *Journal of Animal Science* **73**, 2355-2363.

Bikker P, Verstegen MWA & Campbell RG (1996) Performance and body composition of finishing gilts (45 to 85 kilograms) as affected by energy intake and nutrition in earlier life: II. Protein and lipid accretion in body components. *Journal of Animal Science* **74**, 817 – 826.

Birkett S & de Lange K (2001*a*) Limitations of conventional models and a conceptual framework for a nutrient flow representation of energy utilisation by animals. *British Journal of Nutrition* **86**, 647 – 659.

Birkett S & de Lange K (2001*b*) A computational framework for a nutrient flow representation of energy utilisation by growing monogastric animals. *British Journal of Nutrition* **86**, 661-674.

Birkett S & de Lange K (2001*c*) Calibration of a nutrient flow model of energy utilisation by growing pigs. *British Journal of Nutrition* **86**, 675-689.

Bishop SC & Stear MJ (2003) Modeling of host genetics and resistance to infectious diseases: understanding and controlling nematode infections. Veterinary Parasitology **155,** 147 – 166.

Bisset SA, Vlassoff A, Douch PGC, Jonas WE, West CJ & Green RS (1996) Nematode burdens and immunological responses following natural challenge in Romney lambs selectively bred for low or high faecal egg counts. *Veterinary Parasitology* **61,** 249 – 263.

Black JL (1974) Manipulation of body composition through nutrition. *Proceedings of the Australian Society of Animal Production* **10,** 211-218.

Black JL & Griffiths DA (1975) Effects of live weight and energy intake on nitrogen balance and total N requirement of lambs. *British Journal of Nutrition* **33,** 399 – 413.

Black JL, Davies GT, Bray HJ, Giles LR & Chapple RP (1986) Simulation of energy and amino acid utilisation in the pig. *Research and Development in Agriculture* **3,** 121-145.

Black JL, Davies GT, Bray HJ, Giles LR & Chapple RP (1995) Modelling the effects of genotype, environment and health on nutrient utilisation. pp 85-105. In *Proceedings of IVth*

International Workshop on Modelling Nutrient Utilisation of Farm Animals [Danfaer A, Lescoat P, editors). National Institute Of Animal Science, Denmark,.

Black JL and de Lange CFM (1995) Introduction to the principles of nutrient partitioning for growth. In *Modelling growth in the pig, EAAP publication* no. 78, pp. 33-45 [PJ Moughan, MWA Verstegen & MI Visser-Reyneveld, editors]. Wageningen: Wageningen Press.

Black JL, Bray HJ, & Giles LR (1999) The thermal and infectious environment. In A quantitative biology of the pig, pp. 71-97 [I Kyriazakis, editor]. Wallingford: CAB International.

Black JL, Giles LR, Wynn PC, Knowles AG, Kerr CA, Jones MR, Strom AD, Gallagher NL & Eamens GJ (2001) Factors limiting the performance of growing pigs in commercial environments. pp 9-36 *In 'Manipulating pig production VIII'* (Ed. PD Cranwell). Australasian Pig Science Association: Werribee, Vic.

Blatteis CM (2003) Fever: pathological or physiological, injurious or beneficial? *Journal of Thermal Biology* **28,** 1-13.

Boisen S, Hvelplund T & Weisbjerg MR (2000) Ideal amino acid profiles as a basis for feed protein evaluation. *Livestock Production Science* **64**, 239 – 251.

Bown MD, Poppi DP & Sykes AR (1991) The effect of post-ruminal infusion of protein and energy on the pathophysiology of *Trichostrongylus colubriformis* infection and body composition in lambs. *Australian Journal of Agricultural Research* **42**, 253 – 267.

Bradley SF & Kauffman CA (1988) Protein malnutrition and the febrile response in the Fischer rat. *Journal of Leukocyte Biology* **43,** 36 – 40.

Brailsford TJ & Mapes CJ (1987) Comparison of *Heligmosomoides polygyrus* primary infection in protein-deficient and well-nourished mice. *Parasitology* **95,** 311 – 321.

Broussard SR, Zhou JH, Venters HD, Bluthe RM, Freund GG, Johnson RW, Dantzer R & Kelley KW (2001) At the interface of environment-immune interactions: Cytokine and growth-factor receptors. *Journal of Animal Science* **79,** (E suppl.) E268 – E284.

Calder PC (1995) Fuel utilization by cells of the immune system. *Proceedings of the Nutrition Society* **54,** 65 – 82.

Campbell RG & Dunkin AC (1983*a*) The effects of energy intake and dietary protein on nitrogen retention, growth performance, body composition and some aspects of energy metabolism of baby pigs. *British Journal of Nutrition* **49**, 221 – 230.

Campbell RG & Dunkin AC (1983*b*) The influence of dietary protein and energy intake on the performance, body composition and energy utilisation of pigs growing from 7 to 19 kg. *Animal Production* **36,** 185 – 192.

Campbell RG & Taverner MR (1988) Relationships between energy intake and protein and energy metabolism, growth and body composition of pigs kept at 14 or 32 degree C from 9 to 20 kg. *Livestock Production Science* **18**, 289 – 303.

Campbell RG, Taverner MR & Curic DM (1984) Effect of feeding level and dietary protein content on the growth, body composition and rate of protein deposition in pigs growing from 45 to 90 kg. *Animal Production* **38**, 233 – 240.

Campbell RG, Taverner MR & Curic DM (1985*a*) The influence of feeding level on the protein requirement of pigs between 20 and 45 kg live weight. *Animal Production* **40**, 489 – 496.

Campbell RG, Taverner MR & Curic DM (1985*b*) Effects of sex and energy intake between 48 – 90 kg live weight on protein retention in growing pigs. *Animal Production* **40**, 497 – 503.

Campbell RG, Taverner MR & Rayner CJ (1988) The tissue and dietary protein and amino acid requirement of pigs from 8 to 20 kg live weight. *Animal Production* **46**, 283 – 290.

Carroll JA, Matteri RL, Dyer CJ, Beausang LA & Zannelli ME (2001) Impact of environmental temperature on response of neonatal pigs to an endotoxin challenge. *American Journal of Veterinary Research* **62,** 561 – 566.

Cooper KE (2002) Molecular biology of thermoregulation: Some historical perspectives on thermoregulation. *Journal of Applied Physiology* **92,** 1717 – 1724.

Csapo'-Kiss Zs, Stefler J, Martin TG, Makray S & Csapo' J (1995) Composition of mares' colostrum and milk. Protein content, amino acid composition and contents of macro- and micro-elements. *International Dairy Journal* **5,** 403 – 415.

Chapman HD, Fernandes DL & Davison TF (1982) A comparison of the effects of infection with *Eimeria maxima* and dietary restriction on weight gain, plasma metabolites and liver glycogen in the immature fowl, *Gallus domesticus*. *Parasitology* **84,** 205 – 213.

Chung TK & Baker DH (1992) Efficacy of dietary methionine utilisation by young pigs. *Journal of Nutrition* **122**, 1862 – 1869.

Conn CA, McClellan JL, Maassab HF, Smitka CW, Majde JA & Kluger MJ (1995) Cytokines and the acute phase response to influenza virus in mice. *American Journal of Physiology and Regulatory Integrated Comparative Physiology* **37,** R78-R84.

Crompton DWT (1984) Influence of parasitic infection on feed intake. *Federation Proceedings* **43,** 239 – 245.

Close WH, Mount LE & Brown D (1978) The effects of plane of nutrition and environmental temperature on the energy metabolism of the growing pig. 2. Growth rate, including protein and fat deposition. *British Journal of Nutrition* **40,** 423 – 431.

Close WH & Stanier MW (1984) Effects of plane of nutrition and environmental temperature on the growth and development of the early-weaned piglet. 1. Growth and body composition. *Animal Production* **38**, 211 – 220.

Colin AJ, van Milgen J, Dubois S & Noblet J (2001) Effect of high temperature on feeding behaviour and heat production in group-housed young pigs. *British Journal of Nutrition* **86**, 63-70.

Coltman DW, Pilkington J, Kruuk LEB, Wilson K & Pemberton JM (2001) Positive genetic correlations between parasite resistance and body size in a free-living ungulate population. *Evolution* **55**, 2116 – 2125.

Colditz IG (2002) Effects of the immune system on metabolism: implications for production and disease resistance in livestock. *Livestock Production Science* **75**, 257 – 268.

Coop RL & Kyriazakis I (1999) Nutrition-parasite interactions. *Veterinary Parasitology* **84**, 187 – 204.

Coop RL & Kyriazakis I (2001) Influence of host nutrition on the development and consequences of nematode parasitism in ruminants. *Trends in Parasitology* **17**, 325 – 330.

Coop RL, Sykes AR & Angus KW (1976) Subclinical trichostrongylus in growing lambs produced by continuous larval dosing. The effect on performance and certain plasma constituents. *Research in Veterinary Science* **21**, 253 – 258.

Coop RL, Sykes AR & Angus KW (1982) The effect of three levels of intake of *Ostertagia circumcincta* larvae on growth rate, feed intake and body composition of growing lambs. *Journal of Agricultural Science Cambridge* **98**, 247 – 255.

Coop RL, Field AC, Graham RB, Angus KW & Jackson F (1986) Effect of concurrent infection with *Ostertagia circumcincta* and *Trichostrongylus vitrinus* on the performance of lambs. *Research in Veterinary Science* **40**, 241 –245.

Curnow RN (1978) A smooth population response curve based on an abrupt threshold and plateau model for individuals. *Biometrics* **29**, 1-10.

Cutler RS (2001) A review – The prevalence and eradication of pig diseases in Australia. In Manipulating Pig Production VIII (ed P.D. Cranwell), pp 125 – 140. Australasian Pig Science Association, Werribee, Victoria.

Datta FU, Nolan JV, Rowe JB & Gray GD (1998) Protein supplementation improves the performance of parasitised sheep fed a straw-based diet. *International Journal for Parasitology* **28,** 1269 – 1278.

Daugschies A, Burger H-J & Akimaru M (1998) Apparent digestibility of nutrients and nitrogen balance during experimental infection of calves with *Eimeria bovis*. *Veterinary Parasitology* **77,** 93 – 102.

de Greef KH (1992) Prediction of production: Nutrient induced tissue partitioning in growing pigs. *PhD Thesis*. Department of Animal Nutrition, Wagening Agricultural University.

de Greef KH, Kemp B & Verstegen MWA (1992) Performance and body composition of fattening pigs of two strains during protein deficiency and subsequent realimentation. *Livestock Production Science* **30,** 141 – 153.

de Greef KH & Verstegen MWA (1993) Partitioning of body protein and lipid deposition in the body of growing pigs. *Livestock Production Science* **35**, 317-325.

de Greef KH & Verstegen MWA (1995) Evaluation of a concept on energy partitioning in growing pigs. In *Modelling growth in the pig*, *EAAP publication* no. 78, pp. 137-149 [PJ Moughan, MWA Verstegen & MI Visser-Reyneveld, editors]. Wageningen: Wageningen Press.

de Lange CFM (1995) Framework for a simplified model to demonstrate principles of nutrient partitioning for growth in the pig. In *Modelling growth in the pig, EAAP publication* no. 78, pp. 71-85 [PJ Moughan, MWA Verstegen & MI Visser-Reyneveld, editors]. Wageningen: Wageningen Press.

de Lange CFM, Gillis AM & Simpson GJ (2001) Influence of threonine intake on whole-body protein deposition and threonine utilisation in growing pigs fed purified diets. *Journal of Animal Science* **79,** 3087 – 3095.

Demas GE, Chefer V, Talan MI & Nelson RJ (1997) Metabolic cost of mounting an antigen-stimulated immune response in adult and aged C57BL/6J mice. *American Journal of Physiology* **273,** R1631 – R1637.

Deschepper K & de Groote G (1995) Effect of dietary protein, essential and non-essential amino acids on the performance and carcase composition of male broiler chickens. *British Poultry Science* **36,** 229 – 245.

Dividich JI & Noblet J (1984) Effect of colostrum intake on metabolic rate and plasma glucose in the neonatal pig in relation to environmental temperature. *Biology of the Neonate* **46,** 98-104.

Dividich JI, Vermorel M, Noblet J, Bovier JC & Aumaitre A (1980) Effects of environmental temperature on heat production, energy retention, protein and fat gain in early weaned piglets. *British Journal of Nutrition* **44,** 313-323

Douch PGC, Green RS, Morris CA, McEwan JC & Windon RG (1996) Phenotypic markers for selection of nematode resistant sheep. *International Journal For Parasitology* **8/9,** 899 – 911.

Dritz SS, Owen KQ, Goodband RD, Nelssen JL, Tokach MD, Chengappa MM & Blecha F (1996) Influence of lipopolysaccharide-induced immune challenge and diet complexity on

growth performance and acute-phase protein production in segregated early-weaned pigs. *Journal of Animal Science* **74,** 1620 – 1628.

Dunkin AC & Black JL (1985) The relationship between energy intake and nitrogen balance in the growing pig. In *Energy metabolism of farm animals: Proceedings of the 10th symposium, EAAP publication* no 32, pp 110-113 [PW Moe, HF Tyrrell & PJ Reynolds, editors]. Totowa, N: Rowman & Littlefield.

Eckersall PD (2000) Recent advances and future prospects for the use of acute phase proteins as markers of disease in animals. *Revue Medicine Veterinaire* **151,** 577 – 584.

Edwards HM & Baker DH (1999) Maintenance sulphur amino acid requirements of young chicks and efficiency of their use for accretion of whole-body sulphur amino acids and protein. *Poultry Science* **78**, 1418-1423.

Edwards HM, Baker DH, Fernandez SR & Parsons CM (1997) Maintenance threonine requirement and efficiency of its use for accretion of whole-body threonine and protein in young chicks. *British Journal of Nutrition* **78**, 111 – 119.

Edwards HM, Fernandez SR & Baker DH (1999) Maintenance lysine requirement and efficiency of using lysine for accretion of whole-body lysine and protein in young chicks. *Poultry Science* **78,** 1412 – 1417.

Elsasser TH, Klasing KC, Filipov N & Thompson F (2000) The metabolic consequences of stress: Targets for stress and priorities for nutrient use. In *The biology of animal stress.* pp 77-110 [Moberg GP & Mench JD, editors]. CAB international press, New York.

Emmans GC (1988) Genetic components of potential and actual growth. In *Animal Breeding opportunities. British Society of Animal Production Occasional Publication* **12,** pp. 153 – 181. [RB Land, G Bulfield & WG Hill, editors].

Emmans GC (1989) The growth of turkeys. In Recent Advances in Turkey Science. Poultry Science Symposium No 21. pp 135 – 166. [C Nixey and T.C. Grey, editors]

Emmans GC (1994) Effective energy – A concept of energy utilisation applied across species. *British Journal of Nutrition* **71,** 801-821.

Emmans GC Fisher C (1986) Problems of nutritional theory. In Nutritional requirements and nutritional theory. pp. 9-57. [C Fisher and KN Boorman, editors).

Emmans GC & Kyriazakis I (1997) Models of pig growth: problems and proposed solutions. *Livestock Production Science* **51**, 119-129.

Emmans GC & Kyriazakis I (2000) Issues arising from genetic selection for growth and body composition characteristics in poultry and pigs. In *The challenge of genetic change in Animal Production.* British Society of Animal Science Occasional Publication no **27,** pp 39 – 53. [WG Hill, SC Bishop, B McGuirk, JC McKay, G Simm & AJ Webb]. British Society of Animal Science, Edinburgh, UK.

Emmans GC & Kyriazakis I (2001) Consequences of genetic change in farm animals on feed intake and feeding behaviour. *Proceedings of the Nutrition Society* **60,** 115-125

Eraud C, Duriez O, Chastel O & Faivre B (2005) The energetic cost of humoral immunity in the Collared Dove, Streptopelia decaocto: is the magnitude sufficient to force energy-based trade-offs. *Functional Ecology* **19,** 110 – 118.

Escobar J, van Alstine WG, Baker DH & Johnson RW (2002) Growth performance and whole-body composition of pigs experimentally infected with Mycoplasma hyopneumoniae. *Journal of Animal Science* **80,** 384 – 391.

Escobar J, van Alstine WG, Baker DH & Johnson RW (2004) Decreased protein accretion in pigs with viral and bacterial pneumonia is associated with increased myostatin expression in muscle. *Journal of Nutrition* **134,** 3047 – 3053.

Faggioni R, Fuller J, Moser A, Feingold KR & Grunfeld C (1997) LPS-induced anorexia in leptin-deficient (ob/ob) and leptin receptor-deficient (db/db) mice. *Am. J. Phys. Reg. Int.Comp. Phys.* 273, R181 – R186.

Ferguson NS, Gous RM & Emmans GC (1994) Preferred components for the construction of a new simulation model of growth, feed intake and nutrient requirements of growing pigs. *South African Journal of Animal Science* **24,** 10-17.

Ferguson NS & Gous RM (1997) The influence of heat production on voluntary food intake in growing pigs given protein-deficient diets. *Animal Science* **64**, 365 – 378.

Ferguson NS, Gous RM & Emmans GC (1997) Predicting the effects of animal variation on growth and food intake in growing pigs using simulation modelling. *Animal Science* **64**, 513 – 522.

Fern EB, McNurlan MA & Garlick PJ (1985) Effect of malaria synthesis on rate of protein synthesis in individual tissues of rats. *American Journal of Physiology* **249,** E485 – E493.

Field CJ, Johnson IR and Schley PD (2002) Nutrients and their role in host resistance to infection. *Journal of Leukocyte Biology* **71,** 16 – 32.

Fisher C, Morris TR & Jennings RC (1973) A model for the description and prediction of the response of laying hens to amino acid intake. *British Poultry Science* **14,** 469 – 484.

Fleck A (1989) Clinical and nutritional aspects of changes in acute-phase proteins during inflammation. *Proceedings of the Nutrition Society* **48,** 347 – 354.

Fox MT, Gerrelli D, Pitt SR, Jacobs DE, Gill M & Gale DL (1989) Ostertagia-ostertagi infection in the calf – effects of a trickle challenge on appetite, digestibility, rate of passage of digesta and live weight gain. *Research in Veterinary Science* **47,** 294 – 298.

Forster MR (2000) Key concepts in model selection: Performance and generalizability. *Journal of Mathematical Psychology* **44,** 205-231.

Fuller MF & Crofts RMJ (1977) The protein-sparing effect of carbohydrate. 1. Nitrogen retention of growing pigs in relation to diet. *British Journal of Nutrition* **38,** 479-488.

Fuller MF, McWilliam R, Wang TC & Giles LR (1989) The optimum dietary amino acid pattern for growing pigs. 2. Requirements for maintenance and for tissue accretion. *British Journal of Nutrition* **62,** 255-267.

Fuller MF & Garthwaite P (1993) The form of response of body protein accretion to dietary amino acid supply. *Journal of Nutrition* **123,** 957 – 963.

Fuller MF, Franklin MF, McWilliam R & Pennie K (1995) The responses of growing pigs, of different sex and genotype, to dietary energy and protein. *Animal Science* **60,** 291-298.

Fuller MF, Franklin MF, McWilliam R & Pennie K (1995) The responses of growing pigs, of different sex and genotype, to dietary energy and protein. *Animal Science* **60**, 291 – 298.

Gahl M.J, Crenshaw TD & Benevenga NJ (1994) Diminishing returns in weight, nitrogen, and lysine gain of pigs fed 6 levels of lysine from 3 supplemental sources. *Journal of Animal Science* **72,** 3177 – 3187.

Graham AL, Allen JE & Read AF (2005) Evolutionary causes and consequences of immunopathology. *Annual Reviews Ecology Systems* **36,** 373 – 397.

Grimble RF & Grimble GK (1998) Immunonutrition: Role of sulphur amino acids, related amino acids, and polyamines. *Nutrition* **14,** 605-610.

Green DM & Whittemore CT (2003) Architecture of a harmonized model of the growing pig for the determination of dietary net energy and protein requirements and of excretions into the environment (IMS Pig). *Animal Science* **77,** 113-130.

Greer, A. W., M. Stankiewicz, N. P. Jay, R. W. McNulty, and A. R. Sykes. 2005. The effect of concurrent corticosteriod induced immuno-suppression and infection with the intestinal parasite *Trichostrongylus colubriformis* on feed intake and utilisation in both immunologically naïve and competent sheep. Anim. Sci. 80: 89 – 99.

Greiner LL, Stahly TS & Stabel TJ (2000) Quantitative relationship of systemic virus concentration on growth and immune response in pigs. *Journal of Animal Science* **78,** 2690 – 2695.

Gross WB (1972) Effect of social stress on occurrence of Marek's disease in chickens. *American Journal of Veterinary Research* **33,** 2275 – 2279.

Grunfeld C, Zhao C, Fuller J, Pollock A, Moser A, Friedman J & Feingold KR (1996) Endotoxin and cytokines Induce expression of leptin, the Ob Gene product, in hamsters: A role for leptin in the anorexia of infection. *Journal of Clinical Investigation* **97,** 2152–2157.

Hale OM. (1986) The influence of internal parasite infections on the performance of growing finishing swine. *Research Bulletin, Georgia Agricultural Experiment Stations* 341, 1-5.

Hale OM & Marti OG (1983) Influence of an experimental infection of swine kidneyworm (*Stephanus dentatus*) on performance of pigs. *Journal of Animal Science* **56,** 616-620.

Hart BL (1988) Biological basis of the behaviour of sick animals. *Neuroscience and Biobehavioural Reviews.* **12,** 123 – 137.

Hasday JD, Garrison A, Singh IS, Standiford T, Ellis GS, Rao S, He Ju-Ren, Rice P & Frank M (2003) Febrile-range hyperthermia augments pulmonary neutrophil recruitment and amplifies pulmonary oxygen toxicity. *American Journal of Pathology* **162,** 2005 – 2017.

Heegard PMH, Klausen J, Nielsen JP, Gonzalez-Ramon N, Pineiro M, Lampreave F & Alava MA (1998) The porcine acute phase response to infection with actinobacillus

pleuropneumoniae. Haptoglobin, C-reactive protein, major acute phase protein and serum amyloid A protein are sensitive indicators of infection. *Comparative Biochemistry and Physiology* **119B**, 365 - 373.

Hein H (1968) The pathogenic effects of Eimeria acervulina in young chicks. *Experimental Parasitology* **22,** 1-11.

Heger J, van Phung T & Krizova L (2002) Efficiency of amino acid utilisation in the growing pig at sub-optimal levels of intake: lysine, threonine, sulphur amino acids and tryptophan. *Journal of Animal Physiology and Animal Nutrition* **86,** 153 – 165.

Heger J, van Phung T, Krizova L, Sustala M & Simecek K (2003) Efficiency of amino acid utilisation in the growing pig at sub-optimal levels of intake: branched chain-chain amino acids, histidine and phenylalanine + tyrosine. *Journal of Animal Physiology and Animal Nutrition* **87,** 52 – 65.

Henken AM & Brandsma (1982) The effect of environmental temperature on immune response and metabolism of the young chicken. 2. Effect of the immune response to sheep red blood cells on energy metabolism. *Poultry Science* **61,** 1667 – 1673.

Henken AM, Ploeger HW, Graat EAM & Carpenter TE (1994a) Description of a simulation model for the population dynamics of *Eimeria acervulina* infection in broilers. *Parasitology* **108,** 503 – 512.

Henken AM, Ploeger HW, Graat EAM & Carpenter TE (1994b) Description of a model to simulate effects of *Eimeria acervulina* infection on broiler production. *Parasitology* **108,** 513 – 518.

Henryon M, Berg P, Jensen J & Andersen S (2001) Genetic variation for resistance to clinical and sub-clinical diseases exists in growing pigs. *Animal Production* **73,** 375 – 387.

Hoshen MB, Heinrich R, Stein WD & Ginsburg H (2000) Mathematical modelling of the within-host dynamics of *Plasmodium falciparum*. *Parasitology* **121,** 227 – 235.

Houdijk JGM & Athanasiadou SL (2003) Direct and indirect effects of host nutrition on ruminant gastrointestinal nematodes. *Proceedings of the VI International Symposium on the nutrition of herbivores*: Matching herbivore nutrition to ecosystems biodiversity. [t'Mannetje, L; Ramirez-Aviles, L; Sandoval-Castro, CA; Ku-vera, JC, eds] Merida, Mexico.

Houdijk JGM, Jessop NS & Kyriazakis I (2001) Nutrient partitioning between reproductive and immune functions in animals. *Proceedings of the Nutrition Society* **60,** 515 – 525.

Houdijk JGM, Anderson DH & Kyriazakis I (2005) Induction of experimental sub-clinical post-weaning colibacillosis in pigs. Proceedings of the British Society of Animal Science **47,** 84.

Hudson, DJ (1966) Piece-wise linear regression analysis. *Journal of Americal Statistics Association* **61,** 1097

Huff GR, Huff WE, Balog JM, Rath NC, Anthony NB & Nestor KE (2005) Stress response differences and disease susceptibility reflected by heterophil to lymphocyte ratio in turkeys selected for increased body weight. *Poultry Science* **84,** 709 – 717.

Hulten C, Johansson E, Fossum C & Wallgren P (2003) Interleukin 6, serum amyloid A and haptoglobin as markers of treatment efficacy in pigs experimentally infected with Actinobacillus pleuropneumoniae. *Veterinary Microbiology* **95,** 75 - 89.

Humphrey BD & Klasing KC (2004) Modulation of nutrient metabolism and homeostasis by the immune system. *World's Poultry Science Journal* **60,** 90-100.

Hunter EAL & Grimble RF (1997) Dietary sulphur amino acid adequacy influences glutathione synthesis and glutathione-dependent enzymes during the inflammatory response to endotoxin and and tumour necrosis factor-α in rats. *Clinical Science* **92,** 297 – 305.

Jackson F, Greer AW, Huntley J, McNulty RW, Bartley DJ, Stanley A, Stenhouse L, Stankiewicz M & Sykes AR (2004) Studies using *Teladorsagia circumcincta* in an in vitro direct challenge method using abomasal tissue explants. *Veterinary Parasitology* **124,** 73 – 89.

Johnson W, Roof M, Vaughn E, Christopher-Hennings J, Johnson CR & Murtaugh MP (2004) Pathogenic and humoral immune responses to porcine reproductive and respiratory syndrome virus (PRRSV) are related to viral load in acute infection. *Veterinary Immunology and Immunopathology* **102,** 233 – 247.

Jones HE, Amer PR, Lewis RM & Emmans GC (2004) Economic values for changes in carcass lean and fat weights at a fixed age for terminal sire breeds of sheep in the UK. *Livestock Production Science* **89,** 1-17.

Jiang Q, Cross AS, Singh IS, Chen TT, Viscardi RM & Hasday JD (2000) Febrile core temperature is essential for optimal host defense in bacterial peritonitis. *Infection and Immunity* **68,** 1265 – 1270.

Johnson RW (1998) Immune and endocrine regulation of food intake in sick animals. *Domestic Animal Endocrinology* **15,** 309 – 319.

Kambara T & McFarlane RG (1996) Changes in T cell subpopulations of sheep due to age and dietary protein intake; association with protective immunity to Trichostrongylus colubriformis. *Veterinary Immunology and Immunopathology* **51,** 127 – 135.

Kamerman PR, Brooksbank R, Pitts N & Laburn HP (2001) Heat stress increases the rate of tolerance development to lipopolysaccharide in rats. *Journal of Thermal Biology* **26,** 589 – 594.

Kanis E, de Greef KH, Hiemstra E & van Arendonk JAM (2005) Breeding for societally important traits in pigs. *Journal of Animal Science* **83,** 948 – 957.

Kaplanski J, Magazanik A, Hadas I, Sod-Moriah U & Fraifeld V (2000) Effect of lipopolysaccharide on body temperature and plasma zinc and iron concentrations in rats exposed to different ambient temperatures. *Journal of Thermal Biology* **25,** 35 – 38.

Kemm EH, Siebrits FK & Barnes P (1990) A note on the effect of dietary protein concentration, sex, type and live weight on whole body amino acid composition of growing pig. *Animal Production* **51**, 631 – 634.

Kerr CA, Eamens GJ, Briegel J, Sheehy PA, Giles LR & Jones MR (2003). Effects of combined *Actinobacillus pleuropneumoniae* challenge and change in environmental temperature on production, plasma insulin-like growth factor-1 (IGF-1), and cortisol parameters in growing pigs. Australian Journal of Agricultural Research **54,** 1057-1064.

Kimambo AE, McRae JC, Walker A, Watt CF & Coop RL (1988) Effect of prolonged subclinical infection with Trichostrongylus colubriformis on the performance and nitrogen metabolism of growing lambs. *Veterinary Parasitology* **88,** 191 – 203.

Klasing KC (1998) Avian macrophages: Regulators of local and systemic immune responses. *Poultry Science* **77,** 983 – 989.

Klasing KC & Austic RE (1984) Changes in plasma, tissue, and urinary nitrogen metabolites due to an inflammatory challenge. *Proceedings of the Society for Experimental Biology and Medicine* **176,** 276 – 284.

Klasing KC & Barnes DM (1988) Decreased amino acid requirements of growing chicks due to immunological stress. *Journal of Nutrition* **118,** 1158 – 1164.

Klasing KC & Calvert CC (1999) The care and feeding of an immune system: an analysis of lysine needs. pp 253 – 263 *In 'Protein metabolism and nutrition'* (Eds Lobley GE, White A, MacRae JC) Wagening Press.

Klasing KC, Laurin DE, Peng RK & Fry M (1987) Immunologically mediated growth depression in chicks: Influence of feed intake, corticosterone and interleukin-1. *Journal of Nutrition* 117, 1629 – 1637.

Klir JJ, Shahbazian LM, Matteri RL, Fritsche KL & Becker BA (1997) Effects of thermal environment on response to acute peripheral lipopolysaccharide challenge exposure in neonatal pigs. *American Journal of Veterinary Research* **58,** 364-369.

Knap PW (2000) Stochastic simulation of growth in pigs: relations between body composition and maintenance requirements as mediated through protein turn-over and thermoregulation. *Animal Science* **71,** 11-30.

Knap PW & Bishop SC (2000) Relationships between genetic change and infectious disease in domestic livestock. *Proceedings of the British Society of Animal Science: Occasional Publication* **27,** 65 – 80.

Knap P, Roehe R, Kolstad K, Pomar C & Luiting P (2002) Characterisation of pig genotypes. *Journal of Animal Science* **80** (E. Suppl. 2), E187-E195.

Knox MR & Steel JW (1999) The effects of urea supplementation on production and parasitological responses of sheep infected with *Haemonchus contortus* and *Trichostrongylus colubriformis*. *Veterinary Parasitology* **83,** 123 – 135.

Knura-Deszczka S, Lipperheide C, Petersen B, Jobert JL, Berthelot-Herault F, Kobisch M & Madec F (2002) Plasma haptoglobin concentration in swine after challenge with Streptococcus suis. *Journal of Veterinary Medicine Series B* **49,** 240-244.

Koutsos EA & Klasing KC (2001) Interactions between the immune system, nutrition and productivity of animals. Pages 173-190 In *Recent Advances in Animal Nutrition* [PC Garnsworthy and J Wiseman, eds] Nottingham University Press, Nottingham, UK.

Koyama K, Chen G, Lee Y & Unger RH (1997) Tissue triglycerides, insulin resistance, and insulin production: implications for hyperinsulinemia of obesity. *American Journal of Physiology Endocrinology and Metabolism* **273,** 708-713.

Kreukniet MB & van der Zijpp (1989) Effects of different doses of sheep erythrocytes on the humoral immune response of chicken lines selected for high or low antibody response. *Poultry Science* **69,** 608 – 614.

Kringel H & Roepstorff (2006) Trichuris suis population dynamics following a primary experimental infection. *Veterinary Parasitology* **139,** 132 – 139.

Książek A, Konarzewski M, Chadzińska M & Cichoń M (2003) Costs of immune response in cold-stressed laboratory mice selected for high and low basal metabolism rates. *Proceedings of the Royal Society of London Series-B.* **270,** 2025 – 2031.

Kyriazakis I (2003) The control and prediction of feed intake in sickness and in health. In Perspectives in pig science, pp. 381 - 403 [J Wiseman, MA Varley and B Kemp, editors]. Nottingham: Nottingham University Press.

Kyriazakis I & Emmans GC (1992*a*). The effects of varying protein and energy intakes on the growth and body-composition of pigs. 1. The effects of energy-intake at constant, high protein-intake. *British Journal of Nutrition* **68,** 603-613.

Kyriazakis I & Emmans GC (1992*b*) The effects of varying protein and energy intakes on the growth and body-composition of pigs. 2. The effects of varying both energy and protein-intake. *British Journal of Nutrition* **68,** 615-625.

Kyriazakis I, Emmans GC & McDaniel R (1993) Whole body amino acid composition of the growing pig. *Journal of Science Food and Agriculture* **62,** 29 – 33.

Kyriazakis I, Dotas D & Emmans GC (1994) The effect of breed on the relationship between feed composition and the efficiency of protein utilisation in pigs. *British Journal of Nutrition* **71**, 849 – 859.

Kyriazakis I, Oldham JD, Coop RL & Jackson F (1994) The effect of subclinical infection on the diet selection of growing sheep. *British Journal of Nutrition* 72, 665 – 677.

Kyriazakis I & Emmans GC (1995) The voluntary feed-intake of pigs given feeds based on wheat bran, dried citrus pulp and grass meal, in relation to measurements of feed bulk. *British Journal of Nutrition* **73,** 191-207.

Kyriazakis I, Emmans GC & with the technical assistance of D.H. Anderson (1995) Do breeds of pig differ in the efficiency with which they use a limiting protein supply? *British Journal of Nutrition* **74**, 183 – 195.

Kyriazakis I, Anderson DH, Oldham JD, Coop RL & Jackson F (1996) Long-term subclinical infection with *Trichostrongylus colubriformis*: effects on feed intake, diet selection and performance of growing lambs. *Veterinary Parasitology* **61,** 297 – 313.

Kyriazakis I, Anderson DH, Coop RL, Jackson F (1996) The pathophysiology and development of immunity during long-term subclinical infection with Trichostrongylus colubriformis of sheep receiving different nutritional treatments. *Veterinary Parasitology* **65,** 41 – 54.

Kyriazakis I, Tolkamp BJ & Hutchings MR (1998) Towards a functional explanation for the occurrence of anorexia during parasitic infections. *Animal Behaviour* **56,** 265 – 274.

Langhans W (2000) Anorexia of infection: current prospects. *Nutrition* **16,** 996 – 1005.

Le Floc'h N, Melchior D & Obled C (2004) Modification of protein and amino acid metabolism during inflammation and immune system activation. *Livestock Production Science* **87,** 37 – 45.

Le Jambre LF (1995) Relationship of blood loss to worm numbers, biomass and egg production in Haemonchus infected sheep. *International Journal of Parasitology* **25,** 269 – 273.

Lee KA & Klasing KC (2004) A role for immunology in invasion biology. *TRENDS in Ecology and Evolution* **19,** 523 – 529.

Lee JE, Austic RE, Naqi SA, Golemboski KA & Dietert RR (2002) Dietary arginine intake alters avian leukocyte population distribution during infectious bronchitis challenge. *Poultry Science* **81,** 793 – 798.

Leibholz J (1985) An evaluation of total and digestible lysine as a predictor of lysine availability in protein concentrates for young pigs. *British Journal of Nutrition* **53,** 615 – 624.

Lewis RM, Emmans GC, Dingwall WS & Simm G (2002) A description of the growth of sheep and its genetic analysis. *Animal Science* **74,** 51 – 62.

Li Z, Nestor KE, Saif YM & Anderson JW (2000) Antibody responses to sheep red blood cell and *Brucella abortus* antigens in a turkey line selected for increased body weight and its randombred control. *Poultry Science* **79,** 804 – 809.

Lipsitch M, Herre EA & Nowak MA (1995) Host population structure and the evolution of virulence: a 'law of diminishing returns'. *Evolution* **49,** 743 – 748.

Liu SM, Masters DG & Adams NR (2003) Potential impact of nematode parasitism on nutrient partitioning for wool production, growth and reproduction in sheep. *Australian Journal of Experimental Agriculture* **43,** 1409 – 1417.

Lochmiller RL & Deerenberg C (2000) Trade-offs in evolutionary immunology: just what is the cost of immunity? *Oikos* **88,** 87 – 98.

Loughmiller JA, Spire MF, Dritz SS, Fenwick BW, Hosni MH & Hogge SB (2001) Relationship between mean body surface temperature measured by use of infrared thermography and ambient temperature in clinically normal pigs and pigs inoculated with Actinobacillus pleuropneumoniae. *American Journal of Veterinary Research* **62,** 676 – 681.

Lovatto PA, Sauvant D (2003) Modeling homeorhetic and homeostatic controls of pig growth. *Journal of Animal Science* **81,** 683-696

Mackowiak PA, Bartlett JG, Borden EC, Goldblum SE, Hasday JD, Munford RS, Nasraway SA, Stolley PD & Woodward TE (1997) Concepts of fever: Recent advances and lingering dogma. *Clinical Infectious Diseases* **25,** 119 – 138.

Magnusson U, Bosse J, Mallard BA, Rosendal S & Wilkie BN (1997) Antibody response to Actinobacillus pleuropneumoniae antigens after vaccination of pigs bred for high and low immune response. *Vaccine* **15,** 997 – 1000.

Magnusson U, Wilkie BN, Mallard BA, Rosendal S & Kennedy B (1998) Mycoplasma hyorhinis infection of pigs selectively bred for high and low immune response. *Veterinary Immunology and Immunopathology* **61,** 83 – 96.

Magnusson U, Wilkie BN & Mallard B (1999) Interferon-alpha and haptoglobin in pigs selectively bred for high and low immune response and infected with Mycoplasma hyorhinis. *Veterinary Immunology and Immunopathology* **68,** 131 – 137.

Mahan DC & Shields Jr RG (1998) Essential and non-essential amino acid composition of pigs from birth to 145 kilograms of body weight, and comparison to other studies. *Journal of Animal Science* **76**, 513 – 521.

Mansour MM, Rowan TG, Dixon JB & Carter SD (1991) Immune modulation by Ostertagia ostertagi and the effects of diet. *Veterinary Parasitology* **39,** 321 – 332.

Mansour MM, Rowan TG, Dixon JB & Carter SD (1992) Modulation of calf immune responses by Ostertagia ostertagi: the effect of diet during trickle infection. *Veterinary Immunology and Immunopathology* **33,** 261 – 269.

Martin LB, Scheuerlein A & Wikelski M (2002) Immune activity elevates energy expenditure of house sparrows: a link between direct and indirect costs? *Proceedings of the Royal Society of London Series B* **270,** 153 – 158.

Mashaly MM, Heetkamp MJW, Parmentier HK & Schrama JW (2000) Influence of genetic selection for antibody production against sheep blood cells on energy metabolism in laying hens. *Poultry Science* **79,** 519 – 524.

Marquardt DW (1963) An Algorithm for Least Squares Estimation of Parameters. *Journal of the Society of Industrial and Applied Mathematics* **11**, 431-441.

May, R. M. and M. A. Nowak. 1995. Coinfection and the evolution of parasite virulence. Proc. R. Soc. Lond. B. Biol. Sci. 261: 209 – 215.

McCarty DO, Kluger MJ & Vander AJ (1984) The role of fever in appetite suppression after endotoxin administration. *American Journal of Clinical Nutrition* **40,** 310 – 316.

McRae JC, Smith JS, Sharman GAM and Corrigall W (1982) Energy metabolism of lambs infected with Trichostrongylus colubriformis. In *Energy metabolism of Farm Animals* (ed A. Ekern & RF Sundstol). EEAP Publication No **29** p112-115.

McRae JC (1993) Metabolic consequences of metabolism. *Proceedings of the Nutrition Society* **52,** 121-130.

Miller DS & Payne PR (1961) Problems in the prediction of protein values of diets. *British Journal of Nutrition* **15,** 11 – 19.

Miller FM, Blair HT, Reynolds GW & Revell DK (1998) The role of cysteine in the increased parasite susceptibility of Romney sheep selected for hogget fleece-weight. *Proceedings of the New Zealand Society of Animal Production* **58,** 150 – 153.

Miller FM, Blair HT, Birtles MJ, Reynolds GW, Gill HS & Revell DK (2000) Cysteine may play a role in the immune response to internal parasites in sheep. *Australian Journal of Agricultural Research* **51,** 793 – 799.

Miyajima A, Hara T & Kitamura T (1992) Common subunits of cytokine receptors and the functional redundancy of cytokines. *Trends Biochem. Sci* **17,** 378 – 382.

Mohn S, Gillis AM, Moughan PJ & de Lange CFM (2000) Influence of dietary lysine and energy intakes on body protein deposition and lysine utilisation in the growing pig. *Journal of Animal Science* **78**, 1510 – 1519.

Mohn S & de Lange CFM (1998) The effect of body weight on the upper limit to protein deposition in a defined population of growing gilts. *Journal of Animal Science* **76,** 124 – 133.

Moughan PJ & Smith WC (1987) Whole body amino acid composition of the growing pig. *New Zealand Journal of Agricultural Research* **30**, 301 – 303.

Moughan PJ, Smith WC & Pearson G (1987) Description and validation of a model simulating growth in the pig (20-90 kg liveweight). *New Zealand Journal of Agricultural Research* **30,** 481-489.

Moughan PJ (1999) Protein metabolism in the growing pig. In *A quantitative biology of the pig* pp 299-331 (I Kyriazakis, editor). Wallingford, CAB International.

Moughan PJ (2003*a*) Simulating the partitioning of dietary amino acids: New directions. *Journal of Animal Science* **81,** E60-E67.

Moughan PJ (2003*b*) Amino acid availability: aspects of chemical analysis and bioessay methodology. *Nutrition Research Reviews* **16**, 127 – 141.

Murata H, Shimada N & Yoshioka M (2004) Current research on acute phase proteins in veterinary diagnosis: an overview. *The Veterinary Journal* **168,** 28 – 40.

Newsholme P (2001) Why is L-glutamine metabolism important to cells of the immune system in health, postinjury, surgery or infection. *Journal of Nutrition* **131,** 2515S – 2522S.

Nieuwhof GJ & Bishop SC (2005) Costs of the major endemic diseases of sheep in Great Britain and the potential benefits of reduction in disease impact. *Animal Science* **81,** 23 – 29.

Noblet J, Fortune H, Shi XS & Dubois S (1994) Prediction of net energy value of feeds for growing pigs. *Journal of Animal Science* **72,** 344-354.

NRC (1998) *Nutrient requirements of swine*. 10th edition. National Academy Press, Washington DC.

Ots I, Kerimow AB, Ivankina EV & Ilyina A (2001) Immune challenge affects basal metabolic activity in wintering great tits. *Proceedings of the Royal Society of London Series B* **268,** 1175 – 1181.

Paling RW, Moloo SK, Scott JR, Gettinby G, Mcodimba FA & Murray M (1991) Susceptibility of N'Dama and Boran cattle to sequential challenges with tsetse-transmitted clones of Trypanosoma congolense. *Parasite Immunology* **13,** 427 – 445.

Papet I, Ruot B, Breuille D, Walrand S, Farges M-C, Vasson M-P & Obled C (2002) Bacterial infection affects protein synthesis in primary lymphoid tissues and circulating lymphocytes of rats. *Journal of Nutrition* **132,** 2028 – 2032.

Parkins JJ & Holmes PH (1989) Effects of gastrointestinal helminth parasites on ruminant nutrition. *Nutrition Research Reviews* **2,** 227 – 246.

Parkins JJ, Taylor LM, Holmes PH, Bairden K, Skalman SK & Armour J (1990) pathophysiological and parasitological studies on a concurrent infection of *Ostertagia ostertagia* and *Cooperia oncophora* in calves. Research in Veterinary Science **48,** 201 – 208.

Parks JR (1982) A theory of feeding and growth of animals. Springer Verlag, Berlin, Germany.

Pernthaner A, Stankiewicz M, Bisset SA, Jones WE, Cabaj W & Pulford HD (1995) The immune responsiveness of Romney sheep selected for resistance or susceptibility to gastrointestinal nematodes: lymphocyte blastogenic activity, eosinophilia and total white blood cell counts. *International Journal of Parasitology* **25,** 523 – 529.

Pilorz V, Jackel M, Knudsen K & Trillmich F (2005) The cost of a specific immune response in young guinea pigs. *Physiology & Behaviour* **85,** 205 – 211.

Pomar C, Kyriazakis I, Emmans GC & Knap, PW (2003) Modeling stochasticity: Dealing with population rather than individual pigs. *Journal of Animal Science* **81**, E178 – E186.

Poppi DP, McRae JC, Brewer A & RL Coop (1986) Nitrogen transactions in the digestive tract of lambs exposed to the intestinal parasite, Trichostrongylus colubriformis. *British Journal of Nutrition* **55,** 593 – 602.

Powanda MC, Wannemacher RW & Cockerell GL (1972) Nitrogen metabolism and protein synthesis during pneumococcal sepsis in rats. *Infection and Immunity* **6,** 266 – 271.

Powanda MC, Cockerell GL, Moe JB, Abeles FB, Pekarek RS and Canonico PG (1975) Induced metabolic sequelae of tularemia in the rat: correlation with tissue damage. *American Journal of Physiology*. **229,** 479 – 483.

Powanda MC (1977) Changes in body balance of nitrogen and other key nutrients: description and underlying mechanisms. *American Journal of Clinical Nutrition* **30,** 1254 – 1268.

Powanda MC & Beisel WR (2003) Metabolic effects of infection on protein and energy status. *Journal of Nutrition* **133,** 322S-327S.

Praharaj NK, Dunnington EA & Siegel PB (1995) Growth, immunoresponsiveness, and disease resistance of diverse stocks of chickens reared under two nutritional regimens. *Poultry Science* **74,** 1721 – 1729.

Quiniou N, Noblet J, van Milgen J & Dourmad J-Y (1995) Effect of energy intake on performance, nutrient and tissue gain and protein and energy utilisation in growing boars. *Animal Science* **61,** 133 – 143.

Quiniou N, Dourmad J-Y & Noblet J (1996) Effect of energy intake on the performance of different types of pig from 45 – 100 kg body weight. 1. Protein and lipid deposition. *Animal Science* **63,** 277 – 288.

Raina N, Matsui J & Jeejeebhoy KN (2000) Nutritional and metabolic effects of the endotoxin bacterial lipopolysaccharide in orally and parenterally fed rats. *American Journal of Clinical Nutrition* **71,** 835 – 843.

Rauw WM, Kanis E, Noordhuizen-Stassen EN & Grommers FJ (1998) Undesirable side effects of selection for high production efficiency in farm animals: a review. *Livestock Production Science* **56**, 15-33

Reddy NR, Wilkie BN, Borgs P & Mallard BA (2000) Cytokines in Mycoplasma hyorhinis-induced arthritis in pigs bred selectively for high and low immune responses. *Infection and Immunity* **68,** 1150 – 1155.

Reeds PJ, Fjeld CR & Jahoor F (1994) Do the differences between the amino acid compositions of acute-phase and muscle proteins have a bearing on nitrogen loss in traumatic states? *Journal of Nutrition* **124,** 906 – 910.

Rigby MC, Hechinger RF & Stevens L (2002) Why should parasite resistance be costly? *TRENDS in Parasitology* **18,** 116 – 120.

Rivest J, Bailleul J & Pomar, C. (1996). Incorporation of a net energy system into a dynamic pig growth model. *Journal of Animal Science* **74,** (suppl. 1) 196 (abstr.).

Roberts NE & Almond GW (2003) Infection of growing swine with porcine reproductive and respiratory syndrome virus and Mycoplasma hyopneumoniae – Effects on growth, serum metabolites, and insulin-like growth factor-1. *Canadian Veterinary Journal* **44,** 31-37.

Ruot B, Breuille D, Rambourdin F, Bayle G, Capitan P & Obled C (2000) Synthesis rate of plasma albumin is a good indicator of liver albumin synthesis in sepsis. *American Journal of Physiology and Endocrinology and Metabolism.* **279:** E244-E251.

Sakamoto M, Fujisawa Y & Nishioka K (1998) Physiologic role of the complement system in host defense, disease, and malnutrition. *Nutrition* **14,** 391 – 398.

Sakumoto R, Kasuya E, Komatsu T & Akita T (2003) Central and peripheral concentrations of tumour necrosis factor-α in Chinese Meishan pigs stimulated with lipopolysaccharide. *Journal of Animal Science* **81,** 1274 – 1280.

Sanchez-Cuenca J, Martin CM, Pellicer A, & Simon C (1999) Cytokine pleitropy and redundancy – gp130 cytokines in human implantation. *Immunology Today* **20,** 57 – 59.

Samuels SE & Baracos VE (1992) Catch-up growth following Escherichia coli infection in weanling rats. *Metabolism* **41,** 208 – 215.

Sandberg FB, Emmans GC & Kyriazakis I (2005a) Partitioning of limiting protein and energy in the growing pig: description of the problem, possible rules and their qualitative evaluation. *British Journal of Nutrition* **93,** 205 – 212.

Sandberg FB, Emmans GC & Kyriazakis I (2005b) Partitioning of limiting protein and energy in the growing pig: testing quantitative rules against experimental data. *British Journal of Nutrition* **93,** 213 – 224.

Sandberg FB, Emmans GC & Kyriazakis I (2006) A model for predicting food intake of growing animals during exposure to pathogens. *Journal of Animal Science* **84,** 1552 – 1566.

Sato T, Meguid MM, Quinn RH, Zhang L & Chen C (2001) Feeding behaviour during sialodacryoadenitis viral infection in rats. Physiology Behaviour **72,** 721 – 726.

Schinckel AP & de Lange CFM (1996) Characterisation of growth parameters needed as inputs for pig growth models. *Journal of Animal Science* **74,** 2021-2036.

Schinckel AP, Li N, Richert BT, Preckel PV & Einstein ME (2003) Development of a model to describe the compositional growth and dietary lysine requirements of pigs fed ractopamine. *Journal of Animal Science* **81,** 1106 – 1119.

Schinckel AP, Spurlock ME, Richert RT & Weber TE (2003) Modelling health status-environmental effects on swine growth. Book of Abstracts of the 54th Annual Meeting of the European Association for Animal Production pp 355.

Schwartz GJ (2002) Neural-Immune Gut-Brain communication in the anorexia of disease. *Nutrition* **18,** 528 – 533.

Siegel HS & Gross WB (1965) Social grouping, stress and resistance to coliform infection in cockerels. *Poultry Science* **44,** 1530 – 1536.

Sell KM, Crowe SF, Kent S (2003) Lipopolysaccharide induces biochemical alterations in chicks trained on the passive avoidance learning task. *Physiology Behaviour* **78,** 679-688.

Siebrits FK, Kemm EH, Ras MN & Barnes PM (1986) Protein deposition in pigs as influenced by sex, type and live mass. 1. The pattern and composition of deposition. *South African Journal of Animal Science* **16,** 23 – 27.

Siegel PB & Gross WB (1980) Production and persistence of antibodies in chickens to sheep erythrocytes. *Poultry Science* **59,** 1-5.

Sheldon BC & Verhulst S (1996) Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. *TREE* **11,** 317 – 321.

Spurlock ME (1997) Regulation of metabolism and growth during immune challenge: An overview of cytokine function. *Journal of Animal Science* **75,** 1773 – 1783.

Spurlock ME, Frank GR, Willis GM, Kuske JL & Cornelius SG (1997) Effect of dietary energy source and immunological challenge on growth performance and immunological variables in growing pigs. *Journal of Animal Science* **75,** 720 – 726.

Stamataris C, Kyriazakis I & Emmans GC (1991) The performance and body-composition of young pigs following a period of growth retardation by food restriction. *Animal Production* **53,** 373-381.

Steel JW, LEA Symons, and WO Jones (1980) Effects of level of larval intake on the productivity and physiological and metabolic responses of lambs infected with Trichostrongylus colubriformis. *Australian Journal of Agricultural Research* **31,** 821 – 838.

Steel JW, Symons LEA & Jones WO (1982) Effects of a concurrent infection of *Trichostrongylus colubriformis* on the productivity and physiological and metabolic responses of lambs infected with *Ostertagia circumcincta*. *Australian Journal of Agriculture* **33,** 131 – 140.

Steiger M, Senn M, Altreuther G, Werling D, Sutter F, Kreuzer M & Langhans W (1999) Effect of prolonged low-dose lipopolysaccharide infusion on feed intake and metabolism in heifers. *Journal of Animal Science* **77,** 2523 – 2532.

Stewart TB & Hale OM (1988) Losses to internal parasites in swine production. *Journal of Animal Science* **66,** 1548 – 1554.

Susenbeth A (1995) Factors affecting lysine utilisation in growing pigs: an analysis of literature data. *Livestock Production Science* **43,** 193-204.

Sykes AR & Coop RL (1977) Intake and Utilization of Food by Growing Sheep with Abomasal Damage Caused by Daily Dosing with Ostertagia-Circumcincta Larvae. *Journal of Agricultural Science* **88,** 671 – 677.

Sykes AR, Coop RL & Rushton B (1980) Chronic subclinical fascioliasis in sheep: effects on food intake, food utilisation and blood constituents. *Research in Veterinary Science* **28,** 63 – 70.

Sykes AR (2000) Environmental effects on animal production: The nutritional demands of nematode parasite exposure in sheep. *Asian Australasian Journal of Animal Sciences* **13,** 343 – 350.

Sykes AR & Greer AW (2003) Effect of parasitism on the nutritent economy of sheep: an overview. *Australian Journal of Experimental Agriculture* **43,** 1393 – 1398.

Symons LEA & Jones WO (1970) Nematospiroides dubius, Nippostrongylus brasiliensis, and Trichostrongylus colubriformis: Protein digestion in infected mammals. *Experimental Parasitology* **27,** 496 – 506.

Symons LEA & Jones WO (1975) Skeletal muscle, liver and wool protein synthesis by sheep infected by the nematode Trichostrongylus colubriformis. *Australian Journal of Agricultural Research* **26,** 1063 – 1072.

Symons LEA & Jones WO (1978) 5. Trichostrongylus colubriformis: changes of host body mass and protein synthesis in guinea pigs with light and heavy infections. *Experimental Parasitology* **44,** 7 – 13.

Symons LEA, JW Steel, and WO Jones (1981) Effects of larval intake on the productivity and physiological and metabolic responses of lambs infected with Ostertagia circumcincta. *Australian Journal of Agriculture.* **32,** 139 – 148.

Svensson E, Raberg L, Koch C & Hasselquist (1998) Energetic stress, immunosuppression and the costs of an antibody response. *Functional Ecology* **12,** 921-919.

Swiergiel AH, Smagin GN & Dunn AJ (1997) Influenza virus infection of mice induces anorexia: comparison with endotoxin and interleukin-1 and the effects of indomethacin. *Phar.Biochem. Behav* **57,** 389 – 396.

Takhar BS & Farrell DJ (1979a) Energy and nitrogen metabolism of chickens infected with either Eimeria acervulina or Eimeria tenella. *British Poultry Science* **20,** 197 – 211.

Takhar BS & Farrell DJ (1979b) Energy and nitrogen metabolism of chickens subjected to infection and reinfection with Eimeria acervulina. *British Poultry Science* **20,** 213 - 224.

Taylor CS (1980) Live-weight growth from embryo to adult in domesticated mammals. *Animal Production* **31,** 223 – 235.

Taylor LM, Parkins JJ, Armour J, Holmes PH, Bairden K, Ibarra-Silva I, Skalman SK & McWilliam PN (1989) Pathophysiological and parasitological studies on *Ostertagia ostertagi* infections in calves. *Research Veterinary Science* **46,** 218 – 225.

Taylor-Robinson AW (2000) Increased production of acute-phase proteins corresponds to the peak parasitemia of primary malaria infection. *Parasitology International* **48,** 297 – 301.

Timms R, Colegrave N, Chan BHK & Read AF (2001) The effect of parasite dose on disease severity in rodent malaria Plasmodium chabaudi. *Parasitology* 123, 1 – 11.

Tsiagbe VK, Cook ME, Harper AE & Sunde ML (1987) Enhanced immune responses in broiler chicks fed methionine-supplemented diets. *Poultry Science* **66,** 1147 – 1154.

Tuma RA & Pamer EG (2002) Homeostasis of naïve, effector and memory CD8 T cells. *Current Opinions Immunology* **14,** 348 – 353.

Turner JL, Dritz SS, Higgins JJ, Herkelman KL & Minton JE (2002a) Effect of a *Quillaja saponaria* extract on growth performance and immune function of weanling pigs challenged with *Salmonella typhimurium*. *Journal of Animal Science* **80,** 1939 – 1946.

Turner JL, Dritz SS, Higgins JJ & Minton JE (2002b) Effects of *Ascophyllum nodosum* extract on growth performance and immune function on young pigs challenged with *Salmonella typhimurium*. *Journal of Animal Science* **80,** 1947 – 1953.

Valles A, Marti O, Garcia A & Armario A (2000) Single exposure to stressors causes long-lasting, stress-dependent reduction of feed intake in rats. *Am. J. Phys. Int. Comp. Phys* **279,** R1138 – R1144.

van Dam JTP (1996) The interaction between nutrition and metabolism in West African Dwarf Goats infected with Trypanosomes. PhD Thesis, Wageningen University, Wageningen, Netherlands.

van Dam JTP, Schrama JW, Vreden A, Verstegen MWA, Wensing T, van der Heide D, & Zwart D (1997) The effect of previous growth retardation on energy and nitrogen metabolism of goats infected with *Trypanosoma vivax*. *British Journal of Nutrition* **77,** 427 – 441.

van Dam JTP, Hofs P, Tolkamp BJ & Zwart D (1998) The effect of the quality of roughage on the course of *Trypanosoma vivax* infection in West African dwarf goats. 1. Organic matter intake, body weight changes and efficiency of nitrogen metabolism. Livestock Production Science **53,** 69 – 80.

van Diemen PM, Henken AM, Schrama JW, Brandsma HA & Verstegen MWA (1995) Effects of atrophic rhinitis by *Pasteurella multocida* toxin on heat production and activity of pigs kept under different climatic conditions. *Journal of Animal Science* **73,** 1658 – 1665.

van Heugten E, Coffey MT & Spears JW (1996) Effects of immune challenge, dietary energy density, and source of energy on performance and immunity in weanling pigs. *Journal of Animal Science* **74,** 2431 – 2440.

van Houtert MFJ, Barger IA, Steel JW, Windon RG & Emery DL (1995) Effects of dietary protein intake on responses of young sheep to infection with Trichostrongylus colubriformis. *Veterinary Parasitology* **56,** 163 – 180.

van Houtert MFJ & Sykes AR (1996) Implications of nutrition for the ability of ruminants to withstand gastrointestinal nematode infections. *International Journal for Parasitology* **26,** 1151 – 1168.

van Milgen J & Noblet J (1999) Energy partitioning in growing pigs: the use of a multivariate model as an alternative for the factorial analysis. *Journal of Animal Science* **77,** 2154-2162.

van Milgen J, Quiniou N & Noblet J (2000) Modelling the relation between energy intake and protein and lipid deposition in growing pigs. *Animal Science* **71,** 119-1130.

van Milgen J & Noblet J (2003) Partitioning of energy intake to heat, protein, and fat in growing pigs. *Journal of Animal Science* **81,** E86-E93.

Velu JG, Baker DH and Scott HM (1971) Protein and energy utilisation by chicks fed graded levels of a balanced mixture of crystalline amino acids. *Journal of Nutrition* **101,** 1249 – 1256.

Vercruysse J & Claerebout E (2001) Treatment vs non-treatment of helminth infections in cattle: defining the threshold. Veterinary Parasitology **98,** 195-214.

Verhulst S, Dieleman SJ and Parmentier HK (1999) A tradeoff between immunocompetence and sexual ornamentation in domestic fowl. *Proceedings of the National Academy of Sciences* **96,** 4478 – 4481.

Verstegen MWA, Zwart D, van der Hel, W, Brouwer BO & Wensing T (1991) Effect of trypanosoma vivax infection on energy and nitrogen metabolism of west african dwarf goats. *Journal of Animal Science* **69,** 1667 – 1677.

Wagland BM, Steel JW & Dineen JK (1982) Effect of continuous challenge with *Trichostrongylus colubriformis* on live weight gain and wool growth of resistant sheep. Proceedings of Australian Society Animal Production **14,** 39 – 44.

Wagland BM, Steel JW, Windon RG & Dineen JK (1984) The response of lambs to vaccination and challenge with *Trichostrongylus colubriformis*: effect of plane of nutrition on, and the inter-relationships between immunological responsiveness and resistance. Int. J. Para. **14,** 39 – 44.

Wakelin D (2000) Rodent models of genetic resistance to parasitic infections. In Breeding for disease resistance in farm animals, pp. 107 – 126 [RFE Axford, SC Bishop, FW Nicholas and JB Owen, editors]. Wallingford: CABI Publishing.

Walkden-Brown SW & Kahn LP (2002) Nutritional modulation of resistance and resilience to gastrointestinal nematode infection – A review. *Asian-Australasian Journal of Animal Science.* **6,** 912 – 924.

Walkden-Brown SW & Eady SJ (2003) Nutritional influences on the expression of genotypic resistance to gastrointestinal nematode infection in sheep. *Australian Journal of Experimental Agriculture* **43**, 1445 – 1454.

Wang TC & Fuller MF (1989) The optimum dietary amino acid pattern for growing pigs. 1. Experiments by amino acid deletion. *British Journal of Nutrition* **62,** 77-89.

Wannemacher RW, Powanda MC, Pekarek RS & Beisel WR (1971) Tissue amino acid flux after exposure of rats to *Diplococcus pneumoniae*. *Infection and Immunity* **4,** 556 – 562.

Wannemacher RW, Powanda MC & Dinterman RE (1974) Amino acid flux and protein synthesis after exposure of rats to either *Diplococcus pneumoniae* or *Salmonella typhimurium*. *Infection and Immunity* **10,** 60 – 65.

Webel DM, Johnson RW & Baker DH (1998a). Lipopolysaccharide-induced reductions in body weight gain and feed intake do not reduce the efficiency of arginine utilization for whole-body protein accretion in the chick. *Poultry Science* **77,** 1893-1898.

Webel DM, Johnson RW & Baker DH (1998b) Lipopolysaccharide-induced reductions in food intake do not decrease the efficiency of lysine and threonine utilization for protein accretion in chickens. *Journal of Nutrition* **128,** 1760-1766.

Wilkie BN and BA Mallard (1998) Multi-trait selection for immune response: A possible alternative strategy for enhanced livestock health and productivity. Pages 29–38 in Progress in Pig Science. J. Wiseman, ed. Nottingham Univ. Press, Nottingham, U.K.

Wellock IJ, Emmans GC & Kyriazakis I (2003a) Modelling the effects of thermal environment and dietary composition on pig performance: model logic and concepts. *Animal Science* **77**, 255-266.

Wellock IJ, Emmans GC and Kyriazakis I (2003b) Predicting the consequences of social stressors on pig feed intake and performance. *Journal of Animal Science* **81,** 2995 – 3007.

Wellock IJ, Emmans GC & Kyriazakis I (2004) Describing and predicting potential growth in the pig. *Animal Science* **78,** 379 – 388.

Wellock IJ, Fortomaris PD, Houdijk PGM & Kyriazakis I (2006) The effect of dietary protein supply on the performance and risk of post weaning enteric disorders in newly weaned pigs. *Animal Science* **82,** 327 – 335.

Whittemore CT & Fawcett RH (1974) Model responses of the growing pig to the dietary intake of energy and protein. *Animal Production* **19**, 221-231.

Whittemore CT & Fawcett RH (1976) Theoretical aspects of a flexible model to simulate protein and lipid growth in pigs. *Animal Production* **22,** 87-96.

Whittemore CT (1983) Development of recommended energy and protein allowances for growing pigs. *Agricultural Systems* **11**, 159-186.

Whittemore CT (1995) Modelling the requirement of the young growing pig for dietary protein. *Agricultural Systems* **47,** 415-425.

Whittemore CT, Green DM & Knap PW (2001) Technical review of the energy and protein requirements of growing pigs: protein. *Animal Science* **73,** 363 – 373.

Whittemore CT & Green DM (2002) The description of the rate of protein and lipid growth in pigs in relation to live weight. *Journal of Agricultural Science* **138,** 415-423.

Wieland TM, Lin X & Odle J (1993) Utilisation of medium-chain triglycerides by neonatal pigs: effect of emulsification and dose delivered. *Journal of Animal Science* **71,** 1863-1868.

Williams NH, Stahly TS & Zimmerman DR (1997a) Effect of chronic immune system activation on the rate, efficiency and composition of growth and lysine needs of pigs fed from 6 to 27kg. *Journal of Animal Science* **75,** 2463 – 2471.

Williams NH, Stahly TS & Zimmerman DR (1997b). Effect of chronic immune system activation on body nitrogen retention, partial efficiency of lysine utilization, and lysine needs of pigs. *Journal of Animal Science* **75,** 2472-2480.

Williams NH, Stahly TS & Zimmerman DR (1997c) Effect of level of chronic immune system activation on the growth and dietary lysine needs of pigs fed from 6 to 112 kg. *Journal of Animal Science* **75,** 2481-2496.

Williamson JF, Blair HT, Garrick DJ, Pomroy WE, Douch PGC, Green RS & Simpson HV (1995a) Parasitism and production in fleece-weight-selected and control sheep. *New Zealand Journal of Agricultural Research* **38,** 381 – 387.

Williamson JF, Blair HT, Garrick DJ, Pomroy WE, Douch PGC & Green RS (1995b) Parasitological characteristics of fleece-weight-selected and control sheep. *New Zealand Journal of Agricultural Research* **38,** 389 – 397.

Willis GM & Baker DH (1981a) Eimeria acervulina infection in the chicken: A model system for estimating nutrient requirements during coccidiosis. *Poultry Science* **60,** 1884 – 1891.

Willis GM & Baker DH (1981b) Eimeria acervulina infection in the chicken: Sulphur amino acid requirement of the chick during acute coccidiosis. *Poultry Science* **60,** 1892 – 1897.

Windon RG, Dineen JK, Gregg P, Griffiths DA & Donald AD (1984) The role of thresholds in the response of lambs to vaccination with irradiated *Trichostrongylus colubriformis* larvae. *Int. J. Para.* **14,** 423 – 428.

Wu G, Ott TL, Knabe AD & Fuller WB (1999) Amino acid composition of the fetal pig. *Journal of Nutrition* **129**, 1031 – 1038.

Yu F, Bruce LA, Calder AG, Milne E, Coop RL, Jackson F, Horgan GW & MacRae JC (2000). Subclinical infection with the nematode Trichostrongylus colubriformis increases

gastrointestinal tract leucine metabolism and reduces availability of leucine for other tissues. *Journal of Animal Science* **78,** 380 – 390.

Yunis R, Ben-David A, Heller ED & Cahaner A (2000) Immunocompetence and viability under commercial conditions of broiler groups differing in growth rate and in antibody response to Escherichia coli vaccine. *Poultry Science* 79, 810 – 816.

Zeisberger E (1999) From humoral fever to neuroimmunological control of fever. *Journal of Thermal Biology* **24,** 287 – 326.

Zijlstra RT, Donovan SM, Odle J, Gelberg HB, Petschow BW & Gaskins HR (1997) Protein-energy malnutrition delays small-intestinal recovery in neonatal pigs infected with rotavirus. *Journal of Nutrition* **127,** 1118-1127.

Zwart D, Brouwer BO, van der Hel W, van den Akker HN & Verstegen MWA (1991) Effect of trypanosoma vivax infection on body temperature, feed intake, and metabolic rate of west african dwarf goats. *Journal of Animal Science* **69,** 3780 – 3788

Publication List

1) Fredrik B Sandberg, Gerry C Emmans and Ilias Kyriazakis (2005a) Partitioning of limiting protein and energy in the growing pig: Description of the problem, possible rules and their qualitative evaluation. *British Journal of Nutrition* 93, 205 – 212.

(2) Fredrik B Sandberg, Gerry C Emmans and Ilias Kyriazakis (2005b) Partitioning of limiting protein and energy in the growing pig: Testing quantitative rules against experimental data. *British Journal of Nutrition* 93, 213 – 224.

(3) Fredrik B Sandberg, Gerry C Emmans and Ilias Kyriazakis (2005) Predicting the voluntary food intake of growing animals during exposure to pathogens of different kinds and doses. *British Society of Animal Science*, Abstract pp 15.

(4) Fredrik B Sandberg, Gerry C Emmans and Ilias Kyriazakis (2006) A model for predicting food intake of growing animals during exposure to pathogens. Journal of Animal Science, *Journal of Animal Science* 84, 1552 – 1566.

(5) Ilias Kyriazakis and Fredrik Sandberg (2006) The problem of predicting the partitioning of scarce resources during sickness and health in pigs. In *Mechanistic modelling in pig and poultry production*. Edited by C Fisher; R Gous; T Morris, CABI Publishing.